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2 Vineyard soil bacterial diversity and composition revealed by 16S rRNA genes: Differentiation
3 by vineyard management

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Abstract

Little is known about the hierarchical effects of management practices, soil attributes and location factors on structure of vineyard soil microbiota. A hierarchical effect occurs when the specific influence of an experimental factor (e.g. cover crop type, compost application) on soil-borne bacterial communities is greater within a subset composing the larger set but not across the entire set (e.g. bacterial communities only respond to a management practice within a subset of soil types but not across the entire set composed of all soil types). To address this concept, we measured differences in soil bacterial and archaeal diversity in wine-grape vineyard soils throughout Napa Valley, California. We describe how vineyard management practices influence soil resources, which in turn determine shifts in soil-borne bacterial communities. Soil bacterial communities were structured with respect to management practices, specifically cover crop presence and cover crop mix, tillage, and agricultural system designation, i.e. conventional, organic and biodynamic production systems. Distinctions with respect to management were associated with differences in pH and soil resource pools: total carbon and total nitrogen of the <53 and 53-250 μm particulate organic matter fractions, and potentially mineralizable nitrogen. Findings in this study suggest management practices in vineyard production systems directly influence soil microbial community structure, as mediated by shifts in soil resource pools. However, hierarchical effects occur, in which β -diversity is more strongly affected by specific management practices only within certain soil types, tillage or no-till soils or winegrowing region. This work allows for subsequent assessments of interrelationships of vineyard management, microbial biodiversity and their combined influence on soil quality, vine health, and berry quality.

47   **Keywords**

48   16S rDNA, plant-microbe interactions, soil organic matter, adaptive radiation, *Firmicutes*,

49   *Actinobacteria*

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1. Introduction

Vineyard soil microorganisms are affected by winegrowing region, climate and topography, as mediated in part by their suite of impacts on soil properties like pH and soil organic matter pools (Burns et al. 2015). These same soil properties are directly influenced by vineyard management practices. Soil microorganisms also influence their local environment through pathogen suppression; decomposition processes that affect soil organic matter (SOM) mineralization, contribution and preservation of SOM and aggregate stability; and availability of nitrogen and other mineral nutrients (Kögel-Knabner, 2002; Kuzyakov et al., 2009; Grandy and Neff, 2008; Plaza et al., 2013). These processes and their controls on soil structure and nutrient availability reflect the possible indirect effects of soil microorganisms on plant growth, health and fruit development (Garbeva et al., 2004; Compant et al., 2010). Vineyard management practices and production systems that alter the soil environment, and thus may contribute to shaping the microbial community, include: cover crop use, tillage, compost application, and conventional, organic, or biodynamic systems. Here, we focus on establishing a baseline understanding of the relationships between management practices and changes soil microorganisms within winegrowing regions. This represents the baseline from which we can subsequently delineate the ecological roles of specific taxa to elicit desired outcomes in wine grape production. In other words, altering management practices to change soil properties, which in turn shift key individual or consortia of soil microorganisms, could tune interactions among wine grapes, the soil environment, and associated microorganisms to influence wine grape production.

The soil microbial roles discussed above are intrinsically coupled to both soil quality and soil health. Soil quality refers to the fitness of soil for a particular purpose (Doran et al., 1996; Pierce and Larson, 1993), and thus, requires a specific definition for each purpose. In viticulture, soil quality is defined as “the soil’s ability to support the production of a crop while minimizing negative effects on the environment” (Riches et al., 2013). Soil health is subtly distinct from soil quality. Soil health is defined as “the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, maintain the quality of air and water environments, and promote plant, animal, and human health” (Doran et al., 1996). Soil organic matter stability is intrinsically coupled to concepts of soil quality and health. Microorganisms are intimately linked with the cycling and stability of soil organic matter, among other functions related to soil health, and are sensitive to changes in soil attributes and management (e.g. Calderón et al., 2001; Jackson et al., 2003; Giller et al., 1997; Grandy et al., 2008; Cotrufo et al., 2015). Therefore, measurements of soil-borne microbial communities, such as biomass, structure and functions, have been recommended as good indicators of soil quality (e.g. Jackson et al., 2003; Riches et al., 2013; Steenwerth et al., 2003; Chaparro et al. 2012).

In order to implement assessments of soil microbial community structure for soil health monitoring, additional research is needed to understand the link between soil microbial community structure and soil functions, as they relate to soil health. Studies have begun to show empirically that soil microbial community structure and function are linked (Fierer et al., 2012a; Fierer et al., 2012b), and soil biodiversity is assumed to improve ecosystem resilience by offering functional redundancy (Giller et al., 1997). Soil biodiversity is recognized for its importance to agricultural sustainability in an economic, social, and ecological context (Brussaard et al., 2007). By describing the effect of agricultural management practices on the soil

microbial community structure, we aim to form the foundation from which linkages among soil quality, agroecosystem function, and soil biodiversity can be built to better define soil health for wine grape production. Recent work has shown that climate, region, soil type, and wine grape variety can play strong roles in structuring microbial communities in vineyard soil, the vine phyllosphere, must and wine, and that soil microbial activities and wine metabolome are correlated with microbial community structure (Bokulich et al. 2014; Bokulich et al. 2016; Burns et al. 2015; Zarraonaindia et al. 2015). However, no single study examines the vineyard microbiome from soil to wine nor do they examine effects of vineyard management practice on soil microbial communities.

Numerous studies have assessed the effects of land use and agricultural management practices on soil quality, soil properties, and soil microbial communities (e.g. Castañeda et al. 2015; Drenovsky et al., 2010; Steenwerth et al., 2003). Land-use effects on soil microbial communities are thought to be mediated mostly through alteration of soil properties. Soil properties correlated with soil microbial community structure include soil texture, pH, water content, carbon (C) and nitrogen (N) content, and C:N ratio (e.g. Cookson et al., 2006; Drenovsky et al., 2004; Fierer and Jackson, 2006; Fierer et al., 2012a; Hogberg et al., 2007; Lauber et al., 2009). Plants alter many soil properties as well as soil aggregation and soil nutrient status, through root exudation and fine root turnover. In turn, this affects the soil microbial environment, resulting in shifts in the soil microbial community (Angers and Caron, 1998; Berg and Smalla, 2009; Garbeva et al., 2004; Haichar et al., 2014; Kowalchuk et al., 2002; Shamoot et al., 1968; Starkey, 1929). Tillage disturbance also alters the distribution of soil organic matter and soil structure, thereby causing shifts in aggregate size, composition, and stability, and changing soil nutrient availability (Calderón et al., 2001; Elliott, 1986; Giller et al., 1997; Lee et

al., 2009). Compost amendments add labile carbon and nitrogen, nutrients, and active microbial communities to soil (Bossio et al., 1998; Carpenter-Boggs et al., 2000; Pérez-Piqueres et al., 2006). Consequently, these changes mediate shifts in microbial communities and microbial processes (e.g. Calderón et al., 2000, 2001; Doran, 1980; Jackson et al., 2003; Strauss et al. 2015). These practices are embedded within conventional, organic, and biodynamic agricultural management systems, which differ primarily in their methods of fertilization and control of disease, insects, and weeds. Though effects of pesticides and fertilizers on soil microbial communities are well studied with clear effects (Fierer et al., 2012a; Hussain et al., 2009; Imfeld and Vuilleumier, 2012; Jacobsen and Hjelmsø, 2014), studies based on a comparison of conventional, organic, or biodynamic systems, have not been consistent in showing the same effects on soil microbial communities (Bossio et al., 1998; Carpenter-Boggs et al., 2000; Cookson et al., 2006).

