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ISBN

9798189233296

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Publication Date

2026-06-25

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Unraveling AM Fungal-Soil Interactions in Wine Grapes: Effects of Soil Properties, Phenology,
and Management Practices in Vineyards of the Western USA

By

AMANDA N. RODRIGUEZ
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Agricultural and Environmental Chemistry

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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Abstract

Arbuscular mycorrhizal (AM) fungi are well-known microbial symbionts that support nutrient and water uptake, soil structure, and ecosystem resilience. Although research across diverse cropping systems has advanced our understanding of AM fungi, their dynamics across environmental gradients, phenological stages, and management practices remain poorly understood in perennial woody crops such as grapevines. This dissertation employs amplicon sequencing and microscopy to evaluate AM fungal colonization, diversity, and community composition in western U.S. vineyards. The first chapter surveyed 11 vineyards spanning a broad edaphoclimatic gradient from Oregon to California's Central Coast, comparing AM fungal communities across regions, rootstocks, and scions. Our findings suggest that AM fungal communities in grapevines are structured at multiple scales, where broad environmental gradients establish baseline differences in community composition and plant-level traits may refine patterns of taxon abundance. The second chapter investigated AM fungal colonization and community composition across five phenological stages (bud break, bloom, veraison, harvest, and dormancy) in two vineyard sites cultivating different scion-rootstock combinations. AM fungal colonization and community composition varied across phenological stages and between scion-rootstock combinations, with patterns likely driven by host physiological responses to site conditions and phenological development. The third chapter evaluated the short-term effects of regenerative agriculture practices, including no-till, compost application, and sheep grazing on AM fungal colonization, diversity, and community composition in the same vineyard sites as chapter two. Site characteristics, including soil physicochemical properties and scion-rootstock identity, were stronger drivers of AM fungal communities than the practices themselves, with treatment responses varying between sites. Overall, these studies illustrate the importance of considering site-level characteristics and scion-rootstock identity in shaping AM fungal symbiosis, advancing our understanding of the factors that shape these communities in commercial vineyards.

Introduction

Soil biodiversity and plant-microbial interactions are considered to be important outcomes for evaluating the effects of field management practices in sustainable agriculture. Soil microorganisms play pivotal roles in terrestrial ecosystems, performing essential functions such as nutrient cycling, organic matter decomposition, and nutrient release (Jansson et al. 2023; Khangura et al. 2023). Through their diverse metabolic activities and symbiotic associations with plants, soil microorganisms contribute significantly to the overall resilience and productivity of agricultural systems (Jansson et al. 2023; Khangura et al. 2023). As agriculture evolves toward more sustainable practices, understanding and harnessing soil biodiversity and plant-microbial interactions become central to achieving this goal (Jackson et al. 2005; Khangura et al. 2023).

Among the array of microbial interactions, arbuscular mycorrhizal (AM) fungi are a well-known example of microbial symbiosis in agriculture, forming mutualistic associations with over 80% of terrestrial plants (Lee et al. 2013; Smith and Read 2008). Through these partnerships, AM fungi improve nutrient acquisition, water uptake, and stress tolerance in their hosts in exchange for plant-derived carbohydrates and lipids necessary for their survival, growth, and reproduction (Schreiner and Linderman 2005; Smith and Read 2008; Vieira et al. 2020). These benefits, however, can vary with the composition and abundance of AM fungal communities, which are shaped by edaphoclimatic conditions, host plant identity, and agricultural management practices. Yet the influence of these factors on AM fungal communities has been predominantly explored in natural and annual cropping systems, with comparatively less attention given to perennial, woody crops such as grapevines.

Vineyards represent a particularly compelling system for examining these interactions, as grapevines are one of the most economically important perennial fruit crops globally (Alston and Sambucci 2019). Across wine-growing regions, grapevines are cultivated under a diverse range of

edaphoclimatic conditions, varying in temperature, precipitation, soil texture, and fertility, offering a unique opportunity to examine how these factors shape AM fungal communities. In most commercial vineyards, grapevine scions are grafted onto rootstocks that differ in root architecture, drought tolerance, and nutrient acquisition (Ollat et al. 2016). Variation in these traits modifies the soil-root interface, influencing both AM fungal colonization rates and community composition (Lailheugue et al. 2024; Moukarzel et al. 2021; Noceto et al. 2024; Philippot et al. 2013). Scion identity can further influence AM fungal community by shaping the quantity and composition of photosynthates delivered to the root system (Lailheugue et al. 2024; Noceto et al. 2024). Seasonal changes in grapevine growth can further alter photosynthate delivery to roots, a dynamic that has been observed to drive changes in AM fungal communities in other plant systems (Barnes et al. 2016; Bohrer et al. 2004; Buade et al. 2021; Goldmann et al. 2020). However, the extent to which these shifts influence grapevine-AM fungal symbiosis throughout the growing season remains poorly understood.

Agricultural management practices represent another layer of complexity shaping AM fungal communities in vineyards. Practices, such as no-till, compost application, and livestock grazing, are increasingly being adopted in viticulture to support soil health and biodiversity (Lazcano et al. 2020). While the effects of these practices on AM fungal communities have been studied individually (Cavagnaro 2015; Freitas et al. 2011; Kabir 2005), growers rarely implement them in isolation, and the effects of their combined application on AM fungal communities remain poorly understood. As growers increasingly seek to harness AM fungal symbiosis to support vine health and productivity, understanding how these factors collectively influence grapevine-AM fungal symbiosis is critical to leveraging this relationship in commercial vineyards.

To address these knowledge gaps, this dissertation employs amplicon sequencing and microscopy to evaluate AM fungal colonization, diversity, and community composition in western U.S.

vineyards. The first chapter surveyed 11 vineyards spanning a broad edaphoclimatic gradient from Oregon to California's Central Coast, comparing AM fungal communities across regions, rootstocks, and scions. The second chapter investigated AM fungal colonization and community composition across five phenological stages (bud break, bloom, veraison, harvest, and dormancy) in two vineyard sites cultivating different scion-rootstock combinations. The third chapter evaluated the short-term effects of regenerative agriculture practices, including no-till, compost application, and sheep grazing on AM fungal colonization, diversity, and community composition in the same vineyard sites as chapter two. Collectively, this work contributes to a more integrated understanding of the drivers of AM fungal communities in commercial vineyards, providing growers with a framework for promoting grapevine-AM fungal symbiosis across diverse viticultural systems.

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Chapter 1: Arbuscular Mycorrhizal Fungal Communities in Wine Grapes Along an Edaphoclimatic Gradient of the Western USA

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Abstract

Background and Aims: Arbuscular mycorrhizal (AM) fungi are well-known plant microbial symbionts, and in winegrapes have been found to support vine health and fruit development while maintaining fruit quality. Because AM fungal diversity, colonization rates, and community composition are shaped by both environmental conditions (i.e., variation in climate, soil properties) and host traits (i.e., grapevine scion and rootstock genotype), understanding how these factors interact is important for interpreting patterns observed across vineyard systems. To address this, we surveyed 11 vineyards spanning a broad edaphoclimatic gradient from Oregon to California's Central Coast, comparing communities across regions, rootstocks, and scions.

Methods: AM fungal diversity and root colonization were assessed from grapevine roots via ITS-2 amplicon sequencing and microscopy, respectively. Soil properties, vine nutrient status, and yield metrics were measured to evaluate environmental and plant drivers of community structure.

Results: Oregon vineyards (cool, high-rainfall, fine-textured soils) supported higher AM fungal diversity than California vineyards (warm, dry, coarse soils), whereas colonization was highest in Central Coast

California. Genotype-environment interactions were further associated with shifts in the relative abundance of dominant taxa. These effects were driven more strongly by rootstock identity than by scion, as AM fungal diversity and colonization did not differ significantly between scions grown on the same rootstock under similar conditions. Rootstock-environment combinations (e.g., 101-14 in Oregon) that may have reduced vine stress supported more diverse and even AM fungal communities.

Conclusion: Our results show that edaphoclimatic gradients establish baseline AM fungal community structure, while plant-level interactions refine taxon abundance within regions.

1. Introduction

Arbuscular mycorrhizal (AM) fungi are obligate biotrophs that form mutualistic associations with over 80% of terrestrial plants (Lee et al. 2013; Smith and Read 2008). Present in the soil as spores and hyphae, AM fungi colonize host roots and develop arbuscules, which are highly branched intracellular structures that facilitate nutrient exchange between the fungus and plant (Lee et al. 2013; Vieira et al. 2020). In exchange for plant-derived carbohydrates and lipids necessary for their survival, growth, and reproduction, AM fungi improve nutrient acquisition, water uptake, and stress tolerance in their hosts, thus enhancing plant productivity (Schreiner and Linderman 2005; Smith and Read 2008; Vieira et al. 2020). AM fungi are widely distributed across terrestrial ecosystems, and their abundance and diversity are influenced by biotic and abiotic factors, such as soil properties, geographic location, and host plant identity (Kivlin et al. 2021; Öpik et al. 2013; Schreiner 2005; Vieira et al. 2020). At global scales, AM fungal communities exhibit high turnover, with limited overlap in taxa present at different sites (Kivlin et al. 2021). This turnover reflects close associations between AM fungal taxa and local environmental conditions, such as soil fertility, temperature, and moisture (de Mello et al. 2018; Kivlin et al. 2021). Understanding how these environmental and biological factors interact to shape AM fungal communities is especially important in agricultural systems, where soil management, climate, and host genotype collectively influence AM fungal composition and their influence on crop performance (Benami et al. 2020).

Vineyards represent a particularly compelling system for examining these interactions, as grapevines are one of the most economically important perennial fruit crops globally (Alston and Sambucci 2019). Grapevines (*Vitis vinifera* subsp. *vinifera* L.) are characterized by low root density compared to other crops (Schreiner and Linderman 2005) and relatively large-diameter fine roots (Richards 1983), which are traits associated with plants that benefit most from AM fungal symbiosis (Hetrick 1991; Schweiger et al. 1995). These fungi enhance plant nutrient and water uptake and improve stress tolerance in nutrient-poor and water-limited environments typical of many viticultural regions (Augé 2001; Van Der Heijden 1998b). This symbiosis varies substantially across vineyards, with colonization rates ranging from 24-94% (Betancur-Agudelo et al. 2021; Biasi et al. 2023; Likar et al. 2013; Schreiner 2020; Schreiner et al. 2007) and Shannon diversity from 1.6-2.5 (Hernandez and Mendez 2019; Oehl and Koch 2018). The biological and environmental drivers underlying this variability, however, remain poorly understood across vineyard systems.

Across wine growing regions, grapevines are cultivated in a diverse range of soil conditions, from weathered, clay-rich soils to loamy alluvial soils that vary in pH, fertility, and texture. These soil chemical and physical characteristics are well-documented drivers of AM fungal communities, influencing both the availability of nutrients for the host plant and the suitability of the habitat for fungal symbionts (Vieira et al. 2020; Kivlin et al. 2011; Moebius-Clune et al. 2013; Jamiołkowska et al. 2018; Porter et al. 1987). Soil fertility, particularly phosphorus (P) availability, is an important driver of AM fungal colonization and diversity. Root colonization is typically highest at low to moderate nutrient availability and declines as inorganic nitrogen (N) and P inputs increase (Fall et al. 2022; Mirzaei and Moradi 2017). Under elevated concentrations of plant-available N and P, plants allocate fewer carbohydrates to AM fungi, which reduces spore production and hyphal growth (Fall et al. 2022). AM fungal diversity often shows a similar pattern, with lower species richness reported in high-P soils compared to P-limited soils (Mirzaei and Moradi 2017), though effects of nutrient availability on evenness remain poorly characterized. Climate can further shape nutrient dynamics and their influence on AM fungal communities by changing soil moisture and organic matter (OM) accumulation (da Silva et al. 2014; Hazard et al. 2013; Xiang et al. 2016). In regions with higher precipitation, higher soil moisture often supports greater soil OM accumulation (Gill et al. 1999), which can increase nutrient availability and suppress AM fungal colonization. However, the effects of OM

on AM fungal communities are nuanced, as higher OM can also enhance microbial activity and root abundance, creating conditions that promote AM fungal colonization and diversity (Gill et al. 1999). In arid regions, slower OM accumulation can limit microbial activity and root biomass, which can constrain AM fungal colonization and diversity (Gill et al. 1999). Low moisture can further limit AM fungal propagule production, as sporulation is often reduced in dry systems (Gai et al 2006). Temperature exerts an additional influence on AM fungal communities, as colonization is highest under moderate growing-season temperatures (~19.5 °C) and declines in both cooler (<15 °C) and hotter (>30 °C) conditions (Soudzilovskaia et al. 2015). While most studies examining climate effects on AM fungal communities have focused on natural systems, how climate interacts with soil properties to shape AM fungal communities across diverse wine-growing regions remains poorly understood.

In addition to fertility, soil pH is another significant factor influencing AM fungal symbiosis, as colonization typically occurs across a pH range of 4.5 to 7.5, but declines when soils are outside of this range due to effects on spore germination and survival, hyphal growth, root penetration, and new propagule production (Clark and Zeto 1996; Coughlan et al. 2000; Jamiołkowska et al. 2018). AM fungal diversity follows a similar trend, with fewer taxa in acidic soils and more taxa appearing as pH increases, reflecting differences in pH tolerance among individual fungal taxa (Clark and Zeto 1996; Coughlan et al. 2000). Soil texture further influences AM fungal symbiosis by affecting both colonization and diversity by regulating pore structure, aeration, and growth (Aker et al. 2022; Mirzaei and Moradi 2017; Vieira et al. 2020). Coarse-textured, sandy soils often support higher colonization and number of taxa than fine-textured, clay-rich soils, which is hypothesized to be due to greater porosity facilitating root elongation, hyphal growth, and the distribution of fungal propagules (Aker et al. 2022). Despite a growing knowledge of how soil chemical and physical properties shape AM fungal communities, the extent to which these patterns are consistent across large edaphoclimatic gradients characteristic of wine-growing regions remains poorly understood. Understanding these interactions can help interpret broader patterns in AM fungal distribution across vineyards spanning large geographic distances and cultivated with different scion-rootstock combinations, hereafter referred to as grapevine genotype.

In most commercial vineyards worldwide, grapevine scions are grafted onto a rootstock, which comprises the root system and serves as the link between the soil environment and the vine (Ollat et al. 2015). Rootstocks

are selected by growers for their performance under specific soil conditions, disease pressure, and climates (Koepke and Dhingra 2013; Warschefsky et al. 2016). Rootstock identity has been shown to strongly influence vine performance under varying environmental conditions, and are hypothesized to be tied to breeding parentage (Chen et al. 2024; Moukarzel et al. 2021; Moukarzel et al. 2022; Ollat et al. 2016). For example, the 101-14 rootstock, derived from *V. rupestris* and *V. riparia* parentage, is suited to moist, fertile soils but performs poorly in coarse-textured soils and under drought and salinity stress due to its shallower root system (Chen et al. 2024; Christensen 2003; Moukarzel et al. 2022; Pongracz 1983). In contrast, the 140R rootstock, derived from *V. berlandieri* and *V. rupestris*, is well-suited to Mediterranean climates and is often selected for its tolerance to drought, salinity, and lime due to its deeper root system (Bavaresco et al. 1992; Chen et al. 2024; Lambert et al. 2008; Song et al. 2015). These differences in root system architecture among rootstocks, as well as additional variance in carbon allocation and root exudates, can modify the soil-root environment and influence AM fungal symbiosis with grapevines (Lailheugue et al. 2024; Moukarzel et al. 2021; Philippot et al. 2013; Warschefsky et al. 2016). Rootstock identity has been shown to strongly influence AM fungal associations, with distinct fungal assemblages observed across rootstocks (Chen et al. 2024; Moukarzel et al. 2021; Moukarzel et al. 2022; Ollat et al. 2016). Studies on grapevines demonstrate that rootstock genotypes can differ in rhizodeposition, including the amount and composition of root exudates, and have been linked to variation in AM fungal colonization and the recruitment of specific AM fungal taxa (Lailheugue et al. 2024; Philippot et al. 2013). Studies comparing multiple rootstocks grown under the same soil and climate conditions further show that root system traits and stress responses contribute to distinct AM fungal assemblages even in shared environments (Moukarzel et al. 2021). Scion can also influence AM fungal communities by shaping the quantity and composition of photosynthates and signaling compounds delivered to the root system, though these effects are generally modest relative to those of the rootstock and the environment (Lailheugue et al. 2024). Together, these findings highlight that scion-rootstock interactions with the environment may be central in shaping grapevine-AM fungal associations in commercial vineyards. Knowledge of how grapevine genotype, soil, and climate collectively drive these associations across diverse viticultural regions is, therefore, critical for leveraging AM fungal symbiosis in vineyards, yet remains poorly understood.

To address this, we investigated how soil properties, climate, and grapevine genotype jointly shape AM fungal diversity and abundance in grapevines (*Vitis vinifera* subsp. *vinifera* L.) grafted onto multiple rootstocks sampled across 11 vineyards and spanning a 1,300 km edaphoclimatic gradient along the U.S. West Coast. Specifically, our objectives were to: (1) characterize AM fungal abundance and diversity across regions and vineyards; (2) evaluate the influence of soil properties on AM fungal community structure; (3) assess the interactive effects of winegrape genotype and environment on AM fungal community structure; and (4) evaluate correlations between AM fungal community structure and vine nutrition across vineyards. Low-to-moderate fertility soils, neutral pH, and coarser textures were predicted to support greater colonization rates, while AM fungal diversity was expected to be highest in vineyards with greater soil moisture. We also expected soil fertility (nitrate [NO₃⁻], ammonium [NH₄⁺], phosphate [PO₄³⁻] concentration), pH, and texture to be the primary drivers of AM fungal communities, with fertility and pH more strongly influencing diversity and texture being a more important determinant of colonization rates. Finally, we hypothesized that broad differences in AM fungal community structure might correspond with variation in vine nutrient status.

To understand how the environment and the vine work together to shape these communities, we compared 1) a single scion-rootstock combination grown across contrasting environments (Pinot Noir grafted to 101-14 in Oregon and Northern Coast California), 2) different scions grafted onto the same rootstock under comparable environmental conditions (Pinot Noir vs. Chardonnay on 101-14 in Oregon); and 3) different scion-rootstock combinations under comparable environmental conditions (Pinot Noir on 101-14 vs. Chardonnay on 140R in Northern Coast California). Based on known differences in rootstock parentage, root system architecture, and stress tolerance, we hypothesize that grapevines whose rootstock traits aligned with local soil and climate would support higher colonization and more diverse AM fungal communities. In contrast, grapevines whose rootstock traits were less suited to site conditions would experience greater stress and invest less in fungal partners. For example, rootstocks with deeper, more drought-tolerant root architectures (i.e., 140R) were expected to support richer AM fungal communities in warmer, drier Northern Coast California vineyards. Alternatively, shallower, less drought-tolerant rootstocks (i.e., 101-14) were expected to support more diverse AM fungal communities in wetter, more fertile Oregon vineyards than in California. We also hypothesized that scion

effects would not contribute significantly to differences in AM fungal communities compared to the influence of rootstock and the environment. Together, these contrasts provide a framework for assessing the importance of environmental gradients, rootstock identity, and scion identity in shaping AM fungal communities in vineyards, which winegrape growers can use to promote this symbiosis in different regions.

2. Material and Methods

2.1 Study design

This study examined eleven commercial vineyards planted on red and white grape varieties (*Vitis vinifera* L.) established across three geographical regions (i.e., Oregon, Northern Coast California, and Central Coast California) in Western USA (Fig. 1.1). Thirty-year (1991-2020) precipitation and temperature normal values were obtained for each location using the PRISM dataset at an 800 m resolution. Precipitation normals increased along a latitudinal gradient, from Central Coast CA (34.4 cm) to Oregon (130.8 cm), while temperature normals decreased from 14.9 °C to 11.2 °C. Across regions, soils also differed in their physical and developmental characteristics, with Oregon vineyards planted on more weathered, clay-rich soils, Northern Coast California sites on younger alluvial and moderately developed soils, and Central Coast California vineyards on loamy, moderately developed soils. Vineyard age also varied by region, with Oregon sites representing younger vineyards (8 years), Northern Coast California including mid-aged vineyards (5-15 years), and Central Coast California consisting of older, long-established plantings (16-29 years). The study included six rootstocks and three grape varieties cultivated across the eleven sites, representing each region's main rootstocks and grape varieties (Supplementary Information, Table S1.1).

