Assessing soil health across California vineyards: a multidisciplinary and participatory approach

By

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Abstract

Soil health is essential for agricultural adaptability and sustainability to climate change challenges. While soil health has been extensively studied in temperate annual agroecosystems with yield maximization goals, it remains understudied in semi-arid irrigated vineyards with unique production goals. This dissertation includes a participatory and multidisciplinary approach to assess the variability of soil health in vineyards. Integrating grower insights and scientific assessments, these three interconnected studies collectively address the complex relationship and variability of soil health practices, indicators, and microbial diversity in vineyards. The first chapter is composed of a "needs assessment" that evaluated wine grape growers' perceptions and attitudes of soil health through semi-structured interviews. Growers defined vineyard soil health as a balanced, biodiverse, and resilient ecosystem that supports high-quality grape production. Barriers such as economic risks and knowledge gaps hinder the adoption of soil health practices, especially among Late Majority growers, emphasizing the need for targeted outreach and practical, outcome-based research. The second chapter focuses on assessing the variability of soil health indicators, such as those that represent carbon, nutrient and water cycling functions as well as microbial diversity, across grower-defined challenging and ideal soils in vineyards. Soil texture emerged as a key determinant of soil health for the growers due to its influence on water cycling functions and perceived effects on vine balance and grape quality. In contrast, disturbance (till vs no-till) practices and vineyard zone (vegetative cover in the tractor rows vs bare and irrigated vine rows) influenced the variability of several soil health indicators. This work underscores the value of incorporating grower collaboration to link soil health assessments with management decisions, particularly those targeting carbon and water cycling. The third chapter investigates the diversity of soil microbial communities and their relationships with soil health indicators in vineyards. Variability in microbial alpha (Shannon diversity index) and beta (Bray-Curtis dissimilarity) diversity was influenced by soil texture, disturbance, and depth. Key soil health indicators, such as TC, MBC, WAS, and NO_3^--N, correlated with microbial diversity, revealing critical connections between microbial dynamics and soil health functions. Together, these studies illustrate the importance of integrating grower perspectives, soil health assessments, and microbial diversity analyses to enhance sustainable vineyard management, advancing our understanding of grower needs, soil health functions, and their role in viticulture.

Chapter 1: Wine Grape Grower Perceptions and Attitudes about Soil Health

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Abstract

Developing and adopting strategies that preserve soil health from degradation due to drastic changes in climate is critical for securing sustainable viticulture. For example, healthy soils promote water infiltration, nutrient cycling, and retention functions that support grape production. However, little research has evaluated drivers of growers' decision-making processes and actions towards soil management practices that impact soil health in vineyards. The objective of this study was to assess wine grape growers’ perceptions and attitudes of soil health to identify grower’s most important soil health functions and definition, and to understand how these might influence behavior related to soil management practices. Therefore, we conducted semi-structured interviews with 16 wine grape growers to understand current barriers, motivations, and opportunities for adopting and/or maintaining practices for building soil health in vineyards. Most growers described healthy vineyard soils as balanced, biodiverse, self-sustaining, and resilient systems that provide nutrient, and water cycling functions and support high-quality wine grape production. Three categories of growers emerged based on soil health attitudes including Early Adopter (n=3), Early Majority (n=4) and Late Majority (n=9) groups. The main barriers for adoption and maintenance of soil health practices were high costs, potential economic risks, and lack of information on how these practices influence grape production especially for the Late Majority group. Most growers were willing to adopt more soil heath practices if specific and practical information could be provided on outcomes of soil health practices for wine grape production systems—especially economic benefits. The outcomes of this study guide future soil health research and outreach activities to better support growers in building and protecting vineyard soil health while achieving viticultural goals.

1. Introduction

Agricultural sustainability includes ecological soundness or health, social responsibility, and economic viability (Hoffman et al., 2011; Velten et al., 2015). Preserving soil health is imperative for ensuring the sustainability of viticulture and environmental healthiness amid climate-induced challenges in semiarid regions (Cataldo et al., 2024). Among these challenges are intensified rainfall and drought events, which have contributed to erosion and the depletion of soil organic matter, thereby impeding soil infiltration and moisture retention functions that are vital for ecosystem services and resilience. Vineyard soils from Mediterranean semi-arid regions also are strongly susceptible to erosion and degradation under a changing climate (Belmonte et al., 2016; Ferreira et al., 2022). These challenges and risks have increased the wine grape industry interest in soil health. Soil health is defined as the continuous capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans (USDA NRCS). Some of the functions potentially provided by a healthy soil include carbon sequestration to offer carbon offsets for greenhouse gas emissions (Lazcano, et al., 2022a); filtration to improve water quality (Lewandowski & Cates, 2023); habitat to enhance soil biodiversity (Saleem et al., 2019), and more efficient nutrient management (J. Lehmann et al., 2020). Therefore, improving the adoption of practices that build soil health is essential to support vineyard resiliency to degradation, longevity, and sustainability for wine grape production.

1.1. Terroir vs. soil health

Historically, the wine grape industry has valued soils as an essential component of *terroir*, i.e., the interaction of human and natural environment factors such as winemaking, climate, soil, geology, viticultural management practices, and vegetation that influence wine attributes (Van Leeuwen & Seguin, 2006) . Terroir is a common concept in viticulture and enology that focuses on inherent soil properties (e.g., soil texture and mineralogy), climate, and cultivar (van Leeuwen et al., 2004; Van Leeuwen et al., 2018). Inherent or static soil properties, such as textural class, clay minerology, and depth to bedrock, are typically insensitive to management because they are derived from soil forming factors (i.e., climate, organisms, topography, parent material, and time) (Jenny, 1941, 1994). Soil health, on the other hand, focuses on *dynamic* properties, including soil organic matter, biota, nutrient cycling, and aggregate stability, which *are* responsive to soil management practices that benefit soil health. Such practices include the application of compost and other organic amendments (Cataldo et al., 2021), and reduced disturbance for cover crop management like no-till (Belmonte et al., 2018). We posit that uncertainties about the meaning of soil health within a viticultural context, knowledge gaps in the effects of soil management practices on Mediterranean vineyard soil health, grape quality and the expression of terroir (Lazcano et al., 2020) might influence growers' selection and adoption of soil health practices.

1.2. Wine grape production challenges and opportunities for soil health

Wine grape production has unique goals like managing vine balance, berry size and chemistry, and yields for high-quality wine production (Reynolds, 2022). Vine balance aims to achieve a balance of fruit yields with vine leaf area and shoot growth, so vines are neither weak nor over-vigorous (Skinkis 2019; Howell, 2001). Reducing berry size can facilitate the extraction of compounds from grape skin into the fermenting must (Melo et al., 2015) and promote more concentrated phenolic compounds from the skin (W.-K. Chen et al., 2018) for better wine aroma, color and flavor (Li & Sun, 2019). Vine balance and berry size can be manipulated by inducing water stress during key phenological stages using deficit irrigation (Chaves et al., 2010; J. Mirás-Avalos & Araujo, 2021; J. M. Mirás-Avalos & Intrigliolo, 2017; Santesteban et al., 2011; Van Leeuwen et al., 2009; L. E. Williams, 2017; B. Yang et al., 2020). Soil fertility is also often managed for grape quality parameters (i.e., sugars, acidity, and other secondary metabolites) (Verdenal et al., 2021). Soil conservation practices, including cover cropping, mulching, and reduced tillage, also impact vine and berry growth management by influencing soil physical properties and therefore facilitate water infiltration and reduce soil compaction for proper vine root growth (Lazcano et al., 2020). However, divergent conclusions from studies (Cataldo et al., 2021; Steenwerth, McElrone, Calderón-Orellana, et al., 2013) highlight the complexity of soil health management implications for viticultural goals. These mixed findings and recommendations might influence growers' perception of vineyard soil health and soil management and subsequent decision making.

1.3. Knowledge, perceptions, and attitude assessments

Perceptions (how a person or group interprets and provides meaningfulness to information) and knowledge (objective, correct information and skills learned) can inform attitudes—including thoughts and actions (Pickens, 2005). Knowledge and perceptions of growers, who are instrumental managers of natural resources, are particularly relevant because they catalyze changes in soil management decisions and actions (Kenfack Essougong et al., 2020). This is especially important since 52% of U.S. land is used for agricultural production (USDA, 2023). In wine grape production systems, decision-making for use of sustainable practices has been impacted by the level of knowledge of both wine grape growers and outreach professionals (Lubell et al., 2011). Consequently, these decisions and actions on land management practices could influence the current and future state of soil health and security. To date, many studies assess soil health using quantitative methods in diverse agroecosystems (Chahal et al., 2021; Congreves et al., 2015; Devine et al., 2021; Fine et al., 2017; Gabechaya et al., 2023; Jemison et al., 2019; Karlen et al., 2019; Kesser et al., 2023; Nunes et al., 2018; Panicker et al., 2022; Sprunger et al., 2021); however, growers' knowledge and perceptions about soil health are rarely included.

Growers' knowledge and perceptions have a significant influence on farm decision-making and actions (Fantappiè et al., 2020; Nguyen et al., 2016). Biophysical soil health assessments in the U.S. have been performed mostly in the midwestern and northeastern regions (Karlen et al., 2019b), where agronomic grain crop systems dominate, and goals are targeted towards increasing soil fertility and yields (Hoffman et al., 2014; Lamarque et al., 2014). Similarly, the few qualitative studies examining grower knowledge, perceptions, and attitudes about soil health have been conducted for commodity production systems (Bagnall et al., 2020; Carlisle, 2016; Irvine et al., 2023; Klein et al., 2024; Mann et al., 2021; Petrzelka et al., 2023). These studies can help us understand general trends that could influence grower decision making as well as understand barriers and incentives. However, results from qualitative studies based in commodity grain systems may not be applicable for high-value wine grape production due to many key differences in agroecological systems (i.e., perennial vs. annual systems), management, and production goals including grape quality prioritization over yields.

To date, there are no qualitative studies assessing in-depth grower knowledge, perceptions, and attitudes particularly about soil health for vineyard systems. Findings from commodity production systems suggest that farmer knowledge, community perceptions, and competitive pressures exercise great influence on the adoption of soil health practices, particularly cover crops and conservation tillage (Arbuckle & Ferrell, 2012; Bell, 2004; Carlisle, 2016; Carolan, 2005). Further, views of commodity producers about soil health differ slightly from those held by soil scientists, especially since these growers prioritize biological parameters, crop productivity and plant health (Mann et al., 2021). Most qualitative research that indirectly involves soil health in grain production systems is related to the adoption of particular practices like cover crops (Arbuckle & Roesch-McNally, 2015; Marques et al., 2015), integrated livestock systems (Hayden et al., 2018), and conservation tillage (Bossange et al., 2016).

In vineyards, qualitative studies that indirectly involve soil health include topics like cover crop adoption and management (Marques et al., 2015), agroecological practices (Garini et al., 2017), drivers of adoption of sustainable practices (Caffaro et al., 2023), best management practices for groundwater quality (Calliera et al., 2021), and integrated livestock systems (Ryschawy et al., 2021). For instance, growers' perceptions of uncertainties about production outcomes and increased costs from using sustainable soil management practices present challenges to their implementation (Dunn et al., 2016; Hoffman, 2013; Marques et al., 2015; Schütte & Bergmann, 2019). Identifying such gaps in the context of soil health assessments and practices in vineyards could contribute to advancing practical research and outreach efforts, facilitating enhanced understanding, and fostering transparent expectations among wine stakeholders.

The Napa Valley American Viticultural Area (AVA) is a representative place to study vineyard soil health from the growers' perspectives since it is an established and high-value and quality wine growing region (Hira & Swartz, 2014) with high soil and microclimate diversity containing 16 sub-appellations (Title 27, Ch.1 # C.F.R. § 9.21 (1979) https://www.ecfr.gov/current/title-27/chapter-I/subchapter-A/part-9/subpart-C). Other advantages of the Napa Valley AVA that make this wine growing region successful include high levels of social capital and entrepreneurship (Hira & Swartz, 2014) which may influence decision making related to soil management practices. In addition, government efforts as well as certifications for the protection of natural resources and specific management practices might influence vineyard soil decision making. For example, the County's efforts for reducing soil erosion and contamination of water (Napa County Code § 18.108) may incentivize the reduction of soil disturbance with practices such as cover crops and reduced tillage especially in sloped vineyards. Vineyard certification labels that validate organic, regenerative, sustainable, and biodynamic practices could make soil health practices attractive due to the potential added value that these practices could have on grapes (Delmas & Gergaud, 2021). Some examples include the Fish Friendly Farming (FFF) (www.fishfriendlyfarming.org), Napa Green (https://napagreen.org), California Certified Organic Farming (CCOF) (www.ccof.org), and the Certified California Sustainable Winegrowing (CCSW) (www.sustainablewinegrowing.org) certifications. Yet, lack of information and economical challenges have been shown to act as barriers for the adoption or maintenance of practices that protect and build vineyard soil health (Lubell et al., 2011; Marques et al., 2015; Payen et al., 2023).

1.4 Project objectives and hypotheses

We interviewed 16 growers to address three main objectives: (1) assess wine grape growers' current knowledge, perceptions, and attitudes about soil health, (2) identify barriers and motivations for adoption and maintenance of soil health practices, and (3) identify research and outreach opportunities related to soil health in vineyards. The overarching goal of this study is to analyze how Napa Valley wine grape growers can be better supported in improving and/or protecting vineyard soil health. We used the Theory of Planned Behavior (Ajzen, 1991) as an underlying framework to explain actions and intent based on perceptions and attitudes about soil health. Additionally, we used the Diffusion of Innovation Theory (Rogers et al., 1995) to classify and explain emerging groups based on the potential for adoption of soil health practices. In general, we hypothesize that knowledge, perceptions, and attitudes based on wine grape production outcomes will be the main factors to explain behavior and intent related to soil health management decisions.

2.Methods

2.1 Napa Valley wine growing region

This study took place in the American Viticultural Area (AVA) of Napa Valley in California, USA. The AVA is defined as "a delimited grape-growing region with specific geographic or climatic features that distinguish it from the surrounding regions and affect how grapes are grown" (US Department of the Treasury, Alcohol and Tobacco Tax and Trade Bureau). The Napa Valley AVA is in the North Coast region of California situated between approximately 38.4° and 38.6° latitude and -122.3° and -122.5° longitude, spans a diverse topography with elevations ranging from 20 to 2,600 feet, and characterized by geographical features such as the Napa River and the Mayacamas and Vaca Range Mountains. This region has a Mediterranean hot summer from the Modified Köppen Classification system (California Department of Fish and Wildlife, 2021) which consists of cold/rainy winters and hot/dry summers. The rainfall is distributed between the months of November and March. In 2021 (when this study took place), the cumulative annual rainfall in Napa was 286.3mm, and the mean annual temperature was 21.7° C. The monthly mean minimum and maximum temperatures were 1.6°C and 26.7°C respectively for 2021 (UC ANR). However, projected impacts of extreme weather fluctuations due to climate change over the next years present a threat for sustainable wine grape production in California including the region of Napa Valley (Parker et al., 2020). Napa County has a rich soil diversity due to diverse parent materials, microclimates, and topography; for instance, in the mountains and hills, Napa soils are primarily formed from volcanic and marine sediment parent material and in the valley, soils are mostly formed from alluvial deposits (Kunkel and Upson, 1960).

Napa Valley's main agricultural activity is wine grape production, and it is a region recognized for producing high-value wine grapes, particularly varieties like cabernet sauvignon and chardonnay (CDFA Grape Crush Report, 2021). This region has approximately 475 physical wineries, mostly family-owned (Napa Valley Vintners, 2022). Production comprises a total land of 19 thousand hectares (ha), with an average production of 6 thousand kg per ha, total production of 245.1 billion kg, and value of $741.7 million USD (Napa County Agricultural Crop Report, 2021). Napa wine grapes have the highest value ($6,101.84 USD per ton or $6.73 USD per kg) in California and the U.S.A, accounting for 22.7% of the total state revenues from wine grape production (CDFA 2021-2022 report). Red wine grapes (primarily cabernet sauvignon) occupy 14.8 thousand hectares of land and producing an average of 6 thousand kg per ha with a total tonnage of 85.8 million kg and value of $663.4 million (USD) (Napa County Agricultural Crop Report, 2021). White grapes (primarily chardonnay) occupy 3.8 thousand hectares and have an average production of 6.1 thousand kg per hectare, a total production of 23.5 million kg and value of $78.3 million USD (Napa County Agricultural Crop Report, 2021).

Many wine grape growers are organized in grower-focused organizations like the Napa Valley Grape Growers Association (NVGA) and the Napa Valley Vintners. Agricultural trade associations like these promote the implementation of best practices for wine grape production and are a platform for knowledge transfer and co-production among the community, outreach professionals, and academic researchers (Nocco et al., 2020; Sullivan, 2008; Taplin, 2010). The NVGA is a 501(C)3 non-profit organization founded in 1975 with the goal of supporting wine grape production in Napa and represents over 710 Napa County grape growers, vineyard owners, and associate businesses. Another stakeholder that influences knowledge transfer and improvement of practices is the University of California at Davis (UC Davis) with the creation of the viticulture and enology program in 1935 (Bonné, 2013) and the Oakville Research Station (Napa County, CA) for advancements in viticulture knowledge.

2.2 Data Collection

We recruited participants in collaboration with the Napa Valley Grape Growers Association (NVGA) in the summer of 2020. The NVGA contacted all grower members (n=18) and reached out to colleagues that were non-members (n=6) by email with an announcement of our soil health project seeking participants. The NVGA assisted in reaching a total of 24 growers. A total of 18 growers responded with interest in participating in our project, 17 accepted to participate and one declined to participate in the interviews. Out of the 17 interviews, one had to be excluded from data analysis due to issues of low-quality recording and transcription. Finally, this study assessed semi-structured interviews of 16 wine grape growers, leading to a recruitment success of 66.7 %. The number of participants in this study is in alignment with other similar qualitative studies (Garini et al., 2017; Klein et al., 2024; Singh et al., 2024).

From the 16 participants, 13 growers agreed to answer a short survey prior to the interviews to obtain general information about their work. Examples of information collected in the pre-interview survey include number of vineyards managed, their main role and involvement in vineyard management, years of experience, main grape varieties produced, vineyard certifications, acreage of total production, yield quantities, vineyard goals, location (American Viticultural Area) and estimated soil textural class of vineyards (SSURGO, n.d). The platform used to conduct the survey was Google Forms (Google Inc.), and these were distributed through email prior to the interviews (Table 1.A1).

We conducted semi-structured interviews with the wine grape growers consisting of 18 open-ended questions (Table 1.1) upon the approval of the Institutional Review Board (IRB code: 1557520-1) of the University of California Davis. Semi-structured interviews use key predetermined open-ended questions combined with follow-up questions emerging from the dialogue to encourage a more complete understanding of what is being asked (Merriam and Tisdell, 2017). Questions used as guide for the interviews were designed with the interdisciplinary collaboration of UC Davis (professors and extension specialists) and USDA ARS scientists, and the support of the Napa Resource Conservation District (RCD). An example of a key question asked to growers in this study was “what soil management practices do you perform on the vineyard soil and why?”, and a follow up question to a grower answering “no-till and cover cropping” would be “why do you choose to do no-tillage instead of tilling the cover crops?” Questions and follow up questions like these allowed us to better understand the growers’ point of view of soil management decision making.

Before starting the project, practice interviews were completed with the Napa RCD staff to refine the questions and structure of the conversation. During the summer of 2020, interviews with the growers lasted from 45 to 60 minutes, during which growers expressed their thoughts on soil health and soil management practices for wine grape production. All interviews were conducted through video call (Zoom Inc.) except for two that were done by phone call. The interviews were recorded with the permission of the interviewees and transcribed by hand. In general, topics covered in the semi-structured interviews included: ideal soil properties and functions, soil health definitions and assessment for wine grape production, soil management practices, vineyard certifications, soil testing, and soil biology and organic matter.

Table 1.1. *Semi-Structured interview questions*

1. How would you describe an ideal soil for your vineyard goals and why?
2. What soil management practices do you perform on the vineyard floor and why? (examples: cover crops, no-tillage, reduced tillage, tillage, compost)
3. What type of soil management practices have you incorporated or are thinking of incorporating to maintain or receive a certification?
4. Do you test your soils regularly? Why? What do you test? What depths do you sample?
5. For you, what is soil health? How would you describe a healthy vineyard soil?
6. How important is soil health for wine grape production? Why?
7. What is "terroir"? How would you compare it to soil health?
8. What soil properties and functions are more important for your goals?
9. What do you think is the role of soil organic matter in vineyards?
10. What do you think is the role of the soil biology (living organisms) in vineyards?
11. What do you think is the role of the soil microbiome in the vineyard?
12. What are your soil health objectives or goals?
13. How do you detect that soil health is improving or decreasing?
14. Do you think there has been changes in soil health in the vineyards that you manage in the past years? Please explain.
15. What are some soil management practices targeted for improving soil health that you find hard to adopt and/or maintain and why?
16. What will help you decide to adopt more practices for soil health?
17. Do you have the freedom to take the floor management decisions for the vineyard or do you follow someone else's specific visions?
18. Are you concerned about the impacts that soil health practices might have on yields and/or grape quality? Please explain.

2.3 Data Analysis

We summarized the data from the short surveys using descriptive statistics. Before data analysis of the semi-structured interviews, all growers were assigned a random ID number for anonymity. We conducted open (first pass) and descriptive (second and third passes) coding following an inductive coding approach (Saldana, 2015) and using NVIVO software as a coding tool (QSR software, 1.6.2 version). The inductive coding consisted of identifying themes that emerged from the growers' answers (Saldana, 2015). The codes were maintained close to the terms used by growers, and the analysis of these data was adapted to the context of scientific terms. Afterwards, codes from the semi-structured interviews were analyzed using thematic analysis (Cooper et al., 2012; Saldana, 2011) into more general themes by research question.

We assessed growers' knowledge, perceptions and attitudes on soil health following the theories of Planned Behavior (Ajzen, 1991) and Diffusion of Innovation (Rogers et al., 1995). The theory of Planned Behavior is used to study how attitudes might influence behavioral intent and actions based on the perceived outcomes and potential associated risks and/or benefits. The integration of this theory has been successfully used to explain human behavior (Ajzen, 2015) and farmers' intentions towards sustainable/conservation agriculture (Márquez-García et al., 2019; Tama et al., 2021). The Diffusion of Innovation theory is used to study how products or ideas perceived as novel or non-conventional get adopted by a specific population or group and has been widely used to explain adoption of sustainable practices in agriculture (Lavoie et al., 2021; Lubell et al., 2011; Rosário et al., 2022; Senyolo et al., 2018). The Diffusion of Innovation theory describes five adopter categories: Innovators, Early Adopter, Early Majority, Late Majority, and Laggards in order from more likely to least likely to adopt a different practice. The innovators are interested in being the first to develop or adopt a new practice and are comfortable with risks and uncertainty. The Early Adopter represent opinion leaders that embrace change opportunities, are willing to take risks and accept some uncertainty, pursue innovation, and are driven by personal interest and curiosity rather than social norms. The Early Majority adopts new practices usually before the average person of their population and are less comfortable with uncertainty but are willing to adopt new practices if they understand how the innovation works and see its success before adopting. Compared to the Early Adopter, the Early Majority group is strongly influenced by social norms and peer experience and are more risk averse. The Late Majority group is more skeptical of change, uncomfortable with uncertainty, and will only adopt an innovation after it has become well established and widely adopted or if there are regulatory compliance requirements. Finally, the Laggards group are very conservative, do not accept uncertainty, and strongly resistant to change.

In this study, we evaluated how expected outcomes influenced growers' views and current actions to support soil health through sustainable management practices. Then, we evaluated growers' maintenance and/or adoption potential for soil health practices through the Diffusion of Innovation theory adopter categories. Finally, we evaluated factors that influenced growers' decision-making and barriers to adopting or maintaining soil health practices in vineyards.

2.4 Background grower information from pre-interview surveys

Out of the 16 participants, 13 responded to the pre-interview survey intended to gather background information from the growers. Most growers identified themselves as vineyard managers (n=9), two as both owners and managers, and two as owners only. The median of the estimated acreage for the total commercial operation growers manage was 26.3 hectares (minimum = 1.8, maximum = 166.3 hectares, two growers abstained from answering). Around half of the growers have more than 10 years of experience in the industry (median = 14.5, minimum = 2.5, maximum = 30). Most growers (n=11) worked in vineyards with at least one sustainability-related certification not related to organic farming, and four vineyards were certified organic. Some of these certifications were the Fish Friendly Farming (FFF) (www.fishfriendlyfarming.org), Napa

Green (https://napagreen.org), California Certified Organic Farming (CCOF) (www.ccof.org), and the Certified California Sustainable Winegrowing (CCSW) (www.sustainablewinegrowing.org). The most common certification that growers had was the Fish Friendly Farming (FFF) with the purpose of complying with the Napa County ordinance Title 18. Zoning (§18.108) or reducing soil erosion. When asked to select the goals and priorities for their vineyards, all growers selected "achieving high grape quality" (n=13) followed by "building soil health" (n=10), complying with certifications (n=8), and achieving yield goals (n=6). Other goals that growers mentioned were sustainable farming, staying within the client's (owner) budget, and reducing fertilizer inputs.

2.5 Positionality Statement

The author who conducted the interviews is a Ph.D. candidate in soils and biogeochemistry with a B.Sc. in agronomy and M.Sc. in soil science with applications to agricultural ecosystems. The first author understands the research processes from the perspective of an early career soil scientist. Other co-authors include early-career technicians and established research scientists and professors in the disciplines of soil and water science, rural sociology, viticulture, and cooperative extension.

3.Results

3.1 Knowledge, perceptions, and attitudes on soil health

Overall, vineyard soil health was viewed as important by all growers (n=16). Regardless of the attitudes towards soil health, all growers highlighted the importance of soil health especially for preventing soil loss through erosion. Desired soil properties and functions were driven by vineyard production goals. In general, achieving high grape quality was the main priority for the Napa Valley AVA growers and a way this is achieved is through the control of yields (tonnage) and berry size, often requested by grape buyers or wine makers and managed through vine vigor control.

> *"Well, you have to understand that it's a little bit different [to other crops] because we're selling to wineries that are creating ultra-premium wines. So, yields are important but, at the same time it's the quality of the fruit that's actually more important."* - Grower #4

Grower #4 speaks to the point of grape quality attributes being more important than producing high yields. A reason for the focus on high-quality grape production is that these can be sold at higher values. However, the specific attributes that make grapes high-quality ones are highly subjective.

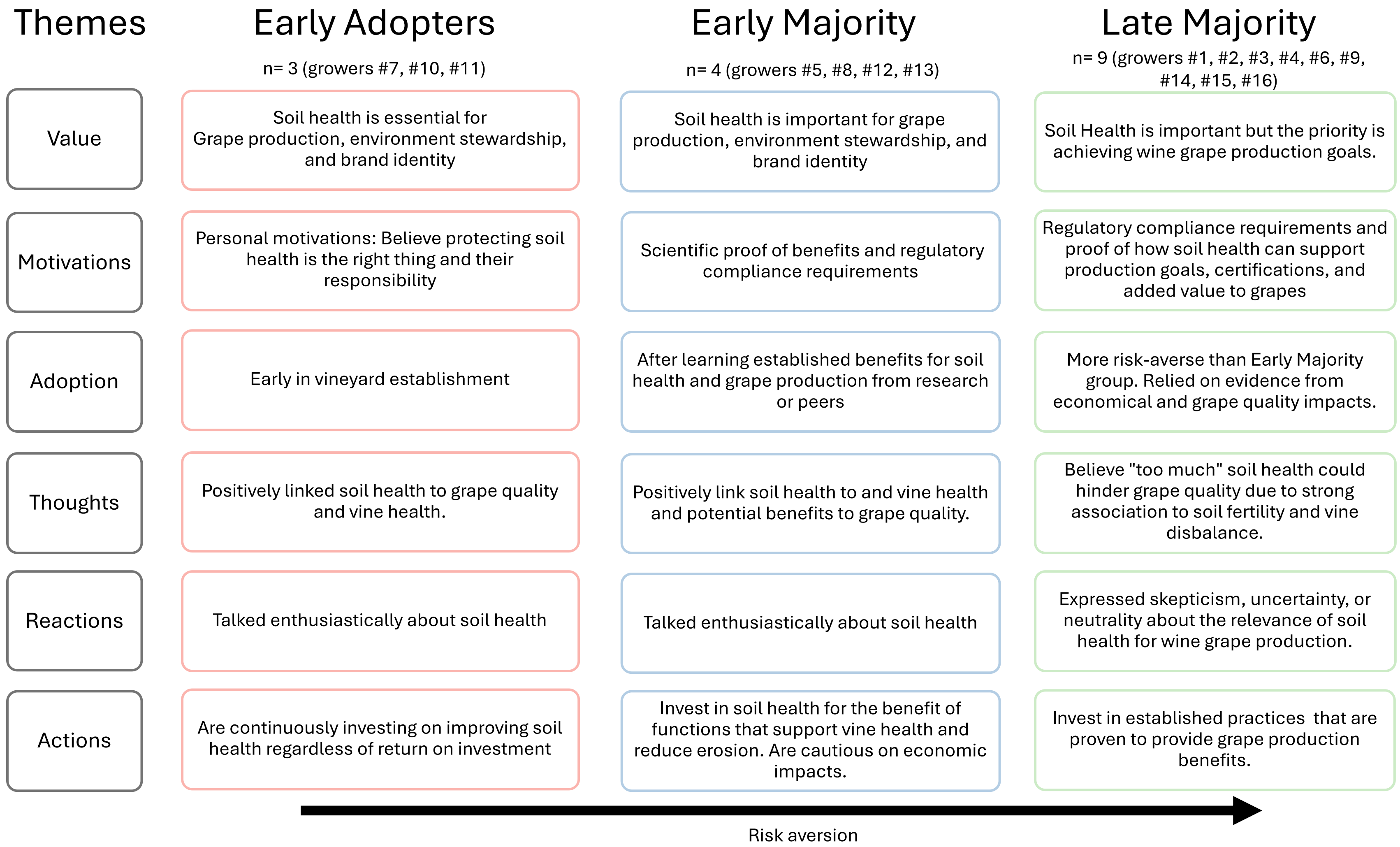
Attitudes towards the importance and relevance of soil health based on expected outcomes (theory of Planned Behavior, Ajzen 1988) allowed us to group growers in three categories from the Diffusion of Innovation Theory (Rogers 1962): Early Adopter (n=3), Early Majority (n=4), and Late Majority (n=9) (Figure 1). No trends were seen in these groups in terms of vineyard certifications, years of experience of each grower, or acreage of total production they manage based on median values of participants (Appendix Figure 1). The three growers in the Early Adopter group (Figure 1: growers #7, #10 and #11) described soil health as an essential part of their production goals and brand; they adopted soil health focused management practices early in their vineyard establishment due to personal motivations. For example, grower #10 expressed they adopted regenerative agriculture practices around 25 years ago, making them one of the first growers to do soil health focused vineyard practices in the area. Growers #7 and #11 were not some of the first ones to adopt soil health practices in the area, but they started their vineyards with a focus of protecting soil health and the environment since they believed it was the correct thing to do. Growers in the Early Adopter group expressed that building soil health and doing sustainable practices for the environment was their responsibility, an investment, and a focal part of their brand identity. Growers in the Early Majority group (Figure 1: n=4; growers #5, #8, #12, #13), like the Early Adopter group, valued soil health as an investment not only for achieving wine grape production goals but also for the stewardship of the environment and natural resources. However, the Early Majority group relied more on sources such as research and outreach, peer experiences, trials, and field observations for making decisions on soil management practices. The main difference between the Early Adopter and Early Majority groups is that the first group is mainly motivated by personal interests and beliefs and is more comfortable with taking risks. Growers in the Early Adopter and Early Majority groups highlighted soil functions like carbon sequestration, nutrient and water cycling that benefited vine health, and vineyard resiliency and longevity.

> *"Soil health is critical. To be a sustainable farmer you have to be a steward on the land in three areas. You have to be the environmental steward, which is passing on your land in better shape than you received it. You have to be socially aware and sustainable by paying a living wage to your workers and including them in the empowerment of creating good soils and a good vineyard. And ultimately you have to be profitable."* – Grower #11

Grower #11 (Early Adopter group) described the essential role that soil health plays in their business values of sustainable wine grape production and stewardship of the land while also promoting the wellbeing and inclusion of farmworkers. Overall, growers in the Early Adopter and Early Majority group associated improving soil health with better vine health and grape quality although no specific details were provided by any growers on how soil health practices would improve the quality of the grape.

> *"I think it [soil health] has to have an effect on the grape quality. There must be correlation between a healthy soil and then a healthy plant and productivity on the plant for fruit."* – Grower #7

Grower #7 (Early Adopter group) expressed their thoughts on the connection between soil health, vine health and grape quality. This grower explained that soil health can improve nutrient cycling and provisioning for good vine health but was unable to expand on specific attributes soil health could influence to grape quality.



Themes	Early Adopters n= 3 (growers #7, #10, #11)	Early Majority n= 4 (growers #5, #8, #12, #13)	Late Majority n= 9 (growers #1, #2, #3, #4, #6, #9, #14, #15, #16)
Value	Soil health is essential for Grape production, environment stewardship, and brand identity	Soil health is important for grape production, environment stewardship, and brand identity	Soil Health is important but the priority is achieving wine grape production goals.
Motivations	Personal motivations: Believe protecting soil health is the right thing and their responsibility	Scientific proof of benefits and regulatory compliance requirements	Regulatory compliance requirements and proof of how soil health can support production goals, certifications, and added value to grapes
Adoption	Early in vineyard establishment	After learning established benefits for soil health and grape production from research or peers	More risk-averse than Early Majority group. Relied on evidence from economical and grape quality impacts.
Thoughts	Positively linked soil health to grape quality and vine health.	Positively link soil health to and vine health and potential benefits to grape quality.	Believe "too much" soil health could hinder grape quality due to strong association to soil fertility and vine disbalance.
Reactions	Talked enthusiastically about soil health	Talked enthusiastically about soil health	Expressed skepticism, uncertainty, or neutrality about the relevance of soil health for wine grape production.
Actions	Are continuously investing on improving soil health regardless of return on investment	Invest in soil health for the benefit of functions that support vine health and reduce erosion. Are cautious on economic impacts.	Invest in established practices that are proven to provide grape production benefits.

Figure 1.1. Categories of growers based on attitudes towards soil health for wine grape growing in Napa Valley (California, USA).

Growers in the Early Adopter and Early Majority groups performed more than one sustainable soil management practice like cover cropping, compost application, reduced or zero tillage, and/or sheep grazing. The third group that emerged for growers in terms of attitudes towards importance and relevance of soil health for wine grape production in Napa Valley was the "Late Majority" group. The Late Majority group consisted of nine growers (Figure 1, n=9: growers #1, #2, #3, #4, #6, #9, #14, #15, #16) and attitudes consisted of conditional interests towards soil health. For example, although growers from this group recognized soil health as important particularly for vine health and preventing soil erosion, practices that allowed consistent desired outcomes related to grape quality were prioritized regardless of how these impacted the soil health.

> *"It's important to have good soil health to grow grapes. But how we do soil health isn't my job, it's to grow the grapes. So, sometimes people get so focused on it. It's not the most important thing, right?"* – Grower #15

As an example of common thoughts in terms of priorities of the Late Majority group, Grower #15 acknowledged the general importance of soil health but reiterated their responsibility and priority of focusing in achieving grape production goals. Additionally, it appears that Grower #15 decouples the connection between wine grape production and soil health.

There were contrasting views across the Late Majority group on the links between soil health, vine health, and grape quality. For instance, some growers from this group agreed that having good soil health could benefit grape quality because having good water cycling could promote the desired water stress for vines (Growers #1 and 4) and benefit vine health especially by providing the vines the nutrients they needed (Grower #16). Late Majority growers also expressed doubts and/or concerns on how improving soil health could disbalance vigor and grape quality.

Here, soil health was often strongly linked to increasing soil fertility and therefore potential vine disbalance (n=5; Grower 1, 3, 4, 9, 14).

> *"I think [soil health] it's important. But I think that sometimes a soil can be too healthy for wine grapes. For high wine grape quality, you need to apply a certain level of stress to the plant. So, think there is a point in which the soils can be almost too healthy that you can't apply that stress that you need."* – Grower #9

Grower #9 associated soil health with soil fertility and perceived unfavorable outcomes on vine balance and grape quality if soil health levels are high. With follow up questions, Grower #9 explained the association of high soil health to high levels of nutrients, organic matter, and available water in soils. Some participants (n=2; growers 14 and 15) explicitly mentioned that vines do not require high levels of nutrients and thrive in less fertile soils.

> *"If you have a soil that literally has more water and nutrients then you should be growing a different crop, not grapes."* - Grower #14

Grower #14 speaks to the point of soils with high nutrient and water cycling being unsuitable for wine grape production. During the conversation, Grower #14 expressed the need for inducing water stress to the vines and not promoting high vegetative growth to achieve the desired yields and berry quality. Additionally, soil health was described as an unclear concept that growers need more information on (Growers 15 and 6).

> *"Soil Health it's not just that you have earthworms in your soil, that is a romantic picture. But because it's a very human-born concept -- health of the soil, what are the healthiness of the soil? I don't know... Second to terroir, soil health is an elusive concept, so it's very widely used, but I don't think that I have a good definition."* – Grower #6

Grower #6 recognizes the complexity of soil properties and expands on their uncertainties on what a healthy soil is for wine grape production. This grower also expresses the lack of understanding that exists for the concept of terroir which, although is widely used in the wine industry, it remains unclear. Finally, several growers in this group (n=4) described soil health's importance as one that only needs to sustain targeted wine grape production goals.

3.1.1. Napa Valley Grower definitions of soil health

When discussing soil health for vineyards, often growers emphasized the uniqueness of wine grape production compared to other crops. Generally, there was an association of soil health with soil fertility and therefore a potential risk for not achieving production goals (i.e. high-grape quality). Particularly, a healthy soil for red wine grape production was mostly described as a balanced soil (n=12; 2 Early Adopter, 3 Early Majority, 7 Late Majority) meaning that it is not a highly fertile nor nutrient depleted soil. A balanced soil was described to promote vine balance. For example, soils that were "too healthy" (i.e. too fertile) were seen as undesirable because it could disbalance vine vigor and hinder grape and wine quality.