Vineyard management in Napa Valley, California, includes this array of management practices and production systems across a range of soil types, allowing us to examine how vineyard floor management practices influence soil bacterial community structure in the context of environmental and edaphic factors. We measured differences in the soil-borne bacterial and archaeal community composition and diversity by sequencing the V4 small subunit ribosomal RNA gene (16S V4 rDNA). We hypothesized that variations in soil bacterial communities, at the landscape scale, result from different agricultural management practices, as mediated through changes in soil properties. The scope is delineated in this manner to extend the observations of Burns et al. (2015), who recently examined the roles of wine growing region, or appellation, climate and topography on soil bacterial communities across this same suite of sites.

2. Materials and Methods

2.1. Overview

The methodology for soil characterization, DNA extraction, library preparation and sequencing has been described in detail in Burn et al. (2015). Distinct from this current effort, data in Burns et al. (2015) were used to examine the effect of geographic region, climate and soil type on soil microbial communities. These sequence data were deposited previously in the QIITA data bank, Study ID 10082.

2.2 Soil sampling and site characterization

Soil samples were collected from 57 sites in 19 wine grape vineyards, with three sites per vineyard, throughout Napa Valley, California, and treated as a completely randomized design. See Burns et al. (2015) for a complete description of the experimental design, approach and details on specific practices at each vineyard. Details of management practices were gathered through interviews with vineyard managers. 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. At each site, three soil samples were collected approximately 2-m apart and mixed into a composite sample. Samples were kept on ice (ca. 2–6 hrs.) 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 .

2.3. Soil characterization

Bulk density by coring, gravimetric soil water content, pH (1:1 in water), inorganic nitrogen (N) pools by colorimetric analysis, dissolved organic carbon (DOC) by filtration and elemental combustion, and potentially mineralizable nitrogen (PMN) by anaerobic incubation and colorimetric analysis were determined for each composite sample, as described in Burns et al. (2015) (Jones and Willett, 2006; Kempers and Kok, 1989; Miranda et al., 2001; Pella, 1990a, b; Rousk and Jones, 2010; Soon et al., 2007; Waring and Bremner, 1964).

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 ($\text{Na}_6\text{O}_{18}\text{P}_6$; 100 mL per 30 g soil) for 18 hours, 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 hours. Total C (TC) and total N (TN) 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).

2.4. Soil DNA extraction, library preparation and sequencing

From each frozen soil sample stored at – 80 °C, DNA from four subreplicates (0.25 g field-moist soil each) per subsample was extracted using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, CA, USA). The manufacturer's protocol was modified slightly by: (1) increasing the vortex time of the PowerBead Tubes to 15 min on a vortex equipped with a 24-place vortex adapter, (2) extending centrifugation of the PowerBead Tubes to 60 s to settle soils with higher clay contents, and (3) 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).

The V4 region of the 16S rRNA gene (Liu et al., 2007) was amplified using the universal primer pair 515F/806R (Bates et al., 2011; Caporaso et al., 2011), following the procedure of Burns et al. (2015), a procedure similar to that of Bokulich et al. (2012).

Following PCR amplification, products were resolved 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), as described in Burns et al. (2015). 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 open-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 phylogenetic tree was generated from the filtered alignment using FastTree (Price et al., 2010). Each subreplicate was collapsed into its composite sample (see Burns et al. 2015). 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 with less than 28,008 sequences following all quality-filtering steps.

Richness was estimated by the number of observed phylotypes (97% similarity OTUs) and by the Chao1 richness estimate (see Burns et al. 2015). 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 (McCune and Grace, 2006; Minchin, 1987; Oksanen et al., 2013).

The impact of vineyard management practices on soil properties, α -diversity and richness, and β -diversity, was determined by examining differences in spread along NMDS axes, using the Kruskal-Wallis rank sum test (non-parametric, one-way ANOVA) or Wilcoxon rank sum (Mann-Whitney) test for the special case of two groupings (Hollander and Wolfe,

1973; R Core Team, 2013). Differences in β -diversity, based on the weighted UniFrac (Lozupone and Knight, 2005) distance matrix, among soil properties and the vineyard management sample groups were also tested using non-parametric multivariate analysis of variance (permutational MANOVA, R vegan ADONIS) (Anderson, 2001) with 999 permutations. Relationships also were investigated within one sub-appellation Rutherford, as it contained the greatest number of samples. The effect of appellation was examined directly by Burns et al. (2015).

To determine which relative taxa abundances differed between vineyard management practices at various levels of taxonomy, one-way analysis of variance (ANOVA) was performed in QIIME. Canonical discriminant analysis (CDA) was performed using the candisc and heplots R packages to graphically reveal differences between sample groups of vineyard management practices and to identify high-level taxa associated with each practice (Fox et al., 2013; Friendly, 2007; Friendly and Fox, 2013; Gittins, 2011).

To help elucidate the relative importance of soil attributes, management, and location in structuring the communities, variation partitioning using canonical correspondence analysis (CCA), a constrained unimodal approach, was performed (Borcard et al., 1992; Drenovsky et al., 2010; Heikkinen et al., 2004) using CANOCO 5 for Windows (Microcomputer Power, Inc., Ithaca, NY, USA; Šmilauer and Lepš, 2014) at a coarse taxonomic level, consisting of phyla, except for Proteobacteria, which were divided into α -, β -, γ -, and δ -classes. This coarse level was selected based on the conclusions of Philippot et al. (2010), which suggested high levels of bacterial taxonomy are ecologically coherent and each different level of taxonomy may offer a different piece of information on the underlying mechanisms driving establishment of bacterial populations. An additional analysis using a fine taxonomic resolution, consisting of genera or the

finest level of classification available for each group, also was conducted. However, the results revealed overall patterns similar to the results of the coarse taxonomic resolution; therefore, details of the finer-level taxonomic analysis are omitted from this report (K.N. Burns, unpubl. thesis 2014).