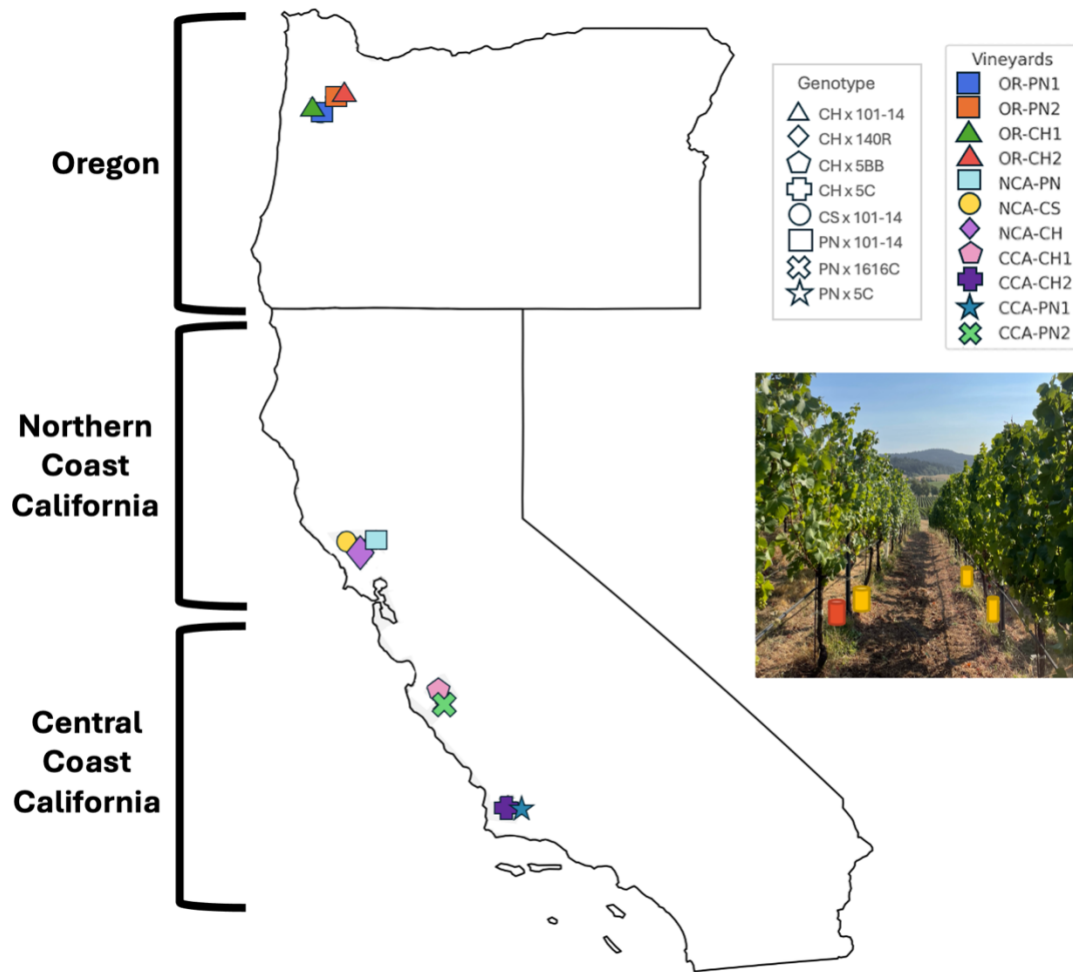


Fig. 1.1 Map of study vineyard locations across three regions along the U.S. West Coast (i.e. Oregon, Northern Coast California, and Central Coast California). Shapes represent grapevine genotypes (scion x rootstock), and colors denote individual vineyard sites. Scion abbreviations are PN = Pinot Noir, CH = Chardonnay, and CS = Cabernet Sauvignon. The inset photo shows a representative vineyard row with a sampling setup, where red cylinders mark soil sampling locations and yellow cylinders indicate root sampling locations

Vineyards were managed according to commercial practices that reflected local growing conditions. To our knowledge, bioinoculants have not been previously applied to any of the vineyards examined in this study. In Oregon, vineyards were planted at high densities ($\approx 6,050$ - $6,250$ vines ha^{-1} ; 1.60 - 1.65 m x 1.00 m spacing) and trained to vertical shoot-positioned (VSP) trellising systems. Vineyards cultivated Pinot Noir (clone 667) or Chardonnay (clone 76) varieties grafted onto the 101-14 rootstock. All sites maintained cover crops, used alternate

tillage (i.e. every other year), and applied herbicides under the vine and foliar fungicides. Drip irrigation was applied from late spring through early fall, typically supplying 8-23 L vine⁻¹ week⁻¹, depending on seasonal water status. Nutrient programs combined annual foliar sprays of boron (B), magnesium (Mg), zinc (Zn), and N with fertigation supplying nitrate (NO₃⁻) and calcium (Ca) at 23-93 L ha⁻¹.

In Northern Coast California, vineyards were planted at moderate densities ($\approx$ 3,077-5,381 vines ha⁻¹; 1.5-2.1 m x 1.2-1.5 m spacing) and trained to VSP trellis systems. Cultivated red varieties included Pinot Noir (clone 667) or Cabernet Sauvignon (clone 337) grafted onto 101-14 rootstock, while white varieties included Chardonnay (clone 4) grafted onto the 140R rootstock. Cover crops, herbicide use under the vine, and foliar fungicide applications were standard practices across vineyards. Tillage practices varied by vineyards, with NCA-PN managed under no-till while NCA-CH and NCA-CS used alternate tillage. Drip irrigation supplied approximately 15-23 L vine⁻¹ week⁻¹ for approximately four months each season. Calcium, ammonium (NH₄⁺), and NO₃⁻ were applied via fertigation at a rate of 28-93 L ha⁻¹, with specific rates adjusted based on foliar nutrient analysis.

In Central Coast California, vineyards exhibited the broadest range of planting densities ($\approx$ 2,241-8,970 vines ha⁻¹; 0.9-2.4 m spacing) and were also trained to VSP trellis systems. Cultivated varieties included Pinot Noir (clone 667) grafted on 5C or 1616C rootstocks, as well as Chardonnay (clones SM4 or 96) grafted onto 5BB or 5C rootstocks. All sites used cover crops, foliar fungicides, and either alternate or full tillage. Herbicides were applied in the vine row for all vineyards in Central Coast California other than in CCA-CH1. Drip irrigation was applied for four to five months, supplying 11-61 L vine⁻¹ week⁻¹ depending on vine spacing and seasonal conditions. Nutrient management included micronutrient foliar sprays and one to two fertigation events per year, supplying Ca, NH₄⁺, NO₃⁻, boron (B), zinc (Zn), potassium (K), sulfate (SO₄²⁻), and phosphate (PO₄³⁻).

2.2 Plant and soil sampling

Before sampling, in spring 2022, we set up between 12-18 experimental plots in each vineyard. A plot was defined as four adjacent vine rows with at least 60 vines per row. Plots were arranged in three blocks, with each block comprising 4-6 plots. From each block, we selected one plot for sampling, giving a total of three sampling plots per vineyard. Soil samples were collected from a single location within each plot, and root samples were from three vines in the same plot (Fig. 1.1).

In March 2022, at budbreak, soil samples (0-30 cm) were collected from the vine row of each sampled plot using a 7.62 cm diameter soil core, yielding approximately 1,370 cm³ of soil per core and a total of 33 soil samples (3 samples x 11 vineyards). In July 2022, three vines were selected from three sampling plots within each vineyard for root and leaf blade sampling. This sampling coincided with grapevine veraison, a stage marking the onset of berry ripening and color change, when grapevines have absorbed the majority of essential soil nutrients. Lateral roots were excavated from the surface soil (0-30 cm) adjacent to the rootstock crown. For each vine, fine root material was collected to fill approximately one-quarter of a standard sandwich-sized polyethylene bag (16.5 x 14.9 cm), providing sufficient tissue for quantification of AM fungal colonization and for DNA extraction for AM fungal community sequencing. A total of 99 root samples were collected across all vineyards (9 vines/vineyard x 11 vineyards). Soil and root samples were placed in polyethylene bags and stored in a 2 °C cold room until processing. Leaf samples were collected from the same node as a fruit cluster and from the upper segment of the shoot to assess vine nutrient status. For each sampling plot, 50 leaf blades were collected and composited (Schreiner and Skinkis 2019), resulting in 33 composite leaf samples (3 samples x 11 vineyards). Leaf samples were then dried at 55 °C and sent to the UC Davis Analytical Lab for analysis of macro (N, P, potassium [K], calcium [Ca], sulfur [S], Mg) and micronutrient (Zn, manganese [Mn], iron [Fe], and copper [Cu]) contents. Nutrient concentrations were determined following nitric acid/hydrogen peroxide microwave digestion and determination by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Meyer and Keliher 1992).

Harvest was conducted jointly by research and vineyard staff between August and September 2022, when fruit reached 23-24 °Brix for red varieties and 22-23 °Brix for Chardonnay. Yield was determined from ten selected vines within each sampling plot by counting all fruit clusters on each vine and recording total cluster weight.

2.3 Analysis of AM fungal abundance and diversity in vine roots

After transport to the laboratory, fine roots were cleaned using a sequential rinsing process to remove adhering soil particles. Initially, roots were rinsed under a stream of DI water to remove large, loosely attached soil particles. Soil tightly adhered to roots was then removed using forceps and a wash bottle. Cleaned roots were then divided into two subsamples: 1) stored in 70% ethanol at 4 °C for root staining; 2) at -80 °C for DNA analysis to

assess AM fungal diversity. Samples preserved in ethanol were stored at 4 °C for approximately six months prior to staining, and frozen subsamples were stored at -80 °C for approximately ten months before DNA extraction.

Grapevine roots preserved in 70% ethanol at 4°C were cleared and stained following Schreiner (2003). AM fungal colonization was quantified using a gridline intersect method at 100x magnification on a Leica DM1000 LED compound microscope. For each sample, stained roots were examined at 100 intersections. At each intersect, the presence or absence of AM fungal structures (i.e., hyphae, arbuscules, and vesicles) crossing the vertical crosshair was recorded. Percent colonization was expressed as the percentage of intersects with AM fungal structures, calculated: $[1 - (\text{intersects with no AM fungal structures} / \text{total intersects})] \times 100$.

In order to characterize arbuscular mycorrhizal community structure, DNA was extracted from roots using DNeasy Plant Pro Kits (Qiagen, Germantown, MD, United States). Amplicon sequencing targeting the fungal internal transcribed spacer region (ITS-2) was performed at the University of California-Davis Host-Microbe Systems Biology Core Facility using the NexF-N3-5.8S_fun and nexR-N3-ITS2_fun primers of variable lengths (Taylor et al. 2016). Libraries were prepared using a two-step PCR analysis. The first PCR step involved an initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation, annealing, and extension, and final storage at 12°C. To incorporate molecular barcodes and Illumina adapters, a second PCR step was carried out, consisting of 8 additional cycles under similar thermal cycling conditions using dual-indexed Nextera XT primers. Amplicons were pooled and quantified for paired-end sequencing (2 x 300 bp) on an Element AVITI instrument (Element Biosciences, San Diego, CA, United States).

After sequencing, raw FASTQ data were processed using the QIIME2 pipeline (version 2023.9; <http://qiime2.org/>). Taxonomic assignment was performed using the QIIME2 feature-classifier plugin trained on the UNITE ITS-2 reference database (Abarenkov et al. 2024). The resulting operational taxonomic unit (OTU) and taxonomy tables were imported into R statistical software and analyzed using the *phyloseq* package (McMurdie and Holmes 2013). All data were filtered to retain only taxa assigned to the phylum Glomeromycota, and downstream analyses were restricted to this group. Alpha diversity metrics, including observed species richness, Chao 1 richness, relative abundance, evenness, and the Shannon diversity index, were used to assess AM fungal community diversity. Observed and Chao1 richness values were identical, so observed richness was reported.

Analyses and interpretation were conducted at the genus level, with taxa of uncertain phylogenetic placement labeled as *gen. incertae sedis* according to the classifier. These designations reflect varying levels of confidence, with some taxa placed to the family level (*Glomeraceae*, *Paraglomeraceae*, *Pervetustaceae*, and *Claroideoglomeraceae*), the order level (*Glomerales* and *Paraglomerales*), or the phylum level (*Glomeromycota*).

2.4 Soil analysis

After collection, soil samples underwent an initial sieving process to 8 mm. Gravimetric soil moisture was measured by oven-drying approximately 10 g of fresh soil at 105 °C for 24 hours. For microbial biomass carbon (MBC) estimation, dissolved organic carbon (DOC) was quantified from both fresh and chloroform-fumigated soil samples extracted with 0.5 M K₂SO₄ (Horwath and Paul 1994), using a Shimadzu TOC-L Total Organic Carbon Analyzer. Microbial biomass C was calculated as the difference in DOC concentrations between fresh and fumigated samples. Potentially mineralizable N (PMN) was determined using a 7-day anaerobic incubation protocol, adapted from Drinkwater et al. (2015). Approximately 8.0 g of fresh soil was combined with 10 mL of deionized water, flushed with nitrogen gas to establish anaerobic conditions, and incubated at 37 °C for 7 days. Both incubated samples and non-incubated controls (8.0 g of fresh soil) were extracted with 0.5 M K₂SO₄. Ammonium-N (NH₄⁺-N) concentrations in both sets of extracts were determined colorimetrically using a modified protocol from Doane and Horwath (2003). Potentially mineralizable N was calculated as the difference in NH₄⁺-N concentrations between incubated and non-incubated samples. Nitrate-N (NO₃⁻-N) concentrations were determined colorimetrically from control samples (Doane and Horwath). Following these analyses, the remaining soil was sieved to 2 mm and air-dried for subsequent physicochemical measurements.

Phosphate-phosphorus (PO₄⁻³-P) concentration was quantified by shaking 2.50 g air-dried soil with sodium bicarbonate (NaHCO₃), followed by colorimetric analysis of the filtered extract (Olsen et al. 1954). Permanganate oxidizable C (POXC) was determined colorimetrically following a revised protocol of Weil et al. (2003), in which 2.50 g of air-dried soil samples reacted with 2 M potassium permanganate solution. Absorbance of the resulting solution was measured using a spectrophotometer (Milton Roy, Houston, Texas, USA). All colorimetric analyses were performed using a Biotek SynergyTM HTX multi-mode microplate reader (BioTek Instruments, Winooski, VT, USA).

Mineralizable C was measured by rewetting 10g of air-dried soil to 50% water-holding capacity with DI water, followed by a 24-hour incubation and quantification of CO₂ production (mg CO₂-C kg⁻¹ soil hr⁻¹) using a LI-COR LI-850 CO₂/H₂O gas analyzer (Lincoln, NE, USA). Soil pH and electrical conductivity (EC) were measured in a 2:1 (soil: water) slurry using a Mettler Toledo pH/conductivity meter (Mettler Toledo, Columbus, Ohio, USA). Soil texture was determined by particle-size distribution analysis using the pipette method (Gee and Bauder 1996). Cation exchange capacity (CEC) was measured using ammonium acetate extraction and quantified using ICP-AES by the University of California-Davis (UCD) Analytical Lab. The University of California Davis Analytical Lab also determined soil micronutrient concentrations (K, sodium [Na], Ca, Mg, Zn, Mn, Cu, Fe) using the diethylenetriaminepentaacetic acid (DPTA) extraction method (Lindsay and Norvell 1978).

2.5 Data analysis

All statistical analyses were performed in R version 4.4.0 (R Core Team 2024). Normality of soil, climate, plant, and AM fungal variables was evaluated using the Shapiro-Wilk and Anderson-Darling tests. As all variables deviated from normality, group differences were assessed using non-parametric tests. Differences in soil, climate, plant, and AM fungal colonization rates and alpha diversity variables were evaluated using Kruskal-Wallis tests, with group comparisons conducted for region, vineyards, or grapevine genotype when applicable. When significant effects were detected, post hoc pairwise comparisons were performed using Dunn's test in the FSA package (v0.10.0) (Ogle et al. 2025). Vineyard and genotype comparisons of AM fungal alpha diversity and colonization accounted for block identity using the coin package (v 1.4.3). For regional comparisons, the limited number of vineyards per regions (n=3-4) precluded this correction, and unrestricted permutations were used. Principal Component Analysis (PCoA) was used to visualize differences in soil, climate, and plant variables across regions and vineyards. The envfit function in the vegan package (Oksanen et al. 2025) was used to identify the environmental variables most strongly associated with the ordination, ranking predictors by the strength of their correlations (R²) with the ordination axes based on permutation tests. Correlations among environmental variables, plant traits, and AM fungal alpha-diversity metrics were evaluated using Pearson correlation coefficients and visualized using the corrplot package (v0.95) (Wei and Simko 2024).

Beta diversity analyses were conducted using the vegan package (v2.6.10) (Oksanen et al. 2025). Permutational multivariate analysis of variance (PERMANOVA) was applied to Bray–Curtis dissimilarities of AM fungal OTUs to test for differences in community composition among regions, vineyards, and genotypes. Non-metric multidimensional scaling (NMDS), a rank-based ordination method that represents multivariate dissimilarities in a reduced-dimensional space, was performed on Bray-Curtis dissimilarities of AM fungal OTUs to visualize patterns in community structure. Environmental variables, including plant, soil, and climate factors, were fitted as vectors onto the NMDS ordination using permutation-based significance testing using the envfit function, which evaluates the strength of correlations (R^2) between each variable and the ordination axes to identify potential drivers of community patterns. SIMPER analysis was performed using the simper function in the vegan package to identify OTUs contributing most to dissimilarities in community composition between regions, vineyards, and genotypes. Variance Partitioning Analysis (VPA) used the varpart function in the vegan package to quantify the proportion of variation in Glomeromycota OTU abundance explained by edaphoclimatic properties, plant traits, and their shared effects. Predictor selection was performed using forward selection via the ordiR2step function in the vegan package, which identifies predictors that best explain OTU abundance while controlling for multicollinearity. Predictor variables for VPA were grouped into soil and climate properties and plant variables. Statistical significance was assessed via permutation tests.

Indicator species analysis was performed using the indicpecies package (v1.8.0) (De Cáceres and Legendre 2009) to identify AM fungal taxa significantly associated with specific regions, genotypes, or vineyard sites. Threshold Indicator Taxa Analysis (TITAN) was performed using the TITAN2 package (v2.4.3) (Baker et al. 2020) to determine directional responses of AM fungal OTUs to continuous gradients in soil properties and plant traits.

3. Results

3.1 Regional Variation in Soil, Climate, and Grapevine Properties

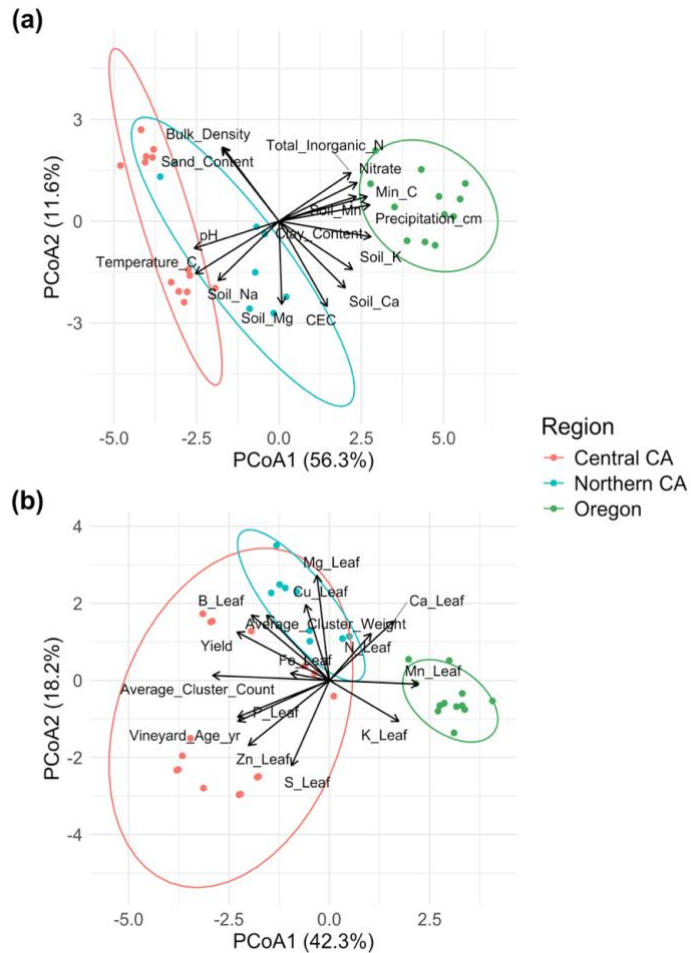


Fig. 1. 2 Principal coordinate analysis (PCoA) showing variability in in (a) soil and climate properties and (b) grapevine characteristics measured in the 11 commercial vineyards used in this study and located in three regions of the Western USA (Central California, Northern California and Oregon). Arrows depict the 15 variables most strongly associated with the ordination axes as determined by envfit R^2 values. Arrow length and direction reflect the strength and orientation of each variable with the PCoA axes. Ellipses represent 95% confidence intervals for each region

Soil and climate properties differed significantly among vineyard regions (Fig 1.2a; Supplementary Information, Table S1.2). A PERMANOVA confirmed significant regional differences in soil and climate variables ($p < 0.001$). Pairwise PERMANOVA tests showed that all regions differed significantly from one another ($p < 0.001$). Variation in edaphoclimatic conditions was primarily driven by differences in precipitation, temperature, soil texture (% clay, % sand), bulk density, CEC, and soil Ca. These variables displayed the largest absolute vector readings, reflecting the strongest associations with the PCoA axes (Table 1.1).

Soils collected in Oregon vineyards were characterized by more clay ($43.1 \pm 1.4\%$) and less sand ($11.6 \pm 1.1\%$) than soils in Central Coast ($17.9 \pm 0.7\%$ clay; $42.5 \pm 2.9\%$ sand) and Northern Coast CA ($24.8 \pm 1.3\%$ clay; $27.4 \pm 1.9\%$ sand). Oregon soils were also more acidic (pH 6.5 ± 0.05) than those in Central Coast (pH 7.7 ± 0.05) and Northern Coast CA (pH 7.4 ± 0.09). Central Coast CA exhibited the highest EC and sodium concentrations, indicating greater salinity levels.