> "*I think that sometimes there can be a misconception about, this will sound weird but, a soil I think can be too healthy for wine grapes. I would say for high wine grape quality, you need to apply a certain level of stress to the plant. So, I do think there is a point in which the soils can be almost too healthy that you can't apply that stress that you need. Vineyard soils need to be healthy but there needs to be also a pretty good balance in the stress that you can apply to the plants.*" - Grower #9

Grower #9 (Late Majority group) expressed concerns on how improving soil health could interfere in the water stress practice needed for vines to support proper wine grape production. During the conversation, Grower #9 explained this could happen since improving soil health increases soil water holding capacity and nutrient availability that could promote high vigor in vines.

> *"It's not about growing a big, healthy, green, plant. It's about producing fruit and so, you try to grow your plants in a way that you have, let's say, seven pounds of fruit for every pound of wood on your plant. And so, we aren't necessarily looking for super fertile soils"*. - Grower #3

Grower #3 reiterates the uniqueness of wine grape production goals of not promoting large plant growth by increasing soil fertility; instead, the goal is to maintain a balance in vine vigor for achieving targeted grape quality parameters. Following these viticultural goals, the functions and properties of a healthy vineyard soils are those that also support vine health (n=10; 1 Early Adopter, 2 Early Majority, 7 Late Majority) and prevent soil erosion like having adequate (not excess) nutrient cycling (n=10; 2 Early Adopter, 2 Early Majority, 6 Late Majority), good structure and aeration (n=9; 1 Early Adopter, 3 Early Majority, 5 Late Majority), and adequate water holding capacity (n=7; 1 Early Adopter, 1 Early Majority, 5 Late Majority) and (Table 1.2). Interestingly, many growers also mentioned soil biodiversity and biological activity (n=7; 3 Early Adopter, 1 Early Majority, 3 Late Majority) as an important component of a healthy vineyard soil (Table 1.2). However, they also expressed lack of knowledge and strong interest in learning more about their role in soil health and benefits for wine production (n=8; 3 Early Majority, 5 Late Majority). These growers were also interested in learning more about the interpretation of novel soil biodiversity tests like DNA sequencing as they become more commercially available.

Table 1.2. Soil health properties and functions for vineyards as described by the growers interviewed in this study.

Soil Health properties and functions	**Total growers**	**Early Adopter**	**Early majority**	**Late majority**	**Examples**
Nutrient cycling	10	2	2	6	"A healthy soil for me has obviously got to be able to hold onto nutrients, it's going to be able to hold onto provide enough water to the vines when necessary but also not have runoff due to issues with any kind of nutrient imbalances." - Grower #1 (LM)
Good structure and aeration	9	1	3	5	"I'd like to see strong soil structure, strong aggregates" - Grower #5 (EM)
Appropriate water holding capacity	7	1	1	5	"What I am looking for are soils that hold moisture, so I don't have to water as much." - Grower #3 (LM)
Soil biodiversity	7	3	1	3	"Soil health to me implies the living part of it, more than the physical-chemical parts of it." - Grower #15 (LM)
Good infiltration	6	1	1	4	"To me a healthy soil captures the rainfall and allows it to get into the groundwater." - Grower #14 (LM)
Adequate soil organic matter levels	6	0	2	4	"Ideally I would like to see high carbon levels." - Grower #16 (LM)
Promotes achievement of targeted grape production	7	0	1	6	"[The goal] is to keep the soil intact I would say. To keep the ability of the soil to produce, to make it as durable as we can... and obviously to produce, what I'm meaning is that we need the soil to still be where, and we can still produce grapes in the quality and quantity that we've been doing for a while." - Grower #6 (LM)
Promotes healthy vines and good root growth	10	1	2	7	"I guess if I took a plant tissue sample and it came back everything nice and even, I would probably think that my soil health is at a place that I want it to be. Then, visually, if I take a look at the vine and it looked healthy and happy." - Grower #2 (LM)
Sequesters carbon	4	1	2	1	"[A healthy soil] would have good levels of carbon. Hopefully, the soil sequestered carbon and we're not just burning up our carbon." - Grower 16 (LM)
Promotes healthy cover	3	0	2	1	"The cover crop grows evenly across. A healthy soil is just not seeing any issues in the vineyard" - Grower #13 (EM)

crop or natural cover					
Prevents erosion	9	2	3	4	"I look to keep the soil healthy. I look to keep a cover crop on the soil, so we don't have the erosion. I want to keep it." Grower #10 (EA)
Vineyard resiliency	2	0	1	1	"This is a crop that's going to be here for the next 25 to 30 years. Hopefully for the next generation. So, within that you want to make sure the site is going to build- have an ability to continue to go along." - Grower #8 (EM)

(EA = Early Adopter group; EM = Early Majority group; LM = Late Majority group)

3.1.2. Vineyard soil properties and management practices to support production goals and soil health

The soil properties that growers of the Napa Valley American Viticultural Area (AVA) (n=16) considered more relevant for wine grape production are mostly soil physical properties related to water cycling functions (Table 1.2). Some examples of static physical soil properties (i.e., those that do not change with management) that were constantly mentioned as important during the interviews were soil texture (especially coarse texture) (n=8) and gravel content (n=7), as well as other vineyard properties like topography (slope; hills vs. valley). Static physical soil properties were important drivers of soil management practices decisions to meet viticultural and soil health goals.

All growers (n=16) practiced cover cropping or maintenance of natural covers (i.e. self-seeding resident or natural vegetation left to grow) in their vineyards, mostly for the purpose of soil erosion control (n=11; 3 Early Adopter, 2 Early Majority, 6 Late Majority) (Table 3). Vine vigor control (n=8; 2 Early Majority, 6 Late Majority) was also an important factor for cover crop management decisions (Table 3). For instance, growers used cover crops to decrease soil moisture levels and induce more water stress to the vines to restrict vine vigor and yields and influence grape quality especially in the valley floor. Other functions growers attributed to cover crops or natural covers in their vineyard included to increase soil health in general (n=5; 1 Early Adopter, 2 Early Majority, 2

Late Majority), improve soil structure (n=3; 1 of each group), and therefore create better water flow and aeration that benefits vine root growth (Table 3). Also, growers highlighted other functions like increasing soil nutrient cycling (n=3; 2 Early Majority, 1 Late Majority), building organic matter (n=2; 1 Early Majority, 1 Late Majority), and minimizing pests (n=1; Early Majority) by having soils covered with living plants (Table 3). Some common cover crop management strategies included tilling or disking (n=5), mowing (n=3), spading (n=2), and sheep grazing (n=1). Other growers opted to establish perennial cover crops (n=3) or left cover crops to senesce as the grape growing season and warm weather progressed.

Table 1.3. Cover crop functions in vineyards as described by the growers interviewed in this study.

Cover Crop functions	**Total growers**	**Early Adopter**	**Early Majority**	**Late Majority**	**Examples**
Erosion control	11	3	2	6	"I look to keep a cover crop on the soil so we don’t have erosion." - Grower #10 (EA)
Vigor control	8	0	2	6	"In the heavier sites (i.e. higher clay content), we leave the cover crop so we can try to reduce the amount of moisture available or water available to the vines throughout the season." - Grower #1 (LM)
General soil health	5	1	2	2	"Anything to do with cover crops would be a benefit to soil health" - Grower 15 (LM)
Improve soil structure	3	2	0	1	"We’ve been doing cover crops, we’ve been using a mix blend on between grasses, legumes... and we used large radishes in order to open up soil pathways (i.e. improve soil structure, infiltration and aeration)." - Grower #7 (EM)
Improve nutrient cycling	3	0	2	1	"For cover crops, if we’re trying to really boost the nitrogen, we’ll do nitrogen fixation cover crops like legumes." - Grower #2 (EM)
Increase organic matter	2	0	1	1	"We are a valley floor vineyard so we have deeper soils that will support more vigorous vines and so we want some competition from the cover crops. That’s part of the picture. The other part is of course building and sustaining organic matter in the soil." - Grower #12 (EM)

Minimize pests	1	0	1	0	"The big benefit of the soil health, by having a permanent cover crop, not only are you minimizing erosion, but dust. So, you're hopefully minimizing other pests that could be present." Grower #8 (EM)

(EA = Early Adopter group; EM = Early Majority group; LM = Late Majority group)

Growers varied tillage intensity depending on conservation or production goals. For instance, most growers mentioned they perform no tillage (in the hills/mountains) and conservation (reduced) tillage like "alternating rows" (tilling alternate rows each year in the valley) (n=13; 3 Early Adopter, 3 Early Majority, 7 Late Majority) mostly for soil erosion control (n=11; 3 Early Adopter, 2 Early Majority, 6 Late Majority) (Table 4). The other purpose of performing minimal, or no tillage was to control vine vigor (n=7; 1 Early Adopter, 1 Early Majority, 5 Late Majority) (Table 4). Particularly, growers explained that they practiced no-till to reduce soil moisture and therefore the vine vigor and achieve desired grape yields in deeper mostly in the valley since these naturally have higher moisture levels. On the other hand, practicing no-till or reduced tillage in areas where soils have lower water holding capacity and therefore vines are more exposed to water stress can difficult the management of vine balance.

> *In our cases, we stop tilling on every single vineyard except other than one or two of them because we're committed to no till from an environmental standpoint. But, we have to irrigate more to keep our vigor up in some of those vineyards because of the decrease in vigor from no till.* – Grower 14

Grower #14 (Late Majority group) explained how doing no-till allows them to achieve their goals of environmental stewardship and as consequence, it results in the challenge of higher irrigation needs to avoid excess water stress and maintain vine balance in some vineyards. Although the main motivations for the Late Majority group were related to economic impacts and regulatory compliance, many of these growers, like Grower #14, adopted practices such as reduced tillage that were proven to significantly minimize soil erosion and degradation (Table 4). Other reasons growers performed conservation or no-tillage include carbon sequestration (n=2; Late Majority), increased soil health in general (n=2; Late Majority), and improved water infiltration (n=2; 1 Early Adopter, 1 Late Majority) (Table 4). While most growers related reduced or no tillage to lower vine vigor due to lower soil moisture levels, one grower (Late Majority group) on the other hand discussed the potential that reducing tillage has on increasing soil water holding capacity.

Table 1.4. Reduced tillage functions in vineyards as described by the growers interviewed in this study.

No-till or reduced tillage functions	**Total growers**	**Early Adopter**	**Early Majority**	**Late Majority**	**Examples**
Erosion control	11	3	2	6	"On the mountain we have no-till for erosion control plan for the county but also we just don't till up on the mountain side." - Grower #13 (EM)
Vigor control	7	1	1	5	"I think when we're doing no-till, we're generally trying to reduce the vigor, which will naturally in turn introduce more sunlight into the canopy or into the fruit zone and improve the flavors in the wine that you're going to make from that. So that's a direct benefit. The idea is that we want to limit the amount of natural vigor on this property and that comes through no till usually." - Grower #1 (LM)
Carbon sequestration	2	0	0	2	"I think our overall approach is to do less tillage. We're trying to be conscientious about carbon sequestration and those type of things are overall, fundamentally what we're about. In terms of global warming, I think it's the right thing to do and it actually does benefit the vineyard long term." – Grower #4 (LM)

General soil health	2	0	0	2	"I think no-till is the practice that allows for the soil to be preserved. Soils are not meant to be tilled, are not meant to be naked, they're meant to be covered... We talked about the soil sustainability and health assumption that it's stable and the closer you are to the natural state of the soil, the probably closer to sustainable you will be. So, I can say that no till is a practice that can be conducive to that stability and soil health." – Grower #6 (LM)
Improve infiltration	2	1	0	1	" I've been trying to figure out how to do no till from an environmental standpoint, carbon footprint standpoint, and water infiltration because water infiltration is so much better in no till soil when you have perennial grasses especially." - Grower 14 (LM)
Improve Water Holding Capacity	1	0	0	1	"You can definitely improve it [soil water holding capacity] by either reducing tillage or adding cover crops that are going to build up biomass." – Grower #1 (LM)

(EA = Early Adopter group; EM = Early Majority group; LM = Late Majority group)

On the other hand, tilling or disking the soil was practiced by several growers (n=5; 2 Early Majority, 3 Late Majority) in the valley soils when they found necessary. For example, contrary to no-till, tilling the soil was used to reduce vine water stress and increase vine vigor (n=3; Late Majority) since it was thought to increase soil moisture (n=4; Late Majority). Also, tillage was used to alleviate water competition from weeds and cover crops (n=4; Late Majority), and for controlling diseases or pests like gophers (n=3; Late Majority). Growers also attributed functions like nutrients increase (n=1; Late Majority), compaction alleviation (n=2; 1 Early Majority, 1 Late Majority), better vine growth (n=2; Late Majority), improved weed and residue control (n=3; Late Majority), and increased soil carbon and yields (n=1; Late Majority). Tilling or disking were also used for terminating cover crops as a fire prevention tool (n=2; Late Majority).

Most of the wine grape growers interviewed practice some kind of compost application in their vineyards (n=14; 2 Early Adopter, 4 Early Majority, 8 Late Majority). Three of these growers (1 of each group) apply compost in the form of "compost tea" through the drip irrigation, the rest apply it in the traditional solid form. The main reason behind the application of compost was to increase nutrients (n=7; 1 Early Adopter, 2 Early Majority, 4 Late Majority) and organic matter (n=5; 1 Early Adopter, 2 Early Majority, 2 Late Majority) in soils (Table 3). Growers also mentioned other benefits related to compost applications including benefit soil health in general (n=4; 1 Early Adopter, 2 Early Majority, 1 Late Majority), improve soil structure (n=3; 1 Early Adopter, 2 Early Majority) and water holding capacity (n=2; 1 Early Majority, 1 Late Majority), benefit vine health (n=4; 1 Early Adopter, 1 Early Majority, 2 Late Majority) and soil microbial diversity (n=3; Late Majority) (Table 5).

> *We don't use compost everywhere. I would say, that's where it goes back to viticulture. If we had a vineyard that was in high vigor and we didn't want the nitrogen input from the compost, then maybe in vineyards like that we probably wouldn't be using it.* – Grower #16

Grower #16 (Late majority group) explained their perceived association of compost application with increased nutrient cycling and availability, particularly nitrogen, that could increase and disbalance vine vigor which is undesired for wine grape quality goals. In general, growers made fertilization and amendment applications based on results from petiole nutrient analysis.

Table 1.5. Compost functions in vineyards as described by the growers interviewed in this study.

Compost functions	**Total growers**	**Early Adopter**	**Early Majority**	**Late Majority**	**Examples**
Nutrient cycling	8	1	3	5	"So, for wine grapes it doesn't usually take that much nitrogen so, if I put on maybe five tons per acre of compost every year it seems to be sufficient to keep an adequate supply in my grapes." - Grower #10 (EA)
Organic matter	5	2	1	2	"We've got the cover crops and also putting in the compost in order to increase organic material because these vines are so old that you know, there's been a lot taken out of the soil and not a lot put back into it over 56 years for some of these vines." - Grower #7 (EA)
Improve soil structure	3	2	1	0	"This last year we put 10 tons per acre [of compost] out for trying to build soil structure and health" - Grower #11 (EA)
Improve Water Holding Capacity	2	0	1	1	"...You can definitely improve it [water holding capacity] by increasing the amount of either reducing tillage or adding cover crops that are going to build up biomass... you can do certain things like amending with compost." - Grower #1 (LM)
Vine health	4	1	1	2	"We've added, umm like a compost tea through the drip line as a fertilizer and we banded compost also, to try to help the struggling vines where their roots clearly aren't healthy or happy." - Grower #13 (EM)
Soil microbial diversity	3	0	0	3	"Besides cover crops the soil amendments, probably the compost is a very good thing for the living part of the soil." - grower #15 (LM)

(EA = Early Adopter group; EM = Early Majority group; LM = Late Majority group)

3.2 Barriers for the adoption and maintenance of soil health practices

The barriers that challenge most growers' adoption or maintenance of soil health management practices were mainly economical (n=11; 2 Early Adopter, 2 Early Majority, 7 Late Majority). Most growers highlighted the high costs associated with these practices, particularly compost applications, made it challenging for maintaining this practice. Compost applications increased costs through transportation, distribution and application logistics that might require acquisition of specialized farm equipment. These and the potential increases in expenses such as time and labor needs made it challenging for them to adopt and/or maintain compost applications as a regular practice.

> *I think the biggest reasons are cost and labor… Obviously we want to add compost, not every year but every two to three years. But it's expensive to truck it. It's expensive to buy the compost in and of itself. It's expensive to either shovel it under an emitter or band it or put it on the spreader. I think, cost wise is the hardest hurdle. Even if we know it would help, we know it will increase the vine health, we know it will make the vines better… The initial cost can be hard from a budgeting standpoint.* – Grower #13

Although Grower #13 (Late Majority group) acknowledged the benefits of compost applications for vine health, they explained the economic and logistical difficulties that prevents them from of adopting and maintaining compost applications as a practice to build soil health in vineyards. Another concern growers highlighted was the quality of the compost. For example, Grower #3 (late majority group) talked about how adding compost bought from an external facility caused pest breakouts in the vineyard which increased pest management costs and prevented them to continue practicing compost applications.

Other practices that growers described as costly were no-till and associated management including managing vegetative residues in the vineyard floor and weeds. Some examples of weed management practices that are difficult to maintain in no-till systems are mulching, increased herbicide spraying to reduce weeds emergence, and increased tractor passes for spraying or mowing. Growers also discussed that no-till would increase their irrigation needs. Ultimately growers agreed that these practices needed in no-till farming would increase labor and costs in areas where they would not see immediate benefits like added value to the fruit.

> *I know tillage is bad for soil health. We've eliminated as much as possible, but the under the vine row has been difficult to adopt because mulching it could cost $25,000 an acre.* – Grower #11

Although Grower #11 (Early Majority group) recognized that tilling the soil can have detrimental impacts in soil health, they explained the difficulty of maintaining no-till practices especially under the vine since alternative practices to manage weeds and keeping soils covered, like mulching, can have substantial increases in costs.

Additional key barriers to adopting, maintaining, and managing soil health practices into vineyards include time and uncertainty around the return on investments.

> *Obviously changing it [soil health] takes time. It takes understanding of the soil, and it can be expensive. So, economically, if you're not getting a return on your investment, it may be cost prohibitive.* – Grower #4

Grower #4 (Late Majority group) explained how not seeing immediate benefits, particularly returns on investments, from soil health practices in wine grape production is a major economical challenge for the adoption and maintenance of these practices. Another important barrier to soil health practice adoption or maintenance was the lack of information (n=4; 1 Early Majority, 3 Late

Majority) on how these practices would influence vine vigor and grape yields and quality. Particularly, growers highlighted the economic uncertainties from the effects of these practices on vine vigor and wine grape yields and quality. An additional barrier for the adoption or maintenance of practices to build soil health in vineyards is the potential for increased irrigation needs (n=2; 1 Early Majority, 1 Late Majority). On the other hand, three growers highlighted the lack of barriers or limitations for them to adopt or maintain healthy soil management practices (n=3; 1 Early Adopter, 1 Early Majority, 1 Late Majority).

> *I guess income and typically net profits are good too. These practices are not out of reach for your average grape grower in Napa Valley. Like they should be able to afford that equipment and cover crop*. – Grower #14

Grower #14 (Late Majority group) expanded on their views on how soil health practices should not be economically difficult for Napa Valley growers to adopt due to the high wine grape values of this region.

3.3 Needs to incentivize the adoption of soil health practices.

In general, growers were willing to adopt more management practices that benefit soil if more targeted and practical information is provided (n=10; 2 Early Adopter, 3 Early Majority, 5 Late Majority) (Table 5). Particularly, growers are interested in learning which soil management practices can help improve soil health and how these could benefit grape quality and economical aspects of wine grape production (Table 6). For example, growers highlighted the need for more information on which practices help increase soil organic matter and water cycling in vineyards. Some of these practices included vineyard cover crop species recommendations and regenerative management strategies like sheep grazing and how these could influence irrigation needs. Other common questions from growers included learning how to better apply solid compost and the effectiveness of compost teas. Finally, growers requested more information on how different gradients of tillage, particularly no-till, could influence soil health functions like water cycling.

Growers, especially in the Late Majority group, indicated that factors such as affordability (n=4), added value to grapes or wine (n=3), and minimal risks for the production (n=1) would motivate increased commitment for more soil health practices. Other factors that would help other growers adopt more soil health practices were the adoption sustainable or organic agriculture related certifications (n=2; Late Majority).

> *I think the promotion of sustainability certificates would promote people to adopt better soil health… When someone farms "organically" with that certificate of farming organic, then usually the grapes fetch a higher price. So, the more money you have, you can afford to invest in everything.* – Grower #2

Grower #2 (Late Majority Group) explained how having organic farming certifications is an investment since it allows growers to get higher value for their wine grapes.

Growers also requested better access to soil health testing and results interpretation (n=2; Late Majority). For example, these growers expressed soil health tests related to biological properties were difficult to interpret for making decisions. Only two growers from the Late Majority group expressed disinterest in changing soil management practices by saying they are "already doing what they can" and two other growers (1 Early Adopter and 1 Early Majority) explained they adopted all the possible soil health practices to their knowledge.

Table 1.6. Soil health information needs for vineyards as described by the growers interviewed in this study.

Grower Group based on Soil Health attitudes	Grower ID	Type of information needed	Example of quote
Early Majority (n=4)	5	Impacts of soil health management practices on relevant soil properties and functions (particularly organic matter); soil biodiversity role in soil health functions	"Seeing organic matter increase every couple of years would be very motivational and if there was a way to, if there were specific fungus or bacteria that we knew contributed, I know it's like we have some idea on the leaders, but the mix of microbes are so different. But if there were tests for that, that would be interesting."
	8	Impacts of soil health management practices on relevant soil properties and functions; soil biodiversity role in soil health functions	"I think better knowledge of if it's actually working right? I think, the other thing is if the microbiome is doing something, and we can find ways to help mine for nutrients or help you utilize less water. If we can see some actual proof to that. I think that would go a long way to umm, adopt other- other kind of, fringe practices."
	12	Impacts of soil health management practices on relevant soil properties and functions	"I think just being able to get more data and being able to understand what's going on better. You know how we're like learning and like okay, what can we do to have the most positive impact. And sometimes that's not always what you think it is. You know, it's just really being able to dig into the research and figure out. I think it's easier for people to buy in if you have good data based on solid science."
	13	Impacts of soil health management practices on relevant soil properties and functions	First and foremost, it kind of starts with me and my level of understanding and knowledge and being comfortable like advocating for it… And that's probably one of the hardest parts. So, if we're going to adopt new things, we're going to have to be able to learn about it first and foremost."
Late Majority (n= 5)	3	Impacts of soil health management practices on relevant soil properties and functions; economic benefits of soil health	"I suppose the knowledge. If I found something I thought would help, that would improve the soil and would be economically feasible."
	9	Soil Health management practicality and impacts in grape quality and yields; economic benefits of soil health	"Umm, if we can command prices for the grapes. I think that would be the main thing. The economics of it"

	14	Impacts of soil health management practices on relevant soil properties and functions	"We're doing everything that we know of for soil health. So, I guess what would help me to decide is finding out something else that I could be doing. We're doing everything we know about, but we don't know about everything, so that would be the thing is to keep learning".
	15	Soil health management impacts in grape quality and yields; economic benefits of soil health	"I guess would be examples… I mean it comes back to meeting the client's goals for production and yield and staying in the budget. So those are the goals, to increase the quality for sure. If it increases the production or decrease the cost, all those things would be positive outcomes."
	16	Impacts of soil health management practices on relevant soil properties and functions	"I think we still need the science to help guide us, to make better decisions because they're still think trying to figure out like, cover crops under California's conditions. We have like a war in the Mediterranean type of climate… are we really increasing organic matter or not? And I still think we're trying to answer a lot of questions and that's why I'm intrigued, I mean I see a lot of interest in the soil heath area, trying to better understand the practices that we're doing like the cultivation of organic matter, till vs no-till…"

4. Discussion

4.1 Perceptions and attitudes of soil health influenced behavioral intent and adoption categories.

This is the first study that assesses growers' perceptions and attitudes for understanding awareness, needs, barriers, and motivations about soil health practices in vineyards. Overall, awareness and generally positive attitudes from Napa Valley wine grape growers may stem from social factors such as increased access to research and outreach efforts from the University of California and other grower-focused stakeholders. Studies have shown that access to outreach programs influenced growers' adoption of sustainable practices (Baumgart-Getz et al., 2012; Lubell et al., 2011; Tilman et al., 2002). For example, the University of California at Davis is a land grant institution that conducts research and outreach at the Oakville Research Station in Napa County and in other commercial vineyards with the collaboration of the wine industry. Other institutions and organizations such as the University of California Cooperative Extension, the Napa Resource Conservation District, the North Coast Soil Hub, the Napa Valley Grape Growers Association, among others, provide educational content in outreach activities for wine grape growers to learn about the latest advancements on soil management practices. Additionally, high peer to peer knowledge transfer has been documented in the Napa Valley AVA, which could be another explanation of why most growers displayed awareness about soil health (Hira & Swartz, 2014; Taplin, 2015).

Attitudes towards soil health and the potential outcomes (i.e., benefits or drawbacks) of soil health management practices on viticultural goals were the main drivers of behaviors, especially decision making. Growers that had more positive attitudes were placed in the Early Adopter and Early Majority group since these were more willing to take risks and adopt and maintain more soil health practices. The main difference between these groups is that the Early Adopter had more personal motivations such as beliefs of the success of soil health practices, while the Early Majority group were more risk averse and needed scientific information that explained and showed benefits from these practices. None of the participants displayed characteristics that fit the other adopter categories from the Diffusion of Innovation theory such as Innovator and Laggards. None of the participants fit the Innovators group because there was no evidence that suggested the invention and establishment of new or unfamiliar soil health practices. We postulate that the soil health focus of this study may have excluded the participation of growers in the Laggard category during the grower recruitment process since it was a voluntary process that might not be of interest to a group that is resistant to soil health. Attitudes from the Early Adopter and Early Majority groups might be due to increased soil health and environmental challenge awareness and connection to viticultural practices as well as peer recommendations (Tran-Nam & Tiet, 2022), and compatibility with existing practices, values and identity (A. Lavoie & Wardropper, 2021) , and current needs.

On the other hand, growers from the Late Majority group were more resistant to the adoption of soil health-focused management practices due to perceived potential economic risks or increases in expenses as well as maintenance costs. Similar findings of potential risks having a strong influence in decision making have been in reported in the adoption of cover crops for soil erosion control in vineyards of central Spain (Marques et al., 2015). Other research has also reported profitability and economic feasibility being key determinants of sustainable management practices decision-making among wine grape growers (Baumgart-Getz et al., 2012; Lubell et al., 2011).

Late Majority attitudes suggested a disconnection between soil health functions and viticulture productivity goals such as grape quality. However, to date, the specific attributes that translate a high-quality grape into a high-quality wine remain unclear and subjective since these depend on not only on environmental conditions but also sensory perception, cultural preferences, and winemaker craftmanship, among other human perceptions and/or cognitive biases (Francis & Williamson, 2015; Marques et al., 2015; Sáenz-Navajas et al., 2016). In contrast, growers from the Early Adopter group "believed" in a positive connection between soil health, vine health, and grape quality and therefore were more willing to take risks with soil conservation practices. This issue complicates predictions on how soil health practices could consistently and objectively impact desired outcomes for wine grape quality. Other studies have also reported grower attitudes to be main drivers of vineyard management practices decision making such as pesticide use (Y. Chen et al., 2022) and cover crops for erosion control (Marques et al., 2015). Therefore, growers' attitudes and their strong influence of on actions should be considered for further research, outreach, and policy efforts on soil health and sustainable vineyard management practices.

4.2 Soil health's unique definition for wine grape production

Growers from all groups, especially from the Late Majority group, associated improving soil health with potential increased soil fertility. This finding was confirmed by a strong emphasis on the need for "balanced" soil health, particularly nutrient and organic matter cycling. The association between soil health and increased soil fertility was stronger for the Late Majority Group where achieving viticultural goals was the main motivation for soil management practices decision-making. The strength of this association decreased for the Early Majority followed by the Early Adopter. Balanced soil health is rooted on the viticultural concept of vine balance (Cataldo et al., 2021). Linking soil health management to vine balance is what distinguishes viticulture soil health from other cropping systems such as grain crops (i.e. maize, soybeans, wheat, oats, among others). For example, one of the main goals of building healthy soils for grain crop production is to increase profitability through increased yields (Irvine et al., 2023). While increasing profitability was important for the participating wine grape growers of our study, high yields were mentioned as not always being conducive to this goal.

However, additional soil health functions highlighted as important by wine grape growers were similar to those of conventional grain agroecosystems like promoting soil biodiversity and biological activity, building resiliency to intense weather events, reducing synthetic inputs, mitigating erosion, improving soil structure and water cycling (Irvine et al., 2023). Also, emphasis on soil physical and chemical properties might be due to the perceived direct link between soil water and vine growth in viticulture (Oliver et al., 2013) and easier access to testing and interpretation of results (Lobry De Bruyn & Andrews, 2016).

4.3. Soil health motivation

In general, soil health management practices were performed with the purpose of complying with policies for soil erosion mitigation and vineyard certifications, and for promoting soil water and nutrient cycling functions that support vine balance. Grower awareness and connection of soil health to viticulture also played a key part in soil health practices adoption and maintenance which has also been seen in other studies (Liu et al., 2018).

4.3.1. Policies for the protection of natural resources

Required management practices from the Napa County's ordinance (Code § 18.108) were strong motivations for growers in the Late Majority group to adopt and maintain cover crops (or natural cover) and no-tillage for erosion control in sites with slopes higher than five percent. These results reflect the success of legislation in the protection of natural resources like soil and water in California which has also been reported in other assessments (Salzman & Thompson, 2001; Vogel, 2019). The extension or creation of more policies for natural resource protection could be an effective method for advancing the adoption of soil health protection practices (Dessart et al., 2019; Prokopy et al., 2019) especially among growers that are more resistant to change. Including growers' perspectives in the development and improvement of these regulations and supporting growers in emerging challenges can make the adoption and maintenance of sustainable agricultural practices a more effective process (Piñeiro et al., 2020). For example, although most growers from the Late Majority adopted soil health practices like no-tillage to comply with the county's ordinance for reducing soil erosion, the maintenance of these practices was challenging, making it a barrier for long-term adoption among these growers. In contrast, growers from the Early Adopter group were the ones who mostly performed practices primarily for the benefit of soil health without regulative obligations because of connection to values, higher awareness of soil health functions, and perceived long-term benefits for vine health and grape production. Similarly, growers in the Early Majority group were not mainly motivated by legislation compliance requirements but they were aware and reiterated that compliance, along with knowledge on the benefits of soil health practices, do influence their soil management decision making.

4.3.2. Vineyard Certifications and potential added value to grapes/wine

Another motivation for adopting soil health practices like compost application, especially in growers from the Late Majority group, was related to the potential increase in value of the grapes and wine from certifications. Sustainability related certifications like sustainable, organic and/or biodynamic farming have been shown to have economic benefits for grapes and wines due to consumer demand (Delmas & Gergaud, 2021). These results support other research findings that incentivizing the adoption of legitimate sustainability-related third-party certifications can be a successful option for promoting the adoption of soil health practices in vineyards (Hillis et al., 2018). Particularly, sustainable agriculture related certifications that might support potential added value to grapes and wine could incentivize growers that are more resistant to changes, such as the Late Majority group of this study, in doing more soil conservation practices.

4.3.3. Vine Balance

Having a balanced soil for the purpose of vine balance was an important factor for most growers and was a stronger motivation for the Late Majority group followed by the Early Majority group. Some of the practices that growers performed for vine vigor control and balance were variations of cover crops, reduced or no-tillage, and compost applications. For example, these growers explained that integrating no-tillage and cover crops in the alleys allowed them to reduce vine vigor in vineyards that had high water holding capacity soils. Specifically, they explained no-till allowed them to improve water infiltration and cover cropping reduces soil moisture from water uptake and competition with vines. However, the effect of soil health practices such as no-tillage and cover crops on vine vigor and grape quality have had diverse results in different studies due to specific management strategies (Gatti et al., 2022; Guerra & Steenwerth, 2012a; Zumkeller et al., 2023). In fact, defining grape quality has been a challenge due to its high subjectivity from human perceptions. Overall, research has shown that practices like cover crops and no-till and can benefit vine health and wine grape quality parameters while supporting other soil health functions (Belmonte et al., 2018b; Guerra & Steenwerth, 2012a; K. Steenwerth & Belina, 2008b) that could benefit vineyard resilience to climate change challenges and longevity. More research, especially long-term trials, and outreach products are needed to better explain and communicate the effects of these soil health focused practices on water use, vine balance, and grape quality parameters in Mediterranean vineyards.

4.3.4. Building Soil Health

Growers from the Early Adopter and Early Majority group were mainly motivated to do practices for the benefit of soil health due to higher awareness and understanding on the benefits of these practices for vine health, vineyard resilience, and positive connection to wine grape production. Other studies have also found growers knowledge to have a strong influence on adoption of practices in vineyards (Lubell et al., 2011). The influence that knowledge has on practice adoption is also evident in the Late Majority group where compost application adoption is high due to awareness and understanding of benefits of this practices for vine health and grape production.

In addition to knowledge, beliefs on the effectiveness of practices without scientific rationale was found among the Early Adopter growers. While these growers are doing practices with the main motivation of building soil health and vineyard resilience, relying in beliefs might be a challenge if these are not supported by scientific evidence. This, along with the other side of the spectrum such as the Late Majority group, presents an opportunity for scientists and outreach specialists to address knowledge gaps and provide helpful information especially to groups with dogmatic or conservative perceptions.

Additional to legislative obligations in sloped vineyards, all growers recognized the importance of and acted on protecting at least the inter-row soils from erosion with cover crops or natural vegetation especially during the rainy season. These results reflect the success of cover crop adoption in Napa Valley especially compared to other regions where the adoption and establishment of this practice has been more challenging and slower due to factors such as doubts on effectiveness, and potential increased costs and water use from growers' perceptions (Cerdà & Rodrigo-Comino, 2021; Marques et al., 2015).

Compost application was adopted and maintained by most growers every few years or as needed, including the Late Majority group. The reason why growers from this resistant group continued compost applications was due to awareness, understanding and witnessing of the benefits that compost applications can have in soil structure and nutrient cycling that benefit vine health and wine grape production. Growers from the Late Majority and Early Majority group relied on soil and petiole nutrient testing for deciding compost application frequency while the growers from the Early Adopter group applied it more frequently as part of their beliefs in increased soil and vine health benefits.

The adoption of compost teas by three growers (one of each group) was done due to the ease of access and application. For example, while regular solid compost can have increased costs and labor including transportation and specialized equipment for dispersion and incorporation (Biala et al., 2021), compost teas were easily applied through the drip. Government efforts such as California's Farmer Equity Act of 2017: Regional Farmer Equipment and Cooperative Resources Assistance Pilot Program (AB522) could provide significant assistant for growers to access equipment needed for the application of composts. Another government effort that could significantly increase the adoption of compost applications in vineyards in California's Organic Waste Law (SB 1383) that would require all commercial edible food generators to reduce organic waste and would potentially increase availability and access of compost.

4.4. Barriers and opportunities to incentivize the adoption and maintenance of soil health practices in vineyards

The main barriers for the adoption and maintenance of soil health practices support findings from other research including lack of information, particularly on potential economic risks, and increase in costs respectively (Carlisle, 2016; Lubell et al., 2011; Marques et al., 2015). These barriers were more evident among growers in the Late Majority group who expressed difficulties accepting risks from potential negative economic impacts. These findings suggest that growers need institutional and structural support to bear the long-term costs of soil health management investments. This has been highlighted as a concern in state advisory panels of the Expert Advisory Committee (EAC) for the assembly bill AB-1757 (2022) which provide recommendations for implementation targets for natural and working lands of California (AB 1757 Expert Advisory

Committee Recommendations, November, 2023). An example of support that could address these barriers include increased financial incentives and support to access funds from soil health and environmental advocacy government programs (Tilman et al., 2002) such as the California Healthy Soils program (CDFA 2017).

Although the effects of soil health practices such as cover crops, compost applications, reduced tillage, and grazing in vineyard soils have been generally well documented (Lazcano et al., 2022a; Lazcano et al., 2022b; K. Steenwerth & Belina, 2008; Wong et al., 2023; Zumkeller et al., 2023), contrasting views on the effects of conservation soil practices suggest that more research and outreach efforts are needed to support growers in the process of adopting and maintaining these. Some of these practices include effects of no-till/tillage and the management of cover crops on soil water cycling, and effectiveness of diverse types and sources of compost. Increasing outreach efforts and grower participation in these have been shown to increase knowledge and incentivize the adoption of sustainable practices in vineyards (Hoffman et al., 2014). Therefore, increasing outreach efforts of soil health practices and implications to viticultural goals can be an effective strategy for the adoption of these practices among more conservative growers and therefore protection of soil health in vineyards.

Also, there is a need for more research that evaluates the combinations of these practices across diverse soil types in Mediterranean vineyards. For example, although benefits of compost tea applications have been reported for nutrient mobility and disease suppression in vineyards (Eon et al., 2023; Evans et al., 2013), the effects on increasing soil organic matter, nutrient cycling and uptake by vines compared to solid composts and synthetic fertilizers remain understudied. Research on compost applications and management is particularly relevant since compost applications are expected to increase due to California’s recent mandatory organic waste collection legislation (Senate Bill 1383). In addition to how these practices influence soil health indicators, more information is needed on how these influence water dynamics (i.e. retention and infiltration) in the soil and how these influence vine health, balance, and grape quality parameters. Particularly, research gaps remain on the economic impacts of soil health practices such as cost-benefit analyses and risk assessments of the implementation and combination of these practices in vineyards. These are excellent opportunities for research institutions to form interdisciplinary collaborations among scientists and the wine grape industry to produce targeted and actionable vineyard soil health knowledge.

In addition to how sustainable soil management practices influence soil health, vines and grape production, growers' strong interest in learning more about soil health testing and interpretation, especially those related to biological properties presents an opportunity for more soil biology focused collaborative research and extension efforts. With increased access to soil biodiversity testing growers need tools and support for interpretation and application of information.