Variation in microbial communities was partitioned among three groups of explanatory variables: soil, management, and location (Drenovsky et al., 2010; Šmilauer and Lepš, 2014). The variables selected for inclusion in the analysis were based on the hypotheses, the results of BEST rankings (Burns et al. 2015), visual relationships and correlations of variables with NMDS axes (Burns et al. 2015), and results of forward selection as implemented in CANOCO 5 for Windows. The first group consisted of soil attributes, including soil pH, soil moisture, soil TC and TN, TC and TN in the 53–250 μm soil fraction, TC and TN in the $< 53 \mu\text{m}$ soil fraction, PMN, an estimate of percent clay content (SSURGO), and soil great group from U.S. Soil Taxonomy (SSURGO). The second group was composed of vineyard alley management practices: tillage ('till') and no tillage ('NT'), presence/absence of compost application in the alley ('compost+' / 'compost-'), and the cover crop mix used, either non-cereal grasses only ('grasses'); legumes and cereals ('leg+cer'); legumes, cereals, and mustards ('leg+cer+mus'); legumes only ('legumes'); mustards only ('mustards'); or only resident vegetation ('no cover crop'). Tillage was further divided to specify recency of tillage ('tillRec+' / 'tillRec-'), relative to the time of soil sampling. A recently tilled soil (tillRec+) included any vineyard alley in which it was evident that it had been tilled in spring 2011, the period of sampling. Location, the third group, consisted of latitude, longitude, elevation, slope, and average annual precipitation. Variation partitioning creates eight fractions: pure effect of soil (*a*); management (*b*); or location attributes (*c*); joint effects of soil and management (*d*); management and location (*e*); soil and

location (*f*); or soil, management, and location attributes (*g*); and unexplained variation (*h*). Simple (marginal) and conditional (unique) effects of each explanatory group and combinations of groups were tested, each using 999 Monte Carlo permutations (Šmilauer and Lepš, 2014).

3. Results

3. 1. Soil physicochemical properties

Soil properties, especially those that reflect resource availability to soil microorganisms, differed as a function of vineyard management practices (Kruskal-Wallis rank sum tests, Tables 1-2, Tabs. S1-S2). TC and TN of the bulk soil differed with respect to cover crop mixes, as did DOC, PMN, soil C:N, the C:N ratios in all the soil fractions ($P < 0.05$, Table 1). In most cases, soils with non-cereal grass cover crops had the highest C, N, C:N ratios, pH, and soil moisture compared to vineyard soils with other cover crops (including ‘no cover crop’). Specifically, TC in the 53–250 μm soil fraction was 4.8-fold higher ($P < 0.001$), TN in the 53–250 μm soil fraction was 4.1-fold higher ($P = 0.001$), and PMN was 3.6-fold higher ($P = 0.003$) than soils supporting all other cover crops. TC in the $<53 \mu\text{m}$ soil fraction also was 1.3- to 3-fold higher ($P < 0.001$) in the non-cereal grass cover crops than other cover crops.

In general, soil C and N pools were highest in no-till soils and lowest in recently tilled soils. Soils from no-till vineyards had 1.6-fold higher soil TC and TN ($<2\text{mm}$). In the soil fractions from no-till vineyards, TC was 1.1 fold higher, TN was 2.0-fold higher, and PMN was 2.4-fold higher PMN than tilled soils (Tab. 2). The pH of soils tilled recently was lower than soils tilled less recently. In this region, vineyard managers add compost to soils that require improvement in organic matter content and fertility. In turn, soils that received compost had lower C and N pools than those that did not receive compost ($P < 0.05$; Table S1). Vineyards that

were under biodynamic or organic management typically had lower C and N pools than those that were under conventional management ($P < 0.05$; Table S2).

3.2. Variation partitioning among soils, management, and locations

Variation among the bacterial and archaeal communities was partitioned into the eight variance fractions (pure and joint effects) to examine the relative importance of: (1) soil attributes, (2) management, and (3) location using canonical correspondence analysis (CCA) (Fig. 1; Fig. S1). Together, pure and joint effects explained 67.3% of the bacterial community variation, leaving 32.7% unexplained. When the variation was partitioned, the largest fraction was accounted for by soil ($a + d + f + g$: 46.0%; Fig. 1), including pure and joint effects. Soil variables alone (a) explained 22.1% of variation. Excluding the joint effect of all three groups (g), the joint effect of soil and management (d : 13.7%; Fig. 1) explained a greater percentage of variation than vineyard management alone (b : 11.6%) or any other remaining fraction (c , e , f , or g). Location variables alone (c) explained 7.5% of variation, and without considering the joint effect of all groups (g), the joint effect of soil and location variables (f : 7.2%) explained nearly as much variation as location's pure effect.

3.3. Diversity and richness of bacterial communities

3.3.1. Vineyard management and bacterial α -diversity patterns

Soil bacterial communities showed highest diversity in vineyards that were tilled less recently, were biodynamic, and had compost application, while soil bacterial communities showed lowest diversity in vineyards that were tilled recently, were organic, and had no compost application. Neither PD nor richness differed by cover crop mix. Specifically, no-till soils and

soils tilled recently had similar mean PDs, while soils that were tilled less recently had a 5% higher mean PD (Kruskal-Wallis $P = 0.045$) than no-till and recently tilled soils. Mean PD was also higher in soils that received compost than in soils that did not (3.9% difference, Wilcoxon $P = 0.027$). Samples from vineyards under biodynamic management had a higher mean PD (1.7% difference) and Chao1 richness (3.1% difference) than those from vineyards under conventional management. Samples from vineyards under other organic management had a lower mean PD (5.6% difference) and richness (6.6% difference) than those from vineyards under conventional management (Kruskal-Wallis $P < 0.005$ for richness comparisons and $P < 0.05$ for PD comparisons). Effectively, conventional and biodynamic PD and richness were more similar to each other compared to organic vineyards.

3.3.2. Vineyard management and phylogenetic bacterial β -diversity patterns

NMDS plots of the weighted UniFrac matrix demonstrate β -diversity patterns of management-related groupings of samples (Fig. 2). The Kruskal-Wallis and Wilcoxon rank sum tests for sample scores along each NMDS axis confirmed the relationships of management with patterns in bacterial community structure (Tabs. 1-2, Tabs. S1-S2). Sample scores along the first NMDS axis show significant separation with compost presence/absence ($P = 0.019$) and with biodynamic, conventional, or organic management ($P = 0.044$) (Fig. 2). Sample scores also show significant separation with the cover crop mix ($P < 0.001$). Specifically, this is evident in the presence/absence of legumes ($P = 0.010$), cereal ($P < 0.001$) or non-cereal grass ($P = 0.001$) along the first axis and by presence/absence of a cover crop ($P = 0.002$). It is also evident in the presence/absence of non-cereal grass ($P = 0.004$) along the second axis. Moreover, the fourth axis has separation by cover crop mix ($P = 0.018$), in particular, with presence/absence of a

cover crop ($P = 0.019$) and the presence/absence of mustards ($P = 0.003$). Finally, no-till vs. tilled samples ($P = 0.008$) and tilled-recently vs. tilled—but not recently vs. no-till ($P = 0.001$) separate on the second and fourth NMDS axes. In general, samples cluster with respect to management groupings along NMDS axes, with separation by compost presence/absence and organic vs. biodynamic vs. conventional along the first axis. Samples also separate with respect to different components of cover crop mix and with tillage or recency of tillage along multiple axes.

ADONIS both confirmed the significance of relationships of vineyard management with patterns of β -diversity and portrayed hierarchical relationships among groups (Tab. 3, Tab. S3). Specific cover crop mix, tillage, recency of tillage, compost application, and conventional vs. organic vs. biodynamic management all exhibited relationships with patterns of β -diversity ($P < 0.010$, Tab. 3).