Nutrient availability and soil C pools also varied significantly by region. Oregon soils exhibited the highest concentrations of NO_3^- -N, NH_4^+ -N, CEC, mineralizable C, MBC, and POXC, reflecting greater overall fertility and microbial activity (Supplementary Information, Table S1.2). In contrast, PO_4^{3-} -P availability was significantly higher in Central Coast CA and Northern Coast CA than in Oregon. Micronutrient concentrations showed region-specific trends, with elevated Fe observed in Oregon and Northern Coast California. Furthermore, Zn was significantly higher in Central Coast California (Supplementary Information, Table S1.2). Calcium, Mn, and K soil concentrations were higher in Central Coast California than in Oregon.

Table 1 Vector loadings and associated p-values of regional soil and climate variables on the first two principal coordinate axes (PCoA1 and PCoA2) from Figure 1.2. Loadings indicate the strength and direction of each variable's contribution to regional dissimilarities in soil and climate characteristics, with higher absolute loading values reflecting stronger influence on axis separation. All loadings and significance values were generated using the envfit function with 999 permutations. Abbreviations include: EC = electrical conductivity; PMN = potentially mineralizable nitrogen; MBC = microbial biomass carbon; POXC = permanganate oxidizable carbon; CEC = cation

exchange capacity; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; Na = sodium; Zn = zinc

Variable	PCoA1	PCoA2	p-value
Normal Precipitation (cm)	2.76	0.48	0.001
Normal Temperature (°C)	-2.53	-1.55	0.001
Bulk Density (g cm ⁻³)	-1.70	2.19	0.001
Clay Content (%)	2.79	-0.46	0.001
Sand Content (%)	-1.73	2.16	0.001
pH	-2.57	-0.80	0.001
EC (μS cm ⁻¹)	-0.61	-1.80	0.001
Nitrate (mg NO ₃ ⁻ -N kg ⁻¹ soil)	2.37	1.14	0.001
Ammonium (mg NH ₄ ⁺ -N kg ⁻¹ soil)	1.56	1.30	0.001
Total Inorganic N (mg N kg ⁻¹ soil)	2.18	1.43	0.001
PMN (mg PMN-N kg ⁻¹ soil)	1.53	-0.61	0.001
Phosphate (mg PO ₄ ³⁻ -P kg ⁻¹ soil)	-0.62	-2.12	0.001
Mineralizable C (mg C kg ⁻¹ soil * hr)	2.67	0.73	0.001
MBC (mg C kg ⁻¹ soil)	2.09	1.28	0.001
POXC (mg C kg ⁻¹ soil)	2.33	-0.25	0.001
CEC (meq/100g)	1.46	-2.51	0.001
Ca (meq/100g)	2.01	-1.97	0.001
Cu (mg Cu kg ⁻¹ soil)	-0.96	-0.29	0.005
Fe (mg Fe kg ⁻¹ soil)	1.83	-1.20	0.001
K (mg K kg ⁻¹ soil)	2.23	-1.42	0.001
Mg (meq/100g)	0.09	-2.45	0.001
Mn (mg Mn kg ⁻¹ soil)	2.34	0.73	0.001
Na (mg Na kg ⁻¹ soil)	-1.84	1.75	0.001
Zn (meq/100g)	-1.88	-0.39	0.001

Differences in grapevine properties were also evident (Supplementary Information, Table S1.3). A PCoA of leaf nutrient content and yield metrics showed clear regional separation (Fig. 1.2b). A PERMANOVA confirmed significant regional differences in plant nutrients and yield metrics ($p < 0.001$), and pairwise PERMANOVAs indicated that all regions significantly differed from one another (Central Coast vs. Northern Coast CA; Central Coast CA vs Oregon; Northern Coast CA vs. Oregon; $p < 0.001$ for each comparison). This separation was mainly driven by variation in yield metrics, vineyard age, and foliar nutrients (Mg, Zn, B, P, S, and Ca), which were identified as the top variables with the highest absolute vector loadings (Supplementary Information, Table S1.4). Because grape variety and rootstock combinations differ in their physiological characteristics and management requirements, plant traits were subsequently analyzed by genotype rather than by region.

3.2 Regional Variation in AM fungal alpha diversity and community composition

Across all samples, 207 AM fungal OTUs spanning 19 genera were detected. Vine roots in Central Coast California sampling locations hosted 93 OTUs across 14 genera, Northern Coast California had 96 OTUs across 15 genera, and Oregon had 99 OTUs across 10 genera. Among these, a core group of AM fungi was shared among all three regions, consisting entirely of taxa within the Glomeraceae family. This group was dominated by *Rhizophagus* (8 OTUs), followed by *Glomus* (3), *Microdominikia* (2), and *Glomeraceae gen incertae sedis* (2) (Fig 1.3d).

Observed AM fungal richness did not differ among regions (Kruskal-Wallis $p = 0.2$) (Fig. 1.3a; Supplementary Information, Table S1.5). Evenness varied significantly across regions ($p < 0.001$), with Oregon exhibiting the most balanced AM fungal communities (0.8 ± 0.01), compared to Northern Coast and Central Coast CA (both ~ 0.70). Evenness was significantly correlated with several edaphoclimatic and plant variables, with the strongest positive associations observed with total inorganic N, NO_3^- , precipitation, mineralizable C, soil Mn, and leaf micronutrients Ca and Mn, and the strongest negative associations with temperature, pH, EC, PO_4^{3-} , soil Na, as well as yield and average cluster count) (Supplementary Information, Table S1.6). Shannon diversity differed significantly among regions ($p = 0.012$), with Oregon supporting the most diverse AM fungal communities (2.0 ± 0.1), followed by Northern Coast CA (1.8 ± 0.1) and Central Coast CA (1.7 ± 0.1) (Fig. 1.3b). Consistent with these regional patterns, Shannon diversity was significantly correlated with numerous edaphoclimatic and plant variables, showing the strongest positive associations with precipitation, clay content, total inorganic N, mineralizable C, POXC, and leaf K and Mn, and the strongest negative associations with temperature, PO_4^{3-} , average cluster count, yield, and leaf Zn (Supplementary Information, Table S1.6).

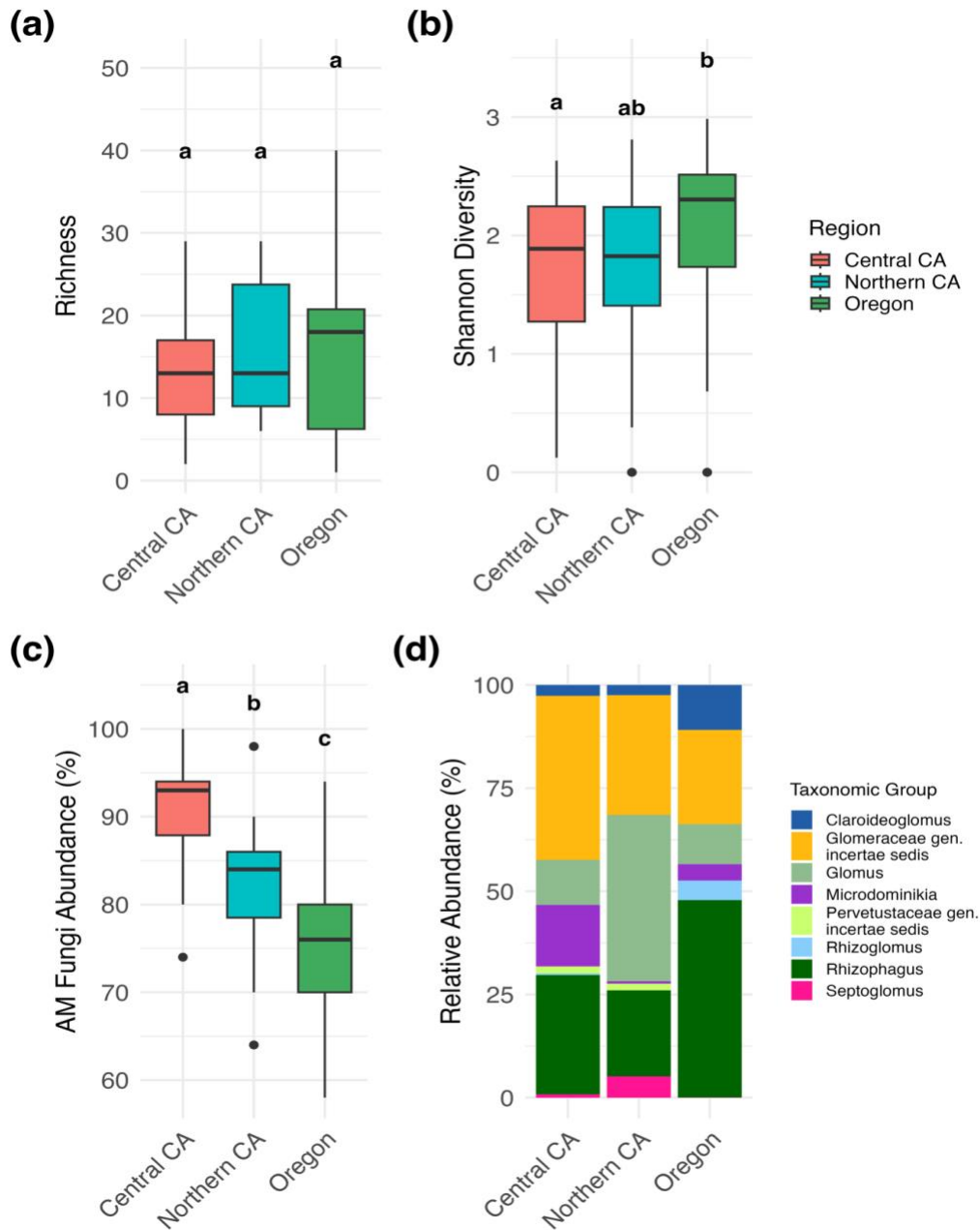


Fig 1.3 Regional patterns of arbuscular mycorrhizal (AM) fungal communities across vineyards. Boxplots show regional variation in AM fungal (a) richness, (b) Shannon diversity, and (c) AM fungi abundance (%). Panel (d) shows the relative abundance of the top 8 AM fungal genera in each region. Different letters above boxplots indicate significant differences between regions based on a Kruskal-Wallis test followed by Dunn's post hoc comparison

A different regional pattern emerged for AM fungal root colonization. Colonization differed significantly among regions (Kruskal-Wallis $p < 0.001$; Fig. 1.3c), with Central Coast CA exhibiting the highest colonization rate ($91 \pm 0.9\%$), followed by Northern Coast CA ($83 \pm 1.5\%$) and Oregon ($75.5 \pm 1.4\%$). Colonization rates were significantly correlated with several edaphoclimatic and plant variables (Supplementary Information, Table S1.6). The strongest positive associations were with temperature, pH, soil Na, and average cluster count, and the strongest negative associations were with precipitation, clay content, N-NO_3^{2-} , total inorganic N, mineralizable C, MBC, POXC, soil micronutrients (Ca, K, and Mn), and leaf micronutrients K and Mn.

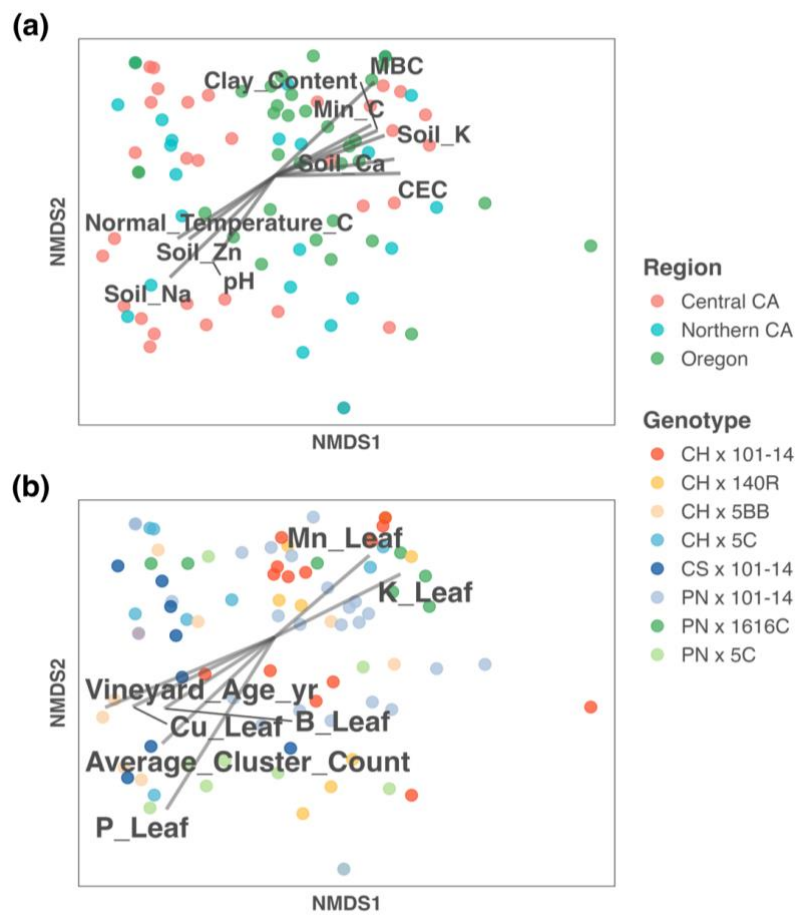


Fig. 1.4 Non-metric multidimensional scaling (NMDS) ordination of arbuscular mycorrhizal (AM) fungi community composition based on Bray-Curtis dissimilarity, with significant (a) soil and climate and (b) grapevine vectors ($p < 0.05$) overlaid using the envfit function. Points in (a) are colored by region to highlight broad-scale edaphoclimatic patterns, while points in (b) are colored by grapevine genotype as plant traits differ by variety and rootstock

combination. Arrow length and direction indicate the strength and orientation of correlations between variables and AM fungal community structure.

PERMANOVA analysis based on Bray-Curtis dissimilarity of Glomeromycota OTUs showed that region, rootstock, and scion significantly influenced AM fungal community composition ($p < 0.001$ for all factors). NMDS ordination was used to further visualize these differences, with separate envfit analysis conducted for soil/climate and plant variables (Fig. 1.4). Several environmental and plant variables were significantly correlated ($p < 0.05$) with AM community differences, including pH, MBC, CEC, mineralizable C, clay content, temperature, soil K, Ca, Na, and Zn, average cluster count, leaf P, K, B, Mn, Cu concentrations, and vineyard age (Fig. 1.4; Supplementary Information, Table S1.7 and S1.8).

SIMPER analysis was performed to identify the specific taxa driving regional differences in community composition, and TITAN2 was used to detect correlations between environmental gradients and shifts in AM fungal taxon abundance (Table 1.2). Northern Coast CA AM communities were enriched in *Glomus*, *Septoglomus*, and *Paraglomerales gen. incertae sedis*, while *Glomeraceae gen incertae sedis* and *Pervestaceae gen incertae sedis* were more prominent in Central Coast CA (Fig. 1.3d). In Oregon, *Rhizophagus*, *Claroideoglomus*, and *Rhizoglomus* dominated, with many rare or absent in Central Coast CA. *Dominikia* and *Claroideoglomeraceae gen incertae sedis* were also unique to Oregon when compared to Central Coast CA. Although differences between Northern Coast CA and Oregon were less pronounced, distinct patterns still emerged, with *Rhizoglomus* more abundant in Oregon and *Paraglomus* and *Paraglomerales gen incertae sedis* more prevalent in Northern Coast CA.

Table 2 Summary for SIMPER analysis results showing arbuscular mycorrhizal (AM) fungal taxonomic groups that contribute significantly to differences in community composition among vineyard regions. Listed are the regions where each taxonomic showed higher relative abundance, the contrasts in which those differences were significant, and their relative contribution (%) to total Bray-Curtis dissimilarity. Diss/SD indicates the consistency of each taxonomic group's contribution across sample comparisons, with higher values reflecting more uniform patterns of difference. Environmental variables associated with negative and positive responders are shown, where negative responders correspond to variables correlated with lower taxon abundance and positive responders correspond to variables correlated with higher taxon abundance. Indicator associations by region, vineyard, or genotype are also provided

Taxonomic Group	Region with greatest abundance	Significant Regional Contrasts	Contribution (%)	Diss/SD	Environmental variables (negative responders)	Environmental variables (positive responders)	Indicator Association (region, vineyard, and genotype)
<i>Glomus</i>	Northern CA	CCA-NCA**	23.0	0.9	---	---	NCA, CH x 140R
<i>Pervestaceae gen incertae sedis</i>	≈	CCA-NCA**	4.9	0.5	---	---	CCA-CH1, CCA-PN1, NCA-CS, CH x 5BB, CS x 101-14, and PN x 5C
<i>Septoglomus</i>	Northern CA	CCA-NCA**	3.2	0.3	Clay content, mineralizable C, POXC, MBC, soil Mn, soil Fe, soil Ca, leaf K	Temperature, sand content, pH, average cluster count	NCA, NCA-CS, CS x 101-14
<i>Paraglomerales gen. incertae sedis</i>	Northern CA	CCA-NCA**, NCA-OR*	2.3-2.4	~0.3	Soil Fe	---	NCA, NCA-CH, NCA-PN, CH x 140R
<i>Paraglomus</i>	Northern CA	CCA-NCA*, NCA-OR*	~0.3	0.3	---	---	NCA, NCA-CH, CH x 140R
<i>Rhizophagus</i>	Oregon	CCA-OR**	35.0	1.3	Temperature, pH, sand content,	Precipitation, clay content, mineralizable C,	OR

					average cluster count	POXC, nitrate, soil Ca, leaf K	
<i>Claroideoglossus</i>	Oregon	CCA-OR**, NCA-OR*	0.6-8.5	~0.7	Temperature, pH, sand content, soil Na, and average cluster count	Precipitation, clay content, mineralizable C, POXC, MBC, nitrate, soil Ca, soil Mn, leaf K	OR
<i>Rhizoglossus</i>	Oregon	CCA-OR**, NCA-OR	2.3-3.8	0.4	Temperature, pH, sand content, soil Na, and average cluster count	Precipitation, clay content, mineralizable C, POXC, MBC, nitrate	OR
<i>Claroideoglossaceae gen. incertae sedis</i>	Oregon	CCA-OR**	0.6	0.3	Mineralizable C, POXC, soil Mn	---	---
<i>Paraglossus</i>	Northern CA	NCA-OR*	0.30	0.30	Soil Na	---	---
<i>Microdominikia</i>	---	---	---	---	Clay content	Sand content	---

Significance levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Regional contrasts: CCA-NCA = Central California vs Northern California; CCA-OR = Central California vs Oregon; NCA-OR = Northern California vs Oregon

"~" indicates that similar abundances between regions

"---" indicates that information was available for this parameter

Indicator species analysis reinforced differences in AM fungal communities across regions, vineyards, and plant genotypes (Table 1.2). *Glomus*, *Septoglomus*, *Paraglomus*, and *Paraglomeraceae gen incertae sedis* were strongly associated with Northern Coast California vineyards, whereas *Rhizophagus*, *Rhizoglomus*, and *Claroideoglomus* were indicative of Oregon vineyards. No region-specific indicator taxa were identified for Central Coast California. At the vineyard scale, *Paraglomeraceae gen. incertae sedis* were strongly associated with NCA-CH and NCA-PN, while *Paraglomus* was specific to NCA-CH and *Septoglomus* to NCA-CS. *Pervestaceae gen. incertae sedis* was strongly associated with multiple vineyards, including CCA-CH1, CCA-PN1, and NCA-CS. Plant genotype-level associations were also detected. While many taxa were linked to genotypes cultivated at sites where vineyard-level associations were observed, *Glomus*, *Paraglomeraceae gen. incertae sedis*, and *Paraglomus* were strongly associated with CH × 140R.

Variance partitioning analysis was used to determine the proportion of variance in AM fungal OTU abundance explained by soil and plant variables, including their shared effects. Predictor variables were selected using MLR to identify those that best explained variation in AM fungal community composition. The final model included soil variables (e.g., pH, EC, texture (% clay, % sand), $PO_4^{3-}P$, Cu, Mg, Mn, and Fe) and plant traits (e.g., average cluster weight and cluster count, foliar K, Ca, P, S, Mg, and Cu concentration), which collectively explained 22% of community variation (adjusted $R^2 = 0.22$). Soil-only and plant-only effects explained 3.5% and 3.8%, respectively, while variation jointly explained by soil and plant predictors accounted for the largest proportion (14.6%).

Table 3 Arbuscular mycorrhizal (AM) fungal diversity and root colonization (%) across three genotype-environment comparisons: (1) the same scion-rootstock combination (Pinot Noir (PN) × 101-14) grown in different vineyard environments (OR-PN1, OR-PN2, NCA-PN); (2) different scions grafted to the same rootstock (Chardonnay × 101-14 vs. Pinot Noir × 101-14) under similar conditions in Oregon; and (3) different scion-rootstock combinations (PN × 101-14 vs. CH × 140R) grown under similar environmental conditions in Northern Coast California. Values represent mean ± standard error (SE). Different letters indicate groups that differ significantly within each scenario ($p < 0.05$).