5. Conclusions

This study was the first to report growers' views and needs on soil health for wine grape production. Grower attitudes were main drivers of decision making related to soil health practices. Wine grape growers defined a healthy soil as one that is balanced, self-sustaining, and resilient that can support viticultural goals such a vine balance. From growers' perceptions and attitudes, we were able to categorize growers in adoption groups using the Diffusion of Innovation Theory including the Early Adopter, Early Majority, and the Late Majority groups. Overall, all growers valued soil health but the growers from the Early Adopter and Early Majority group were more motivated to adopt more soil health practices while the Late Majority group needed regulatory compliance and clear evidence on viticultural benefits. The main barriers for the Late Majority group were lack of information on economic impacts of soil health supportive practices and perceived potential financial risks from adopting diverse soil health focused practices. However, these growers were willing to adopt more soil health practices if more clear and practical information was provided. Our study provided clear information on growers' awareness, perceptions and needs for adopting more soil health supportive practices. More soil health focused research and outreach efforts are needed to support growers in adopting and maintaining practices that protect soil health in vineyards. Additionally, the development of more soil health stewardship legislation as well as the promotion of sustainable wine growing certifications that prioritize soil health and support grape value, could incentivize soil health protection in vineyards.

6. Appendix

Table 1.A1. *Pre-Interview Survey Questionnaire*

1. Vineyard(s)/Company Name(s)?
2. How many vineyards do you manage in Napa Valley?
3. What is your role in these vineyards? (select all that apply)
4. How long have you been working in these vineyards?
5. What is the primary (main) grape variety you produce?
6. What secondary grape varieties do you grow?
7. Do you have any certifications? Which ones? (examples: Certified California Sustainable Winegrowing (CCSW), Certified Organic, Biodynamic Certification, others)
8. What are your main goals in these vineyards? (select all that apply; building soil health, achieve yields, grape quality, comply with certification)
9. What are the typical yields (tons per acre) across the operation, if any? Are you aiming for a specific tonnage or maximizing yields?
10. What is the estimated acreage for the total commercial operation?
11. Please select where your vineyards are in the Napa Valley American Viticultural Area sub appellations
12. Please select the soil types (approximately) that you have in your vineyards based on texture. (select all that apply if possible; Clay, Clay loam, Sandy Clay Loam, Sandy Clay, Sandy, Sandy loam, Silty, Silty Loam, Silty Clay loam, Silty Clay, Loam, Loamy Sand)

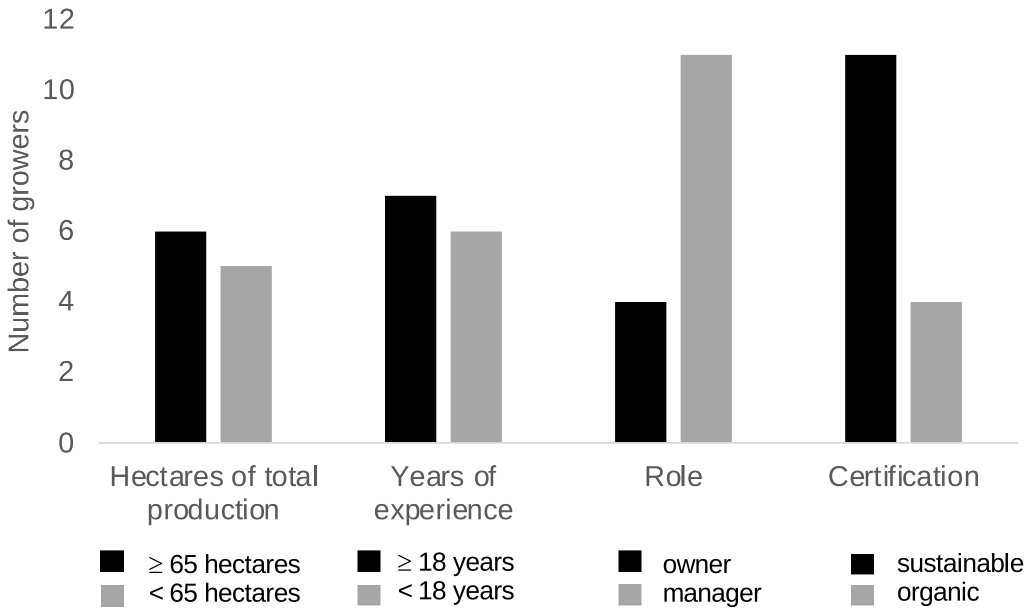


Figure 1.A1. Results from Pre-Interview Survey (n=13). Median values for hectares of total production and years of experience of each grower were 65 ha and 18 years respective

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Chapter 2: What is a healthy soil for wine grape production? A participatory approach for assessing soil health across the landscape

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Abstract

Soil health is critical for sustainable wine grape production, yet few studies have integrated grower participation to align soil health assessments with outcomes of interest. This study aimed to define vineyard soil health by identifying soil health indicators that are associated with wine grape production outcomes and are sensitive to inherent soil properties, management and sampling variability in vineyards. Therefore, this study evaluated the variability of soil health indicators in vineyards across grower ratings of soils based on viticultural productivity, soil textural classes, within vineyard zones, and under varying management practices (till vs. no-till) while incorporating grower insights. Soils were collected across 16 challenging and 16 ideal soils rated by growers based on vine vigor control for vine balance and high grape quality goals. Within these vineyards, soils were collected at two depth intervals (0-10, 10-20 cm), and vineyard zones (vine row, tractor row). The soil health indicators assessed were those representatives of soil carbon, nutrient, and water cycling. Examples of soil health indicators evaluated were total C, permanganate oxidizable carbon (POXC), mineralizable C (Min C), microbial biomass C (MBC), dissolved organic C, total N, plant available N (NO_3^--N and NH_4^+-N), potentially mineralizable N (PMN), pH, EC, bulk density, wet aggregate stability, penetration resistance, and infiltration rate. Our results indicate that soil texture is a key factor influencing soil health, especially carbon and water cycling indicators, across growers' perceptions of challenging and ideal soils as they affect vine vigor control in wine grape production. Indicators such as total carbon (TC), permanganate oxidizable carbon (POXC), total nitrogen (TN), ammonium nitrogen (NH_4^+-N), electrical conductivity (EC), and water-stable aggregates (WAS) were sensitive to growers' perceptions of ideal and challenging soils. Also, when assessing the variability of soil health indicators across vineyards, higher soil health was generally observed in tractor rows with vegetative cover compared to bare vine rows. Also, no-till practices enhanced some carbon (TC, Min C, and DOC) and nitrogen (NH_4^+-N) indicators. Clay content also influenced soil carbon and water cycling indicators. According to this, we determined that indicators like TC, POXC, and Min C, plant-available N, and WAS are adequate indicators of soil health for wine grape production in the Mediterranean-climate region of the north coast of California. Findings from this study highlight the value of integrating growers' views and their participation into soil health research. Grower participation facilitates the identification of relevant indicators linked to management decisions, and in this case, those are related to soil carbon and water cycling functions. Moreover, the study underscores the importance of soil texture as a benchmark for interpreting soil health.

1. Introduction

Numerous studies conducted over the past several decades show that soil heath is essential for supporting ecosystem services, such as carbon (C), nutrient and water cycling, that underpin biodiversity and agricultural sustainability (Lehmann et al., 2020; F. Romero et al., 2024; Smith et al., 2015). Soil health is defined as the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans (USDA NRCS, retrieved on April 10, 2024). However, the concept of soil health remains elusive and difficult to measure objectively due to the challenges of reconciling this vague concept across soil type, cropping system and region (Stewart et al., 2018; Wade et al., 2022). The first challenge for assessing soil health is the selection of indicators and their benchmarks that enable growers to define the health of their soils and success of their management practices (Wade et al., 2022). Soil health assessments often fail to link soil health indicators with agronomic outcomes and provide accessible and targeted information among diverse cropping systems and agricultural regions (Hughes et al., 2023; Wade et al., 2022). Finally, there is often a disconnection between soil health conceptualization and the practical approach for soil health management among researchers and agricultural stakeholders (Wade et al., 2022). These challenges, including perceptions of potential increased production costs, can complicate or disincentivize the adoption of sustainable or regenerative practices to build and protect soil health among growers (Gonzalez-Maldonado et al., 2024).

Common indicators used to assess soil health focus on dynamic soil properties that are sensitive to soil management practices compared to static soil properties that are less sensitive to management practices (Culman et al., 2012; Hurisso et al., 2018; Nunes et al., 2020). Examples of dynamic soil properties include labile C and nitrogen (N) pools from soil organic matter (SOM) (e.g., permanganate oxidizable C, mineralizable C, potentially mineralizable N, among others). These labile C and N pools support soil microbial activity and microbial biomass (Garcia-Pausas & Paterson, 2011), which in turn enhance organic matter mineralization and nutrient cycling (Horwath et al., 2017; Smith et al., 2018). Labile C and N also enhance soil aggregation and structure by promoting biological activity and availability of cementing agents, supporting the physical protection of soil C (Blanco-Canqui & Lal, 2004; Tang et al., 2011). Other soil health indicators are related to physical properties that influence water cycling such as wet aggregate stability, compaction, and infiltration (Bagnall et al., 2023; Karlen et al., 2021b, 2021a; Moebius-

Clune et al., 2016; Stott et al., 2021). Generally, increasing soil organic matter enhances soil C content and aggregate stability protecting soils from erosion (Cantón et al., 2009; Z. Yu et al., 2017).

Many of these indicators have been recommended by relevant organizations such as the Soil Health Institute (Bagnall et al., 2023) and have been included in current soil health assessment frameworks (i.e., structured approaches used to evaluate soil health indicators) like the Cornell Comprehensive Assessment of Soil Health (CASH) (Moebius-Clune et al., 2016) and the soil management assessment framework (SMAF) (Karlen et al., 2003; Wienhold et al., 2009). Nonetheless, both the selection of soil health indicators and establishment of their reference values or targets to enable growers to choose soil management that supports their production goals remain challenging. This stems from the diversity of inherent soil properties (i.e., properties that are minimally influenced by soil management practices) such as texture and/or minerology (Amsili et al., 2021; Fine et al., 2017), but also variation in climate (Fine et al., 2017; Tu et al., 2021), management practices (O. T. Yu et al., 2019), and cropping systems (Amsili et al., 2021; T. Yang et al., 2020). Particularly, most soil health studies offering benchmarks or targets for soil health indicators have been conducted in cool or temperate humid regions in grain crops (Agyei et al., 2024; Amsili et al., 2021; Fine et al., 2017; Norris et al., 2020). As a result, regions like California, with its distinct Mediterranean climate, remain underrepresented in soil health research, facing unique and unresolved challenges. Given California’s role as a leading producer of high-value crops like wine grapes, addressing these gaps is critical to sustain both land productivity and the long-term viability of its agricultural economy. Wine grape production offers a unique opportunity to examine soil health because vineyards span numerous soil types and microclimates. It also supports diverse management practices and unique production goals (Gonzalez-Maldonado et al., 2024). Therefore, this study examines the soil health indicators and benchmarks that are the most relevant for wine grape production in semi-arid Mediterranean-climate regions.

Wine grapes are a woody perennial crop of large economic importance throughout the world. In the USA, more than 80% of wine grapes are produced in California, a Mediterranean-climate region. In contrast to other cropping systems where outcomes of interest focus on augmenting soil fertility for yield maximization (Grassini et al., 2015), the goal in wine grape production is to achieve vine balance for producing high-quality grapes for wine (Gonzalez-Maldonado et al., 2024). Vine balance can be defined as the ratio between vine yield (i.e., amount of grapes a vine produces over the growing season) and vine vegetative growth where the goal is to produce vines that are neither weak nor over-vigorous (Skinkis 2019). Although defining high quality grapes and wine is complex (Charters & Pettigrew, 2007), studies suggest that reducing berry size in order to increase the ratio of skin to pulp can facilitate the extraction of grape skin compounds (Melo et al., 2015). This promotes more concentrated phenolic compounds (Chen et al., 2018) that contribute to better aroma, color and flavor of wines (Li & Sun, 2019). Vine balance can be achieved through control of soil water and nutrient availability to create physiological stress in the vine (Keller, 2005). These goals can be pursued through rootstock selection (Lee & Steenwerth, 2013; Pou et al., 2022; P. Romero et al., 2018; Williams, 2010) and soil type selection (Tramontini et al., 2013; Trought et al., 2008; White, 2015). Additionally, these goals can be pursued through controlled irrigation rates applied to vine rows (Ayars et al., 2017; Buesa et al., 2022; Keller, 2005) and application of distinctive soil management practices in the vine row and under-vine row; for example, tillage and cover crops in the tractor row and weed management and fertilization in the vine row (Guerra & Steenwerth, 2012b; Lee & Steenwerth, 2013; Novara et al., 2018; Steenwerth et al., 2016; Steenwerth et al., 2013; Tezza et al., 2019).

Our previous research revealed distinctions among wine grape growers' perceptions and attitudes about soil health and viticultural outcomes (Gonzalez-Maldonado et al., 2024). In that study, wine grape growers defined a soil with "ideal" soil health by its "balanced" (i.e., not high or low) levels of both nutrients and soil organic matter, high soil microbial biodiversity, and adequate water holding capacity and infiltration that would allow them to achieve vine balance and high grape quality. Also, the growers' perceptions and attitudes influenced their selection of soil management practices that would strongly impact soil health (Gonzalez-Maldonado et al., 2024). Variation in wine grape production has also been linked to soil type and management practices. Higher clay content in soil has been linked to increased plant water uptake and accumulation of sugars in berries (Tramontini et al., 2013), and to higher plant available water that leads to increased vine vigor (Echeverría et al., 2017).

Many soil functions important to growers, such as low compaction, reduced erosion, adequate water holding capacity (WHC), and carbon sequestration (Gonzalez-Maldonado et al., 2024), can be improved by increasing and protecting soil organic matter (SOM). However, increasing SOM may enhance nutrient cycling indicators, like nitrogen pools, raising concerns among wine grape growers about potential vine imbalance, such as over-vigorous vegetative growth (Gonzalez-Maldonado et al., 2024). For example, growers ask if practices that are intended to promote soil health and build SOM can also create excessive water retention and excessive nutrient release, leading to over-vigorous vines (Gonzalez-Maldonado et al., 2024).

In addition to this information gap, another challenge is the disconnection between researchers and agricultural stakeholders during the conceptualization of soil health and the actions taken to build and/or protect it (Wade et al., 2022). Most soil health research, including selection of site, sampling design, and soil health indicators and subsequent interpretation of findings, is led by researchers and excludes growers' participation, potentially limiting the development and adoption of soil health practices (Avriel-Avni & Dick, 2019; Durán et al., 2022; Singh et al., 2024; Terrado et al., 2023). To date, only a few studies have captured growers' perspectives on soil health properties needed to achieve agricultural goals in commodity crop systems but this has not been done yet for vineyard systems (Hermans et al., 2021; Mann et al., 2019; Rekik et al., 2020). Grower participation in quantitative soil health assessments will incorporate their perceptions to advance a pragmatic understanding of soil health, potentially enhancing the adoption of more soil health building and conservation practices. Therefore, the goal of this study was to quantify growers' perceptions of the most relevant soil health indicators to achieve viticultural goals. Furthermore, we aimed to evaluate potential sources of soil health variability in vineyards such as soil texture, vineyard zone (under-vine vs. tractor rows), and soil management (tillage vs. no-tillage). For this, a comprehensive soil health assessment was conducted across vineyards of the Napa Valley wine growing region (CA, USA) with the collaboration of growers.

To select soil health indicators, we used the following criteria following Bagnall et al., (2023): (i) the indicators should be related to grower desired outcomes (vigor control for vine balance), (ii) the indicators should be sensitive to soil management practices (such as tillage and distinctive management practices under-vine and tractor rows), and (iii) the indicator should reflect dynamic soil properties but be influenced by static soil properties such as texture. Therefore, we assessed how soil health indicators varied between contrasting soils rated by growers (i.e., challenging and ideal soil conditions) defined with respect to achievement of important viticultural outcomes such as adequate vine vigor control and high grape quality. Also, we assessed how textural class, management factors such as disturbance (till vs no-till), and heterogeneity in vineyard management (i.e., under the vine vs alleys) influence soil health indicators in a semi-arid Mediterranean climate.

We hypothesized that differences between growers' perceptions of ideal and challenging soils for wine grape production would be primarily reflected in water cycling functions (soil physical indicators) followed by nutrient cycling, carbon cycling, and microbial diversity indicators. Specifically, we hypothesized that ideal soils would have lower compaction as well as greater infiltration and aggregate stability, and greater levels of indicators for carbon and nutrient cycling (particularly total and organic nitrogen pools). We hypothesized also that these indicators would vary across the landscape due to the change in static properties of soil. Specifically, we expected that indicators would be strongly affected by soil texture. Because certain soil health indicators would be sensitive to management and therefore change with soil disturbance (i.e., tillage), we anticipated variability within vineyard zones that are irrigated (i.e., vine row) or rainfed (i.e., tractor row), tilled or no-till, and by soil depth.

2. Materials and Methods

2.1. Study design and soil sampling

This study was carried out in Napa Valley, California (USA), in collaboration with 16 wine grape growers. Grower recruitment and interviews covering topics within soil health and vineyard soil management practices were discussed in Gonzalez-Maldonado et al. (2024). Each participating grower was asked to select two contrasting soils: a challenging and an ideal soil, based on their perceptions and knowledge of best soil properties for achieving outcomes of interest. Growers emphasized soil water infiltration and nutrient levels as key soil properties in their rationale for ideal and challenging soil ratings (Figure 2.1; Tables 2.2A and 2.3A). Here, the main outcome of interest among growers was achieving good vine balance for high-quality grape production (Gonzalez-Maldonado et al., 2024). The process for the collaborative soil health assessment is described in Figure 2.1 and more detailed information about the ideal and challenging soils are found in Appendix (Tables 2.2A and 2.3A).

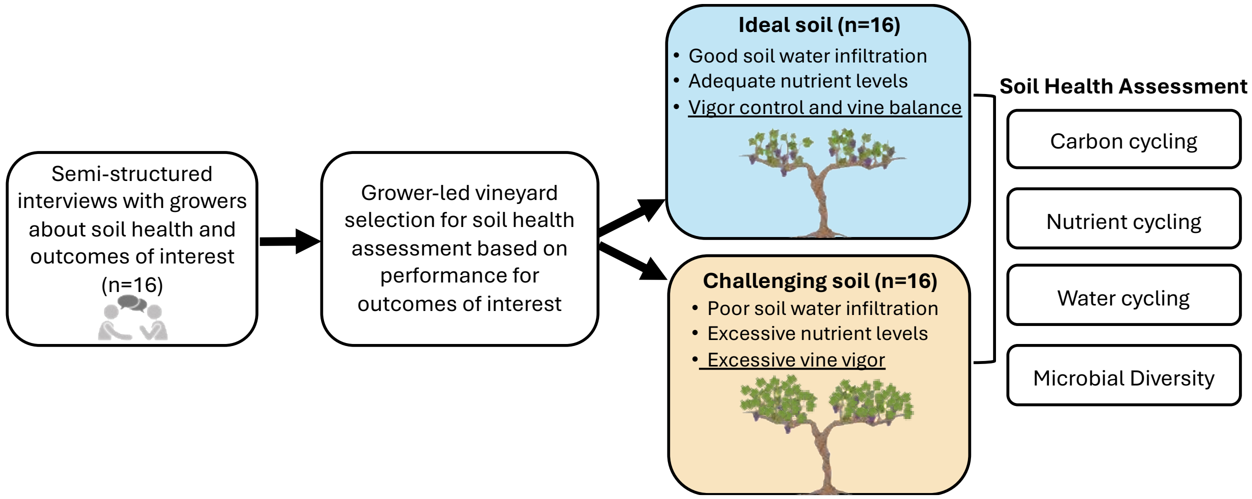

Figure 2.1. Process of the collaborative vineyard soil health assessment with wine grape growers (n=16). Semi structured interviews were reported in Gonzalez-Maldonado et al., (2024).

In total, soil samples were collected from 30 vineyards across the Napa Valley American Viticultural Area (AVA) located in Napa County, and 2 vineyards located in the eastern border of the Sonoma AVA in Sonoma County California, USA (Figure 2.2) between the late winter and spring of 2021 (late February – early May). Sixteen vineyards were rated as ideal and sixteen as challenging. All vineyards were growing red wine grape varieties except for three that grew white varieties (Appendix Tables 2.2A and 2.3A). Napa County has a high soil diversity resulting from diverse parent materials, microclimates, and topography (Kunkel and Upson, 1960). In the mountains and hills, Napa soils are primarily formed from volcanic and marine sediment parent material and in the valley, soils are mostly formed from alluvial deposits (Kunkel & Upson, 1960; Lambert & Kashiwagi, 1978). The climate in Napa Valley is Mediterranean, consisting of cool, wet winters from November to March and dry, hot summers from April to October. The annual cumulative rainfall in 2021 was of 214 mm and an average of 583 mm from 2012-2021 (CIMIS, 2024) (Appendix Table 2.1A). The mean minimum average and maximum temperatures were 6.3°C, 14.2°C and 23°C, respectively, for 2021 (CIMIS, 2024).

The soil subgroups (US Taxonomy) across all vineyards sampled in the Napa Valley AVA were Haploxeralfs (7 vineyards), Haploxerolls (6 vineyards), and Haploxerults (5 vineyards); Vitrixerands, Xerofluvents, and Argixerolls (3 vineyards each); Endoaquerts and Dystroxerepts (2 vineyards each); and Haplohumults (1 vineyard). Across all vineyards, clay content ranged from 4 to 37%, pH ranged from 5.6 to 8.3, and soil organic matter ranged from 0.01 to 7.1%. The dominant representative slope range was 0-1% (12 vineyards) followed by 4-9% and 18-23% in 7 vineyards each, and the 33-53% in 6 vineyards. Detailed information per vineyard is found in Appendix Tables 2.2A and 2.3A.

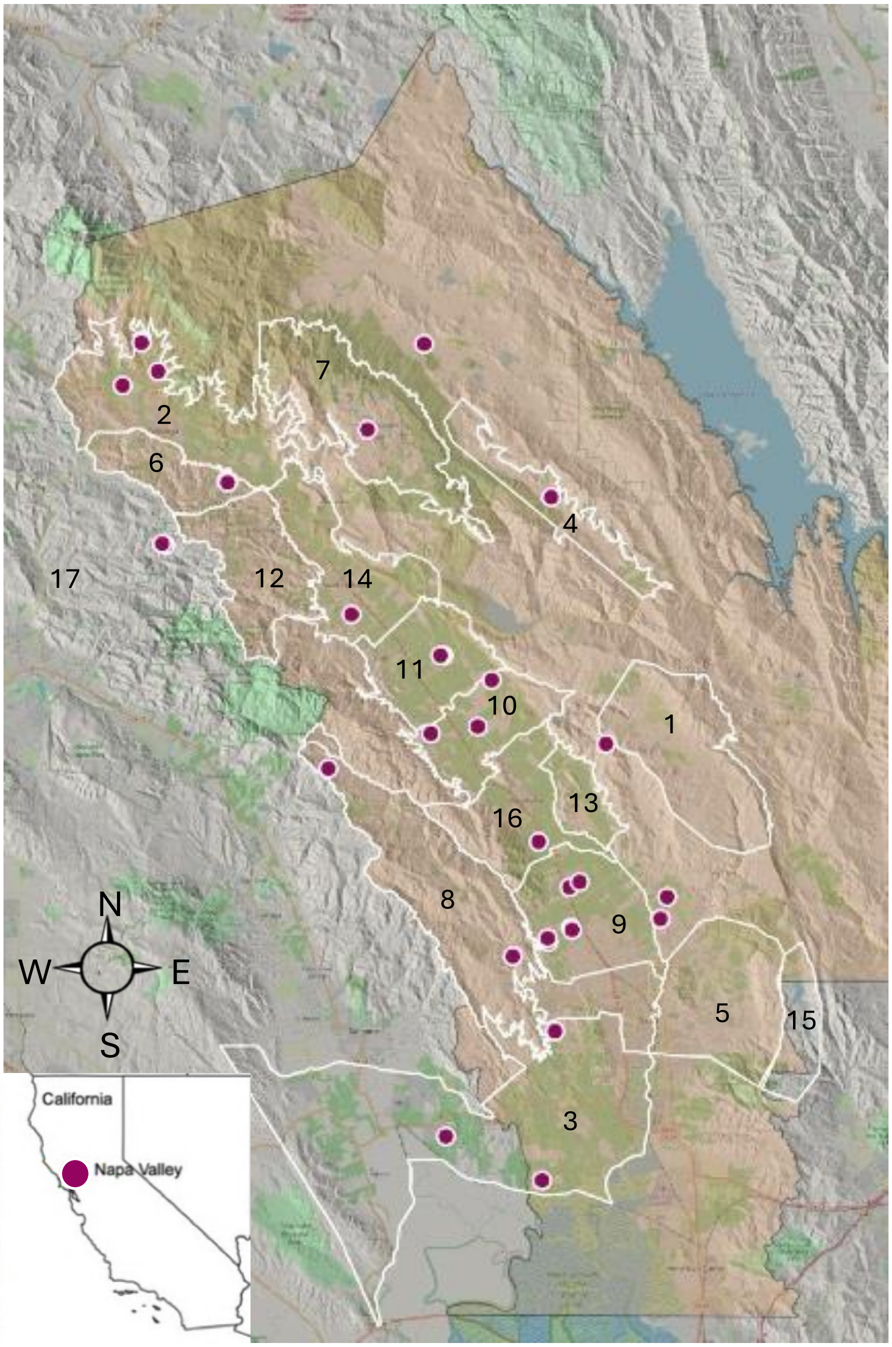

Figure 2.2. Map of the soil sampling sites across the Napa Valley American Viticultural Area (AVA) sub-appellations including Atlas peak (1), Calistoga (2), Carneros (3), Chiles valley (4), Combsville (5), Diamond mountain (6), Howell mountain (7), Mt. Veeder (8), Oak knoll (9), Oakville (10), Rutherford (11), Spring mountain (12), Stags leap district (13), St. Helena (14), Wild horse valley (15), and Yountville (16). Two vineyards sampled were located at the Sonoma AVA sub appellation Fountaingrove (17).

Within each vineyard, soil samples were taken from two vineyard zones: under the vine (vine row, n=3 per depth) and in the interrow (tractor row, n=3 per depth) from March to May of 2021 (Table 2.1). Soil samples were collected from two depth intervals, 0-10 cm and 10-20 cm using a Giddings manual bulk soil core sampler with a diameter of 5 cm (Windsor, CO, USA) (Table 2.1; Figure 2.3). All vine rows were under drip irrigation except for vineyard #20, which was not irrigated (Table 2.3A). Two soil cores were collected approximately 2 meters apart for each replicate. The two cores were subsequently homogenized by depth into a composited replicate. The replicates were collected approximately 12 meters apart in areas that were representative of the vineyard. Immediately after collection, samples were stored in coolers with ice for approximately six hours and then stored in a refrigerator at 4°C until they were processed the next day. The GPS coordinates were recorded for each replicate of every vineyard. All fresh soils were sieved to a size of 8mm to homogenize them and remove gravel. A 100 g subsample of fresh soil was refrigerated (3°C) in sealed plastic Ziploc® bags for subsequent analysis of microbial biomass C, potentially mineralizable N, nitrate, and ammonium (see section 2.2 - 2.3). A subsample of 50 g was frozen (-80°C) in sealed Ziploc® plastic bags for phospholipid fatty acids (PLFA) analysis. The remaining soil was air dried for one week and subsequently ground and sieved to 2mm.

Figure 2.3. Picture indicating the sampled areas in each vineyard replicate including the vine row (i.e., under the vine, in between the vines) and the tractor row (the middle of the alley or interrow).

Soil textural classes were determined from particle size distribution analysis using the Comprehensive Assessment of Soil Health CASH rapid soil texture method (Moebius-Clune et al., 2016); however, verification of this rapid method using the pipette method (Soil Survey Staff, 2014) indicated an underestimation of silt and overestimated clay contents by approximately 30%. Therefore, a subsample of 64 soil samples were run using the soil particle size distribution using the pipette method and corrections were performed using a linear regression model to adjust for the silt and clay content. Soil textural class groups were coarse (n= 36; 4-19% clay; loamy sand and sandy loam), loam (n=204; 12-37% clay; loam and sandy clay loam), silt loam (n=108; 16-27 % clay; silt loam and silt), and fine (n=36; 26-34 % clay; clay loam, silty clay loam, sandy clay), following Amsili et al., (2021).

Detailed information on soil management practices was collected from conversations with growers using online surveys (Google, Inc.) prior to field sampling. Soil disturbance levels were evaluated including tillage (annual disking to an average depth of 20 cm) and no-till (no disking or tilling) for at least 3 years prior to sampling. Information on the factors considered for soil sampling and data analysis (vineyard zone, soil textural class group, and disturbance) in this study are detailed in Table 2.1.

Table 2.1. Soil textural groups, vineyard sampling location, and soil management practices and number of data points associated with each level of the study factors. The total number of soil samples collected were n=384.

Factor	Levels	N
Grower perception (rating)	Challenging soil	192
	Ideal soil	192
Location	Vine row	192
	Tractor Row	192
Depth	0-10 cm	192
	10-20 cm	192
Texture	Coarse	36
	Loam	204
	Silt Loam	108
	Fine	36
Disturbance	Till (annual disking to an average depth of 20 cm)	108
	No-Till	276

2.2 Carbon cycling soil health indicators

Permanganate oxidizable carbon (POXC) was quantified to assess management-sensitive carbon (Culman et al., 2012; Weil et al., 2003). Briefly, 2.5 g of 2mm air-dry and ground soil was combined with 20 mL of a 0.02 M potassium permanganate ($KMnO_4$) solution in 50 mL centrifuge tubes. The tube was shaken for two minutes (180 strokes per minute) using a reciprocal shaker and allowed to settle for 10 minutes. Then, 0.5 mL of supernatant was transferred to another 50mL centrifuge tube containing 49.5 mL of deionized water and mixed briefly and gently by hand for approximately 10 seconds. Finally, the sample absorbance was measured by an Agilent BioTek Epoch 96-well microplate spectrophotometer at 550 nm (Agilent Technologies, Inc., Santa Clara, CA) .

Mineralizable carbon (Min C) was quantified to assess labile carbon that is respired and mineralized by soil microorganisms... We used a method of rewetting 10 g of 8 mm sieved dry soil to 50% gravimetric water holding capacity (WHC) in a 227 mL glass jar (Franzluebbers et al., 2000; Haney et al., 2001; Haney & Haney, 2010). After rewetting, jars were capped tightly with lids containing two silicone septa and incubated at 25°C for 48 hours. After the incubation, input and output syringes were injected into an LI-850 CO_2/H_2O gas analyzer (LI-COR, Biosciences, Lincoln, NE) to determine the concentration of CO_2 in the headspace. Finally, mineralizable C was calculated as the difference between a sample and a blank control using the ideal gas law and the headspace volume.

Microbial biomass C (MBC) was measured to assess the labile carbon that is contained withing soil microorganisms. using the fumigation-extraction method (Horwath & Paul, 1994; Vance et al., 1987). Briefly, 6 g of fresh soil was fumigated with chloroform for 24 h prior to extraction with 0.5 M K_2SO_4. A non-fumigated duplicated subsample was extracted with 0.5 M K_2SO_4. The extracted solutions were diluted in a 4:1 deionized water:solution ratio, and the concentration of dissolved organic C (DOC) was analyzed using the high-salt method for the Shimadzu TOC-L total organic carbon analyzer (Shimadzu Corp.). Microbial biomass C was calculated from the difference in DOC concentrations between fumigated and nonfumigated soil samples with a K_e factor of 0.35 (Horwath & Paul, 1994). Soil total carbon content (TC) was measured by direct combustion (Nelson & Sommers, 1982) with a Costech CHN Elemental Combustion Analyzer (Costech Analytical Technologies Inc., CA, USA).

2.3 Nutrient cycling soil health indicators

Nitrate (NO_3^--N) and ammonium (NH_4^+-N) were assessed colorimetrically in the soil samples to study plant-available N (Doane & Horwáth, 2003; Keeney & Nelson, 1982). Briefly, soils were extracted using 0.5 M K_2SO_4 solution, shaken for 1 h and centrifuged (2,900 RPM) for 2 min. Samples were filtered using a grade 1 Whatman® filter paper and stored in a refrigerator at 4 °C for a maximum of 14 days until next steps could be continued. Samples were read on a spectrophotometer Synergy HTX Multi-Mode Reader, Biotek Instruments ©, VT, USA) at 450 and 640 nm for NO_3^--N and NH_4^+-N, respectively.

Like TC, total soil nitrogen content (TN) was measured by direct combustion. Soil pH and electrical conductivity (EC) were measured in a 2:1 (soil:water) paste using a SevenCompact Duo S213-meter, pH/Ion dual channel benchtop meter (InLab Expert Pro-ISM pH sensor, InLab 731-ISM conductivity sensor; Mettler Toledo, OH, USA).

Potentially mineralizable nitrogen (PMN) was estimated to assess organic matter pools that could be converted to plant-available N by soil microorganisms following Drinkwater et al., (1996) (i.e,. 7-day incubation of 8 g soil in 10 mL of deionized water at 37°C; headspace replaced with N_2 gas in sealed 50mL centrifuge tubes). Then, these incubated samples were extracted (30 mL of 0.67M K_2SO_4), and NH_4^+-N was measured as above. PMN was calculated from the difference between control and incubated samples.

2.4 Water cycling soil health indicators

Soil unsaturated hydraulic conductivity was measured in the field to assess soil water infiltration rates using a mini disk infiltrometer (Meter Group, Inc.) adjusted to -2 cm suction rate to control flow through macropores. The mini disk infiltrometers were placed in a leveled soil area of the tractor row, and measurements from water flow were recorded every five minutes (up to 35 minutes) to obtain a constant change in volume from a minimum of three consecutive readings.

Then, infiltration rate was calculated using the Phillips model (Philip, 1969). Wet aggregate stability was measured with a wet sieving apparatus (Eijkelkamp Agrisearch, Wilmington, NC), following the wet sieving method with a single sieve (Kemper & Rosenau, 2018). Soil bulk density was measured to assess soil compaction by using a 103 cm^3 volume core, drying the soil samples at 105 °C for 24 hours and then dividing the dry mass by the volume of the core (Blake, 1965). Finally, penetration resistance was measured as another form to assess soil compaction using a SpotOn® Digital Compaction Meter (Innoquest Inc.,Woodstock, IL) and gravimetric soil moisture adjustment was calculated following Equations 1 and 2 form Busscher et al., (1997):

$C_i= aW_o^b$ ....Equation 1

Where, C_i is the penetration resistance cone index (MPa), W_o is the water content on a mass basis ($g\ g^{-1}$), and a and b are calculated empirical parameters.

$C_f = C_i + \frac{dC}{dW}(W_c - W_o)$ ....Equation 2

Where, C_f is the adjusted penetration resistance cone index (MPa), C_i is the unadjusted penetration resistance cone index (MPa), W_o is the gravimetric water content ($g\ g^{-1}$) for the C_i at 20 cm soil depth, W_c is the average gravimetric water content ($g\ g^{-1}$) for all treatments at 20 cm soil depth, and dC/dW is the first order derivative for the Equation 1.

2.5 Microbial community structure

Four weeks after collection, frozen (-80°C) composite subsamples were sent overnight on dry ice to a commercial laboratory for phospholipid fatty acids (PLFA) analysis to assess soil microbial community structure (Ward Laboratories Inc.). The PLFA analysis was performed only in the top depth (0-10 cm) for both vineyard zones (n= 64) following Bligh and Dyer (1959) and Buyer and Sasser (2012). The PLFA assigned to the functional groups are found in Appendix Table 2.4A.

Indicators calculated from PLFAs included biomass (ng g^{-1}) for the following functional groups: total (all), total bacterial, actinomycetes, gram negative bacteria, gram positive bacteria, total fungal biomass, arbuscular mycorrhizal fungi biomass, saprophytic fungi biomass, and fungi:bacteria ratio.

2.6. Data analysis

All indicators were tested for normality using the “ggdensity()”, “ggqqplot()”, and “hist()” functions from the *ggpubr* package and base R using RStudio (Version 2023.12.1 + 402) (Kassambara, 2023). Data were transformed when needed to obtain normal distribution using the “log()” function (NH_4^+-N, NO_3^--N, PMN, PLFA indicators, and infiltration rate) and “sqrt()” (MBC, DOC, Min C, TN, and bulk density) functions on severely and moderately skewed variables, respectively. Descriptive statistics were completed by using the *tidyverse* package (Wickham et al., 2019) in RStudio. Soil health indicators were analyzed using a linear mixed model (using the “lmer()” function from the *lme4* package in R) and linear model (using the “lm()” function form the *stats* package in R) analyses in two separate models by research question (Bates et al., 2015; R Core Team, 2024). The purpose of this first model equation (Equation 3), which is a linear mixed model analysis, was to determine growers’ perceptions of challenging and ideal soils with a soil health assessment:

$$Y_{ij} = \beta_0 + \beta_1 * (\text{Grower Rating}_{ij}) + \mu_j + \varepsilon_{ij} \quad \text{.......... Equation 3}$$

Where Y_{ij} represents the soil health indicator for the i^{th} observation within the j^{th} grower, β_0 is the fixed intercept reflecting the mean “soil health indicator” across all “growers” when the Grower Rating is zero; β_1 is the fixed effect of “Grower Rating”; Grower Rating_{ij} is the “Grower Rating” value for the i^{th} observation under the j^{th} grower; μ_j is the random intercept for each grower, accounting for variability in soil health indicators specific to each grower; and ε_{ij} is the residual error.

The purpose of the second model, which was a linear model analysis (Equation 4), was to evaluate the variability of soil health indicators among vineyard sampling zone, soil depth, texture and soil management related to mechanical disturbance (disk vs no-till):

$Y_i = \beta_0+\beta_1 *(\text{Vineyard Zone})_i +\beta_2 * (\text{Depth})_i+\beta_3 *(\text{Texture})_i+\beta_4 *(\text{Disturbance})_i +\varepsilon_i$ .... Equation 4

Where Y_i represents the soil health indicator for the i^{th} observation; β_0 is the intercept; β_1, β_2, β_3, and β_4 are the fixed effects, which represent the effects of Vineyard Zone, Depth, Texture, and Disturbance, respectively, on the soil health indicator; and ε_i is the residual error.