Cover crop mix had a relatively strong relationship with bacterial community structure ($R^2 = 0.29$ and $P < 0.001$), and each cover crop component was significantly correlated by ADONIS ($R^2 > 0.04$ and $P \leq 0.013$, Tab. 3). However, as depicted by relatively lower R^2 -values ($R^2 < 0.12$), the presence/absence of a cover crop or any specific individual cover crop mix component (legumes, cereals, mustards, or non-cereal grasses) did not have particularly strong correlations with bacterial community structure when compared to cover crop mix altogether.

When all samples were considered, tillage had a weak, but significant relationship with community structure ($R^2 = 0.06$ and $P = 0.003$, Tab. 3). The tillage relationship became stronger, as indicated by increasing R^2 -values (for $P < 0.05$, $0.06 < R^2 < 0.24$, Tab. 3 and S3), when other aspects such as other management practices or winegrowing region were held constant. However, selection of a smaller sample size also meant less significant findings for some

subsets. Based on relatively higher R^2 -values ($R^2 > 0.06$), the following are some of the subsets which contained stronger effects of tillage or recency of tillage: only soils within the Rutherford sub-appellation, only soils with cover crops, only soils receiving compost application, and only soils in vineyards under organic management (Tab. 3). Overall, examining β -diversity within groups reveals stronger patterns than looking across all groups, especially for the effect of tillage (see Table 3, Table S3).

3.4. Vineyard management and bacterial taxa abundances

Specific cover crop mix had a complex assortment of relationships ($P < 0.05$) to relative abundances of phyla *Actinobacteria*, *Armatimonadetes*, *Firmicutes*, *OD1*, *Proteobacteria*, and *Verrucomicrobia*, and classes *Betaproteobacteria* and *Planctomycetia* (the most abundant *Planctomycetes*) (Fig. 3, Fig. S2). In some cases, specific taxa at phyla or class level were associated with individual components of a cover crop mix. For example, *Firmicutes* was 1.54-fold higher in the presence of mustards ($P = 0.012$), whereas *Tenericutes* was 3.84 fold higher in their absence ($P = 0.032$). Absence of a legume was associated with 1.29-fold higher relative abundance of the class *Planctomycetia* ($P = 0.037$), but there was no effect at the phyla level. In contrast, while *Verrucomicrobia* relative abundance showed a significant relationship with specific cover crop mix groupings ($P = 0.019$), it did not show significant relationships with any of these cover crop elements individually. Impacts of compost and conventional vs. organic management on bacterial community composition were not visible at the phyla level. However, at each finer level of taxonomy, new significant differences in taxa abundances emerged (Fig. 3, Fig. 4 and Fig. S2).

In the soils with a grass cover crop, *Actinobacteria* ($P < 0.001$) and *WPS-2* ($P = 0.026$) relative abundances were 1.48-fold and 11.20-fold higher than other cover crop groups, respectively. This includes the ‘no cover crop’ group, which actually reflects the presence of resident vegetation. Meanwhile, relative abundances of *Armatimonadetes* (2.1-fold lower, $P = 0.013$), *Chlorobi* (1.93-fold lower, $P = 0.038$), *Gemmatimonadetes* (1.55-fold lower, $P = 0.035$), and *Proteobacteria* (1.12-fold lower, $P = 0.012$) were lower in soils with a grass cover crop compared to their relative abundances in soils with other cover crops and resident vegetation. Among the *Proteobacteria* classes, *Betaproteobacteria* showed the most significant decrease in relative abundance (1.31-fold lower, $P = 0.021$) with the presence of a grass cover crop. The class *Planctomycetia*, on the other hand, increased (1.33-fold higher, also $P = 0.021$) in relative abundance with the presence of a grass cover crop.

Taxa abundances were likewise associated with tillage practices (Fig. 3). Relative abundances of *Armatimonadetes*, *Firmicutes*, and *Gemmatimonadetes*, and *Nitrospirae* were greater (1.52- to 1.73- fold higher, $P < 0.05$) in tilled soils than no-till soils. Relative abundances of these same taxa for soils tilled less recently were intermediate ($P = 0.090, 0.022, < 0.001$, and 0.111, respectively) compared to recently tilled soils (1.09- to 1.34- fold lower in less recently soils) and no-till soils (1.35- to 1.57- fold higher in less recently soils). In general, there is a gradient in relative abundances of these taxa with tillage disturbance as follows: recently-tilled > tilled-but-not-recently > no-till. Relationships of relative taxa abundances with tillage and recency of tillage are also apparent from the results of CDA (Fig. 3). For finer details on these relationships, see Supplemental Results S1.

4. Discussion

4.1. Soil bacterial communities reflect vineyard management practices

The structure of soil-borne microbial communities is influenced by soil properties typically affected by crop management practices (Figs. 1-2, Tabs. 1-2). Management practices or other factors were identified that were more relevant to structuring the microbial community in one subset (e.g. a specific ‘great group’) compared to another subset of that same type. This suggests that the microbial community was more responsive to a given management practice or factor due to inherent characteristics associated with that subset (Tab. 3, Tab. S3). This also suggests that there is a relative hierarchy of effect of these management practices or factors on microbial community structure, where one factor (e.g. cover crop) may have a stronger effect within one subset (e.g. Xeralf within ‘great group’) versus another (e.g. Fluvent within ‘great group’).

Because of the strong relationship of sub-appellation with soil microbial communities, and the great variation and uneven representation among sub-appellations (Burns et al., 2015), it is also useful to look at the influence of the other factors, such as management and soil type, within a single sub-appellation instead of across all sub-appellations. For example, general effects of tillage presence/absence and specific effects that occurred based on its recency of application in the field emerged within the Rutherford American Viticultural Area (AVA), as the best represented sub-appellation (Tab. 3, Tab. S3). These findings suggest that an agricultural practice could have different effects with respect to AVA, which may not be too surprising given that soil attributes also differ with respect to AVA in this region (Burns et al. 2015). If we assumed that tillage, for example, affects all AVAs the same by only examining its overall effect

rather than within individual AVAs, we would overlook important information regarding the structuring of the soil bacterial communities.