Significant differences within each scenario were determined using Kruskal-Wallis tests followed by Dunn's post hoc comparisons

	Observed Richness	Evenness	Shannon Diversity	Colonization (%)
<i>Same rootstock and scion (i.e., PN x 101-14) in different environment</i>				
OR – PN1	19.2 ± 9.0 ^a	0.8 ± 0.07 ^a	2.3 ± 0.4 ^a	71 ± 1.1 ^a
OR – PN2	12.4 ± 7.2 ^a	0.8 ± 0.09 ^a	1.9 ± 0.5 ^{ab}	84 ± 1.4 ^b
NCA – PN	10.7 ± 5.9 ^a	0.7 ± 0.15 ^a	1.6 ± 0.5 ^b	87 ± 2.6 ^b
<i>Different scions and same rootstock in similar environments (i.e., Oregon)</i>				
CH x 101-14	16.3 ± 2.6 ^a	0.8 ± 0.02 ^a	1.9 ± 0.2 ^a	74 ± 2.3 ^a
PN x 101-14	16.2 ± 1.9 ^a	0.8 ± 0.02 ^a	2.1 ± 0.1 ^a	77 ± 1.7 ^a
<i>Different rootstocks and scions in similar environments (i.e., Northern Coast CA)</i>				
PN x 101-14	10.7 ± 5.9 ^a	0.7 ± 0.1 ^a	1.9 ± 0.3 ^a	87 ± 2.6 ^a
CH x 140R	18.2 ± 7.9 ^b	0.7 ± 0.1 ^a	1.6 ± 0.5 ^b	77 ± 2.3 ^b

3.3 Genotype x Environment Interactions: AM fungal community structure variation across vineyards and different rootstock-scion combinations

To assess the influence of environment and grapevine genotype on AM fungal community structure, three comparisons were conducted by subsetting the eleven vineyards such that either environment or genotype (scion x rootstock) varied while the other remained relatively constant. The first comparison examined three vineyards (OR-PN1, OR-PN2, and NCA-PN) cultivating the same scion-rootstock combination (Pinot Noir x 101-14) across different vineyard environments to determine whether AM fungal communities varied with environment when genotype was held constant. PERMANOVA confirmed significant differences in edaphoclimatic variables among the three Pinot Noir vineyards ($p < 0.001$), and a pairwise PERMANOVA indicated that all vineyards differed significantly from one another ($p < 0.001$ for each comparison). Oregon vineyards (OR-PN1 and OR-PN2) had higher precipitation, clay content, soil C pools, and total inorganic N (NO_3^- -N and NH_4^+ -N), along with lower temperature, pH, and PO_4^{3-} -P compared to NCA-PN (Supplementary Information, Table S1.9). Vine performance also varied significantly among Pinot Noir vineyards (PERMANOVA $p = 0.001$), with pairwise PERMANOVA comparisons confirming significant differences between all vineyard pairs ($p = 0.001$ for each comparison; Supplementary Information, Table S1.10).

Observed richness and evenness did not differ among Pinot Noir vineyards ($p > 0.05$) (Table 3; Supplementary Information, Figure S1.1a). Shannon diversity, however, differed significantly ($p = 0.012$), with the highest values in OR-PN1 (2.32 ± 0.37), followed by OR-PN2 (1.93 ± 0.52), and NCA-PN (1.61 ± 0.50) (Table 3; Supplementary Information, Figure S1.1b). Post hoc comparisons confirmed significant differences between OR-PN1 and NCA-PN, while OR-PN2 did not differ significantly from either. Shannon diversity was significantly correlated with several edaphoclimatic variables, with particularly strong positive associations with precipitation, clay, mineralizable C, MBC, POXC, CEC, soil Ca, and negative associations with temperature and leaf P (Supplementary Information, Table S1.6). A contrasting pattern was observed for AM fungal root colonization rate, which differed significantly among Pinot Noir vineyards (Kruskal-Wallis $p = 0.001$), with the highest colonization in NCA-PN ($87 \pm 2.6\%$), followed by OR-PN2 ($84 \pm 1.4\%$), and OR-PN1 ($71 \pm 1.1\%$) (Table 3; Supplementary Information, Figure S1.1c). OR-PN1 differed significantly from OR-PN2 ($p = 0.005$) and NCA-PN ($p < 0.001$), whereas OR-PN2 and NCA-PN did not differ ($p = 1.00$). Several edaphoclimatic and plant variables were significantly correlated with AM fungal colonization rate (Supplementary Information, Table S1.6). Strong positive associations were observed with temperature, sand, and soil micronutrients (Na and Zn), and negative associations with precipitation, clay, NO_3^- , total inorganic N, MBC, and leaf micronutrients (K and S).

Community composition differed among Pinot Noir vineyards (Supplementary Information, Figure S1.1d), with NCA-PN dominated by *Glomeraceae gen incertae sedis* (48.1%), *Rhizophagus* (25.5%) and *Glomus* (21.0%), and Oregon vineyards dominated by *Rhizophagus* (OR-PN1: 48.7%; OR-PN2: 48.1%). SIMPER analysis identified strongest compositional differences between NCA-PN and OR-PN2, as well as between the two Oregon Pinot Noir vineyards (Supplementary Information, Table S1.11). *Glomeraceae gen incertae sedis* and *Paraglomeraceae gen incertae sedis* were more prevalent in NCA-PN, while OR-PN1 exhibited a higher relative abundance of *Rhizoglomus* and *Rhizophagus* compared to OR-PN2.

NMDS ordination confirmed that both region ($p = 0.006$) and vineyard ($p = 0.028$) significantly explained variation in AM fungal community composition among Pinot Noir vineyards (Supplementary Information, Figure 1.2). Several environmental and plant variables were significantly associated with differences in community composition (Supplementary Information, Table S1.12 and S1.13). The most influential soil factors were total

inorganic N and soil Na concentration ($p < 0.001$), while significant ($p < 0.01$) plant variables included average cluster count, leaf K and Cu concentrations, and vineyard age. To complement these patterns, TITAN2 was used to identify correlations between individual AM fungal taxa and environmental gradients (Supplementary Information, Table S1.11). Variance partitioning analysis showed that soil and plant variables together explained 24% of the variation in OTU abundance (adjusted $R^2 = 0.24$), with shared effects accounting for the majority (19%). Soil- and plant-only contributions were 4.4% and 0.6%, respectively. Predictors included soil temperature, $\text{NH}_4^+\text{-N}$, CEC, vineyard age, and leaf Fe content.

The second scenario examined the effect of scion identity on AM fungal communities. To do so, we examined AM fungal communities across four Oregon vineyards cultivating grapevines with the same rootstock (101-14) but different scions: Pinot Noir (OR-PN1 and OR-PN2) and Chardonnay (OR-CH1 and OR-CH2). PERMANOVA confirmed significant differences in soil composition ($p < 0.001$) and plant properties ($p < 0.001$) among Oregon vineyards, with pairwise comparisons confirming significant differences between all vineyard pairs ($p < 0.006$) (Supplementary Information, Table S1.14 and S1.15). Despite these differences in soil properties and plant properties, AM fungal richness, evenness, Shannon diversity, and root colonization rate did not differ significantly between scions (Kruskal-Wallis $p > 0.05$; Table 3). SIMPER analysis did not identify any taxa contributing significantly to differences in community composition between scions. TITAN2 identified that *Claroideoglomus* was positively associated with soil Na, with no other significant relationships detected between AM fungal taxa and environmental or plant variables.

The third comparison evaluated the effect of different scion-rootstock combinations (PN x 101-14 vs CH x 140R) in two Northern Coast California vineyards, NCA-PN and NCA-CH. By selecting vineyards in close proximity (~500 ft apart) with similar climatic conditions, this comparison aimed to assess the influence of grapevine genotype on AM fungal community composition and diversity while minimizing environmental variation. PERMANOVA identified significant differences in edaphoclimatic variables between NCA-CH and NCA-PN ($p < 0.001$). NCA-CH was characterized by higher sand content, NH_4^+ , and total inorganic N, while NCA-PN had higher bulk density, clay content, PO_4^{3-} , and soil Ca and K (Supplementary Information, Table S1.16). PERMANOVA also confirmed significant differences in plant properties between genotypes ($p < 0.001$), with NCA-CH (CH x 140R)

characterized by higher average cluster count, average cluster weight, yield and leaf concentrations (i.e., K, Mn, N, and S), and NCA-PN (PN x 101-14) by higher leaf Ca and Fe (Supplementary Information, Table S1.17). In terms of AM fungal community diversity and colonization, significant differences were detected (Kruskal-Wallis $p < 0.05$; Table 3). Richness was significantly higher in NCA-CH (18.2 ± 7.9) than in NCA-PN (10.7 ± 5.9), while Shannon diversity was significantly higher in NCA-PN (1.9 ± 0.3) compared to NCA-CH (1.6 ± 0.5) (Table 3). Richness and Shannon diversity were significantly correlated with several edaphoclimatic and plant variables (Supplementary Information, Table S1.6). The strongest positive associations with richness were with sand content, vine performance metrics (average cluster count, average cluster weight, and yield), and leaf B, and the strongest negative associations were with bulk density, clay content, and soil micronutrients Ca and K. PMN and average cluster count were most strongly, positively associated with Shannon diversity, while leaf Ca showed the strongest, negative association. Evenness did not differ significantly between vineyards. Similar to Shannon diversity, AM fungal colonization rates were significantly higher in NCA-PN ($87\% \pm 2.6$) than in NCA-CH ($77\% \pm 2.3$) (Table 3). Colonization was significantly correlated with several edaphoclimatic and plant variables (Supplementary Information, Table S1.6). Bulk density, clay, sand, pH, and soil Ca showed the strongest positive associations, whereas soil NO_3^- concentration, average cluster count, average cluster weight, yield, and leaf micronutrients (B, K, Mn, and S) showed the strongest negative associations. SIMPER analysis identified *Rhizophagus* as the primary contributor to dissimilarity between vineyards, with higher abundance in NCA-CH. Additionally, TITAN2 did not identify any significant correlations between individual AM fungal taxa and environmental gradients.

4. Discussion

4.1 Alpha diversity and colonization in relation to environmental drivers

This study investigated how environmental gradients and grapevine genotype collectively shape AM fungal diversity, community composition, and root colonization in vineyard soils. Regional patterns in Shannon diversity and evenness were broadly consistent with our hypothesis that vineyards with greater soil moisture would support more diverse AM fungal communities. However, species richness did not differ significantly across regions or when the same rootstock-scion combination was evaluated in different regions, suggesting that not all components of alpha diversity responded similarly to these gradients.

Across all regions, Shannon diversity values were within the typical ranges reported for managed croplands (1.72 - 2.45) (Maurer et al. 2014; Oehl et al. 2003) and vineyards (1.56 - 2.5) (Hernandez and Mendez 2019; Oehl and Koch 2018), though often on the lower end. Across both the regional and same scion-rootstock (PN x 101-14) comparisons, both metrics were positively associated with N availability and negatively associated with P availability. While P availability has been consistently observed to suppress AM fungal diversity (Mirzaei and Moradi 2017; Treseder 2004), the influence of N availability on diversity metrics is less characterized. Elevated soil N availability has been observed to decrease AM fungal richness and shift community composition in natural systems (Zhang et al. 2024; Xiao et al. 2019). However, these studies involved N addition rates far exceeding those typically applied in vineyards. Thus, N availability in our vineyards may not have reached the concentrations necessary to elicit a suppressive effect on AM fungal diversity. In both regional and same scion-rootstock comparisons, evenness was also positively associated with precipitation, clay content, and mineralizable C. Clay-rich soils typically have high CEC and nutrient retention, while greater precipitation and cooler temperatures enhance organic matter accumulation, elevating potentially plant available nutrients, mineralizable C, and microbial activity (Liu et al. 2009; Mi et al. 2015; Wang et al. 2019). In low-fertility vineyard systems, such conditions likely promoted AM fungal colonization and diversity by enhancing root abundance and microbial activity rather than suppressing symbiosis. Overall, these patterns highlight that, even when plant genetics are held constant, environmental variability plays a key role in shaping AM fungal communities in these vineyards.

In contrast to diversity metrics, root colonization was highest under conditions characteristic of drier, more nutrient-limited environments, consistent with our hypothesis that coarser, low-to-moderate fertility soils would support greater colonization. However, soil P, Zn, and Cu concentrations were exceptions to this fertility pattern, as higher colonization was detected even where their concentrations were elevated. Betancur-Agudelo et al. (2021) reported a similar pattern in Brazilian vineyards, where AM fungal colonization was positively correlated with soil P, Cu, and Zn. This indicates that elevated concentrations of these elements do not necessarily suppress colonization in vineyard systems, although the mechanisms underlying this response in our study remain unclear.

While soil fertility, pH, and texture emerged as important drivers of both metrics, their effects on diversity and colonization were opposing rather than distinct as originally hypothesized. Among the soil properties that

promoted diversity in the regional and same scion-rootstock comparisons, variables such as precipitation, clay content, mineralizable C, and total inorganic N were negatively correlated with colonization, while temperature, sand content, pH, and soil Na were positively associated with colonization but negatively associated with diversity metrics. Several of these relationships are consistent with previous vineyard studies. High clay content has been linked to suppressed colonization rates through reduced aeration and increased physical constraints on hyphal spread (Bouffaud et al. 2016). Similar to our study, colonization in vineyards tends to increase under drier conditions, as water deficit can stimulate grapevines to establish more interactions with AM fungi to compensate for the lower density of fine roots (Schreiner 2003; Schreiner and Mihara 2017). In contrast, conditions that improve soil structure, such as porosity, aeration, and water-retention capacity, can promote higher AM fungal species richness and diversity in vineyards (Fors et al. 2023).

Overall, these findings suggest that the environmental conditions favoring high AM fungal diversity differ from those supporting greater colonization. An inverse relationship between colonization and diversity has also been observed, though the conditions driving it differed, with nutrient-rich soils favoring colonization but reducing richness (Betancur-Agudelo et al. 2021; Wang et al. 2024). This inverse relationship is not universal, however, as colonization and diversity have also been observed to increase together (Sharma et al. 2025). Such contrasts suggest that the conditions structuring colonization and diversity are system dependent. One possibility is that high colonization reflects dominance by a few highly colonizing taxa that occupy much of the root system and limit the establishment of others (Frew et al. 2024), which may explain the lower diversity observed in our study. To our knowledge, this mechanism has not been directly tested in grapevines.

4.2 Influence of Edaphoclimatic and Plant Variables on AM Fungal Beta Diversity

4.2a AM fungal beta diversity in relation to environmental and plant drivers

Vector fitting with permutation-based significance testing identified significant correlations between community composition and soil and plant variables. Across both regional and scion-rootstock (PN x 101-14) comparisons, soil Na was a significant predictor of OTU variation, which is consistent with previous studies that identified elevated soil Na concentration as an important driver of AM fungal community composition and colonization under field conditions (Eskandari et al. 2018; Garcia and Mendoza 2007). As most vineyard and field

surveys report drivers at the community or diversity level rather than resolving taxon-specific responses, we are therefore not aware of studies that have examined associations between individual AM fungal genera and specific soil or plant properties, which limits direct comparison of the following taxon-level patterns with prior work.

In both comparisons, soil microbial biomass C (MBC) and soil Fe also emerged as key factors associated with variation in AM fungal OTUs. High MBC and soil Fe were associated with lower relative abundance of *Septoglomus*, while high MBC was correlated with higher relative abundances of *Rhizoglomus*, *Rhizophagus*, and *Claroideoglomus*. Higher soil Fe was additionally correlated with greater relative abundance of *Paraglomerales gen. incertae sedis*. Correlations with MBC may reflect shared environmental conditions that promote both AM fungal abundance and MBC.

When comparing the same scion-rootstock combination, a broad set of explanatory factors was associated with AM fungal OTU variance, including bulk soil density, NH_4^+ , total inorganic N, clay, PO_4^{3-} , and soil Ca. In contrast, fewer significant edaphoclimatic drivers were detected when considering all vineyard regions, likely due to greater heterogeneity in edaphoclimatic conditions and grapevine genotype obscuring individual effects. Among the predictors identified, only clay and soil Ca impacted genus-level relative abundance. Higher levels of both were correlated with lower relative abundance of *Septoglomus* and higher relative abundance of *Rhizophagus*, *Rhizoglomus*, and *Claroideoglomus*. Elevated soil Ca also correlated to the relative abundance of *Glomeraceae gen incertae sedis*, while *Microdominikia* had lower relative abundance in soils with higher clay content. The associations with clay are likely indirect, reflecting broader patterns of soil fertility rather than a direct influence of clay content. Genera such as *Rhizophagus*, *Rhizoglomus*, and *Claroideoglomus* appeared to favor more fertile, clay-rich soils, while *Septoglomus* was more abundant in lower-fertility, coarser soils.

When comparing OTU variability in the same scion-rootstock combination, vine performance metrics (average cluster count, cluster weight, and yield) and leaf nutrient concentrations (K, B, Ca) were most strongly correlated with AM fungal community structure. Notably, higher yields were associated with lower abundance of *Rhizophagus*, *Rhizoglomus*, and *Claroideoglomus*, but with increased *Septoglomus* abundance. Higher concentrations of leaf K and Ca were positively associated with higher relative abundances of *Claroideoglomus*, while higher leaf K concentrations were further associated with higher *Rhizophagus* and lower *Septoglomus*.

relative abundance. However, associations with plant variables should be interpreted cautiously, as they may reflect differences in vine management across different sites and indirect effects of soil and climate rather than direct plant-fungal interactions.

4.2b Genus-Level Patterns and Environmental Associations

Given our hypothesis that soil moisture, pH, and texture would be the primary drivers of AM fungal community structure, we examined correlations of soil properties with AM relative abundance at the genus-level to evaluate how dominant taxa varied across these gradients. *Rhizophagus* and *Glomus*, from the Glomeraceae family, were the two most dominant genera shared among all vineyards, as previously reported in other studies (Bentancur-Agudelo et al. 2021). These results likely reflect their competitive life-history strategies and broad compatibility with diverse environments, grapevine genotypes, and management practices (Berruti et al. 2014; Chagnon et al. 2013; Fors et al. 2023; Sýkorová et al. 2007; Trouvelot et al. 2015). *Rhizophagus* was most abundant in Oregon vineyards, accounting for nearly half (~47%) of all AM fungal OTUs. Although its biomass is typically short-lived and considered “low-cost,” these colonization strategies can become C-intensive in arid, low-input systems (Chagnon et al. 2013), which may explain its reduced competitiveness in the drier, saline conditions of Central Coast CA vineyards. TITAN2 analysis reinforced this interpretation, consistently linking *Rhizophagus* abundance and multiple soil C pools (i.e., POXC, mineralizable C, and MBC) across both regional and scion-rootstock (PN x 101-14) comparisons. In regional analysis, broader associations with precipitation, clay content, and soil NO₃⁻ concentration further aligned with the higher *Rhizophagus* abundance observed in Oregon’s wetter, more fertile vineyards. Although we are not aware of any studies linking soil carbon pools directly to *Rhizophagus*, these conditions may stimulate microbial activity and organic matter turnover that can foster a soil environment conducive to greater AM fungal symbiosis (Freitas et al. 2011; Montiel-Rozas et al. 2017).

Glomus is widely recognized as a generalist genus with broad ecological tolerance, enabling it to survive across a wide range of soil and climate conditions (Stürmer 2012). Consistent with this ecological breadth, it showed no strong associations with environmental variables but was particularly abundant in Northern Coast CA vineyards (~40%). Within this region, *Glomus* comprised 55% of the AM fungal community in NCA-CH compared to 20% in NCA-PN. This variation may reflect differences in P availability between sites, as *Glomus* abundance is

known to be sensitive to soil P concentration (Yan et al. 2025). In contrast, the dominant taxon in vines grown in high P soils was *Glomeraceae gen. incertae sedis*. This group includes taxa within the Glomeraceae family that cannot be assigned to a specific genus due to limited sequencing resolution, which limits more specific ecological interpretation, as it may encompass taxa with differing P responses.

Microdominikia was also consistently detected across all vineyard regions, contributing to the core AM fungal community in this study. While its ecological role remains poorly understood, this recently described genus has been found globally in association with various plant hosts (Alguacil et al. 2012; Corazon-Guivin et al. 2019; Magurno et al. 2014; Yang et al. 2013). In our study, the positive association between *Microdominikia* and sand content likely contributed to its higher abundance in Central Coast CA vineyards. However, this relationship was not consistent across all sites, suggesting that soil texture alone does not fully explain its distribution and additional factors like rootstock adaptability, microclimate, or management practices might also influence its abundance. Reduced competition from dominant Glomeraceae taxa, such as *Rhizophagus* or *Glomus*, may have further contributed to its higher relative abundance in Central Coast CA.

Claroideoglossum and *Rhizoglossum* were both predominantly found in Oregon vineyards, where their abundance was strongly correlated with higher clay content, precipitation, and soil C pools. Higher clay content and precipitation in Oregon soils compared to California likely promoted organic matter retention, supporting the abundance of both genera and their contribution to driving regional AM fungal community differences. The positive relationship between *Claroideoglossum* and soil C pools aligns with previous findings showing this genus responds well to systems with sustained carbon inputs (Pellegrino et al. 2020; Sommermann et al. 2018).