An analysis of variance (ANOVA) was then run on the models to obtain F statistics and p values. Post hoc analyses were done using the *emmeans* package (Lenth, 2024). Data visualization (boxplots) was done using the *emmeans* and *ggplot2* (Wickham, 2016) packages. A principal component analysis (PCA) was conducted using the *FactoMineR*, *factoextra*, and *ggplot2* packages to evaluate the distribution and correlations of soil health indicators (Kassambara & Mundt, 2020; Lê et al., 2008). A Permutational multivariate analysis of variance (PERMANOVA), which tests for differences in multivariate data by partitioning variation among groups based on a distance matrix, was done to evaluate the effect of vineyard sampling zone, soil depth, texture, and soil disturbance on soil health indicators using Bray-Curtis dissimilarity distances with the *vegan* package (Oksanen et al., 2024). Pearson correlations were conducted to evaluate relationships between soil health indicators. For all the analyses, the $p<0.05$ level was used to explain variables that differed from each other.

3. Results

3.1. Variability in soil health indicators across Napa Valley vineyards

The soil health indicators showed different levels of variability across all vineyards in the Napa Valley wine growing region (Table 2.2). The highest variability across carbon cycling indicators occurred in microbial biomass C (MBC) (coefficient of variation, CV=80.5%) and mineralizable C (Min C) (CV=78.4%), and the lowest variability occurred in total C (TC) (CV=39.9%) and active C (POXC) (CV=36.2%). Min C and MBC ranged from 0.3 to 122 mg kg^{-1} and 3.4 to 646 mg kg^{-1}, respectively. POXC and TC ranged from 117 to 1156 mg kg^{-1} and 0.04 to 41 g kg^{-1}, respectively. Among nutrient cycling indicators, soil NH_4^+-N showed the highest variability (CV = 88.32%), ranging from 0.6 to 17.6 mg kg^{-1}. Soil NO_3^--N (CV=74.4 %) had the second highest variability followed by potentially mineralizable nitrogen (PMN) (CV=64.8 %), electrical conductivity (EC) (CV = 61.6%), total nitrogen (TN) (CV=48.8%), and pH (CV=7.5%). Total nitrogen (TN) values ranged from 0.13 to 4.6 g kg^{-1}, NO_3^--N values ranged from 0.26 to 5 mg kg^{-1}, and PMN values ranged from 0.66 to 94 mg kg^{-1}. Among water cycling indicators, infiltration rate had the highest variability (CV=105%), ranging from 0.3 to 14.5 cm hr^{-1}, and bulk density had the lowest (CV= 12.7%), ranging from 0.63 to 1.8 g cm^{-3}. Saprophytic fungi biomass (CV=125%), had the highest variability of the microbial diversity indicators (CV=127%), ranging from 0 to 618 ng g^{-1}, and fungi:bacteria ratio had the lowest (CV=54%), ranging from 0 to 0.58. Descriptive statistics and CVs for every soil health indicator are shown in Table 2.2.

Table 2.2. Global summary of back-transformed data including mean, standard deviation (sd), minimum, maximum, median, 95% confidence intervals (CI), and coefficients of variation (CV) of soil health indicators across all 32 vineyards sampled in Napa Valley (California, USA).

Functions/ Properties	Indicators	mean	sd	min	max	lower CI	upper CI	CV (%)
Carbon cycling (n=384)	Total C (g kg^{-1})	19.27	7.68	0.04	40.95	18.50	20.04	39.87
	POXC (mg kg^{-1})	591.9	214.4	117.2	1156	570.5	613.4	36.2
	Min C (mg kg^{-1}day^{-1})	23.48	18.40	0.29	122.0	21.64	25.32	78.37
	MBC (mg kg^{-1})	125.4	101.0	3.38	645.8	115.3	135.5	80.52
	DOC (mg kg^{-1})	13.28	8.40	0.03	40.40	12.44	14.12	63.30
Nutrient cycling (n=384)	Total N (g kg^{-1})	1.42	0.69	0.13	4.62	1.36	1.49	48.77
	NO_3^--N (mg kg^{-1})	1.46	1.09	0.26	4.96	1.35	1.57	74.39
	NH_4^+-N (mg kg^{-1})	3.70	3.27	0.58	17.64	3.37	4.02	88.32
	PMN (mg kg^{-1} wk^{-1})	24.51	15.89	0.66	93.98	22.92	26.10	64.83

	pH	7.01	0.52	5.60	8.30	6.96	7.07	7.45
	EC (uS cm^{-1})	114.8	70.23	33.46	532.0	107.8	121.9	61.16
Water cycling (n=384)	Bulk Density (g cm^{-3})	1.20	0.15	0.63	1.80	1.19	1.22	12.73
	PR (kPa)	2846	911	689	5176	2755	2937	32.02
	WAS (%)	51.57	12.79	20.07	85.51	50.29	52.85	24.81
	Infiltration (cm hr^{-1}) (n=96)	2.25	2.38	0.29	14.55	2.02	2.49	105.37
	Sand %	35.55	12.47	12.02	67.48	34.31	36.80	35.06
	Silt %	43.47	9.22	28.35	67.17	42.55	44.40	21.21
	Clay %	20.97	5.64	3.94	37.01	20.41	21.53	26.91
Microbial diversity (n=64)	Total PLFA (ng g^{-1})	1816	1563	131.4	9170	1545	2087	86.07
	Total Bacteria (ng g^{-1})	565.6	469.2	36.99	2431	484.3	646.9	82.96
	Actinomycetes (ng g^{-1})	84.67	65.76	3.10	314.7	73.28	96.06	77.67
	Gram N bacteria (ng g-$^{-1}$)	279.9	267.9	22.48	1424	233.5	326.3	95.71
	Gram P bacteria (ng g^{-1})	285.7	210.3	14.51	1007	249.3	322.2	73.59
	Total Fungi (ng g^{-1})	162.2	186.0	0.00	837.2	130.0	194.5	114.6
	Saprophytes (ng g^{-1})	103.7	131.4	0.00	617.7	80.96	126.5	126.7
	AMF (ng g^{-1})	58.52	69.13	0.00	305.6	46.55	70.50	118.1
	Fungi:-Bacteria ratio	0.23	0.12	0.00	0.58	0.21	0.25	54.06

Carbon (C), nitrogen (N), permanganate oxidizable C (POXC), mineralizable C (Min C), dissolved organic C (DOC), potentially mineralizable N (PMN), electrical conductivity (EC), penetration resistance (PR), wet aggregate stability (WAS), gram negative (N) bacteria, gram positive (P) bacteria, Arbuscular Mycorrhizae Fungi (AMF).

3.2. Soil health indicators associated with desired viticultural outcomes

Soil health indicators were evaluated by growers' perceptions of what constitutes a challenging and ideal soil for achieving viticultural goals of vigor control and high grape quality. Challenging and ideal soils differed significantly in six out of 24 soil health indicators (Table 2.3). All indicators with significant differences were carbon, nutrient, and water cycling indicators: TC, POXC, TN, EC, NH_4-N, and wet aggregate stability (WAS) (Table 2.3). None of the six soil microbial diversity indicators differed between grower ratings (Table 2.3). Static soil properties like sand, silt and clay content were also significantly different between grower ratings (Table 2.3).

Among carbon cycling indicators, TC and POXC mean values were 16% and 17%, respectively, higher in the challenging soils compared to the ideal soils (Table 2.3). Mineralizable C tended to be higher in the challenging soils, but differences were not significant (p=0.05). Soil DOC and MBC did not differ between grower ratings. Soil C levels corresponding to ideal vineyard soil conditions have the following values (mean ± sd): 17.9 ± 7.9 g TC kg^{-1} and 546 ± 223 mg POXC kg^{-1} (n=192). In contrast, the C levels that represent a challenging include 20.6 ± 7.3 g TC kg^{-1} and 638 ± 196 kg POXC kg^{-1} (n=192). Soil Min C, MBC and DOC did not differ between challenging and ideal soils (Table 2.3).

Among the nutrient cycling indicators, TN and EC mean values were 15% and 21%, respectively, higher in the challenging soils, whereas soil NH_4-N mean values were 9% higher in the ideal soils. Soil NO_3^--N, PMN, and pH did not differ by grower rating (Table 2.3). N levels in the soils rated as ideal by growers have the following means (mean ± sd): 1.3 ± 0.7 g TN kg^{-1}, and 6.8 ± 13.21 mg NH_4^+-N kg^{-1}(n=192). Ideal soils had EC mean and standard deviation of 104 ± 63 μS cm^{-1} (n=192). In contrast, the N levels in the challenging soils from growers' perceptions were: 1.52 ± 0.7 g TN kg^{-1} and 5.58 ± 6.8 mg NH_4^+-N kg^{-1} (n=192). The challenging soils had EC mean and standard deviation of 126 ± 75.5 μS cm^{-1} (n=192). Soil NO_3^--N, PMN and pH were not different between grower ratings; the mean values across all soils were 1.46 ± 1.09 mg NO_3^--N kg^{-1}, 24.5 ± 15.9 mg PMN kg^{-1}, and 7.01±0.52, respectively (n=384).

Three out of the six water cycling indicators differed between grower ratings, including greater wet aggregate stability (5.5%), silt (4%), clay (14%) in the challenging soils, and greater sand content (12%) in the ideal soils (Table 2.3). Bulk density, penetration resistance (PR) and infiltration rate did not differ by grower rating. Therefore, the water cycling indicators that represented an ideal soil from growers' perspectives had the following means (mean ± sd): 50.2 ± 12.5 % WAS, 38 ± 14 %

sand, 42.6 ± 9.8 % silt, and 19.6 ± 6.6 % clay (n=192). In contrast, water cycling indicator values that represented a challenging soil from growers' perspectives had the following means (mean ± sd): 53 ±13 %WAS, 33.3 ± 10.2 % sand, 44.3 ± 8.6 % silt, and 22.3 ± 4 % clay (n=192).

Table 2.3. F statistics and p values from ANOVA, means, standard deviation (sd), and 95% confidence intervals (CI), from grower ratings based on their perceptions of challenging and ideal soils for wine grape production goals across 32 vineyards in Napa Valley (California, USA). Bolded numbers are used to represent higher means when significant differences between challenging and ideal were detected.

			Challenging[1]				Ideal[2]			
Soil Indicators	F	sig.	Mean	sd	lower CI	upper CI	Mean	sd	lower CI	upper CI
Total C (g kg^{-1})	16.4	***	**20.7**	7.3	19.6	21.7	17.9	7.9	16.8	19.0
POXC (mg kg^{-1})	20.1	***	**638**	196	610	666	546	223	514	577
Min C (mg kg^{-1} day^{-1})	3.6	NS	25.2	19.9	22.4	28.0	21.7	16.7	19.4	24.1
MBC (mg kg^{-1})	0.2	NS	125	91.3	112	138	126	110	110	141
DOC (mg kg^{-1})	1.4	NS	13.7	8.2	12.5	14.8	12.9	8.6	11.7	14.1
Total N (g kg^{-1})	11.9	***	**1.5**	0.7	1.4	1.6	1.3	0.7	1.2	1.4
NO_3^--N (mg kg^{-1})	0.3	NS	1.5	1.2	1.3	1.7	1.4	1.0	1.3	1.6
NH_4^+-N (mg kg^{-1})	8.1	**	3.5	3.4	3.0	4.0	**3.9**	3.2	3.4	4.3
PMN (mg kg^{-1})	0.002	NS	24.7	16.8	22.4	27.1	24.3	15.0	22.2	26.4
pH	1.2	NS	7.0	0.6	6.9	7.1	7.0	0.5	7.0	7.1
EC (µS cm^{-1})	24.1	***	**126.1**	75.5	115.4	136.8	104.1	63.1	95.1	113.0
Bulk Density (g cm^{-3})	2.5	NS	1.2	0.1	1.2	1.2	1.2	0.2	1.2	1.2
PR (kPa)	0.05	NS	2838	984	2698	2977	2855	837	2736	2973
WAS (%)	7.7	**	**52.9**	13.0	51.1	54.8	50.2	12.5	48.4	52.0
Infiltration (cm hr^{-1})	0.02	NS	2.3	2.6	2.0	2.7	2.2	2.2	1.9	2.5
Sand (%)	32.1	***	33.3	10.2	31.9	34.8	**37.8**	14.0	35.8	39.8
Silt (%)	10.5	**	**44.3**	8.6	43.1	45.6	42.6	9.8	41.2	44.0
Clay (%)	44.8	***	**22.3**	4.0	21.8	22.9	19.6	6.6	18.7	20.6
Total PLFA (ng g^{-1})	1.87	NS	1756	1860	1300	2212	1876	1224	1576	2175
Total Bacteria (ng g^{-1})	0.54	NS	548	519	421	675	583	421	480	687
Actinomycetes (ng g^{-1})	0.55	NS	80.9	69.6	63.8	98.0	88.4	62.5	73.1	103.8
Gram P bacteria (ng g^{-1})	1.73	NS	271	300	197	345	289	235	231	346
Gram N bacteria (ng g^{-1})	0.34	NS	277	225	222	332	295	198	246	343
Total Fungi (ng g^{-1})	0.36	NS	166	215	113	218	159	156	120	197

Saprophytes (ng g^{-1})	1.92	NS	111	160	72.1	151	96.1	96.4	72.5	120
AMF (ng g^{-1})	0.79	NS	54.6	71.1	37.2	72.0	62.5	68.0	45.8	79.1
Fungi:Bacteria ratio	0.58	NS	0.2	0.1	0.2	0.3	0.2	0.1	0.2	0.3

Codes for significance (sig.): '***' = $p < 0.001$; '**' = $0.001 < p < 0.01$; '*' = $0.01 < p < 0.05$; 'NS' = $p > 0.05$

Carbon (C), Nitrogen (N), Permanganate oxidizable C (POXC), mineralizable C (Min C), dissolved organic C (DOC), potentially mineralizable N (PMN), electrical conductivity (EC), penetration resistance (PR), wet aggregate stability (WAS), Arbuscular Mycorrhizae Fungi (AMF).

[1] n= 192 for challenging soils

[2] n= 192 for ideal soil

3.3. Variability in soil health indicators due to textural class

Several soil health indicators differed among some textural classes (Table 2.4). Examples of carbon cycling indicators influenced by textural class were TC, POXC, MBC, and DOC. In general, soil carbon cycling indicators were higher in the finer soil texture groups like silt loam and/or fine soils. For example, TC was highest in the silt loam soils (n=108) compared to the loam (+20%) (n=204) and coarse (+36%) (n=36) soils. Soil POXC was highest in the silt loam soils (+14% compared to the fine soils (n=36), +13% compared to the loam soils, +25% compared to the coarse soils). Microbial biomass C (MBC) was highest in the fine textured soils followed by the loam and coarse soils. It was the lowest in the silt loam. Soil DOC was highest in the loam and silt loam soils. Mineralizable C (Min C) was not different among soil textural classes.

Most nutrient cycling indicators were affected by soil textural class (Table 2.4); however, trends were variable. Soil NO_3^--N was higher in the loam and fine textured soils compared to the silt loam (+34%) and coarse (+90%) soils. Soil NH_4^+-N was higher in the loam soils compared to silt loam (+133%) and fine (+42%) soils; also, NO_3^--N was 46% higher in the coarse soils compared to the silt loam soils. Soil TN and NH_4^+-N did not differ among soil textural classes. Soil pH was approximately 3% lower in the silt loam soils compared to the other textures. Finally, EC was higher in the loam soils compared to the silt loam soils. Overall, no consistent trends were found among the nutrient cycling indicators with increasing clay content among soil texture classes across the landscape.

The water cycling indicators that differed by textural class were bulk density, PR, WAS, and infiltration (Table 2.4). For instance, bulk density was lowest in the fine soils compared to coarse and loam soils. Penetration resistance was highest in the silt loam soils (+38% compared to coarse, +20% compared to loam, +26% compared to fine soils) and WAS was highest in the fine soils (WAS = 62%), followed by loam soils (WAS = 53.8%) and lowest in the coarse (WAS= 47.6%)

and silt loam soils (WAS = 45.3%). Infiltration rate was 173% higher in the coarse soils compared to the silt loam soils only. Finally, the microbial diversity biomass indicators from PLFA did not differ among soil textural classes across the landscape; however, most of these indicators tended to be higher in the loam soils.

3.4. Variability in soil health indicators by vineyard zone

We explored variability in soil health indicators within the vineyard by comparing soils collected in the tractor row and vine row. These two zones typically receive different management, and therefore variability in soil health indicators could reflect differences due to long-term management. Most soil health indicators differed between vineyard zones (Table 2.4; Figure 2.4). Most mean values of carbon cycling indicators were higher in the tractor row compared to the vine row (Table 4, Figure 2.4). Soil TC, POXC, MBC, and Min C were 10%, 17%, 23%, and 20% higher, respectively, in the tractor row compared to the vine row. Among nutrient cycling indicators, NH_4^+-N and PMN were higher in the tractor row (22% and 28%, respectively) while NO_3^--N, pH, and EC were higher in the vine row (22%, 3%, and 15%, respectively). Among water cycling indicators, higher levels of bulk density (3%) are seen for the vine row and higher levels of PR (12%), and WAS (6%) were measured for the tractor row.

Most soil microbial diversity indicators were higher in the tractor row, including total bacterial biomass (54%), actinomycetes biomass (51%), gram positive bacteria (50%), and arbuscular mycorrhizal fungi (AMF) (111%) compared to the vine row (Table 2.4; Figure 2.4). Generally, soil health indicators were higher in the tractor rows compared to the vine rows.

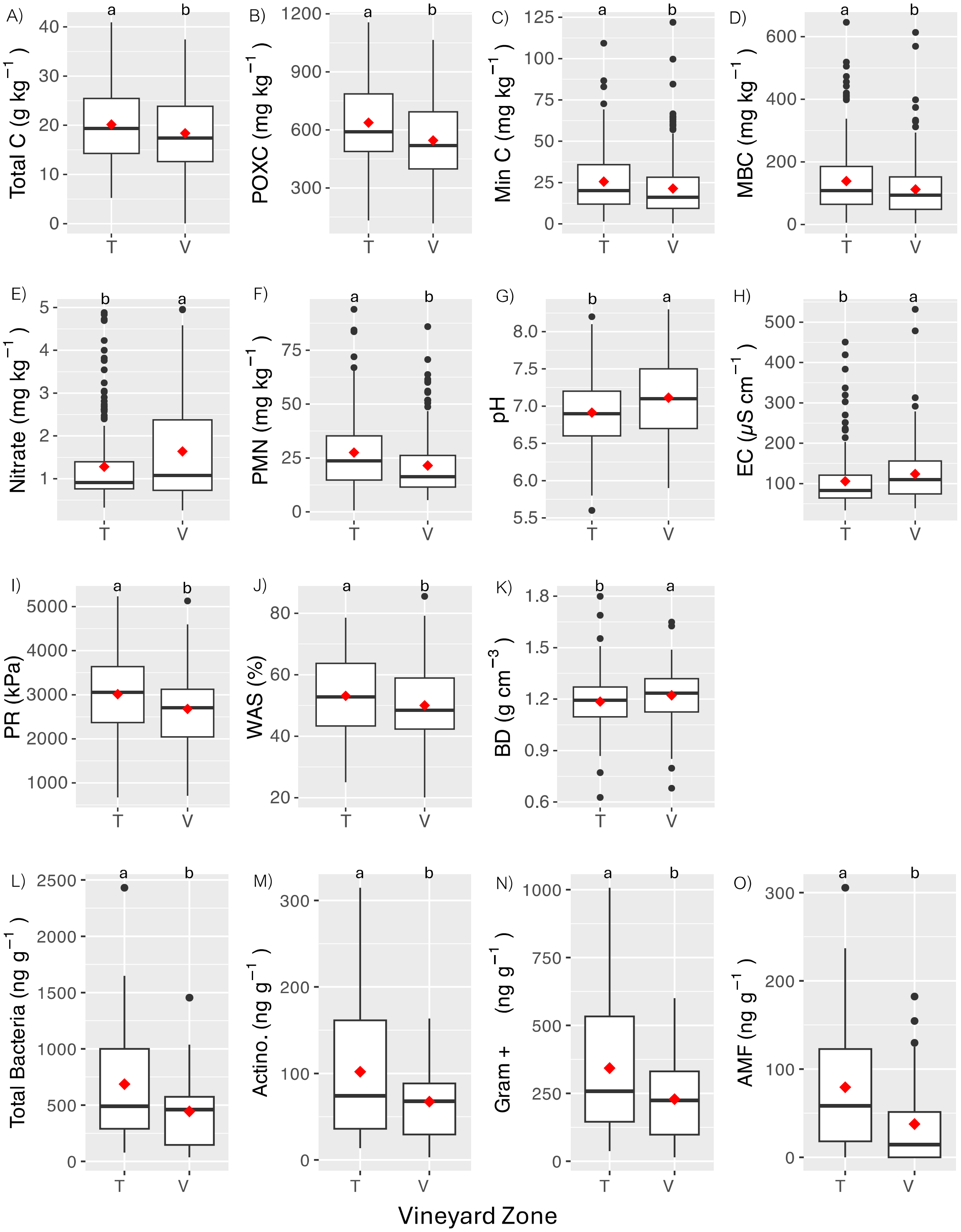

Figure 2.4. Boxplots of soil health indicators significantly different ($p<0.05$) between vineyard zones [tractor (T) vs. vine (V) rows] across 32 vineyards in Napa Valley (California, USA). Means are represented by a red diamond (tractor row n= 192; vine row n=192). Different letters represent significant ($p<0.05$) differences between Vineyard Zone levels with “a” representing higher means and “b” representing lower means.

Table 2.4. Linear mixed model ANOVA F statistics and significance ($p<0.05$), and means for soil health indicators across vineyard zone, texture, and disturbance for 32 vineyards sampled across Napa Valley (California, USA). Bolded values are used to indicate higher mean values.

	Vineyard zone[1]				Disturbance[2]				Textural class group[3]									
Soil health indicators	F	Sig	T mean	V Mean	F	Sig	Till mean	NT Mean	F	Sig	Coarse mean		Loam mean		Silt Loam mean		Fine mean	
Total C (g kg^{-1})	6.2	*	**20.2**	18.4	7.6	**	17.7	**21.1**	9.1	***	16.2	b	18.4	b	22	a	19	ab
POXC (mg kg^{-1})	33.7	***	**638**	546	1.4	NS	648	634	8.8	***	523	b	576	b	652	a	570	b
Min C (mg kg^{-1}day^{-1})	8.9	**	**25.6**	21.4	6.7	**	20.6	**27.5**	1.2	NS	22.4	a	22.4	a	25.8	a	20.8	a
MBC (mg kg^{-1})	10.4	***	**138.7**	112.1	2	NS	124	144	11.7	***	128.9	b	128.9	b	98.3	c	201.5	A
DOC (mg kg^{-1})	0.01	NS	13.17	13.38	17	***	**15.72**	12.32	12.5	***	7.9	b	14.9	a	13.4	a	9.0	b
Total N (%)	1.3	NS	1.46	1.39	.001	NS	2.37	2.48	0.7	NS	1.3	a	1.4	a	1.5	a	1.4	a
NO_3^--N (mg kg^{-1})	9.8	**	**1.28**	1.64	0.1	NS	1.03	1.4	9.6	***	0.9	b	1.6	a	1.2	b	1.7	a
NH_4^+-N (mg kg^{-1})	3	NS	3.92	3.47	5.6	*	3.1	**4.2**	13.2	***	3.9	ab	4.4	a	2.5	c	3.5	bc
PMN (mg kg^{-1} wk^{-1})	26.2	***	**27.54**	21.49	0.02	NS	29.1	27.0	2.3	NS	25.9	a	25.9	a	22.7	a	20.6	a
pH	15.0	**	6.91	**7.11**	0.1	NS	6.92	6.91	4.3	**	7.1	a	7.0	a	6.9	b	7.2	a
EC (µS cm^{-1})	12.3	***	105.8	**123.9**	0.3	NS	113	103	3.3	*	104.6	ab	123.1	a	101.7	b	118.8	ab
Bulk Density (g cm^{-3})	5.8	*	1.19	**1.22**	3.1	NS	1.21	1.17	4.9	**	1.2	a	1.2	a	1.2	ab	1.1	b
PR (kPa)	7.2	**	**3013**	2680	0.4	NS	3066	2989	7.4	***	2378	b	2742	b	3280	a	2605	b
WAS (%)	6.7	*	**53.1**	50	0.1	NS	52.52	51.20	24.1	***	47.6	c	53.8	b	45.3	c	62.0	a
Infiltration (cm hr^{-1})	-	-	2.25	NA	0.2	NS	2.49	2.16	3.0	*	4.1	a	2.4	ab	1.5	b	2.0	ab
Total PLFA (ng g^{-1})	1.2	NS	2003	1629	1.1	NS	2241	1910	2.7	NS	1239	ab	2192	ab	1307	b	1787	a
Bacteria (ng g^{-1})	4.5	*	**686.6**	444.6	3.7	NS	554	739	0.8	NS	306.1	a	680.3	a	521.5	a	307.6	a
Actinomycetes (ng g^{-1})	4.1	*	**102.0**	67.4	2	NS	85.2	108	2.5	NS	43.1	a	100.3	a	81.3	a	48.1	a
GN bacteria (ng g^{-1})	3.8	NS	343.7	216.0	3.8	NS	260	376	0.7	NS	143.5	a	336.4	a	266.2	a	137.0	a
GP bacteria (ng g^{-1})	4.7	*	**342.9**	228.6	2.4	NS	293	362	2.4	NS	162.6	a	343.9	a	255.3	a	170.6	a
Fungi (ng g^{-1})	3.7	NS	207.9	116.6	0.6	NS	196	213	0.6	NS	87.7	a	202.3	a	146.5	a	57.0	a
Saprophytes (ng g^{-1})	1.7	NS	128.5	79.0	0.3	NS	144	122	1.9	NS	50.5	a	131.2	a	90.4	a	41.4	a
AMF (ng g^{-1})	8.4	**	**79.4**	37.6	2.9	NS	51.8	90.2	0.2	NS	37.3	a	71.2	a	56.0	a	15.6	a
Fungi:Bacteria ratio	2.6	NS	0.252	0.203	0.3	NS	0.28	0.24	1.5	NS	0.276	a	0.2	a	0.2	a	0.2	a

Codes: ‘***’ = $p < 0.001$; ‘**’ = $0.001 < p < 0.01$; ‘*’ = $0.01 < p < 0.05$; ‘NS’ = $p > 0.05$; ‘-‘ = no data

Permanganate oxidizable C (POXC); mineralizable C (Min C); microbial biomass C (MBC); dissolved organic C (DOC); Nitrate (NO_3^--N); ammonium (NH_4^+-N); potentially mineralizable N (PMN); electrical conductivity (EC); penetration resistance (PR); wet aggregate stability (WAS); gram negative bacteria (GN); gram positive bacteria (GP); arbuscular mycorrhizae fungi (AMF); tractor row (T); vine row (V); no-till (NT); significance (Sig)

[1] n=192 for vine row and n=192 for tractor row

[2] n=108 for till and n= 276 for no-till

[3] n = 36 for coarse, n=204 for loam, n = 108 for silt loam, n = 36 for fine

3.5. Variability in soil health indicators by soil depth

All soil carbon and nutrient cycling indicators were significantly higher in the top 0-10 depth ($p<0.05$) compared to the 10-20 cm depth except for soil pH that did not differ between depths ($p>0.05$). The carbon cycling indicators in the top depth had mean values of 21.6 g TC kg^{-1}, 728 mg POXC kg^{-1}, 30 mg Min C kg^{-1},156 mg MBC kg^{-1}, and 15.3 mg DOC kg^{-1}. In the bottom depth (10-20 cm) these carbon indicators had mean values of 16.9 mg TC kg^{-1}, 456 mg POXC kg^{-1}, 16.1 mg Min C kg^{-1}, 95 mg MBC kg^{-1}, and 11.3 g DOC kg^{-1}. The nutrient cycling indicators in the top depth had mean values of 1.6 mg TN kg^{-1}, 1.6 mg NO_3^--N kg^{-1}, 4.4 mg NH_4^+-N kg^{-1}, and 21.8 mg PMN kg^{-1}. In the bottom depth (10-20 cm) the nutrient cycling indicators had the following mean values: 1.25 mg TN kg^{-1}, 1.3 mg NO_3^--N kg^{-1}, 2.99 mg NH_4^+-N kg^{-1}, and 17.2 mg PMN kg^{-1}. Soil bulk density was the only soil physical indicator evaluated across depths and mean values did not differ ($p>0.05$). Soil microbial diversity indicators were not evaluated across depths but only for the 0-10cm depth.

3.6. Variability in soil health indicators due to soil disturbance

Soil carbon indicators were the most affected by soil disturbance (till vs. no-till) (Table 2.4). Soil TC, Min C, and NH_4^+-N mean values were 12%, 18%, and 18%, respectively, higher in the no-till than in the tilled soils (Table 2.4, Figure 2.5). In contrast, DOC was 27% higher in the tilled soils compared to the no-till soils. Although microbial diversity indicators were not significantly different between tilled and no-till soils, most of these indicators tended to be higher in the no-till soils.

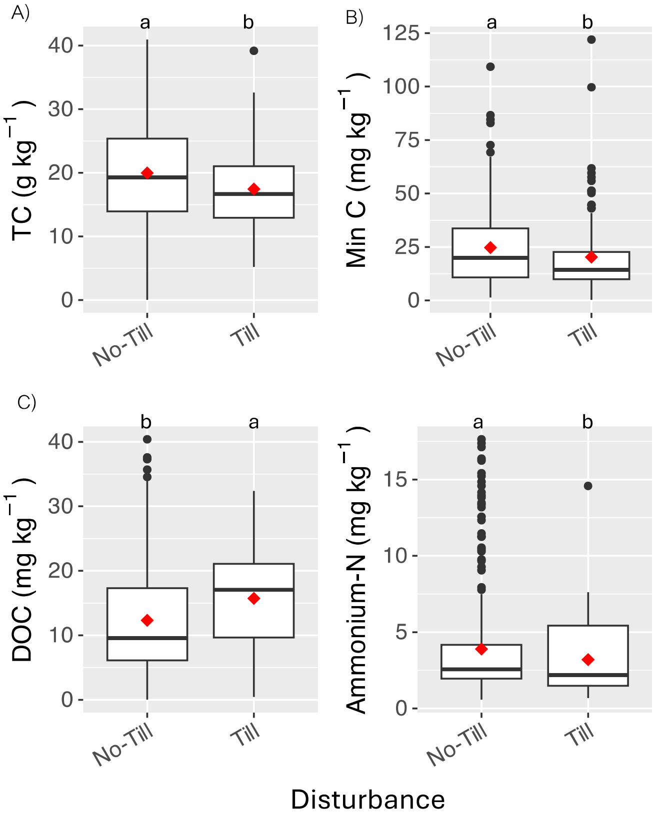

Figure 2.5. Boxplots of soil health indicators significantly affected ($p<0.05$) by soil disturbance (till vs no-till) across 32 vineyards in Napa Valley (California, USA). No-till consisted of no mechanical disturbance with tillage implements, but cover crops were mown or controlled with herbicides, and till consisted of 2-3 passes per year to an average depth of 20cm. Means are represented by a red diamond (n=108 for tilled soils; n= 276 for no-till soils). Different letters represent significant ($p<0.05$) differences between Disturbance levels with “a” representing higher means and “b” representing lower means.

3.7. Correlations of soil health indicators

Pearson correlations were conducted to assess the general relationships between all soil health indicators in the 0-10 cm depth (Figure 2.6). For example, sand content was positively correlated with infiltration rate, and negatively correlated with clay content, TC, POXC, and NH_4^+-N. Conversely, clay content showed positive correlations with MBC, DOC, TN, and wet aggregate stability (WAS). All soil C cycling indicators were positively correlated with PMN except for DOC. Soil TC was positively correlated with POXC and negatively correlated with bulk density. In contrast, soil POXC showed more correlations with other soil health indicators including positive relationships with Min C, MBC, and TN. Soil POXC was negatively correlated with pH and bulk density. Soil MBC followed similar trends to POXC except that there were no significant correlations with Min C and that MBC was negatively correlated with DOC and NO_3^--N only. Soil DOC showed no other significant correlations with other dynamic soil health indicators.

Like POXC, Total N was negatively correlated with soil pH. Similar to MBC, TN was positively correlated with WAS. In contrast to TN, NO_3^--N was positively correlated with soil pH. Also, NO_3^--N and pH were positively correlated with bulk density, and PMN was negatively correlated with bulk density. Both NO_3^--N and NH_4^+-N were positively correlated with PR and soil EC was negatively correlated with EC. Soil WAS and infiltration rate were positively correlated. Soil microbial diversity biomass indicators such as total PLFA, total bacteria, actinomycetes, gram negative, gram positive, total fungi, and saprophytes were positively correlated with POXC, MBC, and TN. Soil NO_3^--N was positively correlated with total fungi, AMF, and fungi:bacteria ratio and soil PMN was positively correlated with gram positive bacteria. In contrast, NH_4^--N was negatively correlated with these soil microbial diversity biomass indicators except for saprophytes. All microbial diversity indicators from PLFA were positively correlated between each other. Pearson correlations between soil health indicators for the 0-20cm depth tended to be similar to the 0-10cm depth with the addition of greater levels of TC and POXC across the 0-20 cm dept (Appendix Figure 2.2).

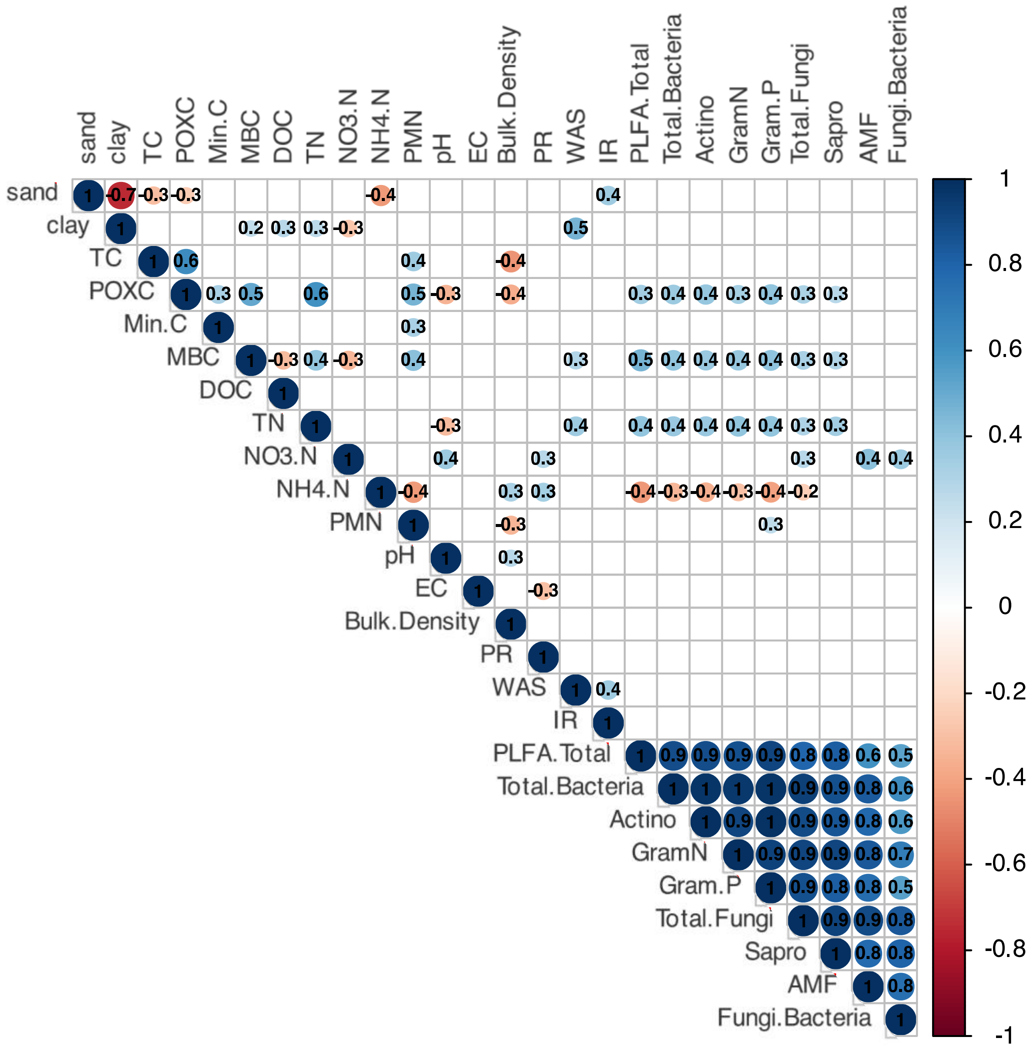

Figure 2.6. Significant ($p<0.05$) Pearson correlations among carbon, nitrogen and water cycling soil health indicators as well as soil microbial diversity biomass from PLFA analysis from 0-10 cm soil samples collected from 32 vineyards in Napa Valley (California, USA). Soil analyses averaged across field replicates (3) to match microbial biodiversity samples that were composited across field replicates (n=64). Abbreviations for soil health indicators include Permanganate oxidizable C (POXC); total C (TC); mineralizable C (Min C); microbial biomass C (MBC); dissolved organic C (DOC); total N (TN); nitrate (NO_3^--N); ammonium (NH_4^+-N); potentially mineralizable N (PMN); electrical conductivity (EC); penetration resistance (PR); wet aggregate stability (WAS); bulk density (BD); and phospholipid fatty acids (PLFA) biomass including total PLFA biomass, total bacteria, actinomycetes, gram negative (N) bacteria, gram positive (P) bacteria, total fungi, saprophytes, arbuscular mycorrhizae fungi (AMF), and fungi:bacteria ratio.