4.1.1. Influences of cover crops

It is known that plants differentially affect soil structure, and hence, the soil microbial environment. Plants differ in contributions to labile soil C and soil organic matter, and hence, the soil microbial resources through differential root exudation and fine root turnover, as suggested by distinctions in total C and N and the <53 μm fractions among cover crops in this study (Tab. 1) (Angers and Caron, 1998; Berg and Smalla, 2009; Haichar et al., 2014; Shamoot et al., 1968). Plant-soil-microbe interactions are particularly pronounced in the rhizosphere, where soil microbial community compositions are often plant-specific and distinct from the bulk soil (Berg and Smalla, 2009; Garbeva et al., 2004; Haichar et al., 2014; Kowalchuk et al., 2002). For example, *Firmicutes* had high relative abundance in association with mustard (*Brassica* spp.) cover crops, similar to that observed in *Brassica juncea* (Mowlick et al. 2013; Mowlick et al. 2014). These plant-soil-microbe interactions enable cover crops to distinctly affect the soil, soil microbial communities, and microbially-mediated soil processes (Ingels et al., 2005; Reicosky and Forcella, 1998). Here, we observed an effect of cover crop mix and the general presence of cover crop on soil physicochemical properties (Section 3.1, Tab. 1). In turn, soil bacterial community structure, taxa abundances and soil C and N pools differed by cover crop mix, suggesting that there is an interaction among the cover crop type, soil resource pools, and the microbial community (Tab. 2, Fig. 2 and Fig. S1-S2). For example, relative abundance of *Actinobacteria* can be associated with enriched C and N pools (Li et al., 2014), as observed here in the grass cover crop soils with the highest soil C and N content. However, the opposite was

observed in vineyard soils in Spain enriched in soil C pools after 13 years of compost application (Calleja-Cervantes et al. 2013). Effects of cover crops on soil microorganisms and microbially-mediated processes (e.g. C and N cycling) have been observed in other vineyard studies (Ingels et al., 2005; Steenwerth and Belina, 2008a, b) and in annual cropping systems (Bossio et al., 1998). The effect of cover crop presence on microbial community structure was also greater in some soil great groups, such as the Xerales, indicating that attributes associated with soil development (e.g. clayey soil have more soil organic matter than sandy soils) can amplify effects of specific management practices like cover cropping. However, an isolated group of samples from alleys supporting grasses, legumes + cereals or mustards were clustered together and associated with higher values in soil pH and clay content. All measured soil attributes also explained the most variation in microbial community structure in the variation partitioning analysis (Fig. 1, S1). Together, these findings suggest that soil chemical attributes and C and N pools played strong roles in structuring soil microbial communities (Fig S1) (Burns et al. 2015).

4.1.2. Influences of tillage and compost

Bacterial communities tended to separate weakly, although significantly, with respect to presence and absence of tillage, but when looking within group factors through ADONIS a stronger effect of tillage emerged (Tab. 3, Tab. S3). For example, within soils with planted cover crops there was a stronger effect of tillage compared to across all soils (weak effect). This may be due to the pre-plant preparation of soil by disking and rolling as well as incorporation of the cover crop into the soil. Similarly, within organic vineyards, tillage played a stronger role compared to conventional vineyards (no effect) or across all vineyards (weak effect), corresponding to lower soil C pools in organic vineyards (Table S2). In this region, organic

growers will till alleys to reduce competition between vines and cover crops and for weed control, and in at least one organic vineyard in this study, alleys had been tilled 4-5 times per vine growing season. Nonetheless, vineyard soils in Napa are not intensively tilled in comparison to annual cropping systems with conventional tillage practices. This presents challenges in comparing effects of tilled treatments on taxa among annual and perennial cropping systems. However, in Australia, a similar effect on *Firmicutes* was observed, in which no-till soils that had been tilled just once exhibited enrichment in *Firmicutes* (Liu et al. 2016). Furthermore, tillage seemed more important within Xeralfs (stronger effect) compared to Fluvents (no effect) or across all soils (weak effect) (Tab. 3). For vineyards in Napa Valley, soil type tends to influence choice of tillage practice, in which Xeralfs and Fluvents are typically tilled while Xerolls and Xerults are not. Also, in Napa Valley, vineyards at higher elevations tend to be no-till due to rocky conditions and steeper slopes. Therefore, the effects of tillage and soil type are linked.

Tillage disturbance creates shifts in soil nutrient availability and in aggregate size, composition, and stability, thereby changing the physical environment and resource availability experienced by soil microorganisms (Calderón et al., 2001; Giller et al., 1997; Lee et al., 2009). This then can lead to shifts in soil microbial communities and microbially-mediated processes (Calderón et al., 2000; Doran, 1980; Jackson et al., 2003). One soil organic matter pool that reflects both tillage disturbance and microbially-mediated processes is that associated with the fine soil fraction (<53 µm). As it is not always mineral associated (Plaza et al. 2013), we will refer to it 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). In our case, no till soils had distinct microbial community structure and the greatest concentrations of total and fine SOM and PMN compared to tilled soils, regardless of the time since tillage occurred (Tab. 2, Figs. 2-3). However, less recently tilled soils had highest diversity and richness, higher than no-till and recently-tilled soils (Tab. 2). At the same time, taxa that exhibited significant differences with tillage (Till) or time since tillage had occurred (tillRec+, tillRec-) had greater relative abundances in tilled (or recently tilled) soils, suggesting that these taxa either had a greater stability in response to tillage disturbance or were selected in response to tillage, as compared to other taxa across all sites. At the phyla level across all sites, no specific taxa had consistent decreases in relative abundance with tillage, suggesting that taxa most sensitive to tillage were consistent among sites. Interestingly, there was no clear overlap between soil microbial communities supporting specific kinds of cover crops and tillage status, suggesting that these practices had relatively independent effects on soil microbial communities (data not shown). However, total cover crop biomass was not collected, and so any correlation of cover crop biomass production and its total contributions to labile soil resources with pre-plant tillage in fall, spring incorporation of cover crops by tillage or no-till is indeterminate.

Further supporting the idea that soil resources drive diversity, compost addition was associated with an increase in overall bacterial diversity and changes in community composition. Increased diversity and relative abundances of certain taxa under compost application may be a response from increased availability of resources, especially as soils that received compost had lower C pools, or from microbial introductions from compost itself (Bossio et al., 1998; Calleja-Cervantes et al. 2015). Compost has varied origins and is derived from diverse materials, which

might explain the lack of a consistent effect across all vineyards on particular taxa abundances, despite the consistent increase in phylogenetic diversity.

As an example of the hierarchy of effects observed within groups (see section 4.1), recency of tillage had a greater impact than tillage presence, in general, on structuring soil bacterial communities, especially when excluding no-till soils (Tabs. 3 and S3). An effect of recency of tillage was even resolved for groups in which tillage in general did not have an effect, such as within conventional vineyards and within Fluvents. Presumably, all vineyards were cultivated during their conversion from previous land-use types. Because tillage is known for its long-lasting impacts, even on soil microbial communities (Buckley and Schmidt, 2001), all vineyards could be considered disturbed ecosystems. Therefore, it might not be a surprise that recency of tillage has a greater influence in structuring soil bacterial communities than the practice of tillage, in general. Tillage also is known for its short-term effects on soil resources and soil microorganisms, as shown in intensively cultivated vegetable crop soils and annual grasslands (Calderón et al., 2001; Jackson et al., 2003). In our case, less recently tilled soils had highest diversity and richness, above that of no-till and recently-tilled soils. This lends some support to the ecological concept of adaptive radiation, where an event (such as a disturbance or mass extinction) gives rise to many new species (or perhaps, for bacteria, the increase of rarer species to detectable levels) expanding into new habitats or ecological roles in a relatively short time (Cain et al., 2008). Other studies have supported the idea of adaptive radiation of bacteria particularly when species were absent from the medium prior to inoculation with the experimental bacterium (Gomez and Buckling, 2013; Koeppel et al., 2013), which could be analogous to adaptive radiation following the tillage-associated reduction of microbial biomass,

richness, and diversity. However, further research is required to confirm the accuracy and wide applicability of such ecological concepts to microbial ecology.