Septoglossum and *Pervetustaceae gen. incertae sedis* shared similar environmental associations, as both taxa were positively correlated with warmer, drier, and sandier conditions. *Septoglossum*' preference for sandy soils and arid environments has also been observed in other studies (Guardiola-Márquez et al. 2022; Chimal-Sánchez et al. 2020). While *Pervetustaceae* is a recently described taxon that is rarely reported and remains poorly understood, prior studies have reported *Pervetustaceae* taxa to enhance plant performance under stressful conditions (Dirks and Jackson 2020), suggesting its detection in CA vineyards may enhance grapevine performance under abiotic stress.

Paraglomeraceae gen. incertae sedis and *Paraglomus* were both primarily associated with Northern Coast CA vineyards. Both genera belong to the order Paraglomerales, an early-diverging AM fungal lineage characterized by heavy investment in extraradical hyphae and lower root colonization rates, which could reduce their resilience under disturbance and abiotic stress (Hempel et al. 2007; Powell et al. 2009; Säle et al. 2021; van der Heijden et al. 1998a). Despite their low relative abundance, their persistence in arid, low-input vineyards suggests tolerance of competitive pressure from other dominant AM fungi (i.e., *Glomus* and *Rhizophagus*). Conversely, Oregon's wetter, cooler climate with higher C and N availability may promote symbiosis with faster-growing, resource-demanding AM fungi, such as *Rhizophagus* and *Claroideoglomus*, potentially outcompeting *Paraglomerales* members due to their slower colonization strategy. While *Paraglomeraceae gen. incertae sedis* was strongly associated with both NCA-PN and NCA-CH, *Paraglomus* was strongly associated with only NCA-CH and the CH x 140R plant genotype, unique to this field. This pattern suggests that host identity may exert a stronger influence on the distribution of *Paraglomus* than environmental variables, a topic explored further in the next section.

4.3 Influence of Plant Genotype on AM Fungal Beta Diversity

Across all scenarios, the variance shared between plant and edaphoclimatic variables explained more compositional differences in AM fungal communities than either factor alone, indicating that much of the variation could not be uniquely captured by either group and likely reflects their intertwined influence on grapevine-AM fungal symbiosis. While we are not aware of any studies partitioning plant and environmental contributions to AM fungal community variation in grapevine systems, much of the variation in a grassland system was attributable to plant allelic composition shared with soil chemical variables (Van Geel et al. 2021), suggesting that their combined influence on AM fungal communities is not unique to vineyard systems. The remaining variance may reflect factors operating at finer spatial scales than our sampling captured, such as small-scale variation in root growth and turnover or localized variation in AM fungal propagule availability. Additional unmeasured site-level factors, including differences in vineyard management, vine age, and vineyard layout, may also contribute to the remaining variance. In vineyards, plant genotype plays a central role in shaping these interactions, particularly through the influence of rootstock selection. Grapevine rootstocks have been shown to strongly influence AM fungal communities and are typically chosen for their compatibility with soil conditions, climate adaptability, and disease

resistance (Chen et al. 2024; Koepke et al. 2013; Moukazel et al. 2021; Moukarzel et al. 2022; Ollat et al. 2016; Warschefsky et al. 2016). Well-selected rootstocks have been shown to reduce vine stress under challenging environmental conditions (Chen et al. 2024), which can help stabilize C allocation to root symbionts (Salmeron-Santiago et al. 2022; Sapes et al. 2020). We hypothesize that this stability may enable a broader array of AM fungal taxa to be supported, particularly under low-resource conditions. In contrast, genotype-environment mismatches may heighten physiological stress (Chen et al. 2024), limit C availability to symbionts (Salmeron-Santiago et al. 2022; Sapes et al. 2020), and potentially favor community shifts toward dominance by fewer AM fungal taxa. While these individual pathways have been documented, we are not aware of studies that explicitly link them to explain taxon-level differences in AM fungal communities. These interpretations suggest that plant genotype may influence AM fungal communities by shaping taxon dominance, rather than merely reflecting environmental gradients.

To further explore these dynamics, we compared AM fungal communities in NCA-CH and NCA-PN, whose close proximity (~500 ft apart) may help minimize the influence of environmental variation. The 140R rootstock is well-suited to NCA-CH conditions because of its deeper root system and ability to tolerate drought and salinity (Bavaresco et al. 1992; Chen et al. 2024; Lambert et al. 2008). These traits may help reduce vine stress under local edaphic conditions and could support more stable C allocation to symbionts, potentially favoring generalist and competitively dominant taxa *Glomus* and *Paraglomus*. This may contribute to the higher relative abundance of *Glomus* in NCA-CH (55.3%) compared to NCA-PN (20.5%). The most dominant genus in NCA-PN was *Glomeraceae gen incertae sedis* (46.8%), whereas it accounted for only 5.3% of the AM fungal community in NCA-CH. At the NCA-PN sites, vines are grafted onto the 101-14 rootstock, which performs poorly in Northern Coast CA vineyard conditions due to its shallow root system and low drought and salinity tolerance (Chen et al. 2024; Christensen 2003; Lambert et al. 2009; Pongracz 1983). This mismatch between rootstock and environment likely increases vine stress and constrains host C allocation to fungal partners, potentially favoring taxa such as *Glomeraceae gen incertae sedis* that may be more tolerant of low C inputs or reduced symbiotic investment. *Glomeraceae gen incertae sedis* is a broadly tolerant taxon found in Central Coast CA vineyards (39.7%) and Oregon vineyards (22.9%). This ecological flexibility likely confers a competitive advantage in stressful environments, such as in

Central Coast CA, where higher temperatures, low precipitation, and salinity can limit AM fungal colonization and symbiosis (Eskandari et al. 2018; Gai et al. 2006; García and Mendoza 2007; Soudzilovskaia et al. 2015).

In Oregon vineyards where rootstock selection appeared well-aligned with local environmental conditions, AM fungal communities still varied in composition across sites, with genera such as *Rhizoglossum*, *Claroideoglossum*, and *Rhizophagus* differing in relative abundance. While scion identity may have contributed to compositional differences across Oregon vineyards, studies have generally reported scion effects on AM fungal communities to be modest relative to those of rootstock (Lailheugue et al. 2024; Noceto et al. 2024). Despite differences in AM fungal Shannon diversity and colonization detected across vineyards cultivating the same scion-rootstock combination, these metrics did not differ significantly between scions grown on the same rootstock in Oregon. Rootstock-soil interactions may therefore have been a stronger driver of compositional differences across sites. To our knowledge, no prior study has demonstrated soil-mediated rootstock effects on AM fungal communities under field conditions. Although Moukarzel et al. (2021) found rootstock to be a primary driver of AM fungal communities, that study compared rootstocks within individual vineyards and did not separate rootstock effects from those of the broader soil environment. A greenhouse study did isolate these factors but found no rootstock-soil interaction (Fors et al. 2023). However, regular watering and fertilization typical in greenhouse studies may suppress rootstock-soil interactions that can occur under field conditions.

Specifically, site-level variation in soil C concentrations, micronutrient concentrations, and leaf nutrient status may have contributed to these patterns. For example, *Rhizoglossum* was more abundant at sites with higher labile C and CEC, while *Claroideoglossum* was more prevalent at sites with lower soil Cu and elevated leaf K. While these observations are correlative and unmeasured site-level factors may also contribute, they raise the possibility that soil-mediated variation in rootstock function may shape taxon-level AM fungal responses in systems where environmental stress is minimized.

5. Conclusion

Our results reveal diverse and abundant AM fungal communities across vineyards in the U.S. West Coast, with 207 OTUs spanning 19 genera detected in grapevine roots. These communities varied in diversity and abundance across broad edaphoclimatic gradients and local soil-plant interactions. AM diversity was highest in

Oregon, the northernmost end of the gradient, consistent with our hypothesis that moister, fertile, and carbon rich soils would support more diverse communities. Higher diversity in Oregon was driven in part by more even AM fungal communities, which likely supported a broader range of taxa and reduced dominance by only a few groups. AM fungal colonization rate showed a different regional pattern than diversity, with the highest rates in soils of the Central Coast of California, the southernmost end of the gradient, and the lowest colonization rates in Oregon. This pattern is consistent with our hypothesis that colonization would be greater in arid, coarse-textured, lower-fertility soils where greater porosity facilitates root and hyphal growth. These findings suggest that soil conditions favoring high colonization may limit overall community diversity. Although our study cannot resolve the underlying mechanism, this may occur either by narrowing the range of taxa able to establish or through competition among AM fungi.

Differences in AM fungal communities within regions may reflect how rootstock-specific traits, including root architecture and stress tolerance, shape C allocation to fungal partners under local soil conditions. Rootstocks that appeared better suited to local environmental conditions were associated with more diverse and even fungal communities. For instance, the 101-14 rootstock, characterized by a shallow root system suited to moist, fertile soils, supported more diverse and even communities in Oregon than in Northern Coast CA. Under the drier and elevated soil Na concentrations of that region, 101-14's limited drought tolerance may have constrained C allocation to fungal partners, favoring community shifts toward greater dominance by fewer taxa, such as *Glomeraceae gen. incertae sedis*. In contrast, the deeper root system and greater stress tolerance of 140R rootstock may have supported more stable C allocation to symbionts under these conditions, favoring higher abundances of generalist *Glomeraceae* taxa. Overall, our findings suggest that AM fungal communities in grapevines are structured at multiple scales. Broad environmental gradients establish baseline differences in community composition, while plant-level traits may refine patterns of taxon abundance in the vineyard. Future research examining how rootstock selection and soil management practices influence AM fungal community structure across diverse environments will help translate these findings into strategies to maintain robust AM fungal communities. Additionally, the detection of numerous taxa of uncertain phylogenetic highlights the need for

further characterization of AM fungal diversity in vineyard systems, as they may have commercial significance and warrant further study.

6. Acknowledgments

This research was funded by the Foundation for Food & Agriculture Research (Grant number CA21-SS-0000000193) and the California Department of Food and Agriculture (Grant number 21-0433-021-SF). The authors are grateful to the personnel of Jackson Family Vineyards for their help in establishing and maintaining this field trial.

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8. Supplementary Information

Table S1.1 Summary of 11 vineyard field trials located along an edaphoclimatic gradient in the Western USA. Soil classifications correspond to the official series-level designations according to the USDA Soil Taxonomy and were verified using the NRCS Web Soil Survey

Region	Site	30 Yr Normal Temperature (°C)	30 Yr Normal Precipitation (cm)	Vineyard Age (yr)	Variety (Clone)	Rootstock	Soil Classification
Oregon	OR-PN1	11	>100	8	Pinot Noir (667)	101-14	Fine, mixed, active, mesic Xeric Palehumults
Oregon	OR-PN2	11	>100	8	Pinot Noir (667)	101-14	Fine, mixed, active, mesic Xeric Haplohumults
Oregon	OR-CH1	11	>100	8	Chardonnay (76)	101-14	Fine, mixed, superactive, mesic Aquic Haplohumults
Oregon	OR-CH2	11	>100	8	Chardonnay (76)	101-14	Fine, mixed, active, mesic Xeric Palehumults
Northern Coast California	NCA-PN	14	80-100	10	Pinot Noir (667)	101-14	Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents
Northern Coast California	NCA-CS	14	80-100	15	Cabernet Sauvignon (337)	101-14	Fine, smectitic, thermic Mollic Palexeralfs
Northern Coast California	NCA-CH	14	80-100	5	Chardonnay (4)	140R	Fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents
Central Coast California	CCA-PN1	15	>40	24	Pinot Noir (667)	5C	Coarse-loamy, mixed, superactive, thermic Cumulic Haploxerolls

Central Coast California	CCA- PN2	15	>40	16	Pinot Noir (667)	1616C	Fine-loamy, mixed, superactive, thermic Typic Argixerolls
Central Coast California	CCA- CH1	15	>40	29	Chardo nnay (SM4)	5BB	Fine, smectitic, thermic Pachic Argixerolls
Central Coast California	CCA- CH2	15	>40	24	Chardo nnay (96)	5C	Coarse-loamy, mixed, superactive, thermic Cumulic Haploxerolls

Table S1.2 Regional differences in edaphoclimatic properties in 11 vineyards located along an edaphoclimatic gradient in the Western USA. Values represent means, with standard errors shown in parentheses. Soil samples were collected in 2022. Significance tests were performed by conducting a Kruskal-Wallis test followed by a post-hoc Dunn comparison. Different letters signify significant differences between values. The p-values report the results of the Kruskal-Wallis test for the effect of region on each soil or climate variable. Abbreviations include: EC = electrical conductivity; PMN = potentially mineralizable nitrogen; MBC = microbial biomass carbon; POXC = permanganate oxidizable carbon; CEC = cation exchange capacity; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; Na = sodium; Zn = zinc

Soil/Climate Property	Central CA (n=41)	Northern CA (n=26)	Oregon (n=38)	p-value
Normal Precipitation (cm)	34.4 (1.1) ^a	88.4 (1.6) ^b	130.8 (1.4) ^c	< 0.001
Normal Temperature (°C)	14.9 (0.05) ^a	14.5 (0.07) ^b	11.2 (0.03) ^c	< 0.001
Bulk Density (g cm ⁻³)	1.3 (0.03) ^a	1.3 (0.03) ^a	1.1 (0.01) ^b	< 0.001
Clay Content (%)	17.9 (0.7) ^a	24.8 (1.3) ^b	43.1 (1.4) ^c	< 0.001
Sand Content (%)	42.5 (2.9) ^a	27.4 (1.9) ^b	11.6 (1.0) ^c	< 0.001
pH	7.7 (0.05) ^a	7.4 (0.09) ^b	6.5 (0.05) ^b	< 0.001
EC (μS cm ⁻¹)	197.5 (15.7) ^a	105.0 (5.0) ^b	97.1 (4.8) ^b	< 0.001
Nitrate (mg NO ₃ ⁻ -N kg ⁻¹ soil)	0.4 (0.2) ^a	0.2 (0.02) ^b	4.4 (0.3) ^c	< 0.001
Ammonium (mg NH ₄ ⁺ -N kg ⁻¹ soil)	0.7 (0.1) ^a	0.9 (0.2) ^a	4.6 (0.6) ^b	< 0.001
Total Inorganic N (mg N kg ⁻¹ soil)	1.1 (0.2) ^a	1.0 (0.2) ^a	9.1 (0.7) ^b	< 0.001
PMN (mg PMN-N kg ⁻¹ soil)	4.4 (0.5) ^a	4.7 (1.0) ^a	8.7 (0.8) ^b	0.001
Phosphate (mg PO ₄ ³⁻ -P kg ⁻¹ soil)	21.1 (1.1) ^a	27.2 (4.0) ^a	8.5 (0.5) ^b	< 0.001
Mineralizable C (mg C kg ⁻¹ soil * hr)	0.9 (0.1) ^a	1.2 (0.1) ^b	2.5 (0.1) ^c	< 0.001
MBC (mg C kg ⁻¹ soil)	235.0 (18.1) ^a	222.5 (25.5) ^a	531.3 (18.5) ^b	< 0.001
POXC (mg C kg ⁻¹ soil)	378.1 (19.5) ^a	367.0 (24.4) ^a	641.3 (17.3) ^b	< 0.001

CEC (meq/100g)	15.6 (0.8) ^a	19.3 (1.3) ^b	24.1 (0.7) ^c	< 0.001
Ca (meq/100g)	2232.0 (111.4) ^a	2610.6 (159.3) ^b	4032.8 (120.7) ^c	< 0.001
Cu (mg Cu kg⁻¹ soil)	2.5 (0.2) ^a	2.7 (0.2) ^b	1.5 (0.1) ^c	< 0.001
Fe (mg Fe kg⁻¹ soil)	6.0 (0.3) ^a	32.8 (3.2) ^b	32.1 (1.9) ^b	< 0.001
K (mg K kg⁻¹ soil)	126.3 (3.6) ^a	285.7 (23.9) ^b	404.8 (14.2) ^c	< 0.001
Mg (meq/100g)	457.5 (31.5) ^a	650.6 (71.8) ^b	450.3 (23.3) ^a	0.019
Mn* (mg Mn kg⁻¹ soil)	17.3 (0.7) ^a	20.3 (1.1) ^b	56.4 (2.7) ^c	< 0.001
Na (mg Na kg⁻¹ soil)	81.9 (4.8) ^a	47.4 (2.1) ^b	10.3 (0.5) ^c	0.247
Zn (meq/100g)	4.1 (0.2) ^a	2.5 (0.3) ^b	1.8 (0.1) ^c	< 0.001

Table S1.3 Genotypic differences in plant properties measured in 2022 in 11 vineyards located along an edaphoclimatic gradient in the Western USA. Values represent means, with standard errors shown in parentheses. Grapevine properties were measured in 2022. Significance tests were performed by conducting a Kruskal-Wallis test followed by a post-hoc Dunn test. Different letters signify significant differences between values. The p-values report the results of the Kruskal-Wallis test for the effect of genotype on each plant variable. Abbreviations include: B = boron; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; N = nitrogen; P = phosphorous; S = sulfur; Zn = zinc

Plant Property	CH x 101-14 (n=18)	CH x 140R (n=8)	CH x 5BB (n=10)	CH x 5C (n=10)	CS x 101-14 (n=8)	PN x 101-14 (n=30)	PN x 1616C (n=30)	PN x 5C (n=11)	p- values
Average Cluster Count (# of clusters per vine)	11.7 (0.6) ^a	31.1 (0.4) ^{bcd}	33.5 (0.4) ^{bc}	35.5 (0.5) ^c	29.2 (0.4) ^{bd}	13.6 (1.0) ^a	26.9 (1.2) ^d	35.1 (1.8) ^{bd}	< 0.001
Average Cluster Weight (kg per vine)	1.6 (0.1) ^a	5.6 (0.1) ^b	3.7 (0.1) ^{bcd}	3.8 (0.1) ^{bc}	2.45 (0.1) ^{cd}	1.8 (0.1) ^a	2.46 (0.1) ^d	1.7 (0.1) ^a	< 0.001
Yield (kg vine ⁻¹)	21.5 (2.4) ^a	184.4 (2.6) ^b	134.0 (3.2) ^{bcd}	151.6 (4.7) ^{bc}	80.9 (4.4) ^{cd}	30.7 (4.0) ^a	70.2 (7.5) ^d	68.5 (7.2) ^d	< 0.001
B Leaf (mg B kg ⁻¹ leaf)	36.9 (1.8) ^a	74.5 (0.6) ^{bc}	92.1 (2.3) ^b	58.9 (1.1) ^{bce}	52.5 (0.9) ^{cde}	38.6 (2.2) ^{ade}	43.4 (3.9) ^{ade}	50.7 (0.2) ^e	< 0.001
Ca Leaf (mg Ca kg ⁻¹ leaf)	26200 (375) ^{ac}	19300 (808) ^{bcd}	27300 (283) ^{ac}	17000 (263) ^{bd}	24700 (864) ^{acd}	25000 (621) ^{acd}	21900 (471) ^d	16000 (176) ^b	< 0.001
Cu Leaf (mg Cu kg ⁻¹ leaf)	7.4 (0.3) ^{ac}	29.6 (5.6) ^{bd}	82.5 (10.9) ^b	6.4 (0.2) ^a	163.0 (31.5) ^b	9.5 (0.5) ^{cd}	13.2 (4.5) ^d	7.2 (0.2) ^a	< 0.001
Fe Leaf (mg Fe kg ⁻¹ leaf)	300.4 (25.8) ^a	253.6 (7.5) ^a	1582.2 (56.7) ^{bc}	822.6 (5.8) ^{bc}	306.6 (5.9) ^{ac}	320.3 (25.4) ^a	2859.4 (297.1) ^b	556.4 (3.6) ^c	< 0.001
K Leaf (mg K kg ⁻¹ leaf)	13000 (543) ^{adef}	13800 (156) ^{adf}	10400 (196) ^{bdef}	9700 (27.7) ^{bcef}	7400 (493) ^c	12800 (575) ^{def}	10600 (333) ^{ef}	11000 (118) ^f	< 0.001
Mg Leaf (mg Mg kg ⁻¹ leaf)	3980 (135) ^a	4960 (35.7) ^b	4550 (32.6) ^{ab}	2990 (78.9) ^{ab}	5720 (115) ^b	3530 (201) ^a	4080 (83.0) ^{ab}	2600 (15.5) ^c	< 0.001
Mn Leaf (mg Mn kg ⁻¹ leaf)	310.4 (20.2) ^{acd}	95.3 (2.3) ^{bc}	184.3 (5.4) ^{cd}	76.6 (1.6) ^{ade}	115.3 (5.8) ^b	203.9 (24.2) ^{ad}	131.7 (6.8) ^d	69.1 (1.3) ^e	< 0.001
N Leaf (mg N kg ⁻¹ leaf)	23000 (641) ^{ae}	26500 (44.1) ^{bd}	22600 (251) ^{ade}	21100 (221) ^{ce}	22300 (331) ^{ace}	24500 (322) ^d	21100 (397) ^{ce}	21800 (167) ^e	< 0.001