3.8. Soil health indicators reflecting grower perceptions are influenced by soil texture

We performed a principal component analysis (PCA) to assess the relevance of different soil health indicators in explaining variability in the soil samples collected across the Napa Valley (Table 2.5, Figure 2.7). The first two principal components, Dim 1 and Dim 2, explain 31% and 21.9% of the total variance, respectively (Table 2.5). Soil TC, POXC, TN, NH_4^+-N, and EC were positively correlated with the horizontal axis (Dim 1) and vertical (Dim 2) axes. Clay content and WAS were positively correlated with the horizontal axis (Dim 1) and negatively correlated with the vertical axis (Dim 2). Sand content was negatively correlated with the horizontal axis (Dim 1). Clay content had PCA loadings of 0.557 in Dim 1 and -0.702 in Dim 2, reflecting a positive correlation with TC, POXC, TN, NH_4^+-N, EC, and WAS, and a negative correlation with sand content (Table 2.5). Soil NH_4^+-N, clay, and sand content had higher PCA loadings in Dim 2 and WAS and EC had higher PCA loadings in Dim 3 (Table 2.5). Soil NH_4^+-N, WAS, and EC were in the positive direction along the first and second axes.

Table 2.5. Principal component analysis (PCA) loadings for soil health indicators that were significantly different between growers' perceptions of challenging and ideal soils for wine grape production in 32 vineyards from Napa Valley (California, USA).

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
Total C (g kg^{-1})	0.674	0.204	-0.499	-0.053	0.252
POXC (mg kg^{-1})	0.732	0.344	-0.010	-0.166	-0.138
Total N (g kg^{-1})	0.731	0.249	-0.203	-0.097	0.187
NH_4^+-N (mg kg^{-1})	0.273	0.641	0.056	0.473	-0.503
EC (μS cm^{-1})	0.361	0.098	0.632	-0.618	-0.150
WAS (%)	0.482	-0.072	0.611	0.443	0.411
clay (%)	0.557	-0.702	0.093	0.270	-0.184
sand (%)	-0.456	0.780	0.215	0.090	0.240
Eigenvalue	2.479	1.749	1.121	0.923	0.654
percentage of variance	30.982	21.863	14.011	11.538	8.174
cumulative percentage of variance	30.982	52.845	66.856	78.394	86.568

Total carbon (C); Permanganate oxidizable C (POXC); Total nitrogen (N); ammonium (NH_4^+-N); electrical conductivity (EC); wet aggregate stability (WAS)

A PERMANOVA was conducted to determine which factors in this study (e.g., Grower rating, Vineyard zone, Disturbance, and Textural class) had the most substantial influence on all soil carbon, nutrient, and water cycling indicators. This multivariate analysis tests for differences in the composition of these soil health indicators across groups by evaluating the distance between group centroids in multivariate space. While Grower ratings were found to be significant ($p < 0.05$) (Table 2.6), the poor separation between the ellipses representing ideal and challenging soils in multivariate space (Figure 2.7 -A suggests substantial overlap in the variability of soil characteristics within these ratings. Similar to grower ratings, vineyard zone levels (vine vs tractor row) showed significant effects ($p<0.04$) yet poor separation of ellipses (Figure 2.7 -B), and soil disturbance (till vs no-till) had no significant effects ($p=0.3$) (Figure 2.7-C). In contrast, textural class groups significantly influenced soil health indicators ($p<0.05$, Table 2.6), with the PCA biplot showing a more distinct separation of ellipses, particularly between coarse and fine-textured soils (Figure 2.7-D). In general, correlations among soil health indicators observed in the PCA tended to be similar to results from the Pearson correlation analysis (Figure 2.6).

Table 2.6. PERMANOVA results assessing the influence of Grower rating, Vineyard zone, Disturbance, and Textural class on soil carbon, nitrogen, and water cycling soil health indicators in 32 vineyards from Napa Valley (California, USA).

Factors	Df	Sum of squares	R2	F	Pr(>F)	Sig.
Grower rating	1	0.13	0.01	5.91	0.011	*
Vineyard zone	1	0.30	0.03	13.39	0.001	***
Disturbance	1	0.02	0.00	0.91	0.375	NS
Textural class	3	0.99	0.10	14.69	0.001	***
Residual	377	8.48	0.85			
Total	383	9.92	1			

Significance (sig). codes: ‘***’ = p< 0.001; ‘**’= p< 0.01 ;‘*’=P< 0.05; “NS” = p>0.05

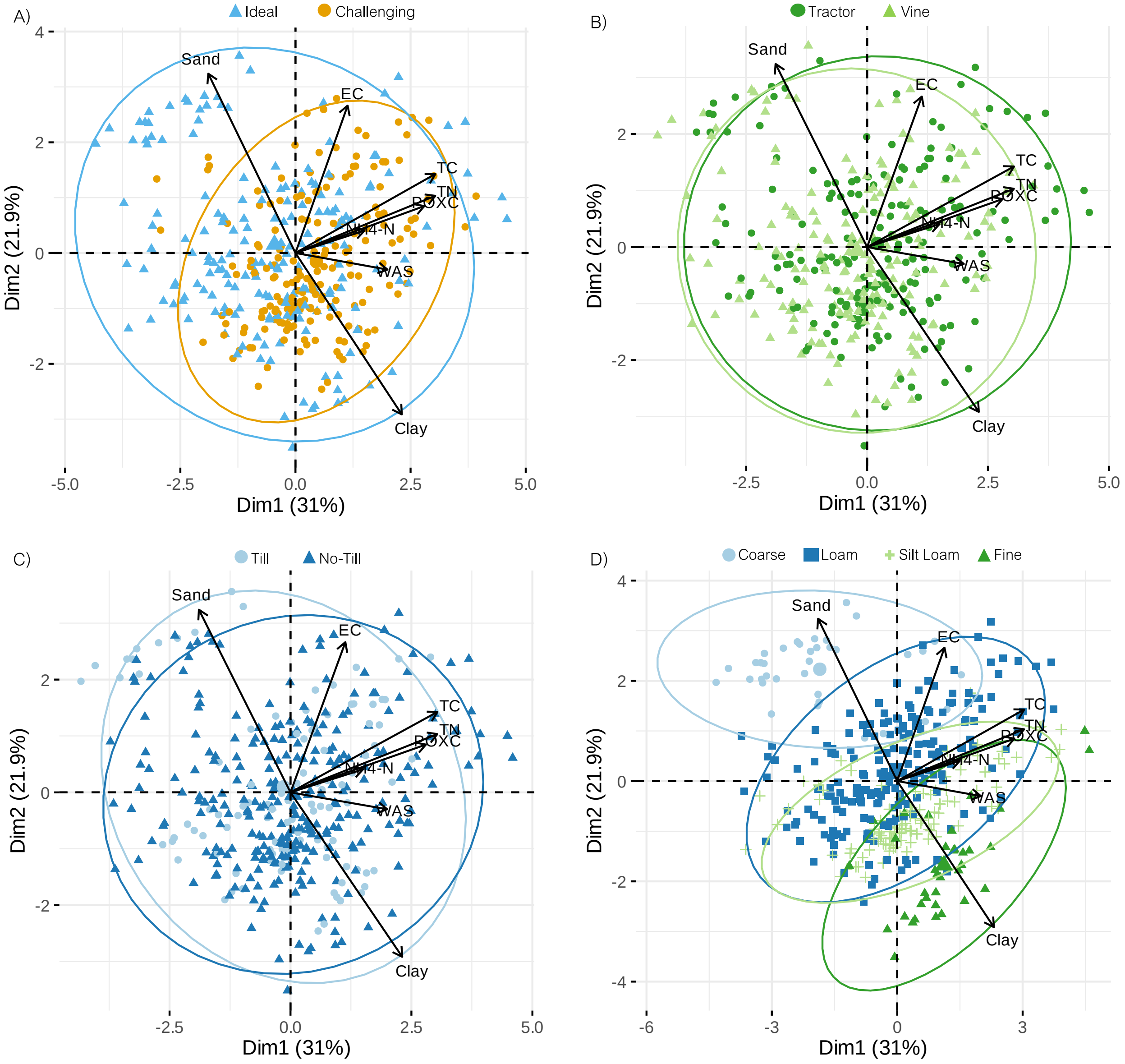

Figure 2.7. Principal component analysis biplots of the soil health indicators that were significantly influenced by grower ratings from the ANOVA color coded by contrasting grower ratings (A) including challenging (orange circles) and ideal (blue triangles) based on their perceptions for achieving viticultural goals; vineyard zones (B) such as tractor row (light green circles) and vine row (light blue triangles); soil disturbance (C) including no-till (dark blue triangles) and disk (light blue circles); and soil textural classes groups (D) including coarse (light blue circles), loam (dark blue triangles), silt loam (light green squares), and fine (dark green plus sign).

4. Discussion

4.1. Soil texture influenced most soil health functions

Soil health indicators were significantly influenced by soil texture, particularly those that represent carbon cycling. Following our hypothesis, finer soil textural classes (silt loam and/or fine) in the vineyards generally corresponded to higher levels of carbon nutrient and water cycling indicators such as TC, POXC, MBC, DOC, NO_3^--N, and WAS. However, some of these indicators, such as DOC and NO_3^--N, which were higher in the silt loam and/or fine soils compared to coarse soils, were not significantly different from loam soils. These findings generally followed similar trends reported by Amsili et al. (2021). Although finer textured soils such as fine and silt loam tended to have higher levels of some C and N indicators, soil compaction was not highest, and infiltration rate was not lowest in the fine soils, as many growers suggested in our recent interviews study (Gonzalez-Maldonado et al., 2024). Also, the magnitude of differences among the texture groups are relatively small, and no consistent patterns of soil health indicators were found among texture groups. These results might be due to the lack of representation of extreme soil textures such as those containing greater than 50% clay and 70% sand contents. Also, these results suggest that these water cycling soil health indicators might be more influenced by other factors such as from soil management.

Soil carbon cycling indicators such as TC, POXC, and MBC were overall higher in soils with finer soil textures like the silt loam and/or fine texture. Soils with greater fine silt and clay content can show greater concentration of C pools and have higher aggregate stability due to their higher surface area, allowing for increased binding capacity and formation of organo-mineral complexes (Feng et al., 2014; Matus, 2021; Schweizer et al., 2019). Other studies have suggested that soil textural classes act as a driver of soil health and function across the landscape (Agyei et al., 2024; Amsili et al., 2021). For example, a study by Amsili et al., (2021) that assessed soil health indicators across annual cropping systems in New York also found that carbon pools such as SOM, POXC, and respiration were higher in fine and/or silt loam soils and that these decreased as soil texture grew coarser. In contrast, Agyei et al. (2023) found poor effects of soil textural classes of soil C, nitrogen and biological soil health indicators across the landscape in temperate grain production systems.

A potential reason why we didn’t see strong differences between soil texture groups might be because not all textureswere represented in our landscape study, especially coarse and fine textures. Even though the high number of vineyards sampled provides uniqueness and robustness for this study, only 36 soil samples represented coarse and fine texture levels each. From these, clay content in the fine texture group did not exceed 39%, and clay contents were similar to those in the silt loam group. Therefore, future research that aims to understand the impact of texture on soil health indicators should include soils that have higher clay content levels. Despite the inconsistent trends of soil health indicators among soil textural classes, using these classes can inform growers’ expectations for baseline values soil health indicators.

4.2. Soil properties linked to viticultural outcomes are related to soil carbon and water cycling indicators

Our study provides insights into how soil health indicators representing soil carbon cycling, nutrient cycling, water cycling, and microbial diversity vary across grower perceptions (ideal vs challenging soils for achieving viticultural goals) in vineyards across the Napa Valley landscape. The high number of sampled vineyards in our study is unique. Only a few studies have evaluated growers’ perceptions of an ideal soil vs a challenging soil for achieving their goals of interest (Mann et al., 2019; O’Neill et al., 2021). Significant differences between ‘good’ and ‘poor’ soils for grain and vegetable production in a temperate region were found for nutrient cycling indicators (Mann et al., 2019), where pH, boron, calcium, and magnesium levels were higher in the ‘good’ soils. Mann et al., (2019) suggest that increasing soil pH and nutrient content in these grain and vegetable production systems are important for growers to achieve yield maximization goals. In the study by O'Neill et al., (2023), scores from a soil health assessment using carbon, biological, and physical parameters, were higher in soils rated as "best" for grain production. These results highlight soil properties that are highly important and relevant for growers to achieve their outcomes of interest which are more focused on maximizing nutrient cycling and yields. The results from our soil health assessment by grower ratings of an ideal and a challenging soil for wine grape production, where goals are not necessarily to increase yields but to stay within a range of grape yields and enhance grape quality attributes, suggests that soil texture is an important soil property for site selection to support vine balance.

Higher clay content, as observed in the challenging soils, led to greater levels of MBC, DOC, TN, EC, and wet aggregate stability in the topsoil (0-10cm), as well as greater levels of TC and POXC across the 0-20 cm depth, as seen from the Pearson correlation results in this study. The reason for this is that clay has increased capacity to retain organic matter and nutrients compared to other soil particles. The high surface area of clay particles allows for enhanced aggregation and protection of soil organic matter through organo-mineral bonds derived from clay's high cation exchange capacity (CEC). Moreover, clay particles enhance soil water-holding capacity due to the higher proportion of small pores, which can reduce the rate of microbial decomposition of soil organic matter (SOM) and increase SOM content under saturated conditions (Keiluweit et al., 2017; Sarkar et al., 2018; Zaffar & Lu, 2015). Similar to other soil health assessments in Midwestern and Northeastern regions of the USA, our study has observed higher levels of carbon and nitrogen pools in soils with higher clay content (Amsili et al., 2021; Geisseler et al., 2024).

Despite growers' preference for avoiding high soil nitrogen levels that could disbalance vine vigor, higher plant-available NH_4^+-N was found in the ideal soils (Gonzalez-Maldonado et al. 2024).

However, nitrogen levels from both challenging and ideal soils, including NO_3^--N, NH_4^+-N, and TN, were overall comparable to vineyards in similar Mediterranean climates (Agnelli et al., 2014; Lazcano et al., 2022). For comparison, the mean mineral-N values from this study are lower than annual and perennial cropping systems in California that require high inorganic N applications, such as tomatoes and woody perennials like almonds (Jahanzad et al., 2020; Lazcano et al., 2015; Nichols et al., 2024). For example, soil nitrate-N content in tomatoes, which is another important crop in California, ranged from approximately 15 to 50 mg NO_3^--N kg^{-1} in 0-20 cm depth soils before planting (Lazcano et al., 2015). Another study in California processing tomatoes reported NH_4^+-N mean values ranging from 0.2 to 20 mg/kg and 2.5 to 35 mg NO_3^--N kg soil. Another common way that soil N content is reported in annual crops like tomatoes is as mineral-N which is the sum of soil NH_4^+-N and NO_3^--N. For our study, mean mineral-N content for the ideal and challenging soils were 5.3 and 5.0 mg kg^{-1} respectively, which are lower than reported mineral N mean values in tomato soils of 15 mg kg^{-1} in the top 30 cm of soil in another recent study (Geisseler et al., 2020). For almonds, soil plant available N content has been shown to range from 12 to 20 mg NO_3^--N kg^{-1} and 1.3 to 22.6 mg NH_4^+ kg^{-1} in California orchards (Jahanzad et al., 2020). In the study conducted by Nichols et al. (2024) nitrogen in almond orchards ranged from approximately 0-35 mg NO_3^--N kg^{-1}, and 0-40 mg NH_4^+-N kg^{-1} during the early season (March-May). In comparison, the nitrogen mean values for the challenging and ideal soils were 1.4 and 1.5 mg NO_3^--N kg^{-1}, respectively, and 3.5 and 3.9 mg NH_4-N kg^{-1}, respectively. These results show that soil nitrogen levels in the vineyards sampled in this study are relatively low compared to other agroecosystems in the region. Based on this single sampling, results suggest that perceived difficulties in vine vigor control in the challenging soils might not be due to higher plant-available nitrogen content but total soil N (despite a small difference of 15%). Although plant available nitrogen, such as soil NH_4^+-N and NO_3^--N in the soil surface has been shown to impact grapevines (King & Berry, 2005; Steenwerth &

Belina, 2010) these N pools are highly dynamic and therefore can drastically change in the short term (hours to days to weeks) (Burger & Jackson, 2003). Also, vine roots have been documented to concentrate deeper in the soil profile and not in the soil surface (Smart et al., 2006), potentially making vine NH_4^+-N and NO_3^--N uptake dependent on N movement deeper through the soil profile.

In this study, we generally observed positive correlations between soil C and N pools and soil WAS. Soil C and N content have been shown to influence aggregate stability (Bird et al., 2002). Wet aggregate stability (WAS) is a measurement of how well soil can resist water impacts such as from rainfall or irrigation. Contrary to our hypothesis, WAS values were higher (a 6% relative difference) in the challenging soils. These results of WAS being higher in the challenging soils and lower in the ideal soils can be a reflection of growers' association of clay content with water cycling (i.e. infiltration and retention) in these irrigated vineyards as highlighted by growers in Gonzalez-Maldonado et al., (2024) and not necessarily because higher aggregation leads to undesired soil conditions. In Gonzalez-Maldonado et al., (2024) growers expressed a preference for coarse soil texture due to associations of sand content with higher infiltration in irrigated vineyards. This is supported by research stating that sand particles increase spaces between pores that can enhance soil water infiltration (J.-L. Yang & Zhang, 2011). Sands have lower capacity to form aggregates due to their low surface area compared to clays that have high surface area and negative charges that enhance their capacity to bind to particles and form aggregates (Amézketa, 1999). Despite the differences, WAS values in both ideal and challenging soils in this study fit the description of medium physical quality (Mukherjee & Lal, 2014). Contrary to our hypotheses, soil compaction, measured using PR and bulk density, was not higher in the challenging soils. This might be due to the shallow sampling of this study, since soil compaction layers could appear in deeper soil horizons. Instead, most vineyards in this study, including ideal and challenging soils, had high compaction levels (>2068 kPa) that can hinder root growth, based on PR readings (Duiker et al, 2002). Also contrary to our hypothesis, similar infiltration rates were documented in ideal and challenging. This may be due to the use of mini disk infiltrometers, which are less sensitive compared to other methods. Specifically, mini disk infiltrometers measure unsaturated hydraulic conductivity and mainly reflect infiltration through the soil micro-pores (Meter Group, inc., 2012; Baker, 1979) . Also, the high variability of infiltration rates can be attribute to other covariates such as gravel, rocks or cobbles content and variability in management practices that drive microporosity and were not evaluated in this study (Biddoccu et al., 2017; Ma et al., 2024). These management practices include cover crop types and duration of establishment and duration of no-tillage that in addition to water infiltration, it can influence soil C content and aggregate stability (Belmonte et al., 2018). Overall, infiltration rate values in this study indicate moderate water infiltration rate class (Hillel, 1982). Finally, contrary to our hypothesis, there were differences for soil microbial biodiversity between soil textures. This might be due to the relatively small differences in soil C and N levels between the challenging and ideal soils. As revealed by Burns et al. (2016), who showed that vineyard soil management practices in Napa drive soil microbial composition, soil microbial properties might be more responsive to diverse soil management practices that were not controlled for in this study such as the ones discussed in section 4.8.

4.3. Soil health indicators are responsive to management

We hypothesized that soil health indicators would be responsive to management practices that disturb the soil and could reduce soil health. Particularly, we expected soil carbon cycling, nutrient cycling, and microbial diversity indicators as well as WAS and infiltration to be negatively affected by tillage. We observed that soil carbon and nitrogen indicators were the most impacted by soil disturbance, as suggested by higher values of TC, Min C, and NH_4^+-N in the no-till soils and higher DOC mean values in the tilled soils. Similar findings of higher C pools in vineyards or semi-arid soils under no-tillage have been reported in other studies (Belmonte et al., 2016; Carbonell-

Bojollo et al., 2015). A potential reason why TC, Min C and NH_4^+-N concentrations were higher in the no-till soils is the protection of soil with cover crops or resident vegetation in the tractor rows in all of the no-till vineyards sampled. For example, cover crop roots and root exudation can contribute organic matter through root exudation and root biomass (Puget & Drinkwater, 2001).

In no-till soils, lack of both physical disturbance of soil and incorporation of the cover crops facilitates physical protection and stabilization of SOM (Lazcano et al., 2022; Peregrina et al., 2010). An increase in total and labile C pools such as Min C indicates higher accumulation of soil organic matter that could enhance mineralization and stabilization processes of soil C (Hurisso et al., 2016). The accumulation of soil organic matter can incentivize microbial activity, and the lack of soil disturbance can facilitate the carbon stabilization processes, where some of its source is the microbial biomass, in the form of aggregates (Bhattacharyya et al., 2022). However, stratification of soil organic matter occurs in no-till soils, where organic C accumulates in the upper depths and decreases with increasing depth (Lazcano et al., 2022). Nonetheless, an increase in both C mineralization and its stabilization in no-till soils supports outcomes of interest described by growers, such as biologically based nutrient cycling that could reduce nutrient inputs, enhance soil aggregation and structure which can protect soil erodibility potential and enhance infiltration and water retention, and benefit soil microbial diversity while reducing C loss (Paul, 2016; Xiao et al., 2018).

In general, growers tilled soils to incorporate cover crops, improve worker access to the vineyards, avoid frost damage and pest issues, and reduce nutrient and water competition with vines (pers. comm., various growers from Gonzalez-Maldonado et al. 2024). Growers' rationale of using tillage was to increase nutrient release from SOM mineralization and to reduce water competition from cover crops in order to improve vine vigor (Gonzalez-Maldonado et al., 2024). However, this rationale was not supported by the results of this study. For example, tilled soils had overall lower mean values of TC, Min C, and NH_4^+-N, and other indicators like MBC, TN, NO_3^--N, and microbial diversity indicators (except for total PLFA and saprophytes biomass) tended to be lower in tilled soils. Also, tilled soils tended to have higher levels of soil compaction (bulk density and penetration resistance) compared to no-till soils. Despite no-differences being recorded for soil biological properties between till and no-till soils, soil NO_3^--N, TN, MBC, and the biomass of soil microorganisms such as total bacteria, gram negative and positive bacteria, actinomycetes, total fungi, and AMF tended to be higher in no-till soils in this study. Higher NH_4^+-N and trends of higher NO_3^--N in the no-till soils opposed growers' perspective that tillage increases soil nutrient availability. This could be due to grower perceptions of tillage being more related to short-term effects of this management practice. The soils in this study were sampled prior to tillage events and represented the effects of tillage after approximately 8-10 months. Therefore, it was difficult to address tillage intensity and frequency in this landscape-level study. While tillage can temporally benefit nutrient and water availability, it can have detrimental long-term consequences in soil health and wine grape production by reducing soil organic matter and microbial biomass (Belmonte et al., 2018). In contrast, reducing soil disturbance with no-till can protect organic matter and enhance soil carbon cycling, nitrogen cycling, and soil microbial diversity as seen in the trends of this and other studies (Bansal et al., 2024; Belmonte et al., 2018; Bonifacio et al., 2024). Overall, our results suggest that that no-till, when combined with cover crops or resident vegetation, can generally enhance soil labile and total carbon content and plant-available nitrogen in the form of ammonium in the top 20 cm depth across the Napa Valley landscape.

4.4. Vineyard zone: where we sample matters

Higher levels of indicators for soil carbon, nutrient, and water cycling, and soil microbial diversity biomass in the tractor row likely resulted from soil protection from the use of cover crops or resident vegetation (see section 4.2, for Discussion). Following local regulations, all vineyards in this study maintained cover crops or resident vegetation in the tractor row from late fall (after harvest) to early spring (until budbreak). The growers that practiced no-till terminated their cover crops mainly by mowing (one grower by sheep grazing). In contrast, the vine rows remained bare through cultivation or herbicide use and received irrigation and inorganic fertilizer through drip irrigation. Despite receiving irrigation and fertigation, vine rows had consistently lower levels of soil health as shown by lower values of most soil health indicators, including carbon, nitrogen, and microbial diversity indicators. Our results suggest that keeping soils covered with plants such as cover crops or resident vegetation is an effective practice for enhancing C, nutrient and water cycling functions that can support goals pertaining to soil health in the tractor row. Similar findings in which cover crops enhance soil health indicators have been documented in other vineyard studies (Belmonte et al., 2018; K. Steenwerth & Belina, 2008b, 2008a; Yu et al., 2019; Z. Yu et al., 2017). These results suggest that maintaining soils protected with plant cover could potentially support carbon sequestration goals in the vineyard tractor rows. Extending soil protection with cover crops or resident vegetation in the vine rows could potentially enhance soil health while maintaining grape quality and viticultural goals as previously observed by Guerra & Steenwerth (2012). The benefits of under-vine cover crops have been highlighted by several studies in irrigated vineyards, including enhanced soil carbon, aggregation, nutrient retention, and microbial biomass and activity and reduced nitrous oxide emissions (Abad et al., 2023; Marks et al., 2022; K. L. Steenwerth & Belina, 2010). Also, our results suggest that soil sampling only in the cover-crop covered alleys might overestimate the levels of soil health across the entire vineyard. Therefore, soil sampling must be performed separately by vineyard zones, vine and tractor rows, to support better soil management decisions (Yu et al. 2019; Belmonte et al. 2018; Yu et al. 2017).

4.5. Practical applications

Overall, the results supported our hypothesis that the differences between growers' perceptions of ideal and challenging soils for wine grape production would be primarily reflected in soil physical properties indicative of water cycling functions. Some of these soil properties were soil texture (sand, silt and clay content) and WAS, and these can have impacts on soil water retention and infiltration. The reason for these differences in physical properties like texture and WAS is that soil water management is highly important for wine grape growers to control vine vigor and achieving high grape quality especially in these irrigated vineyards. It is possible that the access to water irrigation for the participating growers drove a focus on soil drainage instead of water retention properties. Despite this, similar infiltration rate, bulk density, and penetration resistance were found between challenging and ideal soils. Contrary to our hypothesis, lower wet aggregate stability, TC, POXC, TN, and EC in the ideal soils might be a result of these soils containing lower clay and silt content and higher sand content. These results may seem counterintuitive, as higher organic matter, TN, and aggregate stability are typically associated with healthier soils in other cropping systems like grain and vegetable production. However, this is not necessarily detrimental to viticulture goals. Instead, these results highlight the potential influence of soil texture on these dynamic properties and the uniqueness of soil conditions needed for red wine grape production in irrigated mediterranean climate like Napa Valley. It's also possible that soil texture may be overemphasized at the expense of dynamic soil properties that can be managed and changed to enhance soil health functions desired by growers. For example, some of the soil functions of interest wine grape growers discussed in Gonzalez-Maldonado et al., (2024) included improving soil structure through enhanced soil aggregation and infiltration, enhancing (not excessively) water holding capacity and nutrient cycling to reduce irrigation and nutrient input needs, and protecting soil organic matter to promote carbon sequestration and reducing soil erosion. This suggests that growers' emphasis on static properties like texture may overlook the opportunities for improving dynamic soil properties—such as organic matter content, compaction, and infiltration—that can be modified to meet their goals.

Overall, the inclusion of growers in the generation of scientific knowledge allows a deeper, context-specific understanding of current perceptions, knowledge gaps, and needs in a specific cropping system, in this case, a specialty crop like wine grapes. In this study, we identified soil properties that appear more relevant for wine grape growers to achieve production goals such as vigor control for high grape quality. These properties included static soil properties like texture, and dynamic soil properties related to water cycling, soil protection, and nutrient cycling such as WAS, TC, POXC, TN, NH_4^+-N, and EC.

Our findings, alongside those of studies like Mann et al. (2019) and O'Neill et al. (2023), suggest that integrating growers into soil health assessments not only provides insight into their current perceptions but also helps identify areas where management practices could shift focus. For example, the integration of growers in soil health assessments was documented in Mann et al. (2019) where soil chemical properties were the most reflective of growers' ratings of "good" and "poor" soils and are important for achieving vegetable and grain production goals in Canada. Similarly, O'Neill et al. (2023) found that soil carbon, biological and physical properties were the most reflective of growers' "best" and "worst" soils for achieving grain production goals in Michigan (USA). These studies and our results suggest that integrating growers in soil health assessment can not only more effectively expand on current knowledge, examine perceptions, and meet growers' needs to build soil health, but also reflect the importance of soil health testing to guide soil management decisions across diverse agroecosystems.

4.6. Soil health testing recommendations for vineyards

The criteria recommended to select soil health indicators include selecting indicators that are related to outcomes of interest to growers, selecting indicators that are sensitive to soil management practices, and select indicators that reflect dynamic soil properties but that can be influenced by static soil properties (Bagnall et al., 2023). Our results partially diverge from minimum soil health indicators in vineyards that have been recommended by the Soil Health Institute when linking soil health assessment with growers outcomes of interest. Some of these indicators were total C, POXC, and WAS what had lower levels in soils rated as ideal for achieving red wine grape production in irrigated vineyards (Bagnall et al., 2023). These results suggests that other soil health indicators should be explored to find a more relevant way to assess soil health in vineyards. On the other side, these indicators and mineralizable C (Min C) were sensitive to management practices and varied across soil texture. For example, TC and Min C were sensitive to tillage which was the management practice evaluated in this study. In addition, TC, Min C, and WAS, were different between vineyard zones (vine row vs tractor row) that were under different management practices (irrigated and continuously bare vine rows vs. partially protected tractor rows with cover crops or resident vegetation). Although this study did not assess water holding capacity this indicator could be highly informative as it was strongly highlighted by wine grape growers in the interviews from our related study (Gonzalez-Maldonado et al., 2024).

Also, the findings of this study show that the magnitude of soil health indicators vary by vineyard zone, with values being generally higher in the tractor row. For example, soil carbon and nitrogen cycling indicators were generally higher in the tractor row soils that were protected with cover crops or resident vegetation for at least half of the year (late fall and winter). Soil testing only in the tractor rows might be overestimating soil health indicators results compared to the vine row. Therefore, soil sampling for soil health testing in vineyards should be conducted in both tractor and vine rows. In addition, recommendations for soil health testing generally suggest a depth of 0-15 or 0-20 cm especially since this is the depth to where soil management practices generally cover in soils (Moebius-Clune et al., 2016). While this recommendation seems appropriate for wine grape production systems, also including deeper soil depths could enhance the understanding of the soil health assessments in vineyards since the grapevine roots can reach depths of approximately 1-2 meters (Smart et al., 2006).

5. Conclusions

This is the first study to include grower participation to assess soil health in vineyards and links its relevance to growers' outcomes of interest. This study provided helpful information for wine grape growers and stakeholders including ranges of soil health indicators by soil textural class, variability within vineyard zones, and management (till vs no-till). Our results suggests that soil texture is an important indicator for wine grape production in relation to moisture management for vigor control in vineyards. In addition to texture, several soil health indicators such as TC, POXC, TN, NH_4^+-N, EC, and WAS were sensitive or responsive to growers' perceptions of ideal and challenging soils, suggesting that including for growers in soil health research is important for identifying relevant indicators and soil functions to outcomes of interest. Also, many of these indicators were also significantly correlated with soil texture, suggesting that benchmarks or expectations for ranges of soil health indicators should be delineated by soil texture class as well as growers' priorities. Other helpful findings include that soil health indicators varied across vineyard zones and were sensitive to soil management practices. For instance, soil health was generally higher in the tractor rows with vegetation cover compared to the vine rows that are typically left bare, suggesting that soil sampling should occur in both zones for more accurate interpretation of soil health results and management practice decisions. Most importantly, our study advanced the understanding of soil health by showing that soil health indicators can be aligned with grower-desired outcomes (e.g., vine vigor control and grape quality) while being sensitive to management practices and soil heterogeneity in semi-arid Mediterranean vineyards. This highlights the importance of tailoring soil health assessments to specific crops and landscapes to better inform management decisions. Overall, our study demonstrated the value of soil health assessment in collaboration with growers and provided a foundation for defining soil health in irrigated wine grape systems, offering ranges of values of soil health indicators to serve as guide for soil management decisions. Future research should explore the direct links between soil management, soil health indicators, vine vigor, and grape quality to further refine soil health indicator selection and benchmarks for this unique specialty crop.

6. Appendix

Table 2.1A. Napa Valley Rainfall from 2012 to 2021.

Year	Cumulative rainfall (mm)	Min temperature (°C)	Average temperature (°C)	Max temperature (°C)
2012	1037.1	6.0	13.8	22.7
2013	152.4	5.7	14.2	23.8
2014	746.8	7.7	15.3	24.0
2015	316.0	8.8	16.6	25.1
2016	613.2	7.8	13.8	20.2
2017	927.1	7.3	14.9	23.8
2018	268.1	6.5	14.2	23.4
2019	1010.0	7.3	14.5	22.8
2020	222.2	7.0	14.9	24.4
2021	688.3	6.3	14.2	23.1
10-year average	598.1	7.0	14.6	23.3

Table 2.2A. Information for soils rated as Ideal by participating growers.

Grower ID	Site ID	Subgroup	AVA	Grape variety	slope (%)	Elevation (m)	Disturbance	Reasons for ideal soil rating
G4	1	Xerofluvents	St. Helena	Cabernet Sauvignon	1	81	Disk	Good water infiltration, ideal vigor control
G17	4	Haploxerolls	Oakville	Cabernet Sauvignon	4	65	Disk	Good water infiltration
G5	6	Dystroxerepts	Fountaingrove	Cabernet Sauvignon	23	525	No-Till	Good water infiltration, good nutrient levels
G8	8	Vitrixerands	Napa Valley	Cabernet Sauvignon	18	31	No-Till	Good water infiltration
G12	10	Xerofluvents	Rutherford	Cabernet Sauvignon	1	50	No-Till	Good water infiltration, good nutrient levels, no pathogens
G3	12	Haploxerolls	Calistoga	Cabernet Sauvignon	36	187	Disk	More soil uniformity supports viticultural goals
G14	14	Haploxerults	Oak Knoll District	Merlot	1	35	No-Till	Good water infiltration, good nutrient levels
G11	15	Argixerolls	Oak Knoll District	Cabernet Sauvignon	40	60	No-Till	High grape quality production
G2	18	Endoaquerts	Oakville	Cabernet Sauvignon	1	43	No-Till	Good water holding capacity, good nutrient levels
G7	19	Haploxerolls	Calistoga	Cabernet Sauvignon	1	141	No-Till	Good water holding capacity, good nutrient levels
G9	22	Haploxerults	Napa Valley	Cabernet Sauvignon	6	32	Disk	Good water infiltration, rocky, ideal vigor control
G13	24	Vitrixerands	Diamond Mt. District	Cabernet Sauvignon	18	164	No-Till	Good water infiltration, ideal vigor control
G10	26	Haploxeralfs	Mt. Veeder	Cabernet franc	23	108	No-Till	Good vine vigor
G1	28	Haploxeralfs	Oak Knoll District	Cabernet Sauvignon	1	32	Disk	Favorable texture, good infiltration, good vigor control

G16	30	Haploxerolls	Atlas Peak	Cabernet Sauvignon	53	416	No-Till	Good water infiltration, ideal vigor control
G16	32	Haploxerolls	Mt. Veeder	Cabernet Sauvignon	53	414	No-Till	Good vigor control

Grower IDs correspond to participating growers in our previous study Gonzalez-Maldonado et al., (2014); Sub group, soil series, and representative slope information was collected from the Web Soil survey (USDA, NRCS); American viticultural area (AVA) and reason for rating was collected from conversations with participating growers, soil textural class was collected from particle size distribution analysis; elevation was collected using Google Earth (Google inc.).

Table 2.3A. Information for soils rated as Challenging by participating growers.

Grower ID	Site ID	Subgroup	AVA	Grape variety	slope (%)	Elevation (m)	Disturbance	Reason Challenging soil rating
G4	2	Haploxerults	Los Carneros	Pinot noir	6	14	Disk	Poor water infiltration
G17	3	Haploxeralfs	Oakville	Cabernet Sauvignon	1	40	Disk	Poor water infiltration, harder to manage vigor
G5	5	Dystroxerepts	Fountaingrove	Cabernet Sauvignon	23	549	Disk	Poor water infiltration, low nutrients, high clay
G8	7	Haploxeralfs	Chiles Valley District	Cabernet Sauvignon	4	267	No-Till	Poor water infiltration, high clay, serpentine soil
G12	9	Xerofluvents	Rutherford	Cabernet Sauvignon	1	50	No-Till	High vine vigor, high soil Mg, low soil C, higher incidence of pathogens
G3	11	Haploxerolls	Calistoga	Cabernet Sauvignon	53	180	Disk	High heterogeneity in soil
G14	13	Haploxerults	Oak Knoll District	Merlot	1	35	No-Till	Poor water infiltration, compaction
G11	16	Argixerolls	Oak Knoll District	Cabernet Sauvignon	33	61	No-Till	Soil compaction
G2	17	Haploxeralfs	Chiles Valley District	Cabernet Sauvignon	4	203	No-Till	Low water holding capacity and nutrients, poor root growth, weak canopies, compaction
G7	20	Haploxeralfs	Mt. Veeder	Cabernet Sauvignon	23	246	No-Till	Low water holding capacity
G9	21	Haplohumults	Howell Mountain District	Cabernet Sauvignon	9	532	Disk	Poor water infiltration, harder to manage vigor
G13	23	Vitrixerands	Diamond Mountain District	Cabernet Sauvignon	18	164	No-Till	Heavy soil texture, harder to manage high vigor
G10	25	Haploxerults	Los Carneros	Pinot blanc	5	21	No-Till	High shrink clay, salt intrusion, compaction layer
G1	27	Haploxeralfs	Oak Knoll District	Cabernet Sauvignon	1	32	Disk	Heavy soil texture, harder to manage vigor
G16	29	Argixerolls	Calistoga	Sauvignon blanc	0	125	Disk	Hard to control high vigor, soil too fertile and high OM, poor drainage
G16	31	Endoaquerts	Yountville	Cabernet Sauvignon	1	27	Disk	Hard to control high vigor, soil too fertile and high OM, poor drainage

Grower IDs correspond to participating growers in our previous study Gonzalez-Maldonado et al., (2014); Sub group, soil series, and representative slope information was collected from the Web Soil survey (USDA, NRCS); American viticultural area (AVA) and reason for rating was collected from conversations with participating growers, soil textural class was collected from particle size distribution analysis; elevation was collected using Google Earth (Google inc.).