4.1.3. Influences of conventional, organic, and biodynamic vineyard management

Bacterial community composition and diversity differed with conventional, organic, and biodynamic vineyard management. It is commonly known that these systems tend to differ in the types of pesticides and fertilizers used, and that pesticides and fertilizers affect soil microbial communities (Fierer et al., 2012a; Hussain et al., 2009; Imfeld and Vuilleumier, 2012; Jacobsen and Hjelmsø, 2014). However, since fertilizers and herbicides are typically only applied under the vine, and other pesticides are most commonly applied foliarly to vines, we do not expect these factors to play large roles in distinguishing our samples, which were taken from alleyways. Nevertheless, conventional, organic, and biodynamic vineyard management systems in our study also differed with respect to tillage, cover crop, compost application and practices specific to biodynamic like field sprays of cow manure and quartz silica as well as additives to compost (Reeves et al. 2005; see Tabs. 1-2 and Tabs. S1-S2 in Burns et al. 2015), all factors that influence soil physical environment and resources available to microorganisms. Furthermore, differences in soils, climate, and sub-appellation may influence (1) management decisions, impacting likelihood of a grower to adopt certain designations, whether conventional, organic, or biodynamic and (2) observed distinctions among bacterial communities with respect to vineyard management systems.

For vineyards, underlying practices (e.g. tillage) embedded within the conventional, organic, and biodynamic management systems affected bacterial communities both in terms of biodiversity and overall community structure. Growers employ tillage as a water and weed

management practice to prevent competition between vines and cover crops or weeds, particularly in organic and dry-farmed vineyards. Conventional systems were more likely to be no-till—likely due to the lack of a need for tillage as a water management or weed control practice. As previously discussed, tillage was associated with lower soil bacterial α -diversity and richness compared to no-till. Since organic vineyards were associated with tillage, we attribute the lower α -diversity and richness associated with organic vineyards to the impact of tillage. Studies in annual cropping systems have found the opposite effect or no differences between conventional, organic, and biodynamic management (Bossio et al., 1998; Carpenter-Boggs et al., 2000; Cookson et al., 2006; Li et al., 2012). This may be due to the different nature of conventional, organic, and biodynamic systems in vineyards compared to annual cropping systems.

4.2. Conclusion

Soil bacterial communities were structured as a function of vineyard management practices and soil properties. As ranked by the highest ADONIS R^2 -value, cover crop mix was the strongest management factor, but hierarchical effects of recency of tillage and compost additions on soil microbial structure were also noted. Our work supports the paradigm that vineyard management practices affect soil microbial communities through their suite of impacts on soil properties, but mechanistic studies will further elucidate the ecological role of specific taxa identified in these vineyard soils. The identification of distinctive soil bacterial communities related to soil resources and vineyard management indicates that soil bacterial community structures (16S rDNA fingerprinting) can be developed as a biological indicator of soil quality. This may provide a strategy to monitor soil quality or health in vineyard soils. This work also has

opened the door for future assessments of interrelationships of vineyard management, microbial biodiversity, and agroecosystem services, especially as they relate to soil quality, soil health, vine health, and berry quality (Burns et al. 2015).

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Fig. 1. Variation partitioning based on canonical correspondence analysis (CCA) using coarse-level taxa (phyla, except for proteobacteria, which were divided into classes). Variation was partitioned into the eight fractions (pure and joint effects) of the three groups of variables: (1) soil (pH, soil moisture, soil TC and TN, TC and TN in the 53–250 μm and $< 53 \mu\text{m}$ soil fractions, PMN, clay content, and soil great group), (2) management (tillage, compost, cover crop mix), and (3) location (latitude, longitude, elevation, slope, and average annual precipitation). The eight fractions are pure effect of soil (*a*); management (*b*); or location attributes (*c*); joint effects of soil and management (*d*); management and location (*e*); soil and location (*f*); or soil, management, and location attributes (*g*); and unexplained variation (*h*). See Fig. S1 for the CCA ordinations based on the shared effect of the three groups using both coarse- and fine-level taxa. TC, total carbon; TN, total nitrogen, PMN, Potential mineralizable nitrogen.

Fig. 2. Non-metric multidimensional scaling (NMDS) unconstrained ordinations of samples based on the weighted UniFrac distance matrix of pairwise phylogenetic dissimilarities between samples (β -diversity). Stress for this four-dimensional solution is 0.06. Lower dimensional solutions are not shown. The two most highly significant axes (Tables 1–2 and S1–S2) for each vineyard management factor are utilized for the ordinations presented: cover crop with NMDS2 vs. NMDS1 (*a*), general tillage practice with NMDS3 vs. NMDS2 (*b*); recency of tillage with NMDS4 vs. NMDS2 (*c*); compost with NMDS2 vs. NMDS1 (*d*); and conventional, organic, or biodynamic with NMDS2 vs. NMDS1 (*e*). Definition of abbreviated labels in the graph legends are as follows. Cover Crop (*a*): ‘leg’, legumes; ‘cer’, cereals; ‘mus’, mustard; noCovCr, no cover crop that was planted but resident vegetation was present. Tillage (*b*): noTill, no tillage, same as NT; till, tillage present. Tillage (*c*): NT:Rec–, No Till or NT; tillRec–, tilled less recently; tillRec+,

tilled recently, or in the spring when samples were collected. Compost (*d*): Comp⁻, no compost; Comp⁺,
compost present.

Fig. 3. Canonical discriminant analysis (CDA) of relative taxa abundances by cover crop mix
(*a*), recency of tillage (*b*), and agricultural method (*c*). 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. Additional
CDA results are presented in Fig. 4 and Fig. S2. Definition of categorical labels are as follows.
Cover crop mix (*a*): ‘leg’, legumes; ‘cer’, cereals; ‘mus’, mustard; noCovCr, no cover crop that
was planted but resident vegetation was present. Tillage (*b*): NT:Rec⁻, No Till, or NT; tillRec⁻,
tilled less recently; tillRec⁺, tilled recently, or in the spring when samples were collected.

Fig. 4. Canonical discriminant analysis (CDA) of relative taxa abundances by compost
application. Boxplots on the left show CDA scores for compost presence/absence along the first
axis. Vectors on the right show the strength and direction of association of each taxon’s relative
abundance with compost presence/absence. This figure represents the same type of analysis as
shown in Fig. 3, but a 2D plot is not possible for only two groups, so the results are presented in
one dimension. 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. Additional CDA results are presented in Fig. 3 and Fig. S2.

Table 1 Means, standard deviations, and Kruskal-Wallis rank sum test of soil properties, α -diversity, richness, and NMDS scores by cover crop.