P Leaf (mg P kg⁻¹ leaf)	1770 (47.7) ^{ad}	2920 (85.2) ^{bc}	3980 (137) ^{bc}	3500 (99.3) ^{bce}	2580 (83.5) ^{cd}	2170 (93.1) ^d	1970 (147) ^d	4670 (196) ^e	< 0.001
S Leaf (mg S kg⁻¹ leaf)	2460 (61.0) ^a	2240 (17.4) ^{ac}	2550 (24.0) ^a	4950 (104) ^b	2260 (118) ^{ae}	2380 (130) ^a	1690 (95.1) ^e	43400 (31.7) ^e	< 0.001
Zn Leaf (mg Zn kg⁻¹ leaf)	55.7 (2.7) ^a	50.8 (2.5) ^{ac}	77.6 (2.0) ^{bde}	108.8 (1.5) ^{bde}	59.8 (2.4) ^{ad}	46.0 (1.3) ^c	69.4 (0.7) ^{de}	89.1 (6.9) ^e	< 0.001

Table S1.4 Vector loadings associated p-values of regional grapevine variables on the first two principal coordinate axes (PCoA1 and PCoA2) from Figure 1.2b. Loadings indicate the strength and direction of each variable's contribution to genotypic dissimilarities in grapevine characteristics, with higher absolute values reflecting stronger influence on axis separation. All loadings and significance values were generated using the envfit function with 999 permutations. Abbreviations include: B = boron; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; N = nitrogen; P = phosphorous; S = sulfur; Zn = zinc

Variable	PCoA1	PCoA2	p-value
Average Cluster Count (# of clusters per vine)	-2.91	0.13	0.001
Average Cluster Weight (kg per vine)	-1.57	1.70	0.001
Yield (kg vine ⁻¹)	-2.30	1.25	0.001
B Leaf (mg B kg ⁻¹ leaf)	-1.94	1.69	0.001
Ca Leaf (mg Ca kg ⁻¹ leaf)	1.60	1.54	0.001
Cu Leaf (mg Cu kg ⁻¹ leaf)	-0.59	1.96	0.001
Fe Leaf (mg Fe kg ⁻¹ leaf)	-0.96	0.18	0.004
K Leaf (mg K kg ⁻¹ leaf)	1.73	-1.06	0.001
Mg Leaf (mg Mg kg ⁻¹ leaf)	-0.31	2.72	0.001
Mn Leaf (mg Mn kg ⁻¹ leaf)	2.22	-0.09	0.001
N Leaf (mg N kg ⁻¹ leaf)	1.04	1.22	0.001
P Leaf (mg P kg ⁻¹ leaf)	-2.28	-0.95	0.001
S Leaf (mg S kg ⁻¹ leaf)	-0.94	-2.21	0.001
Zn Leaf (mg Zn kg ⁻¹ leaf)	-2.02	-1.69	0.001
Vineyard Age (yr)	-2.28	-1.06	0.001

Table S1.5 Alpha diversity of AM fungi by region, reported as means with standard errors in parentheses for Shannon diversity, observed richness, and evenness in Central California (n=41), Northern CA (n=26), and Oregon (n=38). Significant differences were determined using a Kruskal-Wallis test followed by a Dunn post hoc comparison. Different superscript letters indicate significant pairwise differences

Alpha Diversity	Central CA (n=41)	Northern CA (n=26)	Oregon (n=38)
Shannon Diversity	1.7 (0.1) ^a	1.8 (0.1) ^{ab}	2.0 (0.1) ^b
Richness	12.9 (1.0) ^a	16.0 (1.5) ^a	16.2 (1.6) ^a
Evenness	0.7 (0.03) ^a	0.7 (0.02) ^a	0.8 (0.01) ^b

Table S1.6 Pearson correlation coefficients between soil and plant variables and AM fungal diversity metrics and root colonization across four vineyard comparisons. Four comparisons were made: (1) a regional comparison across all eleven vineyards, (2) the same scion-rootstock combination (PN x 101-14) grown across contrasting environments (Oregon and Northern Coast California), (3) different scions (CH vs PN) on the same rootstock (101-14) within a similar environment, and (4) different scion-rootstock combinations (PN x 101-14 vs. CH x 140R) in similar environments (Northern Coast California). Only significant correlations ($p < 0.05$) are shown, with dashes indicating non-significant relationships. Positive values indicate that higher values of a variable were associated with higher AM fungal diversity or colonization, and negative values indicate the opposite

	Regional Comparison				Same Genotype (PN x 101-14) Comparison				Different Scion (PN vs CH x 101-14) Comparison				Different Genotype (PN x 101-14 vs CH x 140R) Comparison			
	Observed Richness	Evenness	Shannon Diversity	Colonization (%)	Observed Richness	Evenness	Shannon Diversity	Colonization (%)	Observed Richness	Evenness	Shannon Diversity	Colonization (%)	Observed Richness	Evenness	Shannon Diversity	Colonization (%)
Soil Variables																
Normal Precipitation (cm)	0.32	0.29	0.35	-0.69	0.41	0.44	0.54	-0.69	0.42	-	0.44	-	-	-	-	-
Normal Temperature (C)	-0.23	-0.39	-0.36	0.64	-	-0.47	-0.50	0.61	-0.42	-	-0.44	-	-	-	-	-
Bulk Density (g cm ⁻³)	-0.05	-0.12	-0.08	0.44	-	-0.48	-0.47	0.54	-	-	-	-	-0.50	-	-	0.59
Clay Content (%)	0.26	0.27	0.32	-0.64	0.47	-	0.55	-0.80	0.42	-	0.44	-	-0.50	-	-	-0.59
Sand Content (%)	-0.16	-0.11	-0.15	0.49	-0.46	-	-0.49	0.77	-0.42	-	-0.46	-	0.50	-	-	0.60
pH	-0.16	-0.25	-0.22	0.62	-	-0.38	-0.41	0.53	-	-	-	-	-	-	-	0.53

EC	-0.13	-0.36	-0.26	0.38	-	-	0.48	-0.43	-	-	-	-	-	-	-	-
Nitrate (mg NO ₃ ⁻ -N kg ⁻¹ soil)	0.23	0.23	0.27	-0.56	-	0.36	0.41	-0.60	-	-	-	-	-	-	-	-0.55
Ammonium (mg NH ₄ ⁺ -N kg ⁻¹ soil)	0.13	0.29	0.25	-0.48	-	-	-	-0.59	-	-	-	-	-	-	-	-
Total Inorganic N (mg N kg ⁻¹ soil)	0.21	0.30	0.31	-0.60	-	0.39	0.39	-0.68	-	-	-	-	-	-	-	-
PMN (mg PMN-N kg ⁻¹ soil)	0.21	0.19	0.27	-0.28	-	-	0.40	-	-	-	-	-	-	-	0.52	-0.48
Phosphate (mg PO ₄ ³⁻ P kg ⁻¹ soil)	-0.27	-0.26	-0.31	0.32	-	-0.47	-0.44	0.41	-	-	-	-	-0.47	-	-	-
Mineralizable C (mg C kg ⁻¹ soil * hr)	0.23	0.34	0.36	-0.66	0.48	0.38	0.60	-0.57	-	-	-	-	-	-	-	-
MBC (mg C kg ⁻¹ soil)	0.21	0.26	0.27	-0.54	0.49	-	0.56	-0.73	-	-	-	-	-	-	-	-
POXC (mg C kg ⁻¹ soil)	0.24	0.28	0.37	-0.52	0.44	-	0.54	-0.50	-	-	-	-	-	-	-	-
CEC (meq/100g)	0.17	0.14	0.22	-0.43	0.45	-	0.58	-0.39	-	-	0.35	-	-	-	-	-
Ca (meq/100g)	0.20	0.20	0.27	-0.50	0.43	-	0.55	-0.55	0.37	-	0.39	-	-0.54	-	-	0.59
Cu (mg Cu kg ⁻¹ soil)	-	-	-	0.14	-	-	-0.38	-	0.46	-	0.48	-	-	-	0.50	-
Fe (mg Fe kg ⁻¹ soil)	0.16	0.13	0.19	-0.47	-	-	-	0.38	-	-	-	-	-	-	-	-

K (mg K kg ⁻¹ soil)	0.13	0.27	0.25	-0.57	-	-	-	-	-	-	-	-	-0.51	-	-	-
Mg (meq/100g)	-	-	-	-	-	-	-	0.48	-	-	-	-	-	-	-	-
Mn (mg Mn kg ⁻¹ soil)	0.10	0.32	0.24	-0.52	-	0.42	-	-	-	-	-	-	-	-	-	-
Na (mg Na kg ⁻¹ soil)	-0.15	-0.31	-0.25	0.62	-	-0.43	-	0.60	-	-0.38	-	-	-	0.48	-	-
Zn (meq/100g)	-0.25	-0.13	-0.20	0.49	-	-	-	0.61	-	-	-	-	-	-	-	-
<u>Plant Properties</u>																
Average Cluster Count (# of clusters per vine)	-0.18	-0.35	-0.31	0.55	-	-	-	0.55	-	-	-	-	0.58	-	0.61	-0.54
Average Cluster Weight (kg per vine)	-0.10	-0.26	-0.23	0.13	-	-	-	0.54	-	-	-	-	0.57	-	-	-0.63
Yield (kg vine ⁻¹)	-0.14	-0.35	-0.30	0.30	-	-	-	0.50	-	-	-	-	0.57	-	-	-0.64
B Leaf (mg B kg ⁻¹ leaf)	-0.10	-0.18	-0.15	0.29	-	-0.50	-	-	-	-	-	-	0.51	-	-	-0.60
Ca Leaf (mg Ca kg ⁻¹ leaf)	0.15	0.31	0.28	-0.41	-	0.37	-	-0.46	-	-	-	-	-	-0.47	-0.58	-
Cu Leaf (mg Cu kg ⁻¹ leaf)	-	-	-	-	-	-0.53	-0.48	0.55	-	-	-	-	-	-	-	-
Fe Leaf (mg Fe kg ⁻¹ leaf)	-0.26	-0.11	-0.21	0.46	-	-	-	0.47	-	-	-	0.45	-	-	-	0.48
K Leaf (mg K kg ⁻¹ leaf)	0.26	0.28	0.34	-0.51	0.43	-	0.44	-0.66	-	-	0.37	-	-	-	-	-0.64

Mg Leaf (mg Mg kg ⁻¹ leaf)	-	-	-	-	-	-0.53	-	0.42	-	-	-	-	-	-	-	-
Mn Leaf (mg Mn kg ⁻¹ leaf)	0.24	0.33	0.32	-0.55	0.38	-	0.43	0.59	-	-	-	-	-	-	-	-0.51
N Leaf (mg N kg ⁻¹ leaf)	0.19	0.11	0.17	-0.42	0.39	-	0.43	-0.54	-	-	-	-	-	-	-	-0.48
P Leaf (mg P kg ⁻¹ leaf)	-0.13	-0.17	-0.17	0.49	-	-0.54	-0.50	0.44	-	-	-	-	-	-0.54	-	-
S Leaf (mg S kg ⁻¹ leaf)	-	-0.19	-0.13	0.16	0.38	-	0.44	-0.72	-	-	-	-0.40	-	-	-	-0.52
Zn Leaf (mg Zn kg ⁻¹ leaf)	-0.26	-0.23	-0.31	0.49	-	-	-	0.56	-0.37	-	-0.43	-	-	-	-	-

Table S1.7 Vector loadings and associated p-values for soil and climate variables fitted onto the NMDS ordination (NMDS1 and NMDS2) in Fig 1.4b. These loadings characterize the strength and direction of each continuous variable's correlation with AM fungal community dissimilarities from samples collected across all regions, with larger absolute values indicating stronger contributions to separation along NMDS axes. Continuous variables are shown as vectors. For categorical variables (e.g. Vineyard, Region), p-values reflect the significance of group-level differences in AM fungal community structure. All loadings and significance values were generated using the envfit function with 999 permutations. Abbreviations include: MBC = microbial biomass carbon; POXC = permanganate oxidizable carbon; CEC = cation exchange capacity; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; Na = sodium; Zn = zinc

Variable	NMDS1	NMDS2	p-value
Normal Precipitation (cm)	0.90	0.43	0.068
Normal Temperature (C)	-0.84	-0.55	0.019
Bulk Density (g cm ⁻³)	-0.99	-0.15	0.068
Clay Content (%)	0.91	0.41	0.023
Sand Content (%)	-1.00	-0.01	0.081
pH	-0.58	-0.81	0.039
EC (μS cm ⁻¹)	-0.56	-0.83	0.122
Nitrate (mg NO ₃ ⁻ -N kg ⁻¹ soil)	0.69	0.72	0.091
Ammonium (mg NH ₄ ⁺ -N kg ⁻¹ soil)	0.89	0.45	0.191
Total Inorganic N (mg N kg ⁻¹ soil)	0.81	0.59	0.077
PMN (mg PMN-N kg ⁻¹ soil)	1.00	0.01	0.434
Phosphate (mg PO ₄ ³⁻ -P kg ⁻¹ soil)	-0.44	-0.90	0.536
Mineralizable C (mg C kg ⁻¹ soil * hr)	0.88	0.47	0.029
MBC (mg C kg ⁻¹ soil)	0.72	0.69	0.002
POXC (mg C kg ⁻¹ soil)	1.00	-0.04	0.089
CEC (meq/100g)	1.00	0.02	0.010
Ca (meq/100g)	0.99	0.14	0.012
Cu (mg Cu kg ⁻¹ soil)	-0.85	-0.52	0.091
Fe (mg Fe kg ⁻¹ soil)	0.87	0.49	0.082
K (mg K kg ⁻¹ soil)	0.94	0.35	0.014
Mg (meq/100g)	0.88	-0.47	0.195
Mn (mg Mn kg ⁻¹ soil)	0.94	0.33	0.067
Na (mg Na kg ⁻¹ soil)	-0.71	-0.70	0.002
Zn (meq/100g)	-0.80	-0.60	0.032
Vineyard	-	-	0.001
Region	-	-	0.115
Genotype	-	-	0.001

Table S1.8 Vector loadings and associated p-values for grapevine variables fitted onto the NMDS ordination (NMDS1 and NMDS2) in Figure 1.4b. These loadings characterize the strength and direction of each continuous variable's correlation with AM fungal community dissimilarities from samples collected across all regions, with larger absolute values indicating stronger contributions to separation along NMDS axes. Continuous variables are shown as vectors. For categorical variables (e.g. Genotype), p-values reflect the significance of group-level differences in AM fungal community structure. All loadings and significance values were generated using the envfit function with 999 permutations. Abbreviations include: B = boron; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; N = nitrogen; P = phosphorous; S = sulfur; Zn = zinc

Variable	NMDS1	NMDS2	p-value
Average Cluster Count (# of clusters per vine)	-0.72	-0.69	0.007
Average Cluster Weight (kg per vine)	-0.81	-0.58	0.698
Yield (kg vine ⁻¹)	-0.87	-0.49	0.127
B Leaf (mg B kg ⁻¹ leaf)	-0.84	-0.55	0.028
Ca Leaf (mg Ca kg ⁻¹ leaf)	-0.07	1.00	0.321
Cu Leaf (mg Cu kg ⁻¹ leaf)	-0.90	-0.44	0.003
Fe Leaf (mg Fe kg ⁻¹ leaf)	-0.07	1.00	0.202
K Leaf (mg K kg ⁻¹ leaf)	0.89	0.445	0.020
Mg Leaf (mg Mg kg ⁻¹ leaf)	-0.99	-0.16	0.657
Mn Leaf (mg Mn kg ⁻¹ leaf)	0.75	0.66	0.031
N Leaf (mg N kg ⁻¹ leaf)	0.98	-0.19	0.075
P Leaf (mg P kg ⁻¹ leaf)	-0.53	-0.85	0.001
S Leaf (mg S kg ⁻¹ leaf)	-0.40	-0.91	0.286
Zn Leaf (mg Zn kg ⁻¹ leaf)	-0.76	-0.65	0.080
Vineyard Age (yr)	-0.92	-0.39	0.002
Genotype	-	-	0.001

Table S1.9 Differences in edaphoclimatic properties among vineyards cultivating the same genotype (PN x 101-14) under different conditions. Values represent means, with standard errors shown in parentheses. Soil samples were collected in 2022. Significance tests were performed by conducting a Kruskal-Wallis test followed by a post-hoc Dunn test. Different letters signify significant differences between values. The p-values report the results of the Kruskal-Wallis test for the effect of vineyard on each soil and climate variable. Abbreviations include: EC = electrical conductivity; PMN = potentially mineralizable nitrogen; MBC = microbial biomass carbon; POXC = permanganate oxidizable carbon; CEC = cation exchange capacity; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; Na = sodium; Zn = zinc

Soil/Climate Property	NCA-PN (n=10)	OR-PN1 (n=11)	OR-PN2 (n=9)	p-value
Normal Precipitation (cm)	83.2 (0.0) ^a	138.9 (0.0) ^b	121.7 (0.0) ^c	< 0.001
Normal Temperature (C)	14.3 (0.0) ^a	11.1 (0.0) ^b	11.5 (0.0) ^c	< 0.001
Bulk Density (g cm ⁻³)	1.2 (0.0) ^a	1 (0.0) ^b	1(0.0) ^b	< 0.001
Clay Content (%)	29.2 (0.0) ^a	49.6 (0.0) ^b	34.1(0.0) ^c	< 0.001
Sand Content (%)	15.9 (0.0) ^a	5.15 (0.0) ^b	16.7 (0.0) ^c	< 0.001
pH	7.38 (0.0) ^a	6.3 (0.1) ^b	6.39 (0.1) ^b	< 0.001
EC (μS cm ⁻¹)	92.6 (2.8) ^a	120.8 (4.2) ^b	98.0 (9.5) ^a	0.001
Nitrate (mg NO ₃ ⁻ -N kg ⁻¹ soil)	0.1 (0.0) ^a	4.9 (0.5) ^b	3.3 (0.6) ^c	< 0.001
Ammonium (mg NH ₄ ⁺ -N kg ⁻¹ soil)	0.5 (0.1) ^a	8.4 (1.5) ^b	2.6 (0.1) ^c	< 0.001
Total Inorganic N (mg N kg ⁻¹ soil)	0.6 (0.1) ^a	13.3 (1.3) ^b	5.9 (0.6) ^c	< 0.001
PMN (mg PMN-N kg ⁻¹ soil)	4.0 (0.8) ^a	5.1 (0.7) ^{ab}	8.8 (1.1) ^b	0.007
Phosphate (mg PO ₄ ³⁻ -P kg ⁻¹ soil)	50.7 (3.4) ^a	8.3 (0.8) ^b	7.0 (0.4) ^b	< 0.001
Mineralizable C (mg C kg ⁻¹ soil * hr)	1.4 (0.0) ^a	2.5 (0.0) ^b	2.3 (0.1) ^b	< 0.001
MBC (mg C kg ⁻¹ soil)	275.8 (38) ^a	564.3 (9.8) ^b	406.4 (31) ^c	< 0.001
POXC (mg C kg ⁻¹ soil)	420.9 (18) ^a	679.6 (31) ^b	593.5 (49) ^{ab}	< 0.001
CEC (meq/100g)	24.0 (0.4) ^a	27.9 (0.6) ^b	24.7 (1.4) ^{ab}	0.001
Ca (meq/100g)	3417 (17) ^a	4678 (139) ^b	3810 (192) ^a	0.0003
Cu (mg Cu kg ⁻¹ soil)	3.4 (0.1) ^a	2.5 (0.1) ^b	1.0 (0.1) ^c	< 0.001
Fe (mg Fe kg ⁻¹ soil)	36.6 (2.0) ^{ab}	34.8 (2.3) ^a	46.2 (3.2) ^a	0.05
K (mg K kg ⁻¹ soil)	412.3 (13) ^a	415.3 (3.5) ^a	445.3 (14) ^a	0.07
Mg (meq/100g)	687.3 (38) ^a	423.4 (16) ^b	550.8 (53) ^a	< 0.001
Mn (mg Mn kg ⁻¹ soil)	23.4 (0.8) ^a	44.6 (2.2) ^b	68.9 (4.4) ^c	< 0.001
Na (mg Na kg ⁻¹ soil)	48.2 (2.1) ^a	8.9 (0.3) ^b	12.7 (1.8) ^b	< 0.001

Zn (meq/100g)	3.6 (0.3) ^a	2.0 (0.1) ^b	2.6 (0.1) ^c	< 0.001
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Table S1.10 Differences in plant properties among vineyards cultivating the same genotype (PN x 101-14) under different environmental conditions. Values represent means, with standard errors shown in parentheses. Grapevine properties were measured in 2022. Significance tests were performed by conducting a Kruskal-Wallis test followed by a post-hoc Dunn test. Different letters signify significant differences between values. The p-values report the results of the Kruskal-Wallis test for the effect of vineyard on each soil and climate variable. Abbreviations include: B = boron; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; N = nitrogen; P = phosphorous; S = sulfur; Zn = zinc