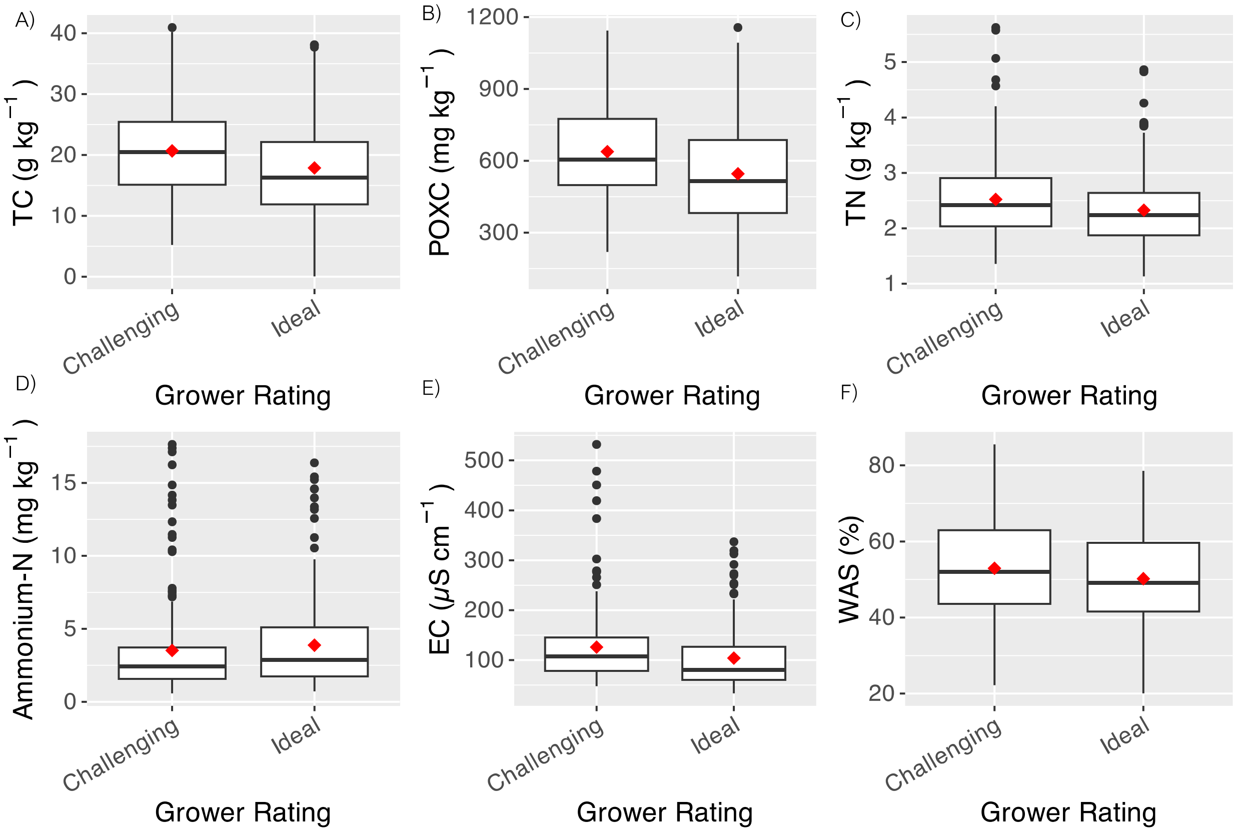

Figure 2.1A. Boxplots of soil health indicators influenced by grower ratings (challenging and ideal soils) across 32 vineyards of Napa Valley (California, USA). The soil health indicators are total carbon (TC) (A), permanganate oxidizable carbon (POXC) (B), total nitrogen (TN) (C), ammonium nitrogen (NH_4^+-N) (D), electrical conductivity (EC) (E), and wet aggregate stability (WAS) (F) across all sampled fields. Red diamonds represent the mean of each indicator by grower rating (n= 192 for challenging soils, n=192 for ideal soils).

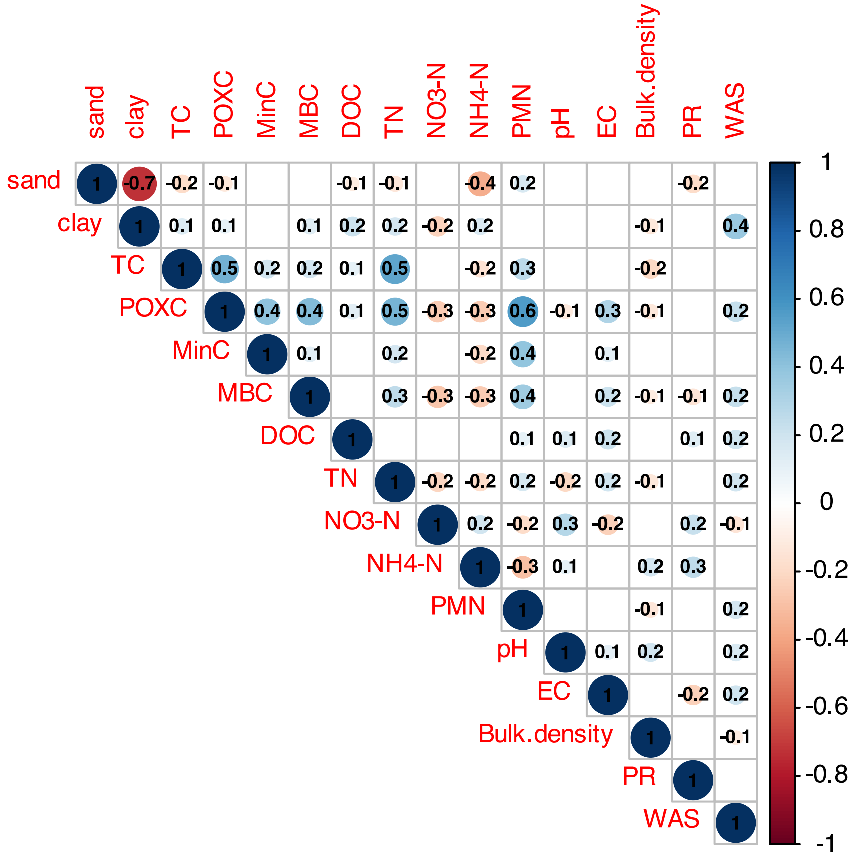

Figure 2.2A. Significant Pearson correlations of 0-20 cm depth carbon, nitrogen and water cycling soil health indicators averaged across field replicates. Abbreviations for soil health indicators include Permanganate oxidizable C (POXC); total C (TC); mineralizable C (Min C); microbial biomass C (MBC); dissolved organic C (DOC); total N (TN); nitrate (NO_3^--N); ammonium (NH_4^+-N); potentially mineralizable N (PMN); electrical conductivity (EC); penetration resistance (PR); wet aggregate stability (WAS) and bulk density (BD).

Table 2.4A. Phospholipid fatty acids biomarkers for soil microorganisms (WARD Laboratories, Inc.)

Group ID	PLFA Biomarkers
Gram – Others	10:0 2OH; 10:0 3OH; 11:0 2OH; 11:0 3OH; 11:0 iso 3OH; 12:0 2OH; 12:0 3OH; 14:0 2OH; 14:0 3OH; 14:0 iso 3OH; 16:1 w7c; 16:1 w7t; 16:1 w9c; 16:0 2OH; 16:0 3OH; 16:1 2OH; 17:0 cyclo; 17:0 CYCLO; 18:1 ω5c; 18:1 ω7c; 19:0 cyclo ω9; 19:0 cyclo ω9c; 19:0 cyclo ω6
Gram + Others	14:0 iso; 15:00; 15:0 iso; 15:0 anteiso; 16:0 iso; 17:00; 17:0 iso; 17:0 anteiso; 19:0 iso; 19:0 anteiso

Rhizobia	13:0 iso 3OH; 15:0 2OH; 19:0 cyclo ω8c; 19:0 cyclo c11-12; 20:2 ω6c; 12:0 iso 3OH; 15:0 iso 3OH; 17:0 iso 3OH
Arbuscular Mycorrhizal	16:1 w5c; 16:1 w11c; 20:1 ω9c; 22:1 ω9c
Actinomycetes	16:0 10-methyl; 17:0 10-methyl; 18:0 10-methyl, TBSA; 18:0 10-methyl
Saprophytes	18:1 ω9c; 18:2 ω6,9c; 18:2 ω6c; 18:3 ω3c; 18:3 ω6c; 18:3 ω6c (6,9,12); 20:5 ω3c
Protozoa	20:2 ω6,9c; 20:2 ω3c; 20:3 ω3c; 20:3 ω6c; 20:4 ω6,9,12,15c; 20:4 ω6c
Gram – Others / Rhizobia	13:0 iso 3OH
Gram – Others / Gram + Others	19:0 iso; 19:0 anteiso
Protozoa / Rhizobia	20:2 ω6c

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Chapter 3: Variability of soil microbial diversity and its relationship with soil health across Mediterranean-climate vineyards

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Abstract

Although soil microorganisms play essential roles in soil biogeochemical processes, we still lack information on the link between microbial diversity with soil health in semi-arid irrigated vineyards. This study evaluated the variability of soil α- and β -diversity across 32 vineyards in the Napa Valley wine growing region and their relationship with soil health indicators. Soil prokaryotic (16S rDNA) and fungal (ITS) communities were assessed using high throughput sequencing. Soil α- and β-diversity were assessed using Shannon Diversity Index and Bray-Curtis Dissimilarity, respectively. The variability of soil microbial diversity was evaluated across soil Depth (0-10, 10-20 cm), Vineyard Zone (vine vs tractor row), Disturbance (till vs no-till), Texture (coarse, loam, silt loam, fine), and Grower Perceptions (challenging vs ideal soil for vigor control). The soil health indicators evaluated were representative of soil C, nutrient, and water cycling functions such as total C and N, permanganate oxidizable C (POXC), mineralizable C (Min C), dissolved organic C (DOC), microbial biomass C (MBC), plant-available N (NO_3^--N and NH_4^+-N), potentially mineralizable N (PMN), pH, EC, particle size distribution, bulk density, penetration resistance (PR), infiltration rate, and wet aggregate stability (WAS). The relationship between soil microbial α- and β- diversity was evaluated by doing Spearman correlations and PCoA ordination. Results showed that soil texture and disturbance influenced microbial β- diversity, especially fungal communities. In contrast, soil depth and disturbance influenced prokaryotic α- diversity and disturbance, and soil texture influenced fungal α- diversity. The soil health indicators that were correlated with soil microbial β-diversity were TC, WAS, and NO_3^--N. In addition, MBC and Min C were also significantly correlated with soil fungal communities. For α- diversity, prokaryotic communities overall had the most significant and positive correlations with soil health indicators like soil labile carbon pools, PMN, pH, EC, and PR indicators. Our results provide insights about how soil microbial diversity varies across the Napa Valley wine growing region landscape and how these relate to soil health indicators.

1. Introduction

Microorganisms are crucial drivers of soil biogeochemical processes, yet the specific links between microbial diversity and soil health in vineyard soils remains unclear (Coller et al., 2019; Sokol et al., 2022). Soil health is defined as the soil's sustained capacity to function as a vital living ecosystem that supports plants, animals, and humans (USDA NRCS). Soil microbial communities are central to soil ecosystems since their activities support nutrient cycling, organic matter decomposition and stabilization, soil aggregation, and disease suppression (Garbeva et al., 2004; Jacoby et al., 2017; Kuypers et al., 2018; J. Lehmann & Kleber, 2015; Paul, 2016). These processes, in turn, enhance plant growth and crop yields for sustainable crop production (Faucon et al., 2017; Romero et al., 2024). While it is often assumed that greater microbial diversity supports these beneficial agronomic outcomes, the relationship may be more nuanced in less disturbed and complex perennial systems with distinctive production goals like wine grapes. For instance, it is well known that there is a tradeoff between vine vigor and grape quality (Poni et al., 2018). Therefore, many producers may choose to stress vines to achieve a certain grape and wine quality, a practice that is unique to this crop and opposite to many annual crops with goals of biomass and yield maximization (Grassini et al., 2015). Under this scenario, it is not clear whether microbial diversity and soil health would be beneficial to produce wine grapes. This study aims to clarify how microbial diversity—both α- and β-diversity— varies and relates to soil health functions in vineyards and explore the implications for sustainable management in these unique and susceptible agroecosystems.

Wine grape growers emphasize the importance of soil functions like good soil structure and infiltration, reduced erosion and compaction, and reduced needs for inputs from enhanced nutrient cycling, which could be influenced by the soil biota (Blankinship et al., 2016; Kuypers et al., 2018). The abundance, diversity, composition, and activity of soil microorganisms play a pivotal role in soil carbon, nutrient, and water cycling processes. For example, microbial activity supports soil aggregate formation through fungal hyphae growth and entangle of particles, labile microbial exudates, and biomass from both fungi and prokaryotes, that act as cementing agents of soil and OM particles (A. Lehmann et al., 2017, 2020). These contributions are essential in vineyards, especially in Mediterranean climate; they are susceptible to challenges from climate change such as extreme weather patterns like heatwaves and droughts. These extremes can exacerbate soil erosion and degradation and put soil ecosystem services at risk (Prosdocimi et al., 2016; Samaniego et al., 2018; Straffelini et al., 2023). Wine grape production has a significant economic value in California (USA). Producing more than 80% of U.S. wine grapes, vineyards cover a large spatial extent in California (ca. 232,694 hectares (CDFA, 2023) and exist on nearly every soil order defined by the U.S. Soil Survey. According to the Wine Institute, 1.1 million people are employed by the wine industry, and $170.5 billion in annual economic activity across the U.S. has been generated by wine production in California (wineinstitute.org/our-industry/, accessed 10/26/2024). Therefore, enhancing and protecting vineyard soil health is particularly important in wine grape production since vineyard longevity (e.g., 20+ years) is economically valuable. Here, we explore connections between microbial diversity and soil health that may be important for sustaining long-term wine grape production (Dubey et al., 2019).

Soil microbial diversity and dynamic soil properties (i.e., highly sensitive to management) used as soil health indicators can be influenced by static or inherent soil properties (i.e., less sensitive to management). Some static soil properties that shape soil microbial communities are pH, EC, and texture (Burns et al., 2015; Holland et al., 2016). For example, our recent study showed that clay content was positively correlated with aggregate stability and some C and N pools (Chapter 2 Results). In addition to texture, dynamic soil properties can also shape soil microbial communities, such as soil organic matter (SOM) and nutrients availability (Novara et al., 2020). The interaction among these properties, along with soil temperature and moisture conditions, can shape regional microbial community composition (Burns et al., 2015, 2016). These interactions in wine growing regions, along with the grape and wine microbiomes that support fermentation, have promoted the concept of “microbial terroir” (Belda et al., 2017; Bokulich et al., 2016; Burns et al., 2015, 2016; Steenwerth et al., 2021). However, the microbial terroir is not only influenced by these regional and soil factors, but it is strongly affected by soil management practices as well.

Soil management practices such as cover crops, tillage, irrigation, and fertilization, have a strong effect in the soil microbiome (Bansal et al., 2024; Burns et al., 2016; Canfora et al., 2018; Chou et al., 2018; Pingel et al., 2023; Vink et al., 2021). Particularly, these practices can affect resource availability for soil microorganisms. For example, cover crops increase SOM and nutrient inputs through plant biomass and root exudates that feed and protect soil microbial communities through the formation of aggregates (Hudek et al., 2022; Seitz et al., 2024). Compared to no-till, the mechanical breakdown of soil and incorporation of cover crops or plant residues from tillage promotes short term aeration and release of soil nutrients that can affect soil microbial groups, differently. For instance, recent studies showed that no-till soils promoted higher mean concentrations of total C, labile C, and ammonium (Chapter 2). Other studies have found that while tillage can increase bacterial diversity and biomass, the opposite results for fungal communities (Pingel et al., 2023). In addition to tillage, irrigation and nitrogen (N) fertilization have also been shown to have significant effects in bacterial community composition . (Canfora et al., 2018; Vink et al., 2021). Irrigation increases water availability under the vines during the growing season especially in semi-arid regions that otherwise would have been under dry conditions until the winter wet season. In Mediterranean-climate vineyards, these practices often vary by vineyard zone, specifically when comparing the vine rows (under the vine) that are commonly irrigated, fertilized, and maintained bare, and the alleys (i.e. tractor rows) that are rainfed and protected with cover crops or resident vegetation (Chapter 2). The differences in management in these vineyard zones can promote variability of soil health indicators that could influence soil microbial diversity (Chapter 2 Results). For example, we recently found that soil health indicators significantly varied between vineyard zones with plant-protected tractor rows having enhanced aggregate stability, and carbon (C) pools, nutrient cycling indicators, and lower compaction levels (Chapter 2 Results). In addition, resource availability in soils can change by soil depth. For example, SOM, which is an important food source for soil microorganisms, often is higher in the top centimeters of soil, supporting higher soil microbial diversity, biomass and activity in the top layer of soil (Bansal et al., 2024; Lazcano et al., 2022). Therefore, all factors that can influence variability in vineyards as affected by from management practices, must be considered when studying the how soil microbial diversity varies and how it relates to soil health.

Our current study offers a way to meet demands by growers to understand how soil health and soil microbial diversity are linked to soil functions and ecosystem services, improve soil management practices that support vineyard resiliency and grape production sustainability. In addition to these research gaps, our recent study documented how wine grape growers are strongly interested in learning actionable information about the connection between soil microbial diversity and soil health for vineyards (Gonzalez-Maldonado et al., 2024). Most studies that assess soil microbial diversity in vineyards focus on changes on microbial taxonomic diversity (community composition) among regions (Burns et al., 2015), soil management practices (Burns et al., 2016; Chou et al., 2018; Vink et al., 2021), and vine phenological stages (Vink et al., 2021). Whereas fewer studies focus on the functional soil microbial diversity in soil ecosystem services (Fritz et al., 2020) and exploring the relationship of diversity indices with total soil C and N pools and microbial respiration (Pingel et al., 2023). Currently, we lack understanding of the relationship of soil microbial diversity with a diverse and comprehensive set of dynamic soil health indicators and their link to outcomes of interest for wine grape growers.

This study assessed the relationship between soil microbial community and soil health indicators across vineyards in Napa Valley (California, USA). Recently, it was documented that growers perceive soil health as a soil with good infiltration and adequate water holding capacity, and “balanced” (i.e. moderate) levels of nutrients and SOM to support vine vegetative vigor control for vine balance and high grape quality production in irrigated vineyards. The extensive diversity of soil types and microclimates within the growing region of Napa Valley added additional complexity to managing the vineyards for these outcomes of vigor control for vine balance. Many growers expressed the need for more practical information about the role of soil microbial diversity (i.e., α- and β-diversity) and activity, its connection to soil health and viticultural outcomes, and what practices can be done to incentivize soil functions that are highly influenced by the soil microbes (Gonzalez-Maldonado et al., 2024).

Therefore, the objectives of this study are to 1) evaluate the relationship of soil microbial diversity with soil health indicators, 2) study how variability in inherent soil properties (i.e. soil texture) drives microbial diversity across the landscape, 3) examine how soil microbial diversity changes across factors that can drive variability in vineyards such as vineyard zone, soil depth, and tillage, and 4) assess how soil microbial diversity varies among growers perceptions of what constitute ideal and challenging soils for wine grape production. We hypothesized that increased soil microbial diversity corresponds to soil health indicators related to C, N, and water cycling. Also, we hypothesized that soil in the tractor rows and under no-till would have higher soil microbial diversity compared to bare vine rows and tilled tractor rows and this would also correspond with higher levels of soil health. We hypothesized that the variability of soil microbial diversity would be influenced by soil texture. Finally, we hypothesized that soil microbial diversity would differ between grower ratings, with ideal soils demonstrating higher soil microbial diversity than challenging soils.

2. Materials and Methods

2.1. Study design

We conducted a landscape study in Napa Valley, California (USA), one of the largest and globally recognized wine growing regions. The region has a high soil diversity resulting from diverse parent materials, microclimates, and topography (Kunkel and Upson, 1960). In the mountains and hills, Napa soils are primarily formed from volcanic and marine sediment parent material and in the valley, soils are mostly formed from alluvial deposits (Kunkel & Upson, 1960; Lambert & Kashiwagi, 1978). The climate in this area is Mediterranean, consisting of cold and wet winters from

November to March and dry and hot summers from April to October. The annual cumulative rainfall in 2021 was 214 mm and the average was 583 mm from 2012-2021. The mean, minimum average, and maximum average temperatures were 6.3°C, 14.2°C and 23°C, respectively, for 2021 (UC ANR, n.d.; CIMIS, 2024). The grower recruitment and site selection are described in Chapter 2, Materials and Methods. Soil samples were collected from 32 vineyards across the Napa Valley American Viticultural Area (AVA) (Figure 2.2) in the spring of 2021. Soil management practices information was collected in interviews and surveys (Google Forms, Google inc.) with participating growers before sampling. Detailed information about the sites sampled can be found in the Appendix of Chapter 2.

2.2. Soil sampling

Soil samples were collected using a Giddings manual bulk soil core sampler with a diameter of 5 cm (Windsor, CO, USA) during March and April of 2021. Three replicates of soil samples were taken to a depth of 0-10 cm and 10-20 and from two locations including under the vine (vine row) and in the interrow (tractor row). At each vineyard and each sampling factor (location and depth), two soil samples (approximately 2 meters apart) were collected and homogenized into a composite sample. Right after collection, samples were stored in coolers with ice for approximately 6 hours until they made it back to the laboratory. GPS coordinates were recorded for each vineyard sampled.

Once in the lab, all fresh soils were sieved to a size of 8mm. A subsample of 100 g fresh soil was stored in sealed plastic bags in a fridge at 4°C for soil biochemical analyses such as microbial biomass carbon (MBC), potentially mineralizable N, nitrate (NO_3^--N), and ammonium. A subsample of 50g was frozen in sealed plastic bags at -80°C for microbial analyses. The remaining soil was air dried for one week.

2.3. Analysis of Soil Microbial Diversity

Soil DNA was extracted using the DNeasy PowerLyzer PowerSoil (QIAGEN inc.) and the soil prokaryotic and fungal microbial communities characterization were subsequently assessed through soil DNA high-throughput amplicon sequencing of 16S rRNA (V4 region) and internal transcribed spacer (ITS) (ITS1 region), respectively, by a commercial laboratory (Biome Makers Inc, CA, USA) as described in Acin-Albiac et al., (2023) and Bansal et al., (2024). Briefly, the libraries for both prokaryotic and fungal communities were prepared using a two-step PCR following Gobbi et al., (2019) and Liao et al., (2019). To ensure zero cross-contamination, negative controls were added in the libraries preparation. Custom primers were used to prepare the 16S rRNA V4 region and the ITS1 region libraries following Becares & Fernandez, (2017) and these libraries were prepared following the two-step PCR Illumina protocol. In this protocol, synthetic DNA sequences were used as positive control and these were then sequenced on an Illumina MiSeq (Illumina, San Diego, CA, USA) using 2 x 300 paired end reads. Then, Cutadapt was used to remove primers (Martin, 2011). These trimmed reads were merged with a minimum overlapping of 100 nucleotides. The sequences were filtered for quality purposes by expected error with a maximum value of 1.0 (Edgar & Flyvbjerg, 2015). Then, amplicon sequencing variants were formed by clustering the readings with single nucleotide differences using Swarm (Mahé et al., 2021). The remaining singletons and De novo chimeras were then removed (Edgar et al., 2011). The taxonomy was assigned from ASVs using a global alignment with 97% identity, against a curated reference database from SILVA 138.1 for 16S sequences and UNITE 8.3 for ITS sequences (Glöckner et al., 2017; Nilsson et al., 2019). The microbiome data was provided in amplicon sequence variants (ASVs). Soil microbial alpha diversity (within vineyards) was assessed by calculating the Shannon diversity index and beta diversity (across vineyards) was calculated using the Bray-Curtis dissimilarity from 16SrRNA and ITS ASVs. Additionally, the biomass of microbial functional groups including total, total bacteria, actinomycetes, gram negative (GN) and positive (GP), total fungi, arbuscular mycorrhizae fungi, saprophytic fungi, and fungi:bacteria ratio were assessed through phospholipid fatty acids (PLFAs) analysis by a commercial laboratory (WARD Laboratories Inc., NE, USA). The PLFA analysis was conducted following Bligh and Dyer (1959) and Buyer and Sasser (2012). The markers used to assess PLFA of the different groups are found in the Chapter 2 Appendix.

2.4. Analysis of Soil Health Indicators

Permanganate oxidizable carbon (POXC) was quantified to assess management-sensitive C (Culman et al., 2012; Weil et al., 2003). Briefly, 2.5 g of soil was reacted with 20 mL of a 0.02 M potassium permanganate ($KMnO_4$) solution in 50 mL centrifuge tubes. The tube was shaken for two minutes (180 strokes per minute) using a reciprocal shaker and allowed to settle for 10 minutes. Then, 0.5 mL of supernatant was transferred and mixed with 49.5 mL of deionized water. Finally, the sample absorbance was read in an Agilent Biotek Epoch 96 well plate reader spectrophotometer at 550 nm (Agilent Technologies, Inc., Santa Clara, CA).

Mineralizable carbon (Min C) was quantified upon rewetting 10 g of 8 mm sieved dry soil to 50% gravimetric water holding capacity (WHC) in a 227 mL glass jar (Franzluebbers et al., 2000; Haney et al., 2001; Haney & Haney, 2010). After rewetting of soils, jars were capped tightly with lids containing two silicone septum and incubated at 25°C for 48 hours. Proceeding incubation, an input and an output syringe were injected into an LI-850 CO_2/H_2O gas analyzer (LI-COR, Biosciences, Lincoln, NE) to determine the concentration of CO_2. Finally, mineralizable C was calculated as the difference between a sample and a blank control using the ideal gas law and the headspace volume.

Microbial biomass C (MBC) was measured using the fumigation-extraction method (Horwath & Paul, 1994; Vance et al., 1987). Briefly, 6 g of fresh soil was fumigated with chloroform for 24 h prior to extraction with 0.5 M K_2SO_4. A duplicated subsample without fumigation was extracted with 0.5 M K_2SO_4. After extractions, the concentration of dissolved organic C (DOC) was analyzed in both fumigated and nonfumigated samples on a Shimadzu TOC-L 680°C combustion catalytic oxidation equipment (Shimadzu Corp.). Microbial biomass C was calculated from the DOC difference of fumigated and nonfumigated soil samples with a Ke factor of 0.35. Soil total carbon (TC) was measured by direct combustion (Nelson & Sommers, 1982) with a CHN analyzer (Costech, Valencia, CA).

Soil total nitrogen (TN) was measured by direct combustion (Nelson & Sommers, 1982) with a CHN analyzer (Costech, Valencia, CA). Nitrate (NO_3^--N) and ammonium (NH_4^+-N) were assessed (Doane & Horwáth, 2003; Keeney & Nelson, 1982); briefly, soils were extracted using 0.5 M K_2SO_4 solution and shaken for 1 h and centrifuged (2900 RPM) for 2 min. Then, extracted samples were read at 450 and 640 nm for NO_3^--N and NH_4^+-N, respectively. Potentially Mineralizable Nitrogen (PMN) was estimated following (Drinkwater et al., 1996) and used a 7 day incubation of 8 grams of soil in 10 mL of deionized water and added N_2 gas to the headspace in sealed 50mL centrifuge tubes. Soil samples were extracted using 0.67M K_2SO_4. Soil NH_4^+-N was measured in the incubated samples and PMN was calculated from the difference between control and incubated samples (Drinkwater et al., 1996; Keeney & Nelson, 1982).

Soil unsaturated hydraulic conductivity was measured using a mini disk infiltrometer (Meter Group, Inc.) adjusted to -2 cm suction rate to control flow through macropores. The mini disk infiltrometers were placed in a leveled soil area of the tractor row, and measurements from water flow were recorded every five minutes up to 35 minutes for a total of seven readings which allowed to obtain a constant change in volume for a minimum of three consecutive readings. Then, infiltration rate was calculated using the Phillips model (Philip, 1969). Wet aggregate stability was measured using the wet sieving method using one single sieve (Kemper & Rosenau, 2018) using an Eijkelkamp Agrisearch wet sieving apparatus. Soil bulk density was measured by using a 103 cm^3 volume core, drying the soil samples at 105 °C for 24 hours and then dividing the dry mass of the soil by the volume of the core used for soil collection (Blake, 1965). Finally, the surface hardness was measured using a SpotOn® Digital Compaction Meter (Innoquest Inc.,Woodstock, IL) to a depth of 20cm.

2.5. Data analysis

Data analysis of the soil microbiome was performed using the *phyloseq*, *vegan* and *betapart* packages in RStudio (Baselga et al., 2023; McMurdie & Holmes, 2013; Oksanen et al., 2024). Microbiome data in amplicon sequence variants (ASVs) from 16s rRNA and ITS were rarefied using the “rrarefy( )” function to the minimum number of reads per sample in the dataset (11086 for 16S and 10781 for ITS) to minimize sample heterogeneity of sequencing. The rarefied ASVs counts in each sample were converted to percentage to calculate Bray-Curtis distances using the “vegdist()” function. Shannon diversity index (SDI) was calculated for each sample using the “diversity()” function. Boxplots for SDI were done using the *ggplot2* package (Wickham, 2016). Due to the non-normal distribution and failed transformation of the residuals of 16S SDI, we analyzed the effect of Location, Depth, Disturbance, and Texture on the SDI of prokaryotes and fungal communities by fitting a generalized linear model (GLM) with an inverse Gaussian distribution and a log link function using a “glm()” function from the *stats* package “*glm(SDI ~ Vineyard zone + Depth + Disturbance+ Texture, data, family = inverse.gaussian (link = "log"))”* (R Core Team, 2024). Also, we performed a Type II analysis of variance (ANOVA) with Chi-square tests to evaluate the significance of each predictor variable. For post hoc comparisons, we calculated estimated marginal means for each level of the Texture factor using the *emmeans* package in R (Lenth, 2024).

Pairwise comparisons between the levels of Texture were conducted, and significance was determined at $p < 0.05$. Compact letter display (CLD) grouping was applied to indicate statistically distinct groups.

To assess the variability of beta diversity by disturbance, texture group, depth and vineyard zone, we used Permutational Multivariate Analysis of Variance (PERMANOVA) alongside Principal Coordinates Analysis (PCoA). For the PERMANOVA, the “adonis2()” function was used for 16S and ITS separately “*adonis2(asvs ~Vineyard zone+Depth+Texture+Disturbance, metadata)”*. A PERMANOVA allowed us to quantify and test the significance of group-level effects in community composition. We selected PCoA for ordination to provide a direct representation of Bray-Curtis dissimilarities between samples. Unlike NMDS, which did not reveal distinct clustering patterns or significant associations with categorical factors in the ordination in preliminary analyses, the PCoA approach enabled a more interpretable visualization of sample relationships and environmental vector associations. The PCoA was conducted using the “vegdist()”, “cmdscale()”, and “envfit()” functions to plot microbial beta diversity as points and soil indicators as vectors.

To assess the relative abundance of major prokaryotic and fungal phyla across the different factors of this study, we calculated mean relative abundances of phyla for each texture group using the aggregate function in R. First, we ensured consistency in sample ordering between the phyla abundance matrix and the metadata (data set containing categorical factors), then filtered metadata to match the phyla matrix. Relative abundance values were then averaged within each texture group using aggregate, and the resulting matrix was used for visualization. For the relative abundance plot, we used the “barplot()” function to create a stacked bar plot to display the proportional abundance of each bacterial phylum across vineyard zone, depth, disturbance, and texture levels. Color palette selection for the relative abundance figure was done using the *RColorBrewer* package (Neuwirth, 2022). A similarity percentage (SIMPER) analysis was conducted on relative abundance data using the "simper()" function from the *vegan* package. This analysis identifies which taxa contribute the most to differences in community composition between groups by calculating the cumulative contributions of each taxon to the overall dissimilarity. The percentages represent the proportional contribution of each taxon to the Bray-Curtis dissimilarity, allowing us to determine which taxa are driving the observed differences in microbial communities between groups.

3. Results

A total of 33,697 and 24,183 amplicon sequence variants (ASVs) were detected for 16S and ITS, respectively. A total of 10 bacterial phyla were identified as the most abundant (at least 1% of the overall relative abundance) including *Actinobacteriota, Proteobacteria, Bacteroidota, Myxococcota, Firmicutes, Planctomycetota, Verrucomicrobiota, Acidobacteriota, Crenarchaeota, Gemmatimonadota,* and *Bdellovibrionota*. For fungi, a total of 4 phyla were identified as most abundant, including *Ascomycota, Basidiomycota, Mortierellomycota* and *Mucoromycota*. Soil total bacterial biomass from PLFA had a minimum value of 37 ng g^{-1}, mean value of 566 ng g^{-1}, and maximum value of 2431 ng g^{-1}. Soil total fungal biomass from PLFA had a minimum value of 0 ng g^{-1}, mean value of 162.2 ng g^{-1}, and maximum value of 837.2 ng g^{-1}.

3.1. Variability of microbial diversity in vineyards

Effects of Vineyard zone, Depth, Disturbance, and Texture were evaluated for soil α- and β-diversity. These factors include vineyard zone (irrigated and bare vine vs rainfed and plant-covered tractor row), soil depth intervals (0-10 vs 10-20 cm), soil disturbance in the tractor row (till vs no-till) and soil texture (coarse, loam, silt loam, and fine).

Results from the PERMANOVA indicated that soil disturbance and texture influenced the variability of prokaryotic and fungal β-diversity ($p<0.05$; Table 3.1). In contrast, vineyard zone and soil depth did not influence the variability of β-diversity in either microbial group ($p > 0.05$; Table 3.1). The Principal Coordinates Analysis (PCoA) of the prokaryotic and fungal community composition revealed that the first two axes (PCoA1 and PCoA2) explain 16.71% and 7.71% of the total variance, respectively, accounting for approximately 24% of the observed variability (Figure 3.1). Prokaryotic communities showed no distinct clustering based on disturbance or texture group within this ordination, consistent with non-significant results from the envfit goodness-of-fit test. Soil fungal community on PCoA showed different visual patterns by Disturbance and Texture from that of prokaryotes, where silt loam soils under no-till, silt loam under tillage, fine soils under tillage, fine soils under no-tillage, coarse soils under no-till, and coarse soils under no-tillage showed clear separation from other samples.

Table 3.1. PERMANOVA on the Bray-Curtis distances of the soil prokaryotic (16S) and fungal (ITS) communities in soil samples collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128).

Factor	**16S**			**ITS**		
	R^2	**F**	**Significance**	**R^2**	**F**	**Significance**
Vineyard zone	0.00349	0.4546	NS	0.00445	0.5867	NS
Depth	0.00484	0.6309	NS	0.00507	0.6686	NS
Texture	0.05235	2.2755	***	0.05457	2.3983	***
Disturbance	0.02669	3.4808	***	0.01817	2.3961	***
Residual	0.91263			0.91774		
Total	1			1		

Significance: $p<0.001$ = “***”; $p<0.01$ = “**”; $p<0.05$ = “*”; $p>0.05$ = “NS”

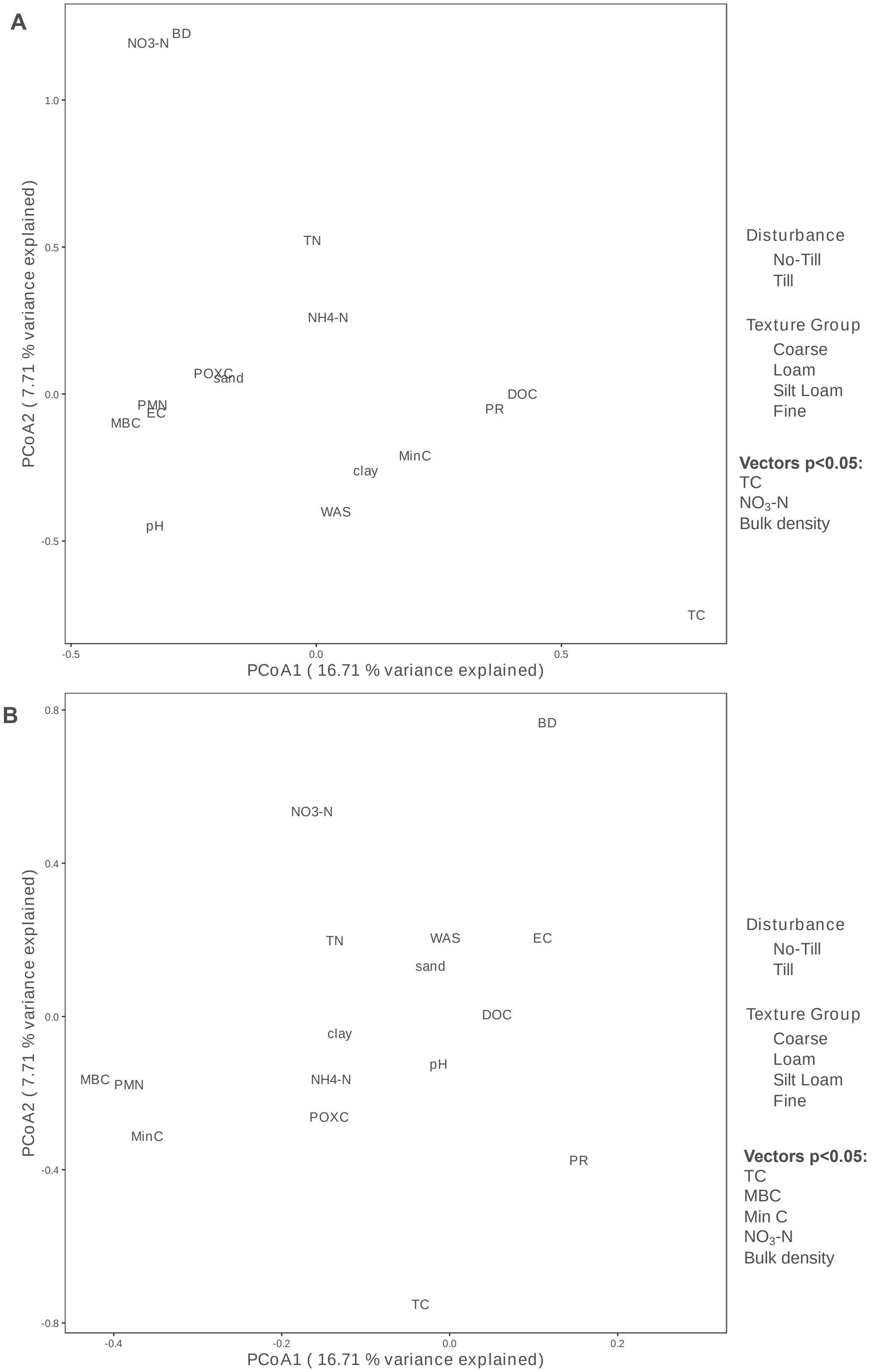


Figure 3.1. PCoA of 16S (A) and ITS (B) Bray-Curtis dissimilarity and soil health variables (vectors) by Disturbance level of No-Till (circles) and Till (triangles) and soil texture levels coarse (blue), loam (orange), silt loam (purple) and fine (green). Soil samples were collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128).