Variable	Grasses	Leg+Cer	Leg+Cer+Mus	Legumes	Mustards	No cover crop	χ^2	df	P
GWC	0.19 ($\pm$ 0.09)	0.15 ($\pm$ 0.05)	0.22 ($\pm$ 0.09)	0.05 ($\pm$ 0.02)	0.11 ($\pm$ 0.09)	0.14 ($\pm$ 0.02)	14.21	5	0.014 *
pH	6.75 ($\pm$ 0.40)	6.57 ($\pm$ 0.28)	6.29 ($\pm$ 0.56)	6.55 ($\pm$ 0.20)	6.55 ($\pm$ 0.70)	6.06 ($\pm$ 0.17)	12.74	5	0.026 *
TC	40.40 ($\pm$ 12.62)	19.09 ($\pm$ 4.29)	22.57 ($\pm$ 4.52)	14.23 ($\pm$ 0.99)	16.47 ($\pm$ 1.43)	17.11 ($\pm$ 2.74)	32.34	5	< 0.001 ****
DOC	130.21 ($\pm$ 51.49)	48.41 ($\pm$ 22.30)	29.51 ($\pm$ 10.16)	45.30 ($\pm$ 4.25)	96.72 ($\pm$ 73.73)	29.34 ($\pm$ 6.04)	35.46	5	< 0.001 ****
TC 250–1000 μ m	48.09 ($\pm$ 21.78)	34.80 ($\pm$ 42.81)	62.18 ($\pm$ 48.48)	18.16 ($\pm$ 3.04)	8.71 ($\pm$ 2.02)	10.32 ($\pm$ 2.74)	29.91	5	< 0.001 ****
TC 53–250 μ m	49.30 ($\pm$ 28.82)	10.91 ($\pm$ 4.88)	10.56 ($\pm$ 3.04)	8.91 ($\pm$ 2.46)	9.62 ($\pm$ 1.91)	8.80 ($\pm$ 2.47)	22.29	5	< 0.001 ****
TC < 53 μ m	33.58 ($\pm$ 7.54)	21.35 ($\pm$ 7.38)	24.31 ($\pm$ 5.98)	13.56 ($\pm$ 0.55)	24.81 ($\pm$ 2.83)	23.09 ($\pm$ 5.81)	26.02	5	< 0.001 ****
TN	3.08 ($\pm$ 1.08)	1.57 ($\pm$ 0.29)	1.80 ($\pm$ 0.28)	1.34 ($\pm$ 0.12)	1.35 ($\pm$ 0.14)	1.40 ($\pm$ 0.17)	24.31	5	< 0.001 ****
PMN	144.29 ($\pm$ 97.30)	41.81 ($\pm$ 36.99)	64.93 ($\pm$ 18.14)	13.56 ($\pm$ 6.30)	22.38 ($\pm$ 24.63)	40.75 ($\pm$ 5.51)	17.64	5	0.003 ***
TN 250–1000 μ m	2.55 ($\pm$ 1.18)	1.89 ($\pm$ 2.16)	3.44 ($\pm$ 2.70)	1.04 ($\pm$ 0.16)	0.57 ($\pm$ 0.13)	0.73 ($\pm$ 0.31)	23.62	5	< 0.001 ****
TN 53–250 μ m	3.04 ($\pm$ 1.88)	0.75 ($\pm$ 0.29)	0.83 ($\pm$ 0.26)	0.65 ($\pm$ 0.16)	0.68 ($\pm$ 0.21)	0.68 ($\pm$ 0.22)	19.80	5	0.001 ***
TN < 53 μ m	2.88 ($\pm$ 0.68)	1.95 ($\pm$ 0.59)	2.16 ($\pm$ 0.36)	1.33 ($\pm$ 0.07)	2.21 ($\pm$ 0.27)	1.97 ($\pm$ 0.39)	22.48	5	< 0.001 ****
C:N ratio	13.40 ($\pm$ 1.20)	12.07 ($\pm$ 0.87)	12.54 ($\pm$ 0.96)	10.67 ($\pm$ 0.54)	12.23 ($\pm$ 0.49)	12.22 ($\pm$ 1.47)	18.20	5	0.003 ***
C:N 250–1000 μ m	18.95 ($\pm$ 2.15)	17.64 ($\pm$ 1.97)	18.04 ($\pm$ 0.87)	17.38 ($\pm$ 0.67)	15.33 ($\pm$ 1.49)	15.30 ($\pm$ 4.16)	13.61	5	0.018 *
C:N 53–250 μ m	16.36 ($\pm$ 1.94)	14.31 ($\pm$ 1.44)	12.91 ($\pm$ 1.30)	13.71 ($\pm$ 0.80)	14.55 ($\pm$ 1.60)	13.17 ($\pm$ 2.04)	18.23	5	0.003 ***
C:N < 53 μ m	11.76 ($\pm$ 1.03)	10.79 ($\pm$ 0.92)	11.15 ($\pm$ 0.84)	10.17 ($\pm$ 0.17)	11.25 ($\pm$ 0.32)	11.62 ($\pm$ 0.98)	13.46	5	0.019 *
PD	232	231	238	221	238	232	2.78	5	0.734 ns
Chao1	5455	5367	5511	5212	5503	5250	2.36	5	0.797 ns
NMDS1	0.07	– 0.03	– 0.09	0.11	0.01	– 0.04	23.09	5	< 0.001 ****
NMDS2	– 0.04	– 0.01	0.05	0.01	0.01	0.08	18.39	5	0.002 ***
NMDS3	0.02	– 0.01	0.01	– 0.02	– 0.03	0.01	8.13	5	0.149 ns
NMDS4	0	0.01	– 0.04	0.01	– 0.02	0.03	13.66	5	0.018 *

**** p < 0.001, *** p < 0.005, ** p < 0.01, * p < 0.05, ns p $\geq$ 0.05

NMDS: Non-metric multidimensional scaling; 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. PD: Faith's phylogenetic diversity; Chao1: Chao 1 richness estimate; 'Leg': Legume; 'Cer': Cereal; 'Mus': Mustard, or *Brassica* spp.; 'No Cover Crop': no planted cover crop but alley supports resident vegetation.

Table 2 Means, standard deviations, and Kruskal-Wallis or Wilcoxon rank sum test of soil properties, α -diversity, richness, and NMDS scores by tillage¹.