Plant Property	NCA-PN (n=10)	OR-PN1 (n=11)	OR-PN2 (n=9)	p-value
Average Cluster Count (# of clusters per vine)	20.6 (1.1) ^a	9.6 (0.2) ^b	10.9 (0.3) ^c	< 0.001
Average Cluster Weight (kg per vine)	2.3 (0.2) ^a	1.4 (0.1) ^b	1.8 (0.0) ^c	< 0.001
Yield (kg vine ⁻¹)	57.4 (5.6) ^a	14.3 (1.0) ^b	21.1 (0.8) ^c	< 0.001
B Leaf (mg B kg ⁻¹ leaf)	53.4 (0.3) ^a	35.7 (0.2) ^b	25.5 (0.6) ^c	< 0.001
Ca Leaf (mg Ca kg ⁻¹ leaf)	20700 (166) ^a	27200 (633) ^b	27200 (425) ^b	< 0.001
Cu Leaf (mg Cu kg ⁻¹ leaf)	13.0 (0.4) ^a	8.0 (0.2) ^b	7.4 (0.2) ^c	< 0.001
Fe Leaf (mg Fe kg ⁻¹ leaf)	276.3 (7.7) ^a	215.9 (10.4) ^b	496.7 (41.7) ^c	< 0.001
K Leaf (mg K kg ⁻¹ leaf)	8530 (183) ^a	15500 (155) ^b	14200 (188) ^c	< 0.001
Mg Leaf (mg Mg kg ⁻¹ leaf)	4950 (93.6) ^a	3110 (42.3) ^b	2470 (172) ^c	< 0.001
Mn Leaf (mg Mn kg ⁻¹ leaf)	42.45 (2.4) ^a	310.1 (30.3) ^b	253.7 (5.7) ^b	< 0.001
N Leaf (mg N kg ⁻¹ leaf)	23200 (235) ^a	26300 (260) ^b	23700 (475) ^a	< 0.001
P Leaf (mg P kg ⁻¹ leaf)	2820 (65.4) ^a	1970 (67.4) ^b	1700 (4.4) ^b	< 0.001
S Leaf (mg S kg ⁻¹ leaf)	177 (25.1) ^{0a}	3240 (112.2) ^b	2020 (32.8) ^c	< 0.001
Zn Leaf (mg Zn kg ⁻¹ leaf)	47.7 (1.6) ^a	38.9 (0.3) ^b	52.7 (2.1) ^c	< 0.001

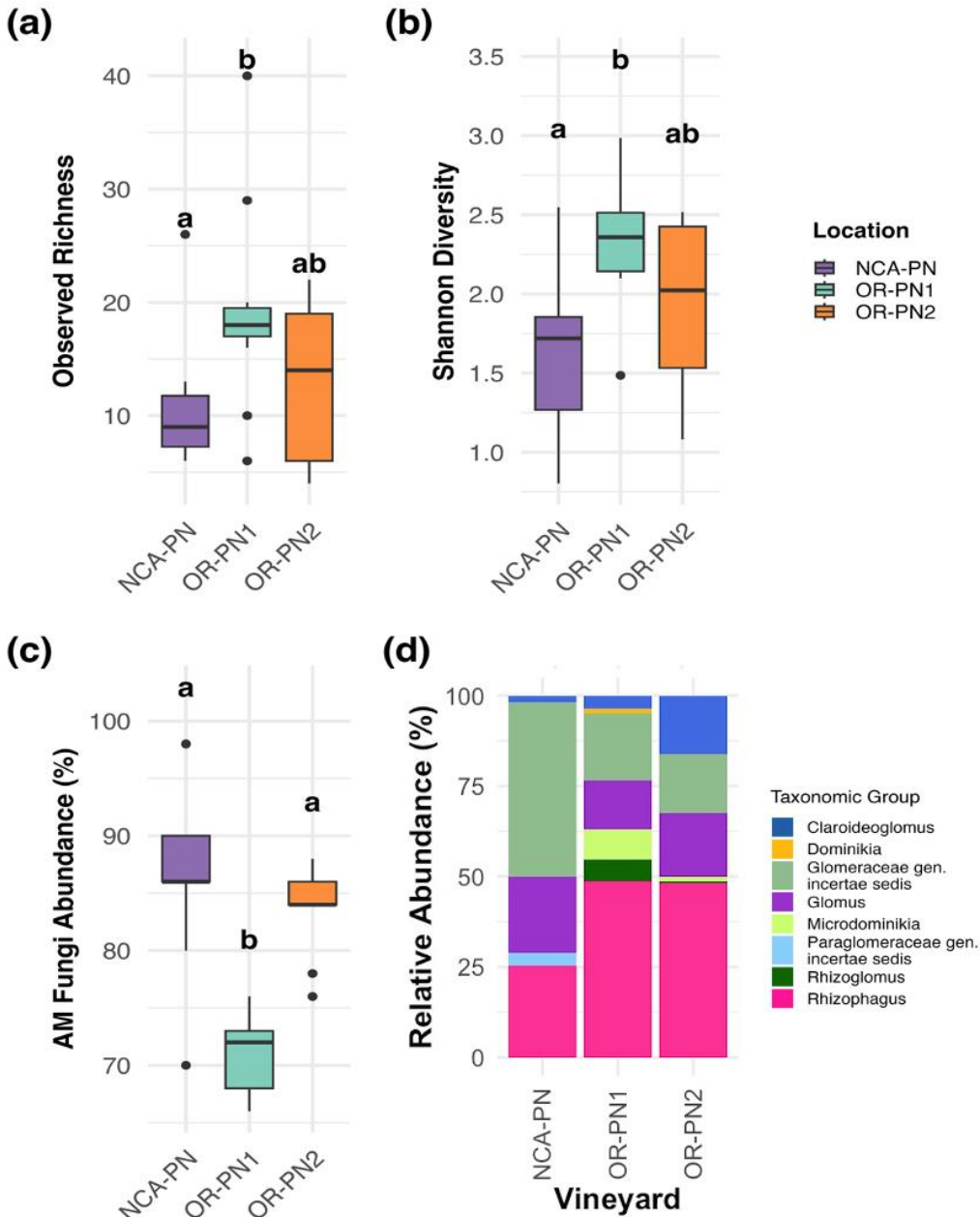


Fig. S1.1 Patterns of arbuscular mycorrhizal (AM) fungal communities across vineyards cultivating the same grapevine genotype (PN x 101-14) on different soils. Boxplots show variation in AM fungal (a) richness, (b) Shannon diversity, and (c) AM fungi abundance (%). Panel (d) shows the relative abundance of the top 8 AM fungal genera in each vineyard. Different letters above boxplots indicate significant differences among regions based on a Kruskal-Wallis test followed by Dunn's post hoc comparison

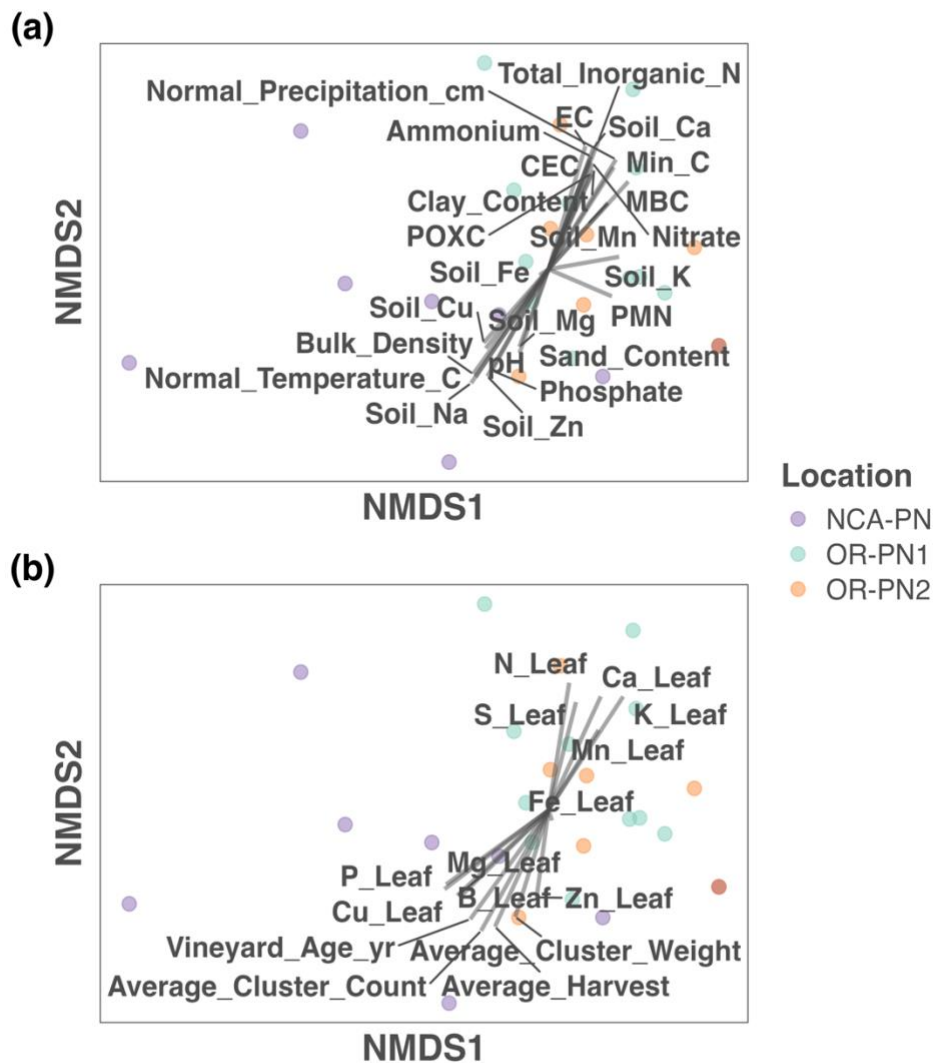


Fig. S1.2 Non-metric multidimensional scaling (NMDS) ordination of arbuscular mycorrhizal (AM) fungi community composition based on Bray-Curtis dissimilarity across vineyards cultivating the same grapevine genotype (PN x 101-14) on different soils. Significant (a) soil and climate and (b) grapevine vectors ($p < 0.05$) were overlaid using the envfit function, which fits variables to the ordination axes and assesses significance by permutation. Arrow length and direction indicate the strength and orientation of correlations between variables and AM fungal community structure

Table S1.11 Summary for SIMPER analysis results showing arbuscular mycorrhizal (AM) fungal taxonomic groups that contribute significantly to differences in community composition among vineyards growing the same grapevine genotype (PN x 101-14). Listed are the vineyards where each taxonomic group showed higher relative abundance, the contrasts in which those differences were significant, and their relative contribution (%) to total Bray-Curtis dissimilarity. Diss/SD indicates the consistency of each taxonomic group's contribution across sample comparisons, with higher values reflecting more uniform patterns of difference

Taxonomic Group	Region with greatest abundance	Significant Comparisons	Contribution (%)	Diss/SD	Environmental variables (negative responders)	Environmental variables (positive responders)	Indicator Association (region, vineyard, and genotype)
<i>Glomeraceae gen incertae sedis</i>	NCA-PN	NCA-PN x OR-PN2*	22.8	0.19	---	POXC, mineralizable C, MBC, precipitation, soil Ca	---
<i>Paraglomeraceae gen incertae sedis</i>	NCA-PN	NCA-PN x OR-PN2**	1.7	0.02	---	---	NCA, NCA-PN
<i>Rhizophagus</i>	OR-PN1	OR-PN1 x OR-PN2**	37.5	0.18	---	POXC, mineralizable C, MBC, soil Ca	---
<i>Rhizoglossus</i>	OR-PN1	OR-PN1 x OR-PN2**	5.4	0.09	---	Soil Ca	---
<i>Claroideoglossus</i>	---	---	---	---	---	POXC, mineralizable C, Ca leaf	---

Significance levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Vineyard contrasts: NCA-PN x OR-PN2 = NCA-PN vs OR-PN2; OR-PN1 x OR-PN2 = OR-PN1 vs. OR-PN2

“---” indicates that information was available for this parameter

Table S1.12 Vector loadings and associated p-values for soil and climate variables fitted onto the NMDS ordination (NMDS1 and NMDS2) in Supplementary Fig 1.2a. These loadings characterize the strength and direction of each continuous variable's correlation with AM fungal community dissimilarities from samples collected in vineyards cultivating the same grapevine genotype (PN x 101-14) on different soils, with larger absolute values indicating stronger contributions to separation along NMDS axes. Continuous variables are shown as vectors. For categorical variables (e.g. Vineyard, Region), p-values reflect the significance of group-level differences in AM fungal community structure. All loadings and significance values were generated using the envfit function with 999 permutations. Abbreviations include: EC = electrical conductivity; PMN = potentially mineralizable nitrogen; MBC = microbial biomass carbon; POXC = permanganate oxidizable carbon; CEC = cation exchange capacity; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; Na = sodium; Zn = zinc

Variable	NMDS1	NMDS1	p-value
Normal Precipitation (cm)	0.53	0.85	0.004
Normal Temperature (C)	-0.57	-0.82	0.003
Bulk Density (g cm ⁻³)	-0.59	-0.80	0.006
Clay Content (%)	0.42	0.90	0.026
Sand Content (%)	-0.33	-0.94	0.108
pH	-0.63	-0.78	0.045
EC (μS cm ⁻¹)	0.29	0.96	0.008
Nitrate (mg NO ₃ ⁻ -N kg ⁻¹ soil)	0.39	0.92	0.014
Ammonium (mg NH ₄ ⁺ -N kg ⁻¹ soil)	0.36	0.93	0.014
Total Inorganic N (mg N kg ⁻¹ soil)	0.37	0.93	0.001
PMN (mg PMN-N kg ⁻¹ soil)	0.92	-0.38	0.247
Phosphate (mg PO ₄ ³⁻ -P kg ⁻¹ soil)	-0.50	-0.87	0.016
Mineralizable C (mg C kg ⁻¹ soil * hr)	0.55	0.84	0.013
MBC (mg C kg ⁻¹ soil)	0.68	0.73	0.015
POXC (mg C kg ⁻¹ soil)	0.42	0.91	0.034
CEC (meq/100g)	0.36	0.93	0.018
Ca (meq/100g)	0.35	0.93	0.008
Cu (mg Cu kg ⁻¹ soil)	-0.67	-0.74	0.050
Fe (mg Fe kg ⁻¹ soil)	-0.84	-0.54	0.966
K (mg K kg ⁻¹ soil)	0.99	0.17	0.248
Mg (meq/100g)	-0.36	-0.93	0.008
Mn (mg Mn kg ⁻¹ soil)	0.68	0.74	0.101
Na (mg Na kg ⁻¹ soil)	-0.57	-0.82	0.001
Zn (meq/100g)	-0.50	-0.86	0.009
Vineyard	-	-	0.028
Region	-	-	0.006

Table S1.13 Vector loadings and associated p-values for grapevine variables fitted onto the NMDS ordination (NMDS1 and NMDS2) in Supplementary Fig 1.2b. These loadings characterize the strength and direction of each continuous variable's correlation with AM fungal community dissimilarities from samples collected in vineyards cultivating the same grapevine genotype (PN x 101-14) on different soils, with larger absolute values indicating stronger contributions to separation along NMDS axes. Continuous variables are shown as vectors. For categorical variables (e.g. Vineyard, Region), p-values reflect the significance of group-level differences in AM fungal community structure. All loadings and significance values were generated using the envfit function with 999 permutations. Abbreviations include: B = boron; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; N = nitrogen; P = phosphorous; S = sulfur; Zn = zinc

Variable	NMDS1	NMDS2	p-value
Average Cluster Count (# of clusters per vine)	-0.50	-0.87	0.001
Average Cluster Weight (kg per vine)	-0.30	-0.95	0.037
Yield (kg vine ⁻¹)	-0.43	-0.90	0.008
B Leaf (mg B kg ⁻¹ leaf)	-0.72	-0.69	0.130
Ca Leaf (mg Ca kg ⁻¹ leaf)	0.43	0.90	0.005
Cu Leaf (mg Cu kg ⁻¹ leaf)	-0.80	-0.59	0.009
Fe Leaf (mg Fe kg ⁻¹ leaf)	0.33	-0.94	0.977
K Leaf (mg K kg ⁻¹ leaf)	0.56	0.83	0.004
Mg Leaf (mg Mg kg ⁻¹ leaf)	-0.74	-0.67	0.014
Mn Leaf (mg Mn kg ⁻¹ leaf)	0.54	0.94	0.920
N Leaf (mg N kg ⁻¹ leaf)	0.16	0.99	0.008
P Leaf (mg P kg ⁻¹ leaf)	-0.82	-0.58	0.011
S Leaf (mg S kg ⁻¹ leaf)	0.25	0.97	0.031
Zn Leaf (mg Zn kg ⁻¹ leaf)	-0.14	-0.99	0.123
Vineyard Age (yr)	-0.59	-0.80	0.006
Vineyard	-	-	0.028
Region	-	-	0.006

Table S1.14 Vector loadings and associated p-values for soil and climate variables on the first two principal coordinate axes (PCoA1 and PCoA2) from vineyards (OR-PN1, OR-PN2, OR-CH1, and OR-CH2) cultivating different scions (CH and PN) grafted on the 101-14 rootstock. Loadings indicate the strength and direction of each variable's contribution to vineyard dissimilarities in soil and climate characteristics, with R² higher absolute loading values reflecting stronger influence on axis separation. All loadings and significance values were generated using the envfit function with 999 permutations. Abbreviations include: EC = electrical conductivity; PMN = potentially mineralizable nitrogen; MBC = microbial biomass carbon; POXC = permanganate oxidizable carbon; CEC = cation exchange capacity; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; Na = sodium; Zn = zinc

Variable	PCoA1	PCoA2	p-value
Bulk Density (g cm ⁻³)	0.06	0.99	0.001
Clay Content (%)	0.98	-0.18	0.001
Sand Content (%)	-0.93	0.36	0.001
pH	-0.84	0.54	0.644
EC (μS cm ⁻¹)	-0.25	-0.97	0.001
Nitrate (mg NO ₃ ⁻ -N kg ⁻¹ soil)	0.94	-0.34	0.052
Ammonium (mg NH ₄ ⁺ -N kg ⁻¹ soil)	0.88	-0.47	0.001
Total Inorganic N (mg N kg ⁻¹ soil)	0.89	-0.45	0.001
PMN (mg PMN-N kg ⁻¹ soil)	-0.47	0.88	0.185
Phosphate (mg PO ₄ ³⁻ -P kg ⁻¹ soil)	-0.62	-0.78	0.106
Mineralizable C (mg C kg ⁻¹ soil * hr)	-0.45	-0.89	0.012
MBC (mg C kg ⁻¹ soil)	0.97	0.24	0.009
POXC (mg C kg ⁻¹ soil)	-0.10	-0.99	0.001
CEC (meq/100g)	0.12	-0.99	0.001
Ca (meq/100g)	0.39	-0.92	0.001
Cu (mg Cu kg ⁻¹ soil)	0.82	-0.57	0.001
Fe (mg Fe kg ⁻¹ soil)	-0.62	-0.78	0.001
K (mg K kg ⁻¹ soil)	-0.55	-0.83	0.001

Mg (meq/100g)	-0.68	-0.73	0.001
Mn (mg Mn kg⁻¹ soil)	-0.99	0.14	0.001
Na (mg Na kg⁻¹ soil)	-0.88	-0.48	0.003
Zn (meq/100g)	-0.75	-0.66	0.001

Table S1.15 Vector loadings and associated p-values for plant variables on the first two principal coordinate axes (PCoA1 and PCoA2) from vineyards (OR-PN1, OR-PN2, OR-CH1, and OR-CH2) cultivating different scions (CH and PN) grafted on the 101-14 rootstock. Loadings indicate the strength and direction of each variable's contribution to genotypic dissimilarities in grapevine characteristics, with higher absolute values reflecting stronger influence on axis separation. All loadings and significance values were generated using the envfit function with 999 permutations. Abbreviations include: B = boron; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; N = nitrogen; P = phosphorous; S = sulfur; Zn = zinc

Variable	PCoA1	PCoA2	p-value
Average Cluster Count (# of clusters per vine)	-0.94	-0.32	0.001
Average Cluster Weight (kg per vine)	-0.80	-0.60	0.001
Yield (kg vine ⁻¹)	-0.90	-0.44	0.001
B Leaf (mg B kg ⁻¹ leaf)	0.66	0.75	0.026
Ca Leaf (mg Ca kg ⁻¹ leaf)	0.19	-0.98	0.752
Cu Leaf (mg Cu kg ⁻¹ leaf)	0.04	-1.00	0.001
Fe Leaf (mg Fe kg ⁻¹ leaf)	-0.88	-0.48	0.001
K Leaf (mg K kg ⁻¹ leaf)	0.98	0.18	0.001
Mg Leaf (mg Mg kg ⁻¹ leaf)	-0.98	-0.21	0.757
Mn Leaf (mg Mn kg ⁻¹ leaf)	-0.05	-1.00	0.003
N Leaf (mg N kg ⁻¹ leaf)	0.30	-0.95	0.001
P Leaf (mg P kg ⁻¹ leaf)	0.89	-0.45	0.001
S Leaf (mg S kg ⁻¹ leaf)	0.78	-0.62	0.001
Zn Leaf (mg Zn kg ⁻¹ leaf)	-0.91	0.42	0.001

Table S1.16 Vector loadings and associated p-values for soil and climate variables on the first two principal coordinate axes (PCoA1 and PCoA2) from vineyards (NCA-PN and NCA-CH) cultivating different grapevine genotypes (PN x 101-14 and CH x 140R). Loadings indicate the strength and direction of each variable's contribution to vineyard dissimilarities in soil and climate characteristics, with R² higher absolute loading values reflecting stronger influence on axis separation. All loadings and significance values were generated using the envfit function with 999 permutations. Abbreviations include: EC = electrical conductivity; PMN = potentially mineralizable nitrogen; MBC = microbial biomass carbon; POXC = permanganate oxidizable carbon; CEC = cation exchange capacity; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; Na = sodium; Zn = zinc