The relative abundance of prokaryotic phyla tended to be similar between tilled and no-till soils, except for tilled soils had lower relative abundance of *Actinobacteriota,* and greater abundance of *Bdellovibrionota* (Figure 3.2-A). For fungal communities, no-till soils had lower relative abundance of the *Mortierellomycota* phylum while the relative abundance of the other fungal phyla, including *Ascomycota*, *Basidiomycota* and *Mucoromycota,* were similar (Figure 3.2-B, Table 3.2).

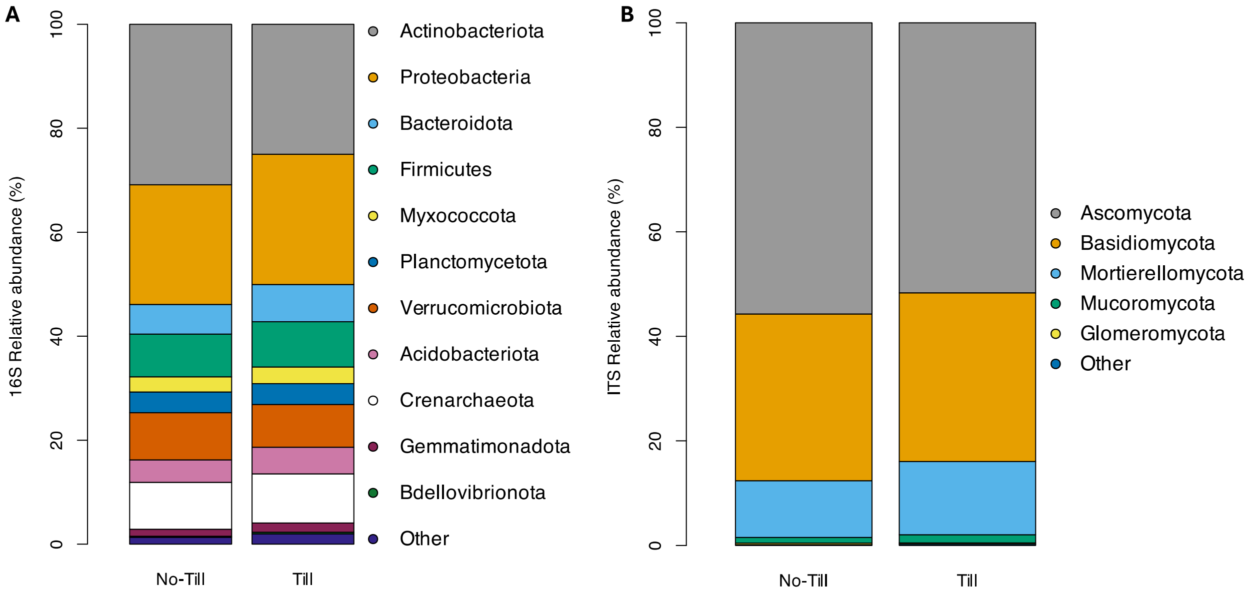


Fig. 3.2. Relative abundance of prokaryotic (A) and fungal (B) phyla by disturbance levels (till vs no-till) in soil samples collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128) .

Table 3.2. Similarity percentage (SIMPER) analysis results for 16S and ITS phyla relative abundance by soil disturbance (till vs. no-till) in soil samples collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128).

Phylum	**Mean Dissimilarity**	**sd**	**ratio**	**Mean No-Till (%)**	**Mean Till (%)**	**Cumulative sum**	**Sig.**
16S							
Actinobacteriota	0.047	0.033	1.42	30.88	25.00	0.21	***
Verrucomicrobiota	0.031	0.029	1.04	9.07	8.21	0.35	NS
Firmicutes	0.030	0.027	1.13	8.21	8.69	0.48	NS
Proteobacteria	0.029	0.023	1.28	23.03	25.05	0.61	NS
Crenarchaeota	0.026	0.021	1.23	9.02	9.44	0.73	NS

Bacteroidota	0.025	0.021	1.17	5.69	7.17	0.84	NS
Acidobacteriota	0.010	0.008	1.24	4.31	5.14	0.89	NS
Planctomycetota	0.008	0.006	1.35	3.98	4.02	0.92	NS
Other	0.006	0.007	0.98	1.31	1.98	0.95	**
Myxococcota	0.005	0.004	1.34	2.94	3.21	0.97	NS
Gemmatimonadota	0.005	0.004	1.37	1.36	1.78	0.99	NS
Bdellovibrionota	0.001	0.001	1.16	0.21	0.31	1.00	*
			ITS				
Ascomycota	0.067	0.050	1.33	55.71	51.66	0.35	NS
Basidiomycota	0.064	0.049	1.30	31.92	32.29	0.69	NS
Mortierellomycota	0.048	0.038	1.26	10.85	14.02	0.94	*
Mucoromycota	0.008	0.012	0.64	1.09	1.56	0.98	NS
Glomeromycota	0.002	0.003	0.66	0.30	0.22	0.99	NS
Other	0.002	0.005	0.32	0.13	0.24	1.00	NS

Significance (Sig.): $p<0.001$ = “***”; $p<0.01$ = “**”; $p<0.05$ = “*”; $p>0.05$ = “NS”

The similarity percentage analysis of the 16S relative abundance of phyla showed that *Proteobacteria, Bacteroidota, and Myxococcota* contributed most to dissimilarities across soil textures, with cumulative contributions to total dissimilarity reaching 55.04% between clay and silt loam and 52.48% between clay and coarse (Table 3.3). For example, the fine textured soils had greater relative abundance of *Verrucomicrobiota*. The coarse textured soils had greater relative abundance of *Proteobacteria,* and *Firmicutes* compared to the fine soils (Figure 3.3-A). The loam and silt loam soils had greater relative abundance of *Actinobacteriota* and lower relative abundance of *Acidobacteriota* compared to the fine soils (Figure 3.3-A, Table 3.3). The loam soils also had lower relative abundance of *Bacterodiota* and *Gemmatimonadota* (Table 3.3).

For fungal communities, although the coarse textured soils tended to have greater relative abundance of *Ascomycota*, and the fine textured soils tended to have greater relative abundance of the *Mortierellomycota* phylum, no significant differences were found (Figure 3.3-B, Table 3.4).

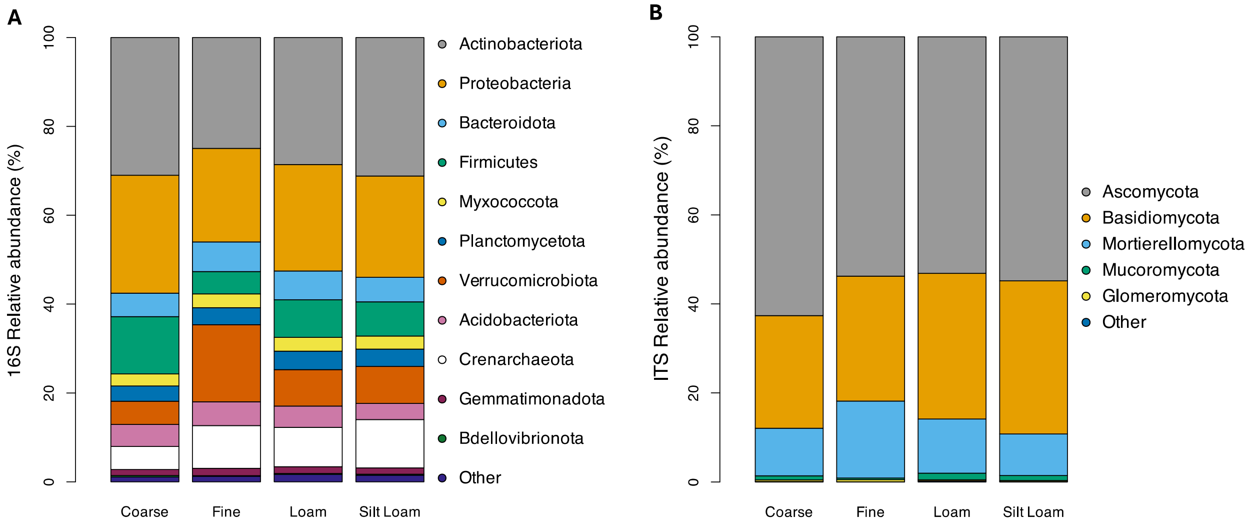


Figure 3.3. Relative abundance of prokaryoticm(A) and fungal (B) phyla by soil texture levels (coarse, fine, loam, and silt loam) in soil samples collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128).

Table 3.3. Similarity percentage (SIMPER)analysis results for 16S phyla relative abundance by soil texture in soil samples collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128).

Contrast	**Phylum**	**Mean Dissimilarity**	**SD**	**Ratio**	**Mean Fine (%)**	**Mean Loam (%)**	**Cumulative sum**	**Sig.**
Loam vs. Fine	*Verrucomicrobiota*	0.057	0.047	1.21	17.33	8.19	0.22	***
	Actinobacteriota	0.049	0.038	1.27	24.97	28.60	0.40	*
	Proteobacteria	0.032	0.024	1.36	21.05	23.96	0.53	NS
	Firmicutes	0.030	0.024	1.22	5.01	8.46	0.64	NS
	Bacteroidota	0.029	0.026	1.10	6.66	6.46	0.75	*
	Crenarchaeota	0.027	0.023	1.16	9.62	8.87	0.86	NS
	Acidobacteriota	0.013	0.012	1.07	5.35	4.80	0.90	*
	Planctomycetota	0.008	0.006	1.37	3.86	4.17	0.93	NS
	Myxococcota	0.006	0.004	1.42	3.11	3.10	0.96	NS
	Gemmatimonadota	0.005	0.004	1.40	1.65	1.50	0.98	*
	Other	0.005	0.005	0.97	1.25	1.64	1.00	NS
	Bdellovibrionota	0.001	0.001	1.08	0.14	0.24	1.00	NS
					Mean Fine	Mean Coarse		
Fine vs. Coarse	*Verrucomicrobiota*	0.064	0.050	1.27	17.33	5.22	0.22	***
	Actinobacteriota	0.050	0.041	1.21	24.97	31.02	0.40	NS
	Firmicutes	0.046	0.039	1.17	5.01	12.88	0.56	**
	Proteobacteria	0.038	0.029	1.31	21.05	26.52	0.69	**
	Crenarchaeota	0.027	0.026	1.07	9.62	5.20	0.79	NS
	Bacteroidota	0.026	0.026	1.00	6.66	5.29	0.88	NS
	Acidobacteriota	0.013	0.012	1.09	5.35	4.95	0.93	NS
	Myxococcota	0.005	0.004	1.34	3.11	2.72	0.95	NS
	Gemmatimonadota	0.005	0.004	1.41	1.65	1.36	0.96	NS
	Planctomycetota	0.005	0.004	1.36	3.86	3.43	0.98	NS
	Other	0.004	0.003	1.39	1.25	1.09	1.00	NS
	Bdellovibrionota	0.001	0.001	1.04	0.14	0.31	1.00	NS

					Mean Fine	Mean Coarse		
Fine vs. Silt Loam	*Verrucomicrobiota*	0.057	0.047	1.21	17.33	8.34	0.22	***
	Actinobacteriota	0.052	0.040	1.31	24.97	31.16	0.43	**
	Crenarchaeota	0.030	0.023	1.33	9.62	10.89	0.54	NS
	Proteobacteria	0.029	0.022	1.35	21.05	22.80	0.66	NS
	Bacteroidota	0.027	0.027	1.00	6.66	5.55	0.76	NS
	Firmicutes	0.024	0.017	1.43	5.01	7.70	0.86	NS
	Acidobacteriota	0.013	0.013	0.97	5.35	3.64	0.91	*
	Planctomycetota	0.007	0.005	1.29	3.86	3.89	0.94	NS
	Myxococcota	0.006	0.004	1.42	3.11	2.92	0.96	NS
	Gemmatimonadota	0.005	0.004	1.42	1.65	1.42	0.98	NS
	Other	0.004	0.005	0.94	1.25	1.47	1.00	NS
	Bdellovibrionota	0.001	0.001	1.17	0.14	0.24	1.00	NS
					Mean loam	Mean coarse		
Loam vs. Coarse	Firmicutes	0.040	0.036	1.13	8.46	12.88	0.19	NS

Significance (Sig.): p<0.001 = “***”; p<0.01 = “**”; p<0.05 = “*”; p>0.05 = “NS”

Table 3.4. Similarity percentage (SIMPER) analysis results for ITS phyla relative abundance by soil texture in soil samples collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128).

Contrast	**Phylum**	**Average Dissimilarity**	**SD**	**Ratio**	**Mean Loam(%)**	**Mean Fine (%)**	**Cumulative sum**	**Sig.**
Loam vs. Fine	*Basidiomycota*	0.065	0.050	1.29	32.72	28.07	0.36	NS
	Ascomycota	0.058	0.044	1.30	53.14	53.77	0.68	NS
	Mortierellomycota	0.048	0.034	1.40	12.18	17.29	0.95	NS
	Mucoromycota	0.006	0.010	0.64	1.47	0.36	0.98	NS
	Glomeromycota	0.003	0.004	0.63	0.25	0.49	0.99	NS
	Other	0.001	0.004	0.30	0.24	0.02	1.00	NS
					Mean Loam	Mean Coarse		
Loam vs. Coarse	*Ascomycota*	0.077	0.055	1.39	53.14	62.63	0.38	NS
	Basidiomycota	0.068	0.053	1.29	32.72	25.30	0.72	NS
	Mortierellomycota	0.048	0.042	1.14	12.18	10.69	0.95	NS
	Mucoromycota	0.006	0.009	0.71	1.47	0.94	0.99	NS
	Glomeromycota	0.002	0.001	1.13	0.25	0.34	0.99	NS
	Other	0.001	0.004	0.36	0.24	0.11	1.00	NS
					Mean Loam	Mean Silt Loam		
Loam vs. Silt Loam	*Basidiomycota*	0.070	0.056	1.26	32.72	34.41	0.37	NS
	Ascomycota	0.069	0.054	1.27	53.14	54.81	0.72	NS
	Mortierellomycota	0.042	0.036	1.17	12.18	9.33	0.94	NS
	Mucoromycota	0.008	0.013	0.61	1.47	1.12	0.98	NS
	Glomeromycota	0.002	0.002	0.78	0.25	0.24	0.99	NS
	Other	0.001	0.004	0.35	0.24	0.09	1.00	NS
					Mean Fine	Mean Coarse		
Fine vs. Coarse	*Ascomycota*	0.070	0.044	1.58	53.77	62.63	0.37	NS
	Basidiomycota	0.057	0.043	1.33	28.07	25.30	0.68	NS
	Mortierellomycota	0.054	0.035	1.55	17.29	10.69	0.97	NS

	Mucoromycota	0.003	0.003	1.35	0.36	0.94	0.98	NS
	Glomeromycota	0.002	0.004	0.66	0.49	0.34	1.00	NS
	Other	0.001	0.001	0.69	0.02	0.11	1.00	NS
					Mean Fine	Mean Silt Loam		
Fine vs. Silt Loam	*Basidiomycota*	0.070	0.054	1.30	28.07	34.41	0.37	NS
	Ascomycota	0.061	0.045	1.33	53.77	54.81	0.70	NS
	Mortierellomycota	0.050	0.034	1.47	17.29	9.33	0.96	NS
	Mucoromycota	0.005	0.011	0.42	0.36	1.12	0.98	NS
	Glomeromycota	0.003	0.004	0.63	0.49	0.24	1.00	NS
	Other	0.000	0.001	0.44	0.02	0.09	1.00	NS
					Coarse	Mean Silt Loam		
Coarse vs. Silt Loam	*Ascomycota*	0.075	0.055	1.35	62.63	54.81	0.38	NS
	Basidiomycota	0.074	0.057	1.29	25.30	34.41	0.75	NS
	Mortierellomycota	0.041	0.040	1.02	10.69	9.33	0.96	NS
	Mucoromycota	0.005	0.011	0.50	0.94	1.12	0.99	NS
	Glomeromycota	0.002	0.002	1.07	0.34	0.24	1.00	NS
	Other	0.001	0.001	0.66	0.11	0.09	1.00	NS

Significance (Sig.): p<0.001 = “***”; p<0. 01 = “**”; p<0.05 = “*”; p>0.05 = “NS”

Prokaryotic and fungal α-diversity did not differ between the soil in the tractor row and the soil under the vine ($p>0.05$; Figure 3.4). In contrast, α-diversity differed between depth intervals; for instance, prokaryotic α-diversity was higher in the 0-10 cm and fungal α-diversity was higher in the 10-20 cm depths ($p<0.05$; Figure 3.4). While fungal α-diversity did not differ between disturbance levels, prokaryotic α-diversity was higher in the tilled soils ($p<0.05$; Figure 3.4). Although prokaryotic α-diversity did not differ between soil texture groups, a significant and positive correlation was seen with clay content and a negative correlation was seen for sand content (Figure 3.5). In contrast, fungal α-diversity was higher in the fine soils compared to the loam soils and no significant correlations were observed for clay and sand contents (Figure 3.4 and Figure 3.5).

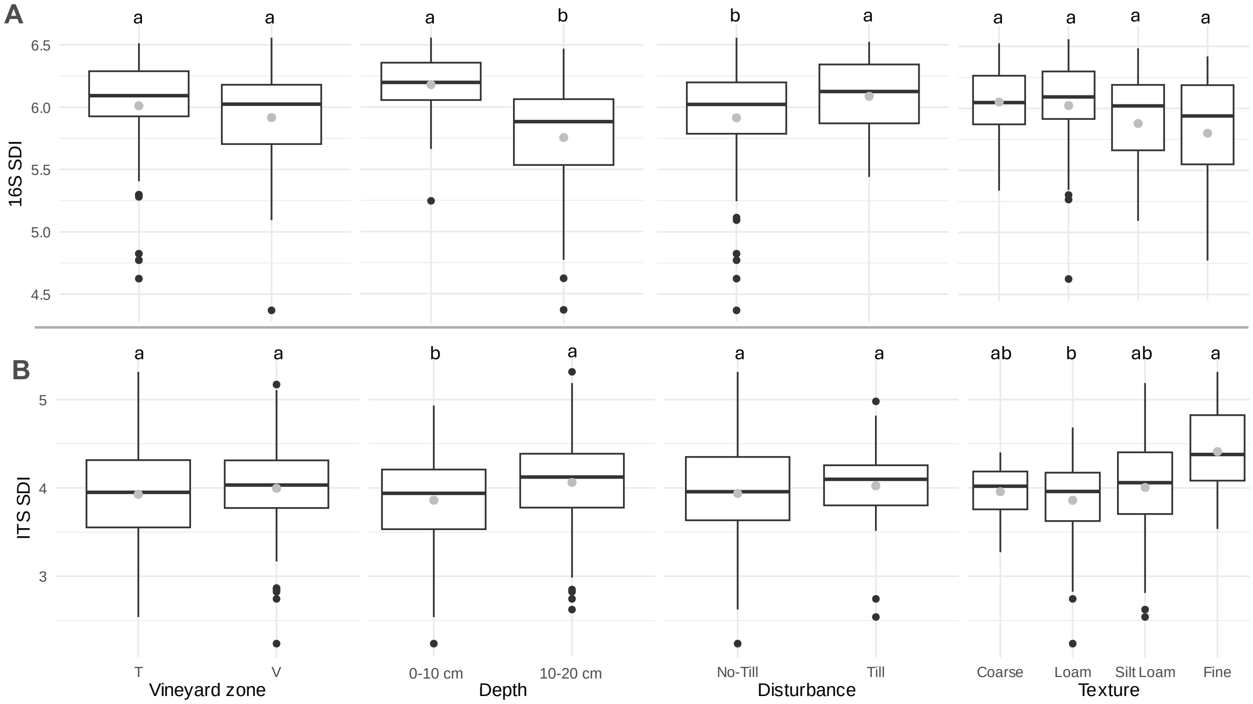


Figure 3.4. Boxplots of 16S (A) and ITS (B) Shannon diversity index values by vineyard zone (vine vs tractor rows; green background), depth (0-10 vs 10-20 cm; orange background), disturbance (till vs no-till; blue background), and textural class (coarse, loam, silt loam, fine; purple background) in soil samples collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128). Letters within each panel indicate significant differences within factor levels at $p<0.05$).

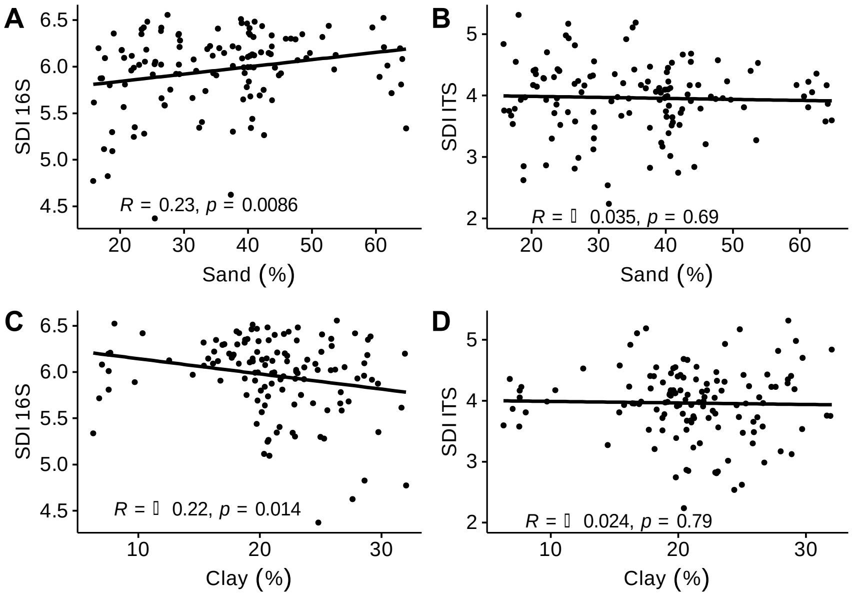


Figure 3.5. Spearman correlations of alpha diversity using Shannon diversity index (SDI) with sand and clay content for prokaryotic communities (A, C) and fungal communities (B, D) in soil samples collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128).

3.2. Relationship between soil health indicators and soil microbial diversity

The relationship between soil microbial α-diversity and soil health indicators was evaluated using Spearman correlations. Three of the four C cycling soil health indicators, including POXC, Min C and DOC, showed significant Spearman correlations ($p<0.05$) with prokaryotic (16S rDNA) and fungal (ITS) α-diversity (Shannon diversity index) (Figure 3.6). These correlations were positive for prokaryotes and negative for fungi. Three of the six soil nutrient cycling indicators, including PMN, pH, and EC, were positively correlated with prokaryotic α-diversity ($p<0.05$; Figure 3.7). In contrast, all nutrient cycling indicators, except for EC, were significantly correlated with fungal α-diversity ($p<0.05$; Figure 3.7). While TN, NO_3^--N, NH_4^+-N and PMN had negative correlations, pH had positive correlation with fungal α-diversity (Figure 3.7). One of the four water cycling indicators, PR was positively correlated with prokaryotic α-diversity ($p<0.05$; Figure 3.8). In contrast, bulk density and PR were correlated with fungal α-diversity ($p<0.05$; Figure 3.8). While bulk density was negatively correlated with fungal α-diversity, PR was positively correlated with fungal α-diversity. The correlations between the biomass of microbial functional groups from PLFA and α-diversity were evaluated, and only fungal biomass was positively correlated with prokaryotic α-diversity ($p<0.05$; Appendix Figure 3.1A).

Soil β-diversity was evaluated through Bray-Curtis dissimilarity and its relationship with soil health indicators was assessed by fitting soil indicators as vectors onto an PCoA plot (Figure 3.6). Soil TC, NO_3^--N and bulk density were correlated with prokaryotic and fungal β-diversity (Figure 3.1). Soil Min C and MBC were also correlated with fungal β-diversity (Figure 3.1-B). Prokaryotic communities were associated with TC across the PCoA1 (horizontal) and with NO_3^--N and bulk density across the PCoA2 axis (horizontal). In the prokaryotic PCoA, NO_3^--N and bulk density were positively correlated, and these appear to be negatively correlated with TC (Figure 3.1-A). In the fungal PCoA, NO_3^--N and PR appear to be negatively correlated while bulk density and TC appear to be negatively correlated (Figure 3.1-B). Fungal communities were associated with labile C pools like MBC, Min C, across the PCoA1 axis (horizontal) and with TC, NO_3^--N, and bulk density across the PCoA2 axis (vertical).

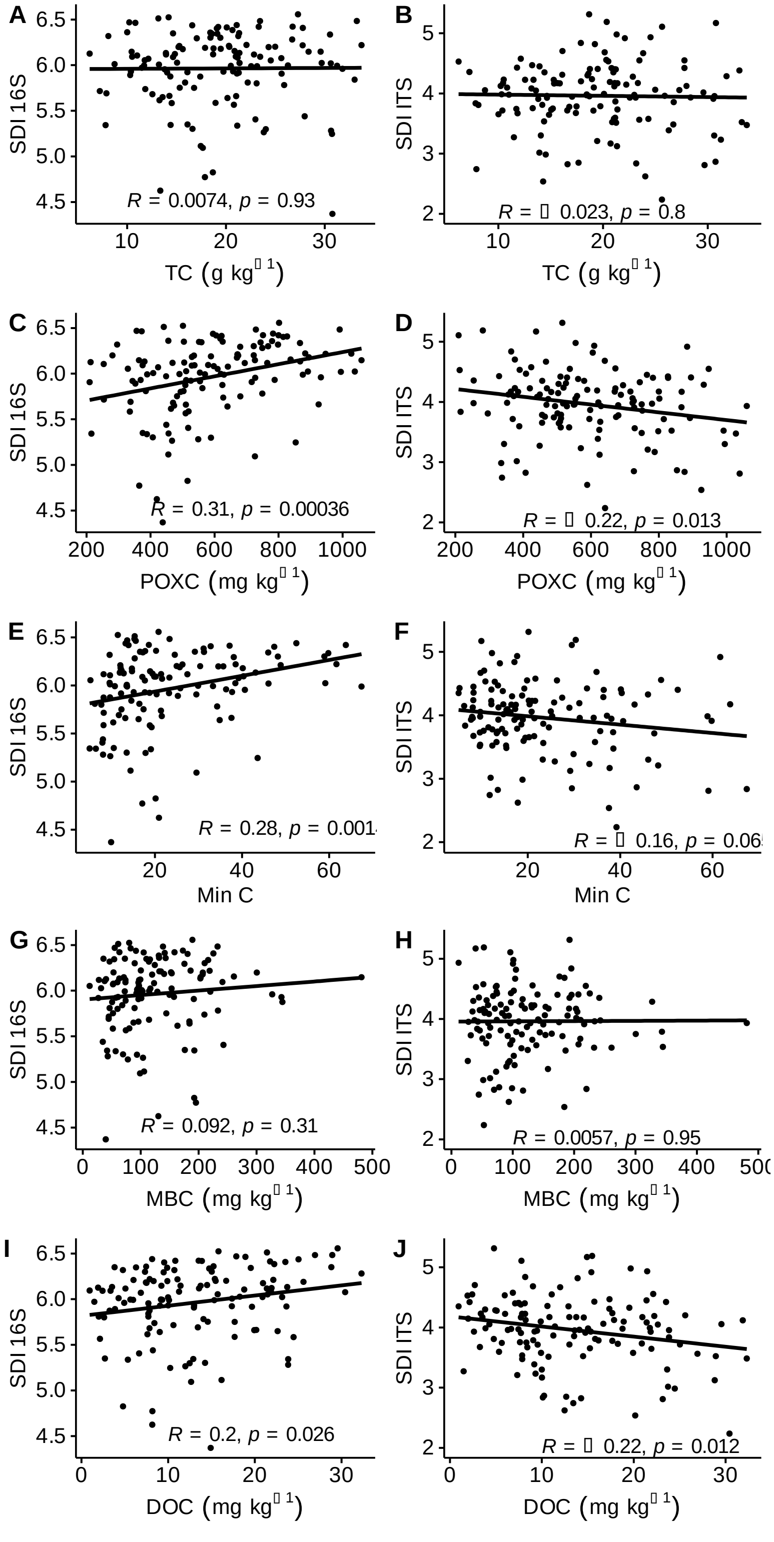


Figure 3.6. Spearman correlations of soil prokaryote (16S) and fungal (ITS) Shannon diversity index (SDI) and carbon cycling indicators total C (TC) (A,B), Permanganate oxidizable C (POXC) (C, D), Mineralizable C (Min C) (E,F), Microbial Biomass C (MBC) (G,H), and Dissolved Organic C (DOC) (I,J) across all samples collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128).

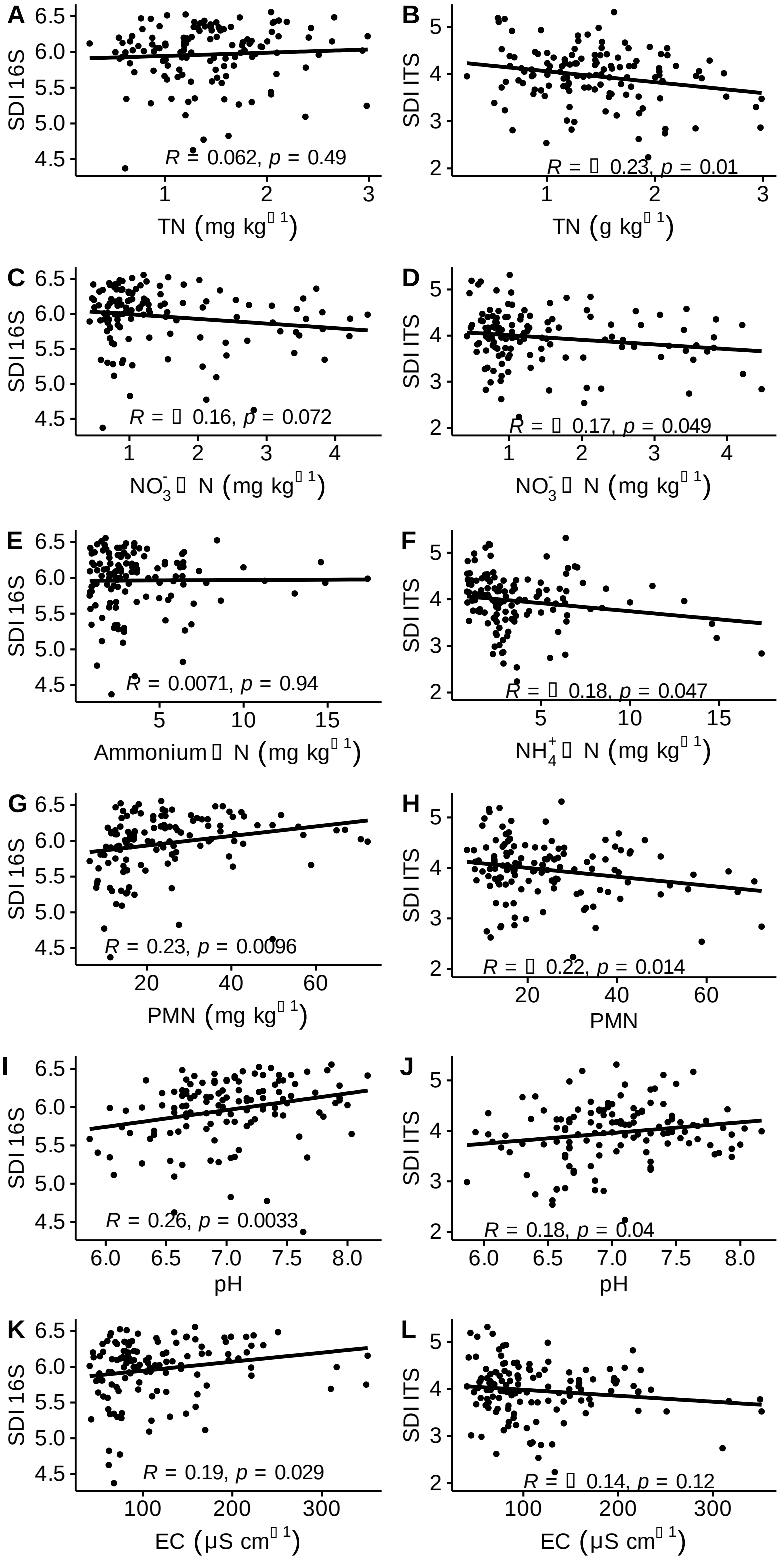


Figure 3.7. Spearman correlations of soil prokaryote (16S) and fungal (ITS) Shannon diversity index (SDI) and nutrient cycling indicators: total N (A,B), nitrate-N (C, D), ammonium-N (E,F), potentially mineralizable N (PMN) (G, H), pH (I, J), and EC (K, L) across all samples collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128).

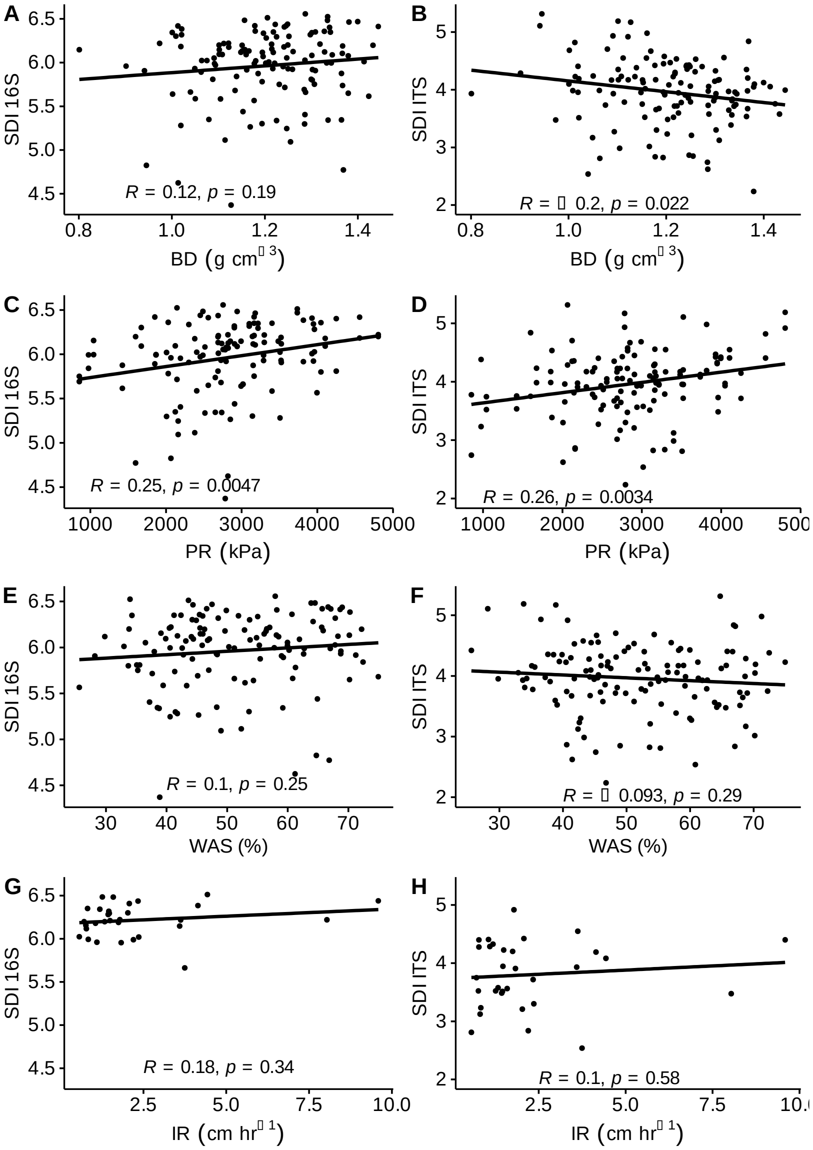


Figure 3.8. Spearman correlations of prokaryotes (16S rRNA) and fungal (ITS) Shannon diversity index (SDI) with water cycling soil health indicators such as bulk density (BD) (A, B), penetration resistance (PR) (C, D). wet aggregate stability (WAS) (E, F), infiltration rate (IR (G,H), sand, and clay content across all samples collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128).

3.3. Variability of microbial diversity by growers' perceptions of ideal and challenging soil conditions for Napa Valley wine grape growing

The variability of soil microbial diversity was evaluated across soils rated as challenging and ideal by growers based on their perceptions for wine grape production in Napa Valley (CA, USA). Results showed that α- β-diversity for 16S and ITS did not differ between grower ratings of challenging and ideal soils (Figure 3.10). Overall, the relative abundance of bacterial and fungal phyla tended to be similar between grower ratings (Figure 3.11). The phyla that were significantly different between the challenging and ideal soils were *Firmicutes* and *Basidiomycota* that were higher in the challenging soils (Table 3.5, Figure 3.11).