Variable	NT	tillRec–	tillRec+	NT/tillRec–/tillRec+				Till	NT/Till			
				χ^2	df	P			χ^2	df	P	
GWC	0.17 ($\pm$ 0.08)	0.16 ($\pm$ 0.09)	0.12 ($\pm$ 0.05)	3.01	2	0.222	ns	0.14 ($\pm$ 0.08)	2.15	1	0.143	ns
pH	6.61 ($\pm$ 0.38)	6.67 ($\pm$ 0.42)	6.15 ($\pm$ 0.39)	10.61	2	0.005	**	6.44 ($\pm$ 0.48)	1.22	1	0.27	ns
TC	29.72 ($\pm$ 14.35)	19.62 ($\pm$ 2.69)	16.72 ($\pm$ 4.62)	15.81	2	<0.001	****	18.33 ($\pm$ 3.89)	12.14	1	<0.001	****
DOC	84.71 ($\pm$ 59.96)	69.77 ($\pm$ 54.06)	37.62 ($\pm$ 8.36)	5.18	2	0.075	ns	55.48 ($\pm$ 43.22)	3.56	1	0.059	ns
TC 250–1000 μ m	36.30 ($\pm$ 29.65)	48.83 ($\pm$ 51.29)	14.36 ($\pm$ 6.20)	7.7	2	0.021	*	33.51 ($\pm$ 41.68)	4.79	1	0.029	*
TC 53–250 μ m	30.02 ($\pm$ 28.14)	10.90 ($\pm$ 4.68)	8.75 ($\pm$ 2.81)	12.39	2	0.002	***	9.94 ($\pm$ 4.05)	11.37	1	<0.001	****
TC < 53 μ m	27.81 ($\pm$ 9.75)	22.69 ($\pm$ 3.65)	20.94 ($\pm$ 7.50)	7.47	2	0.024	*	21.91 ($\pm$ 5.63)	7.3	1	0.007	**
TN	2.33 ($\pm$ 1.09)	1.60 ($\pm$ 0.26)	1.39 ($\pm$ 0.26)	13.38	2	0.001	***	1.50 ($\pm$ 0.27)	10.22	1	0.001	***
PMN	93.64 ($\pm$ 88.43)	39.41 ($\pm$ 36.40)	37.59 ($\pm$ 19.05)	6.9	2	0.032	*	38.60 ($\pm$ 29.46)	6.83	1	0.009	**
TN 250–1000 μ m	2.01 ($\pm$ 1.57)	2.61 ($\pm$ 2.64)	0.83 ($\pm$ 0.29)	8.98	2	0.011	*	1.82 ($\pm$ 2.14)	6.42	1	0.011	*
TN 53–250 μ m	1.91 ($\pm$ 1.75)	0.77 ($\pm$ 0.31)	0.64 ($\pm$ 0.21)	11.63	2	0.003	***	0.71 ($\pm$ 0.27)	10.74	1	0.001	***
TN < 53 μ m	2.45 ($\pm$ 0.79)	2.02 ($\pm$ 0.24)	1.87 ($\pm$ 0.56)	5.92	2	0.052	ns	1.95 ($\pm$ 0.41)	5.64	1	0.018	*
C:N ratio	12.67 ($\pm$ 1.36)	12.36 ($\pm$ 0.67)	11.92 ($\pm$ 1.14)	3.45	2	0.178	ns	12.16 ($\pm$ 0.92)	2.68	1	0.101	ns
C:N 250–1000 μ m	17.54 ($\pm$ 3.00)	18.05 ($\pm$ 1.44)	16.82 ($\pm$ 1.77)	2.52	2	0.283	ns	17.51 ($\pm$ 1.68)	0.63	1	0.429	ns
C:N 53–250 μ m	14.98 ($\pm$ 2.39)	14.39 ($\pm$ 1.56)	13.80 ($\pm$ 0.75)	4.38	2	0.112	ns	14.13 ($\pm$ 1.28)	2.84	1	0.092	ns
C:N < 53 μ m	11.26 ($\pm$ 1.14)	11.20 ($\pm$ 0.80)	11.00 ($\pm$ 0.75)	0.75	2	0.686	ns	11.11 ($\pm$ 0.77)	0.56	1	0.452	ns
PD	230	241	229	6.22	2	0.045	*	235	1.18	1	0.277	ns
Chao1	5356	5524	5350	1.96	2	0.375	ns	5446	0.61	1	0.434	ns
NMDS1	0.01	– 0.02	– 0.01	0.51	2	0.774	ns	– 0.01	0.45	1	0.502	ns
NMDS2	– 0.02	0	0.06	13.45	2	0.001	***	0.03	7.12	1	0.008	**
NMDS3	0.01	– 0.01	– 0.02	5.01	2	0.082	ns	– 0.01	4.86	1	0.027	*
NMDS4	0.01	0.01	– 0.03	6.68	2	0.035	*	– 0.01	1.76	1	0.185	ns

**** p < 0.001, *** p < 0.005, ** p < 0.01, * p < 0.05, ns p $\geq$ 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. PD: Faith's phylogenetic diversity; Chao1: Chao 1 richness estimate.

¹ Comparisons examine the presence and absence of tillage in 'NT/Till' or recency of tillage in 'NT/tillREC-/tillRec+'. NT, No Till; tillRec–, tilled less recently; tillRec+, tilled recently, or in the spring when samples were collected.

Table 3

Selected results from permutational multivariate analysis of variance (ADONIS) of category effects on bacterial diversity patterns.

Group	Factor	ADONIS	
		R ²	P
All ^a	Vineyard ^b	0.716	0.001
All	Tillage	0.056	0.003
Conv ^c	Tillage	0.068	0.092
Organic	Tillage	0.146	0.007
CC+ ^d	Tillage	0.074	0.001
Xeralfs	Tillage	0.169	0.006
Fluvents	Tillage	0.166	0.121
Rfd ^e	Tillage	0.100	0.007
All	Recent Tillage	0.097	0.001
Conv	Recent Tillage	0.191	0.003
Organic	Recent Tillage	0.192	0.014
CC+	Recent Tillage	0.138	0.001
Compost	Recent Tillage	0.136	0.004
Tilled	Recent Tillage	0.099	0.007
Xeralfs	Recent Tillage	0.216	0.008
Fluvents	Recent Tillage	0.655	0.001
Rfd	Recent Tillage	0.231	0.001
All	Compost	0.056	0.010
All	CC +/- ^d	0.063	0.002
All	Cover Crop ^f	0.288	0.001
Conv	Cover Crop	0.329	0.001
Organic	Cover Crop	0.408	0.001
Tilled	Cover Crop	0.344	0.001
NT ^g	Cover Crop	0.268	0.001
Xeralfs	Cover Crop	0.548	0.001
Xerolls	Cover Crop	0.492	0.001
Fluvents	Cover Crop	0.324	0.041
Rfd	Cover Crop	0.400	0.001
All	Grasses +/- ⁱ	0.119	0.001
All	Cereals +/-	0.083	0.002
All	Legumes +/-	0.051	0.009
All	Mustards +/-	0.048	0.013
All	Method ⁱ	0.099	0.003
Tilled	Method	0.156	0.005
NT	Method	0.167	0.007
Xeralfs	Method	0.277	0.003
Xerolls	Method	0.195	0.010
Rfd	Method	0.188	0.001

^a All: All groups

^b Vineyard: each individual vineyard that was sampled in the study

^c Conv: Conventional agricultural practices as identified by the vineyard managers.

^d CC +/-: Presence/absence of a planted cover crop, either with a cover crop (CC+) or no cover crop planted (only resident vegetation; CC-)

^e Rfd: Rutherford American Viticultural Area, a sub-appellation of Napa Valley.

^f Cover Crop: one of the six cover crop groupings.

^g NT: no-till.

^h +/-: Presence/absence of any non-cereal grasses (Grasses +/-), any cereals (Cereals +/-), any legumes (Legumes +/-), or any mustards (Mustards +/-) as components in the cover crop mix.

ⁱ Method: general agricultural method (conventional, organic, or biodynamic) as identified by the vineyard managers.

(a)

(b)

(c)

(d)

(e)