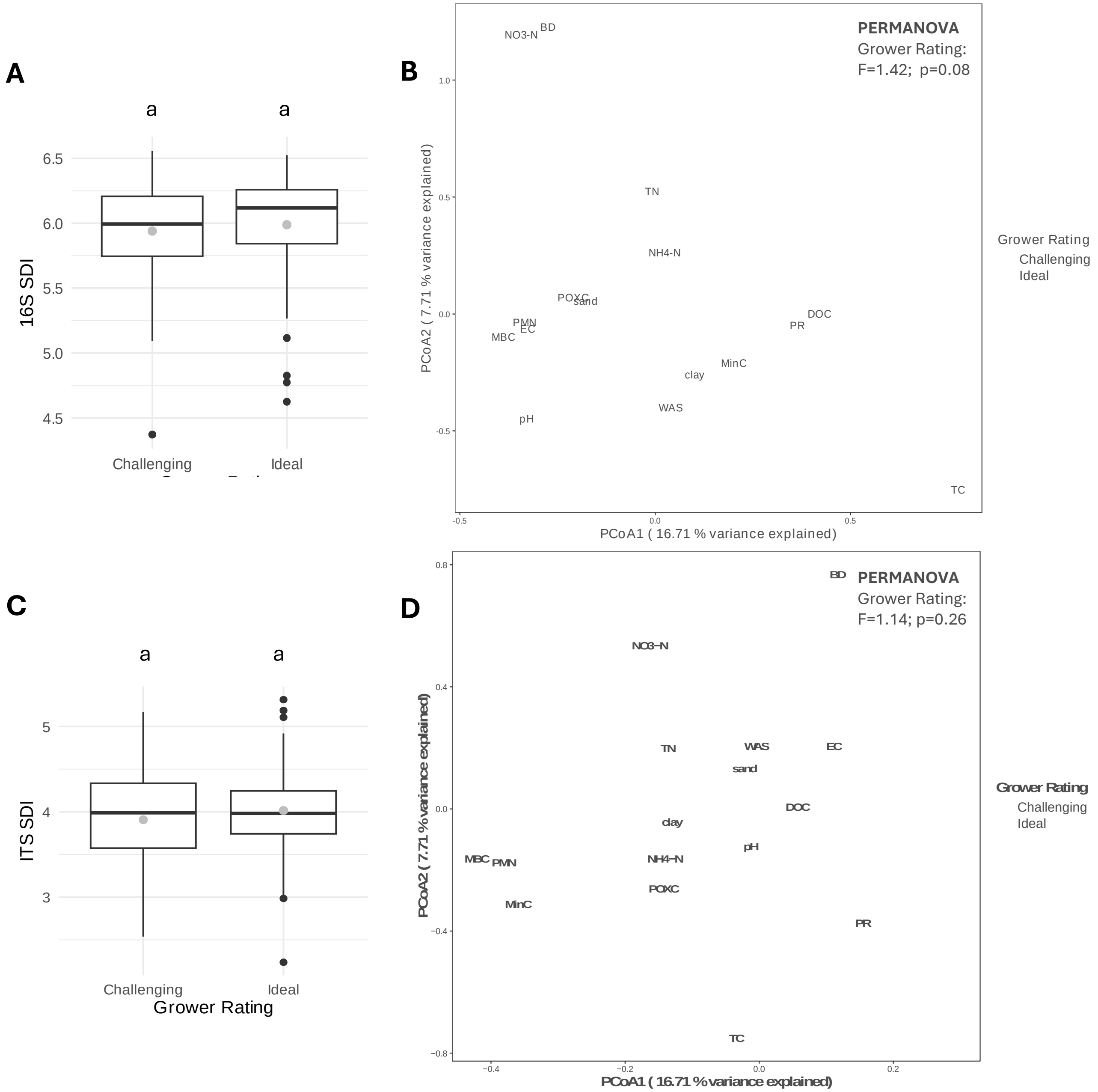


Figure 3.10. Boxplots of Shannon diversity index and PCoA of Bray-Curtis dissimilarity (beta diversity) for soil prokaryotes (A, B) and fungi (C, D) by soil rated as challenging and ideal for wine grape production by growers from soil samples collected from 32 vineyards in Napa Valley (California, USA). Gray circle in boxplots represent the mean (16S n=126; ITS n=128).

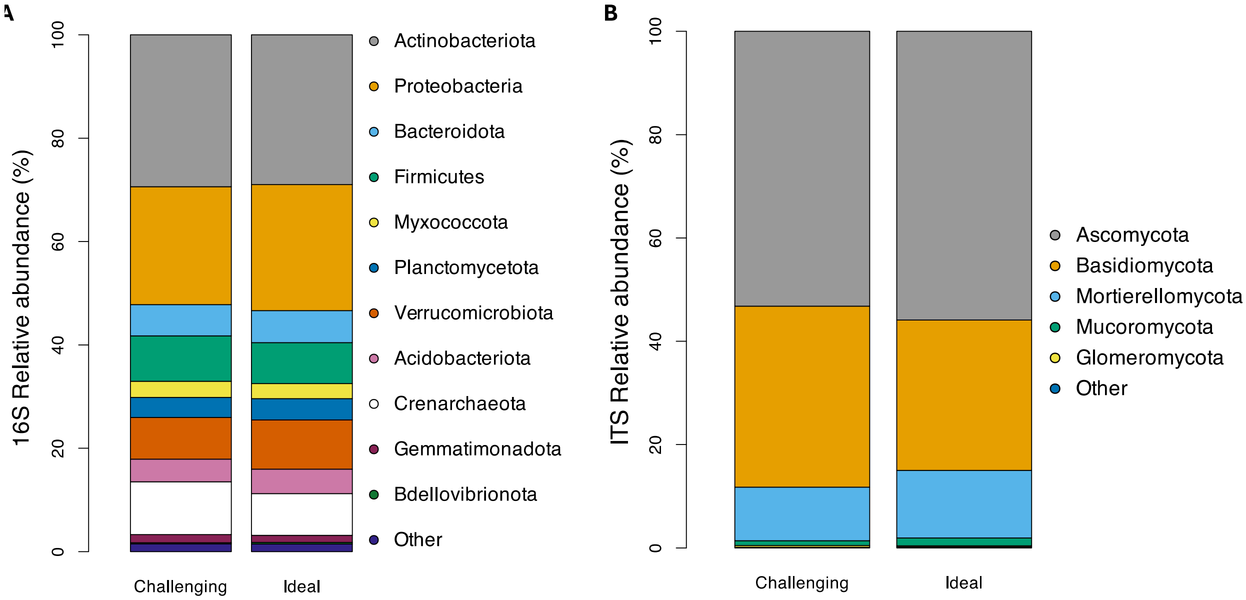


Figure 3.11. Relative abundance plots of prokaryotic(A) and fungal (B) communities by soils rated by growers as challenging and ideal for wine grape production goals in samples collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128).

Table 3.5. Similarity percentage (SIMPER) analysis results for 16S and ITS phyla relative abundance grower ratings (challenging vs ideal soils for wine grape production outcomes) in soil samples collected from 32 vineyards in Napa Valley (California, USA) (16S n=126; ITS n=128).

Phylum	**Mean Dissimilarity**	**SD**	**Ratio**	**Mean Ideal (%)**	**Mean Challenging (%)**	**Cumulative Sum**	**p-value**
				16S			
Actinobacteriota	0.041	0.032	1.30	28.98	29.39	0.19	NS
Verrucomicrobiota	0.033	0.033	1.01	9.55	8.09	0.34	NS
Firmicutes	0.030	0.026	1.16	7.90	8.80	0.48	*
Proteobacteria	0.028	0.021	1.29	24.38	22.83	0.61	NS
Crenarchaeota	0.027	0.022	1.25	8.08	10.22	0.73	NS
Bacteroidota	0.024	0.021	1.16	6.21	6.03	0.84	NS

Acidobacteriota	0.010	0.008	1.15	4.73	4.36	0.89	NS
Planctomycetota	0.008	0.006	1.32	4.11	3.87	0.92	NS
Other	0.006	0.006	0.96	1.49	1.52	0.95	NS
Myxococcota	0.005	0.004	1.32	2.93	3.11	0.97	NS
Gemmatimonadota	0.005	0.003	1.34	1.37	1.59	0.99	NS
Bdellovibrionota	0.001	0.001	1.13	0.28	0.20	1.00	NS
ITS							
Basidiomycota	0.071	0.056	1.27	29.10	35.04	0.36	**
Ascomycota	0.069	0.053	1.30	55.88	53.19	0.71	NS
Mortierellomycota	0.045	0.037	1.21	13.09	10.38	0.95	NS
Mucoromycota	0.007	0.012	0.63	1.51	0.93	0.98	NS
Glomeromycota	0.002	0.003	0.72	0.22	0.34	0.99	NS
Other	0.001	0.004	0.35	0.20	0.13	1.00	NS

Significance (Sig.): $p<0.001$ = “***”; $p<0.01$ = “**”; $p<0.05$ = “*”; $p>0.05$ = “NS”

4. Discussion

Despite the important role that soil microorganisms play in soil ecosystem processes, we lack clear information about the variability of the soil microbiome across vineyards and how microbial diversity is linked to vineyard soil health. This study assessed how the soil microbiome varies across vineyards of Napa Valley (California, USA) and how the soil microbiome interacts with soil health indicators to obtain insights into sustainable vineyard management. Briefly, our results confirmed our hypotheses that soil disturbance (i.e., tillage), texture, and sampling depth influence the variability of prokaryotic and fungal diversity differently. However, contrary to our hypothesis, no differences were observed by vineyard zones. In addition, results confirmed our hypothesis that various soil health indicators indicative of carbon, nutrient, and water cycling were correlated with soil α- and β-diversity for both fungal and prokaryotic communities. These findings align with other studies that examined how the soil microbiome is responsive to management practices like tillage, sampling depth, and relates to soil properties like texture, carbon pools, bulk density, pH, and nutrient availability in vineyards (Bansal et al., 2024; Burns et al., 2015, 2016; Coller et al., 2019; Liang et al., 2019).

4.1. Variability of soil alpha and beta diversity across vineyards

Vineyard soil health is essential for enhancing soil ecosystem functions and supporting sustainable wine grape production. No-tillage practices have been proposed as a soil health practice that could support vineyard adaptation to climate change challenges, as it is frequently associated with the accumulation and protection of soil carbon (Payen et al., 2021). In addition, no-tillage can reduce soil erosion and degradation in vineyards, which is essential for sustaining long-term soil functions (Carretta et al., 2021). Our previous assessment showed that no-tillage increased vineyard TC, Min C, and NH_4^+-N in vineyards across Napa Valley, California (Chapter 2). In addition to no-tillage, our previous study also showed that soil C and N pools were significantly influenced by clay content, highlighting that soil texture plays an important role in determining soils trends for carbon and nitrogen concentrations. As soil C and N pools have been shown to be drivers of soil microbial communities, we hypothesized that Disturbance (i.e., till vs no-till) and texture would influence soil microbial diversity. Results from the current study supported our hypothesis that soil disturbance and texture significantly influenced the clustering of soil β-diversity in vineyards for fungal communities. These findings are consistent with previous studies that have found that no-tillage and soil texture influence soil fungal β-diversity (Hernandez & Menéndez, 2019; Wang et al., 2016). The lack of physical disturbance in soils under no-till leads to stratification of soil organic matter in the topsoil layer (i.e., top 10 to 20 cm). This then encourages preservation of soil aggregates and physical structure which can preserve microhabitats for fungi

(Belmonte et al., 2018; Y. Wang et al., 2010). In contrast, tillage can disrupt soil pore networks and stable fungal hyphal growth, which can reduce the biomass and diversity of fungal communities (Young & Ritz, 2000). In addition to the lack of physical disturbance, soil texture can play an important role in the formation and stability of aggregates, organic matter retention, and pore space. Together, these factors influence the establishment of microbial communities, especially fungi, which further improve soil aggregation and structure (Rashid et al., 2016). For example, soils with higher clay content have the capacity for better aggregate formation, organic matter protection, and water retention that could help of fungal communities thrive (A. Lehmann et al., 2020). Briefly, for soil prokaryotic β-diversity, although results from PERMANOVA for disturbance and texture were significant ($p<0.05$), these differences were not visually apparent as distinct clusters in the ordination plots. These results for prokaryotic β-diversity might be due to high within-group variability, small effect size, multidimensional interactions that might not be reflected in two-dimensional ordination, or heterogeneity in environmental drivers.

The dominance of *Mortierellomycota, Basidiomycota, Ascomycota, Mucoromycota, and Glomeromycota* fungal phyla in soils have been reported in other studies (Darriaut et al., 2022). Despite the significance of physical disturbance on fungal β-diversity, minimal effects were observed for fungal community composition of dominant phyla, suggesting that the overall differences in community structure may be driven by changes in less abundant taxa, specific functional groups, or rare community members rather than widespread shifts in dominant fungal phyla. The significant difference observed in the relative abundance of *Mortierellomycota* between tilled and no-till soils indicates that this phylum may be particularly sensitive to tillage practices. The *Mortierellomycota* phylum are known to include relatively fast-growing, saprophytic fungi that respond to changes in organic matter availability and soil disturbance, suggesting that tillage may create conditions favorable for their proliferation compared to other phyla that are slow-growing and might benefit from no-tillage (Ozimek & Hanaka, 2020).

The dominance of *Actinobacteriota, Verrucomicrobiota, Firmicutes, Proteobacteria, Crenarchaeota, Bacteroidota, Acidobacteriota, Planctomycetota, Myxococcota, Gemmatimonadota,* and *Bdellovibrionota* prokaryotic phyla in soils have been reported in other studies (Darriaut et al., 2022). In contrast to fungi, several prokaryotic phyla such as *Actinobacteriota, Bdellovibrionota,* and less abundant prokaryotic communities (Others) differed significantly between Disturbance levels. Higher levels of *Actinobacteriota* in the no-till soils align with findings from other studies, which suggest that this is due to higher soil carbon pools, similar to what was observed in no-till soils in our study (Wolińska et al., 2019). Higher *Bdellovibrionota* in the tilled soils might be due to abundance of prokaryotic communities that tillage can promote since this phylum is known to be obligate predators that can consume some Gram-negative bacteria (Helgason et al., 2009).

Compared to disturbance, texture had stronger effects on prokaryotic relative abundance with differences being most apparent when compared to fine soils. For example, higher relative abundance of *Verrucomicrobiota* are known to be facultatively anaerobic or anaerobic, and thus their greater abundance in in fine textured soils compared to all other textures might be due to a comparatively higher water holding capacity and retention in fine soils (Chin et al., 2001). Higher relative abundance of *Proteobacteria* and *Firmicutes* in fine soils compared to coarse soils, and higher relative abundance of *Actinobacteriota* and *Acidobacteriota* compared to the silt loam and loam soils, might be due to the higher concentration of C pools in fine soils since these phyla are known to thrive in soils with greater organic matter content (Wolińska et al., 2019). In contrast, the relative abundances of fungal phylae among all texture groups did not differ, suggesting that fungi are more sensitive to management than inherent soil properties like texture.

We hypothesized that soil α-diversity would vary across vineyard zone, depth, disturbance and texture, following similar trends form C, nutrient, and water cycling soil indicators in our previous soil health assessment (Chapter 2). These factors can influence resource availability for microbial communities. Resource availability is a key driver of microbial community composition, as microorganisms rely on accessible carbon, nutrients, and water to sustain growth and activity (Cline & Zak, 2015). In the vineyards studied, tractor rows are enriched in soil carbon pools due to the use of cover crops at least during the dormant season (some vineyards maintained the cover crops the entire year), while the vine rows remain bare but receive frequent irrigation and fertilization in the drip zone during the growing season. In our recent study, most soil carbon and nitrogen pools were higher in the tractor rows compared to the vine rows (Chapter 2). Therefore, we hypothesized that soil microbial community composition would also vary between these vineyard zones. Contrary to our hypotheses and following trends observed in β-diversity, α-diversity of prokaryotic and fungal communities did not differ significantly between vineyard zones. For example, while these contrasting nutrient and water inputs in the vineyard zones could create offsetting effects, where the increased organic carbon in the tractor rows supports microbial activity, the frequent disturbance and resource additions under the vines may also maintain active microbial communities. As a result, the microbial diversity may stabilize across zones despite differences in soil health indicators observed in Chapter 2. This suggests that microbial diversity is shaped by complex interactions among management practices and soil conditions.

For the other factors such as disturbance, sampling depth, and texture, α-diversity trends were variable between prokaryotic and fungal communities. While the prokaryotic α-diversity was higher in tilled soils, no significant differences were observed for fungal α-diversity. Higher α-diversity of prokaryotes in tilled soils followed our hypothesis that tillage could increase the diversity of prokaryotic communities. This may be attributed to increased organic matter breakdown and the disruption of competitive microbial networks by the physical disturbance from tillage. That disturbance could benefit bacterial diversity in vineyard ecosystems by suppressing the proliferation of dominant organisms following the Intermediate Disturbance Hypothesis (Bruggisser et al., 2010; Kazakou et al., 2016; Lienhard et al., 2014; Pingel et al., 2023; Svensson et al., 2012). Prokaryotes, particularly r-selected organisms, may thrive under tilled conditions due to their rapid growth rates, ability to exploit nutrient pulses, and resilience to disturbance (Naylor et al., 2020; Williams et al., 2016). In contrast, fungi are often dominated by K-selected organisms, which are slower-growing and more reliant on stable environmental conditions and complex organic substrates found in no-till systems(Williams et al., 2016). Tillage has been shown to have negative effects in soil fungal hyphae and change fungal abundance and diversity (Bansal et al., 2024; Curaqueo et al., 2011; Jansa et al., 2003). Findings on soil fungal α-diversity not differing between tilled and no-till soils could be due to legacy effects of cover crops especially since these remain in the soil at from the late fall to the early spring (Detheridge et al., 2016) . Also, many confounding factors including tillage frequency, depth, time of sampling since tillage, cover crops, amendments, among others, could be influencing soil fungal α-diversity. Similar results to our study were found in other vineyard studies where tillage incentivized bacterial alpha diversity and, in those cases, decreased fungal alpha diversity in soils (Ibáñez et al., 2024; Pingel et al., 2023).

In contrast, while prokaryotic α-diversity did not differ between texture levels, fungal α-diversity was higher in fine-textured soils compared to loam soils. Higher fungal α-diversity in fine soils aligns with our hypothesis greater clay content would promote greater fungal diversity. These results could be due to the greater moisture retention and organic matter stabilization in fine-textured soils, which create microhabitats for fungal growth (Xia et al., 2020). Both prokaryotic and fungal α-diversity were significantly influenced by soil depth, with prokaryotic diversity being higher in the topsoil (0–10 cm) and fungal diversity being higher in the subsoil (10–20 cm), supporting findings from other studies (Fierer et al., 2003). This pattern might be due to differences in resource availability and environmental conditions. For instance, while the topsoil is enriched with organic matter and oxygen, which support diverse prokaryotic communities, the bottom depth provides more stable micro habitats and less disturbance, favoring the establishment of fungal communities.

These findings suggest that α-diversity of prokaryotic and fungal communities is shaped by distinct environmental drivers, with tillage and soil texture exerting differential effects on community structure. Additionally, the contrasting depth preferences for prokaryotes and fungi highlight their complementary ecological roles in soil processes, such as nutrient cycling and organic matter turnover, across soil depths. The slight differences observed between soil α- and β-diversity results likely reflect the distinct ecological aspects captured by these metrics. While α-diversity emphasizes local richness and evenness, β-diversity highlights compositional differences between communities. These results suggest that environmental factors or management practices may drive shifts in community composition (β-diversity) without necessarily affecting local diversity (α-diversity), underscoring the importance of integrating both measures for a comprehensive understanding of microbial responses.

4.2. Correlations between soil microbial diversity and soil health

We hypothesized that soil health indicators related to carbon, nutrient, and water cycling would be correlated with both microbial α- and β-diversity. Our findings reveal that soil health indicators significantly correlated with the separation of prokaryotic and fungal β-diversity included TC, NO_3^--N, and bulk density, suggesting these factors play a critical role in shaping the soil microbiome. Similar findings for β -diversity in vineyards are reported in Bansal et al., (2024). For prokaryotic communities, TC provides a key energy source for microbial metabolism, while NO_3^--N serves as a readily available nutrient that supports fast-growing, r-selected bacteria (Fuhrmann, 2021; Z. Wang et al., 2021). Specifically, the availability of NO_3^--N facilitates microbial processes such as assimilatory nitrate reduction, in which nitrate is reduced and incorporated into organic nitrogen compounds to support cellular growth and function (Roco et al., 2016).

Bulk density, on the other hand, influences microbial habitat by affecting soil porosity, aeration, and water retention (Or et al., 2007; Young & Ritz, 2000). These indicators, along with labile C pools like Min C (a proxy for microbial respiration) and MBC, likely reflect the availability of organic matter via decomposition and nutrient cycling processes that sustain fungal growth and activity. These results align with findings from other studies, which have demonstrated strong links between soil organic matter, nutrient availability, and microbial community composition and function (Calleja-Cervantes et al., 2015). Together, these relationships highlight the interconnectedness of soil health indicators and microbial diversity, emphasizing the role of key biogeochemical properties in modulating microbial community structure and dynamics.For α-diversity, we hypothesized that soil health indicators related to resource availability would be positively correlated with microbial diversity, except for bulk density and penetration resistance, where negative correlations were anticipated. Our results largely supported this hypothesis for prokaryotes, with POXC, Min C, DOC, PMN, pH, and EC showing positive correlations. Similar positive correlations of soil carbon pools with bacterial alpha diversity have been shown in another study with similar climate (Ramírez et al., 2020). These positive relationships might reflect the role of these indicators in providing energy sources (e.g., POXC, Min C, DOC) and favorable soil conditions (e.g., pH, EC) that support microbial growth and activity (Hu et al., 2014; Zhao et al., 2018). However, contrary to our expectations, penetration resistance was positively correlated with prokaryotic α-diversity, suggesting that some prokaryotes may thrive in compacted soils where their small cell size and metabolic adaptability allow them to persist in restricted pore spaces (Xu et al., 2021).

For fungi, the results showed opposite trends to our initial hypothesis. Indicators such as POXC, Min C, DOC, TN, PMN, pH, and wet aggregate stability were negatively correlated with fungal α-diversity. These negative relationships might be attributed to shifts in fungal community composition favoring a few dominant taxa under resource availability or competitive exclusion dynamics in more nutrient-rich environments. In contrast, some findings aligned with our expectations, such as the negative correlation between fungal α-diversity and soil bulk density, indicating that fungal diversity thrives in less compacted soils with better aeration and structure. These contrasting results highlight the differential ecological responses of prokaryotes and fungi to soil health indicators, underscoring the complexity of soil microbial communities and their interactions with soil properties.

4.3. Linking growers' perceptions to microbial diversity

Our recent study identified soil texture as a key factor in growers' perceptions of soils as either "ideal" or "challenging" for viticultural production (Chapter 2). Wine grape growers in these irrigated vineyards aim to manage soil water to control vine vigor, achieving a balance between vegetative growth and fruit quality, commonly referred to as "vine balance" (Gonzalez-Maldonado et al., 2024). In our previous soil health assessment (Chapter 2), challenging soils were characterized by higher clay content, which was associated with greater carbon pools and wet aggregate stability. Based on these findings, we hypothesized that the higher resource availability (e.g., elevated carbon content) in challenging soils would support greater microbial diversity. However, our results did not support this hypothesis, as there were no significant differences in α- or β-diversity of prokaryotic or fungal communities between challenging and ideal soils. Our findings are comparable to a similar study conducted in a temperate region with annual cropping systems where they did not found significant differences in soil microbial communities from PLFA analysis between soils rated as “good” and “poor” by growers (Mann et al., 2019). findings suggest that while growers define “vine balance” as the distinguishing criterion for soil suitability, this concept may not align with variations in the soil microbiome or other soil biological properties. Instead, the lack of microbial diversity differences between soil types implies that microbial communities are less influenced by the physical and chemical distinctions growers use to classify soils and may be more shaped by overarching management practices, such as irrigation and fertilization, which homogenize microbial habitats across zones. This disconnect highlights the complexity of linking soil microbial diversity to growers’ perceptions and production goals and the need for more research and outreach in the role of soil biology in vineyard soil health.

5. Conclusions

This study evaluated the variability of soil microbial diversity and links to a comprehensive set of soil health indicators across 32 Napa Valley vineyards. Soil texture and disturbance (till vs no-till) played a critical role in shaping microbial β-diversity, especially in fungal communities. Soil depth and disturbance influenced bacterial α-diversity while depth and texture influenced fungal α-diversity. These results suggest that α- and β- diversity of prokaryotic and fungal communities are shaped by distinct environmental drivers, with tillage and soil texture exerting differential effects on community structure. Soil prokaryotic diversity showed more significant correlations with soil health indicators reflective of carbon, nutrient, and water cycling soil functions, compared to fungal communities. Suggesting that prokaryotic communities might be more sensitive to changes in soil health indicators in vineyards. Additionally, soil microbial communities did not differ between growers' perceptions of ideal and challenging soils, suggesting that more research and outreach is needed to better understand the soil microbiome-soil health-and vine vigor control nexus for high wine grape quality production.

6. Appendix

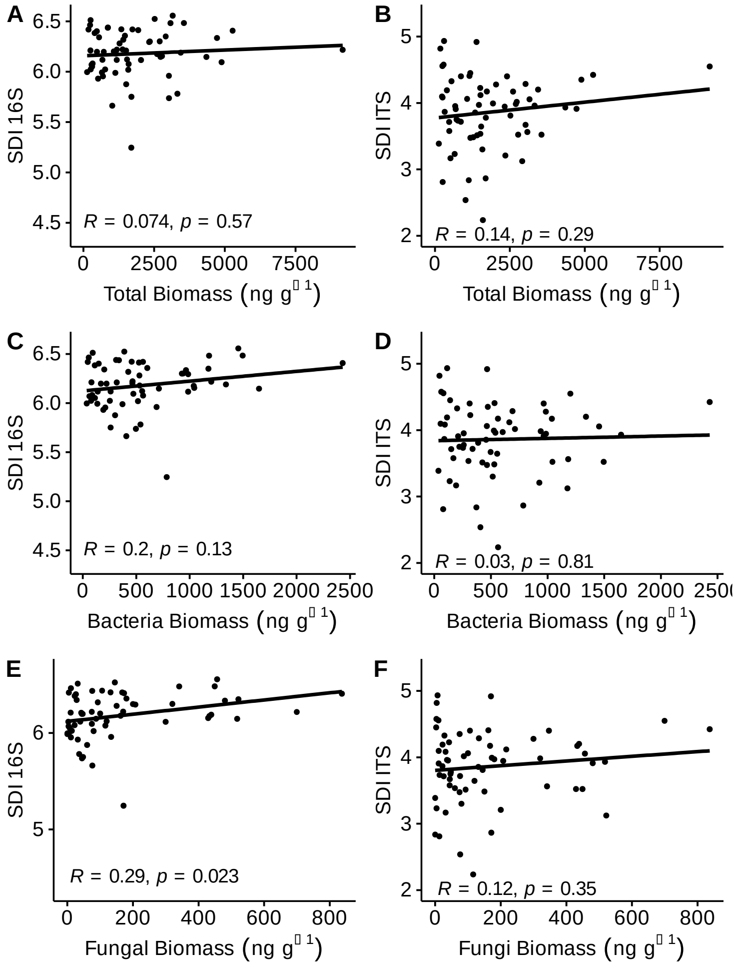


Figure 3.1A. Spearman correlations of prokaryotes (16S rRNA) and fungal (ITS) Shannon diversity index (SDI) with phospholipid fatty acids (PLFA) microbial biomass groups including Total PLFA biomass (A,B), bacteria biomass (C, D), and fungal biomass (E, F).

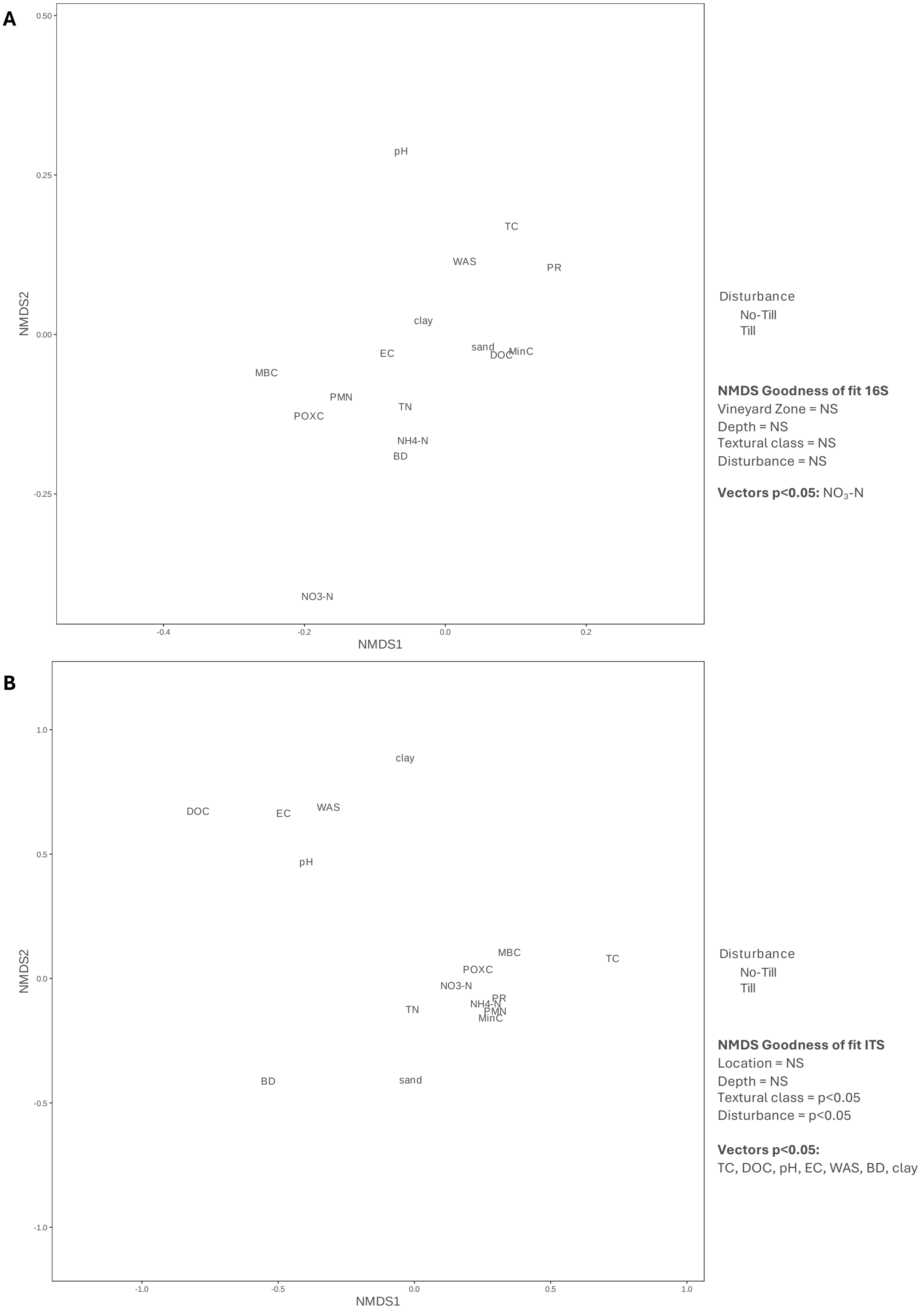
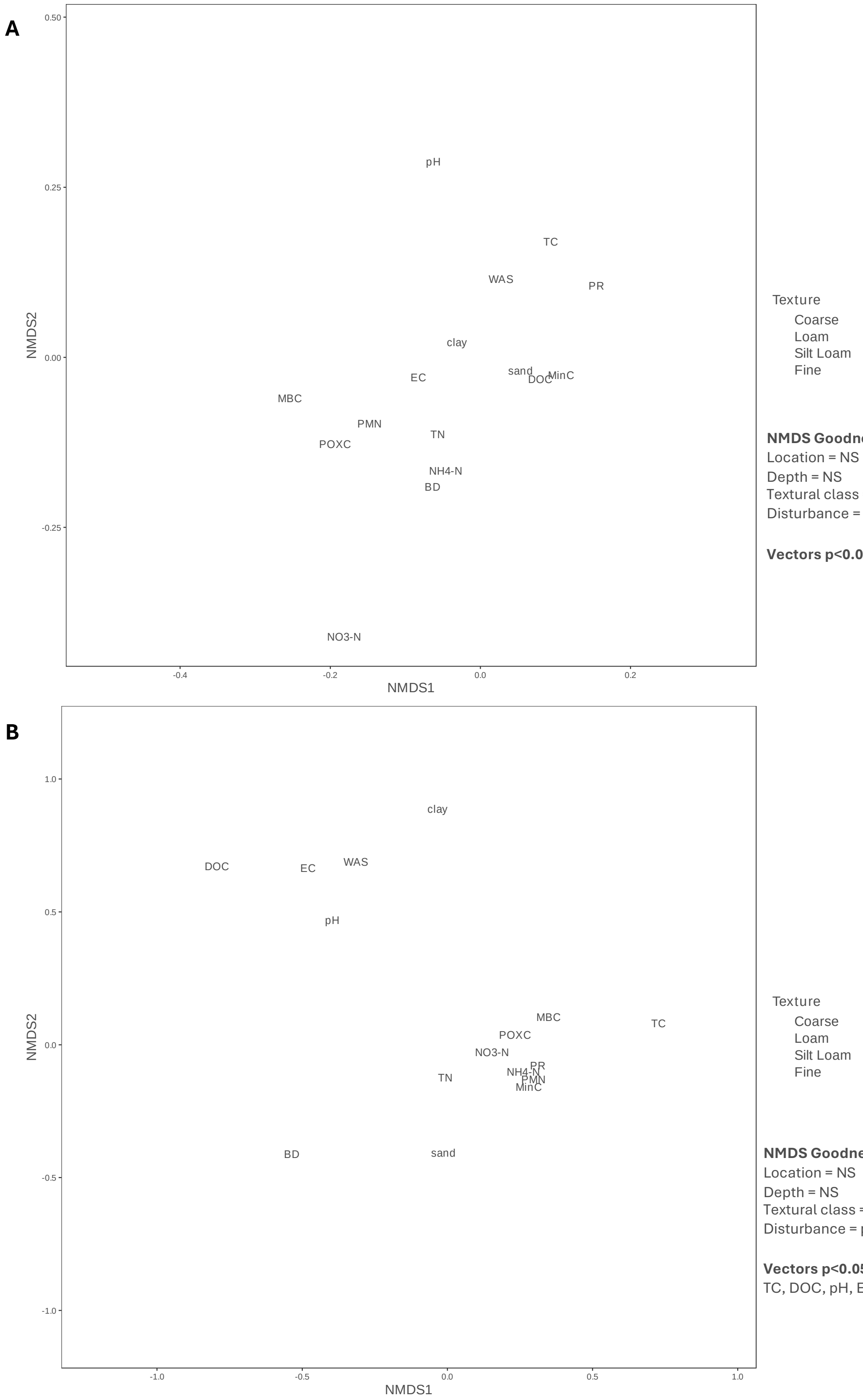


Figure 3.2A. NMDS of 16S rDNA (A) and ITS (B) Bray-Curtis dissimilarity (circles) and soil health variables (vectors) by Disturbance level of No-Till (blue) and Till (yellow).



NMDS Goodness of fit 16S
Location = NS
Depth = NS
Textural class = NS
Disturbance = NS

Vectors p<0.05: NO_3-N

NMDS Goodness of fit ITS
Location = NS
Depth = NS
Textural class = $p<0.05$
Disturbance = $p<0.05$

Vectors p<0.05:
TC, DOC, pH, EC, WAS, BD, clay

Figure 3.3A. NMDS of 16S rDNA (A) and ITS (B) Bray-Curtis dissimilarity (circles) and soil health variables (vectors) by soil texture levels coarse (yellow), loam (blue), silt loam (gray) and fine (green).

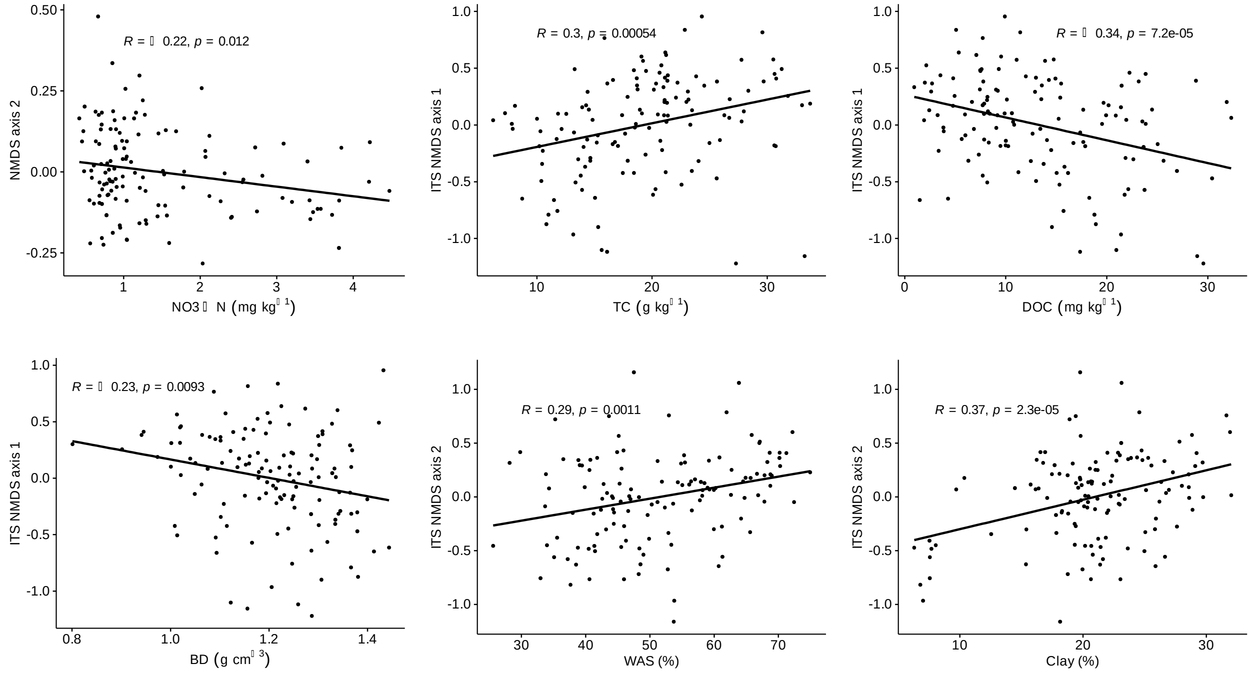


Figure 3.4A. Regressions of NMDS Scores and significant soil indicators from NMDS to explain potential soil drivers of microbiome

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