Variable	PCoA1	PCoA2	p-value
Bulk Density (g cm ⁻³)	-1.00	0.05	0.001
Clay Content (%)	-1.00	0.05	0.001
Sand Content (%)	1.00	-0.05	0.001
pH	-0.87	0.50	0.001
EC (μS cm ⁻¹)	-0.08	-1.00	0.017
Nitrate (mg NO ₃ ⁻ -N kg ⁻¹ soil)	0.70	-0.71	0.001
Ammonium (mg NH ₄ ⁺ -N kg ⁻¹ soil)	0.90	0.44	0.001
Total Inorganic N (mg N kg ⁻¹ soil)	0.93	0.37	0.001
PMN (mg PMN-N kg ⁻¹ soil)	0.32	0.95	0.001
Phosphate (mg PO ₄ ³⁻ -P kg ⁻¹ soil)	-0.96	0.28	0.001
Mineralizable C (mg C kg ⁻¹ soil * hr)	0.45	0.89	0.001
MBC (mg C kg ⁻¹ soil)	0.18	0.98	0.029
POXC (mg C kg ⁻¹ soil)	-0.10	0.99	0.93
CEC (meq/100g)	-0.06	1.00	0.001
Ca (meq/100g)	-0.92	0.38	0.001
Cu (mg Cu kg ⁻¹ soil)	-0.66	0.75	0.001
Fe (mg Fe kg ⁻¹ soil)	0.11	-0.99	0.001
K (mg K kg ⁻¹ soil)	-0.91	0.42	0.001

Mg (meq/100g)	0.54	0.84	0.001
Mn (mg Mn kg⁻¹ soil)	-0.92	0.38	0.648
Na (mg Na kg⁻¹ soil)	0.24	0.97	0.004
Zn (meq/100g)	-0.97	-0.24	0.033

Table S1.17 Vector loadings and associated p-values for for plant variables on the first two principal coordinate axes (PCoA1 and PCoA2) from vineyards (NCA-PN and NCA-CH) cultivating different grapevine genotypes (PN x 101-14 and CH x 140R). Loadings indicate the strength and direction of each variable's contribution to genotypic dissimilarities in grapevine characteristics, with higher absolute values reflecting stronger influence on axis separation. All loadings and significance values were generated using the envfit function with 999 permutations. Abbreviations include: B = boron; Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; N = nitrogen; P = phosphorous; S = sulfur; Zn = zinc

Variable	PCoA1	PCoA2	p-value
Average Cluster Count (# of clusters per vine)	0.92	0.39	0.001
Average Cluster Weight (kg per vine)	1.00	0.06	0.001
Yield (kg vine ⁻¹)	1.00	0.10	0.001
B Leaf (mg B kg ⁻¹ leaf)	0.98	0.17	0.001
Ca Leaf (mg Ca kg ⁻¹ leaf)	-0.30	-0.95	0.001
Cu Leaf (mg Cu kg ⁻¹ leaf)	0.47	-0.88	0.001
Fe Leaf (mg Fe kg ⁻¹ leaf)	-0.31	-0.95	0.001
K Leaf (mg K kg ⁻¹ leaf)	0.99	-0.13	0.001
Mg Leaf (mg Mg kg ⁻¹ leaf)	0.00	-1.00	0.760
Mn Leaf (mg Mn kg ⁻¹ leaf)	1.00	-0.01	0.001
N Leaf (mg N kg ⁻¹ leaf)	0.97	0.25	0.001
P Leaf (mg P kg ⁻¹ leaf)	0.13	-0.99	0.001
S Leaf (mg S kg ⁻¹ leaf)	0.94	-0.33	0.001
Zn Leaf (mg Zn kg ⁻¹ leaf)	0.35	-0.94	0.095

Chapter 2: Arbuscular mycorrhizal fungal colonization and community composition vary across grapevine phenological stages and scion-rootstock combinations

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Abstract

Arbuscular mycorrhizal (AM) fungi are well-known microbial symbionts that support plant nutrient and water uptake, soil structure, and ecosystem resilience. In wine grape production, AM fungi may support vine health and fruit development without promoting excessive vegetative growth that can compromise fruit quality. Root colonization dynamics and AM community composition are shaped by environmental conditions and host traits, and may vary across the growing season in perennial crops as a response to changes in crop needs. However, seasonal dynamics of AM fungal symbiosis across grapevine phenological stages and among different scion-rootstock combinations, remain poorly understood. In this study, we investigated AM fungal colonization and community composition across five phenological stages (bud break, bloom, veraison, harvest, and dormancy) in two vineyard sites, each cultivating a different grapevine scion-rootstock combination (PN: Pinot Noir x 101-14 and CH: Chardonnay x 140R, respectively). Site strongly structured AM fungal colonization, diversity, and community composition across phenological stages, with PN exhibiting higher colonization while CH exhibited greater Shannon diversity across several phenological stages. These patterns may reflect physiological differences in rootstock rooting depth and physiological responses to site conditions. Scion-specific differences in berry demands may have further contributed to these patterns. AM fungal colonization and community composition varied significantly across phenological stages in both sites. In both sites, colonization rates were highest at dormancy, coinciding with

elevated vesicle occurrence. Shannon diversity remained stable across phenological stages in both sites.

Community composition also differed between bud break and all subsequent stages, as well as between bloom and veraison, in both scion-rootstock combinations, likely driven by shifts in host C allocation associated with grapevine physiological development.

1. Introduction

Arbuscular mycorrhizal (AM) fungi are a well-known example of microbial symbiosis in agriculture, forming mutualistic associations with the roots of more than 80% of terrestrial plants (Redecker et al. 2000; Smith and Read 2008). Through these associations, AM fungi enhance plant nutrient uptake and water use efficiency in exchange for plant-derived carbohydrates and lipids required for fungal survival, growth, and reproduction (Lee et al. 2013; Schreiner and Linderman 2005; van der Heijden et al 1998; Vieira et al. 2020). AM fungi colonize host roots by attaching to and penetrating root cells with their fungal hyphae, within which they form specialized intraradical structures (Tikhonovich and Provorov 2007). Within root cells, fungi develop arbuscules, which are highly branched structures that facilitate nutrient exchange between the fungus and the plant (Tikhonovich and Provorov 2007), as well as vesicles that primarily function as nutrient storage structures (Souza 2005). Seasonal variation in host plant carbon (C) allocation and environmental conditions can influence both AM fungal root colonization and broader patterns in community composition (Dumbrell et al. 2011).

Numerous studies focused on diverse host plant communities have observed that AM fungi exhibit pronounced seasonal patterns in root colonization and intraradical structure formation (Barnes et al. 2016; Bohrer 2004; Buade et al. 2021; Cera et al. 2021). Shifts in AM fungal colonization are thought to reflect interactions between soil nutrient availability and plant nutrient demand, which together regulate C allocation from the host to their fungal symbionts (Buade et al. 2021; Hoeksema et al. 2010; Jakobsen et al. 2003). Arbuscules tend to be most abundant during periods of high plant nutrient demand, when rates of nutrient exchange between fungi and host plants are elevated (Allen 1983; Mullin and Schmidt 1993). At other times, arbuscules may be reduced or absent (Allen 1983; Mullin and Schmidt 1993). In contrast, vesicles are more commonly observed during periods of reduced plant nutrient acquisition (Abbot et al. 1984). Rather than being directly associated with specific phases of plant nutrient demand, hyphae typically appear early in the AM fungal symbiotic interaction, playing a central role

in establishing and maintaining nutrient and water transfer between the soil and the hosts' roots (Brundrett 2009; Souza 2025). Collectively, variation in the abundance of intraradical AM fungal structures can provide insight into fungal activity in relation to host plant dynamics (Jakobsen et al. 2003).

AM fungal community composition also exhibits pronounced seasonal patterns, which are hypothesized to arise from seasonal fluctuations in abiotic conditions or from host-mediated shifts in plant C allocation associated with growth and phenology (Barnes et al. 2016; Bennett et al. 2013; Buade et al. 2021; Dumbrell et al. 2011; Escudero and Mendoza 2005; Goldmann et al. 2020; Pereira e Silva et al. 2012). Soil properties can modulate seasonal patterns by shaping fungal habitat conditions, including nutrient availability, moisture dynamics, and oxygen supply (Goldmann et al. 2020). Relatively stable soil characteristics, such as soil texture, have been shown to influence seasonal variation in AM fungal richness in grasslands by mediating the physical and chemical environments experienced by fungi (Goldmann et al. 2020). Seasonal changes in host plant phenology have also been observed to alter AM fungal community structure (Barnes et al. 2016; Dumbrell et al. 2011). For example, in soybean cultivation, higher diversity occurred during early and late developmental stages, whereas dominance by fewer taxa increased during intermediate stages (Buade et al. 2021). The latter was attributed to greater host root selective pressure exerted during these stages (Buade et al. 2021). These shifts in AM fungal community structure can be partitioned into two components, turnover and nestedness. Turnover refers to the replacement of taxa between sampling points, while nestedness is the gain or loss of taxa from one time point to the next (Goldmann et al. 2020). While drivers of AM fungal community turnover remain inconsistent across systems (Gao et al. 2019; Goldmann et al. 2020), nestedness has been linked to environmental filtering, where differences in habitat suitability (i.e. nutrient availability, management intensity) result in the loss or gain of specific taxa (Verbruggen et al. 2012).

In the existing literature, most studies examining seasonal variation in AM fungal communities have focused on annual or herbaceous systems (Buade et al. 2021; Barnes et al. 2016; Bohrer et al. 2004; Goldmann et al. 2020), with comparatively fewer studies addressing perennial, woody species such as grapevines. Grapevines are deciduous perennial crops with a distinct annual growth cycle comprising key phenological stages: bud break, bloom, veraison, harvest, and dormancy (Mullins et al. 1992). Bud break marks the onset of the growing season,

when new shoot growth is sustained by carbohydrate reserves stored in perennial woody tissues, including the trunk, cordons, and roots, before leaves are fully developed and photosynthesis is established (Giese et al. 2020; Mullins et al. 1992). Bloom is characterized by the opening of flower clusters (Giese et al. 2020). During this stage, canopy development increases photosynthetic capacity, allowing vines to shift from reliance on stored reserves to photosynthetically derived C (Mullins et al. 1992). Root growth also peaks at this stage as excess photosynthate becomes available (Mullins et al. 1992; Schreiner 2005). Veraison marks the onset of fruit ripening, when berries begin to soften and accumulate sugar, drawing heavily on leaf photosynthesis as they replace shoots as the dominant C sink (Giese et al. 2020; Mullins et al. 1992). This period of high physiological activity continues through harvest, where berries reach physiological maturity as measured by sugar accumulation (Giese et al. 2020; Rafique et al. 2023). However, the degree of sugar accumulation varies by cultivar, reflecting differences in the wine styles traditionally associated with each variety (Giese et al. 2020). Following harvest, the removal of berries increases C flow to the root system, stimulating a second major flush of root growth (Mullins et al. 1992; Schreiner et al. 2005). As day length decreases and temperatures fall, vines enter dormancy and cease vegetative growth until the following spring (Kovaleski and Londo 2018; Mullins et al. 1992). As AM fungi are obligate biotrophs dependent on host-derived C, these phenological shifts in host C source and allocation may drive changes in AM fungal colonization and community composition. Yet, the extent to which these shifts influence grapevine-AM fungal symbiosis across the growing season remains poorly understood.

While some studies have examined AM fungal symbiosis in relation to grapevine development, they have generally been limited in scope, often relying on a small number of sampling time points within the growing season. Cesaro et al. (2021) examined AM fungal colonization and diversity at two grapevine phenological stages, flowering and early fruit development, and reported no significant differences in colonization or Shannon and Simpson diversity indices between sampling periods. Similarly, Schreiner and Mihara (2009) reported no temporal change in AM fungal diversity between bloom and veraison. Schreiner (2005) conducted a longer-term study across two growing seasons, in which AM fungal colonization and diversity were assessed at monthly intervals over a 7-month sampling period. Although no consistent seasonal trend was observed in root colonization, arbuscule occurrence showed a clear seasonal pattern, increasing early in the growing season and remaining elevated until

leaf senescence (Schriener 2005). Collectively, these findings suggest that sampling at a limited number of phenological stages may obscure important shifts in AM fungal symbiosis over the course of vine development. Studies examining grapevine-AM fungal interactions across a broader range of phenological stages are therefore needed. Additionally, changes in AM fungal community composition across the growing season, and the relative contributions of turnover and nestedness to these changes, are poorly understood.

In most commercial vineyards worldwide, growers graft a grapevine scion onto a rootstock, which comprises the root system and serves as the link between the soil environment and the vine (Ollat et al. 2016). Through differences in breeding parentage and associated functional traits, rootstocks can influence multiple aspects of vine physiology, such as root architecture, scion vigor, and nutrient and water uptake (Basso et al. 2002; Fichtl et al. 2023; Tandonnet et al. 2010). For example, the 140R rootstock, with *V. berlandieri* and *V. rupestris* parentage, is characterized by a deeply developed root system that facilitates access to water and nutrients from deeper soil layers, making it well-suited to dry, Mediterranean-like conditions (Chen et al. 2024). In contrast, the 101-14 rootstock, with *V. riparia* and *V. rupestris* parentage, develops a shallow, less extensive root system suited to heavy, moist clay soils and is less tolerant of drought and saline conditions (Lambert et al. 2008; Pongracz 1983). Variation in root system architecture and root exudate composition among rootstocks can alter the soil-root interface and shape AM fungal colonization and the recruitment of specific taxa (Lailheugue et al. 2024; Moukarzel et al. 2021; Philippot et al. 2013; Warschefsky et al. 2016). Scion can also influence AM fungal communities by shaping the quantity and composition of photosynthates and signaling compounds delivered to the root system, though these effects are generally modest relative to those of the rootstock (Lailheugue et al. 2024; Noceto et al. 2024). Despite growing evidence that scion-rootstock identity shapes AM fungal communities, the role of scion-rootstock combinations in shaping these dynamics across the growing season remains largely unexplored.

In this study, we investigated AM fungal colonization and community composition in two Northern California vineyard sites, CH and PN, cultivating two different scion-rootstock combinations (Chardonnay x 140R and Pinot Noir x 101-14, respectively). Our objective was to assess differences in AM fungal colonization rates and community structure between the two scion-rootstock combinations across five phenological stages: bud break,

bloom, veraison, harvest, and dormancy. We hypothesized that AM fungal colonization would differ significantly across phenological stages, but that the relative abundance of intraradical fungal structures would vary with the plant developmental stage. In particular, we expect arbuscules to be most prevalent during bloom and vesicles to be more abundant during dormancy. We further hypothesized that AM fungal species richness, evenness, and Shannon diversity would vary across phenological stages in response to shifts in host C allocation. During stages of increased C flow to the root system, such as bloom and dormancy, we expected these metrics to be higher. In contrast, we expected a decline in these metrics at veraison and harvest, when C is predominantly redirected toward berry development and away from the root system. AM fungal community composition was also expected to vary across phenological stages, with turnover dominating during periods of pronounced change in vine C dynamics, such as between bud break and bloom, bloom and veraison, or harvest and dormancy. Nestedness was hypothesized to predominate during periods of limited variability in C dynamics, such as between veraison and harvest. We also hypothesized that these phenological shifts in AM fungal colonization and community composition would manifest differently between scion-rootstock combinations. The shallower root system of Pinot Noir x 101-14 grapevines, combined with its limited drought and salinity tolerance, was expected to heighten physiological stress under site conditions, constraining C availability to symbionts and favoring colonization by a few competitive AM fungal taxa. In contrast, the deeper root system of the Chardonnay x 140R grapevines was expected to mitigate vine stress, supporting more stable C allocation to symbionts and harboring a more diverse AM fungal community. By linking seasonal grapevine development with AM fungal colonization and community dynamics, this study offers a framework for understanding how below-ground symbioses align with periods of vine nutrient demand that are critical for maintaining vine balance and fruit quality.

2. Materials and Methods

2.1 Study Design

A controlled, replicated trial was established in 2022 at two sites within a commercial vineyard in the Russian River Valley, Sonoma County, California: PN (Pinot Noir on 101-14 rootstock) and CH (Chardonnay on 140-R rootstock). Vine spacing and planting density differed between sites, with PN planted at 5 x 4 ft spacing (2178 vines ac⁻¹) and CH planted at 6 x 5 ft spacing (1452 vines ac⁻¹). Both sites utilized a vertical shoot-positioned (VSP)

trellising system. Grapevine age also differed between sites, with PN established 11 years prior to sampling and CH established 6 years prior to sampling.

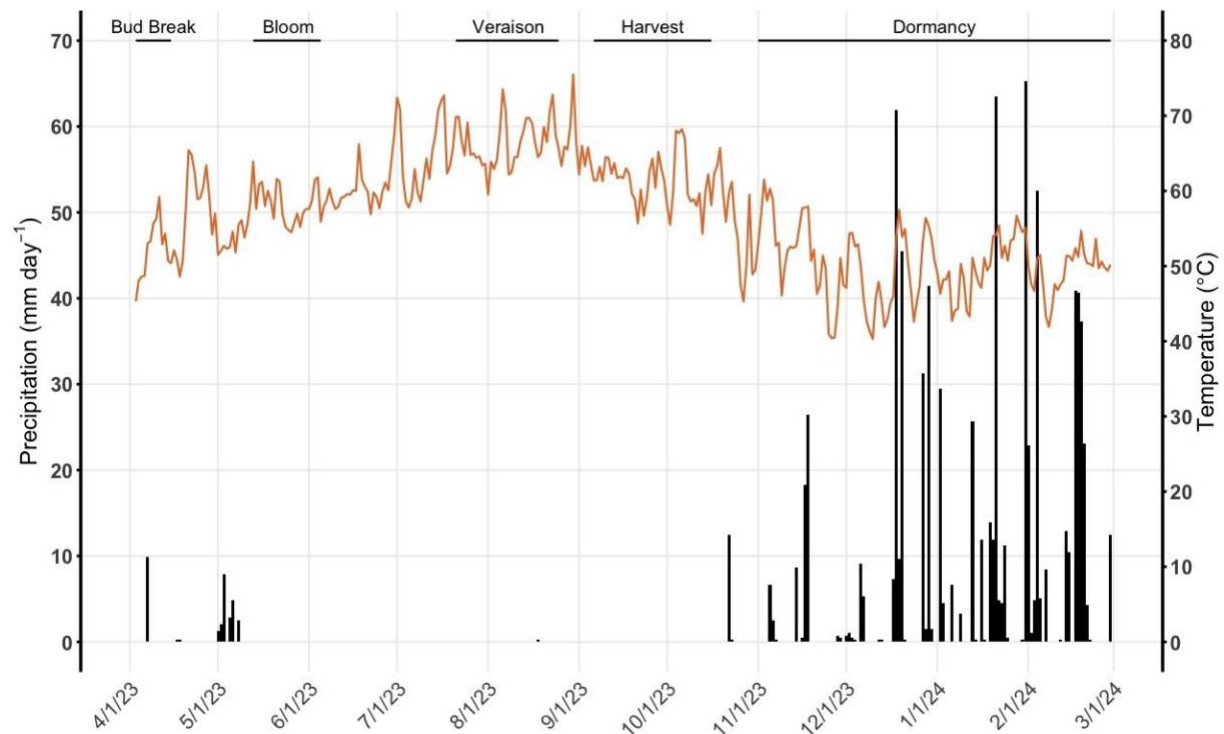


Fig 2.1 Daily precipitation (black bars, mm day⁻¹; left axis) and mean daily temperature (orange line, °C; right axis)

during the 2023–2024 growing season. Phenological stage ranges (Bud Break, Bloom, Veraison, Harvest, Dormancy) are shown in the top panel. Ranges from Bud Break through Harvest were determined from vineyard records spanning 2020–2024 to supplement incomplete 2023 observations. Dormancy was defined as the period after fruit removal until the following bud break. Observations begin on April 3, 2023, corresponding to the start of available climate records, and may not capture earlier bud break events in late March

The sites were located within ~500 m of one another and, therefore, experienced comparable climatic conditions. A weather station located adjacent to the sites recorded daily temperature and precipitation. Weather data were summarized by grapevine phenological stage across the 2023–2024 growing season (Fig 2.1). Grapevine phenological stages were determined in collaboration with vineyard managers using the modified Eichhorn-Lorenz

(E-L) system, with sampling occurring at approximately stages 4 (bud break), 23 (bloom), 35 (veraison), 38 (harvest), 45 (dormancy) (Coombe 1995). Mean temperature for each phenological stage was calculated from hourly air temperature measurements collected on the corresponding sampling date. Precipitation was calculated as the total rainfall accumulated during the month each phenological stage was sampled. Mean temperature increased progressively from bud break (12.4 °C) through bloom (16.2 °C) and veraison (22.7 °C), then declined the rest of the season from harvest (19.8 °C) to dormancy (10.1 °C) (Fig 1). Precipitation remained low throughout the growing season, from bud break (10.4 mm), bloom (21.3 mm), veraison (0.3 mm), and harvest (12.7 mm), before increasing substantially during dormancy (277.4 mm) (Fig 2.1).

Soil properties were described for both sites and phenological stages. According to the NRCS Web Soil Survey, soils at CH are classified as Yolo clay loam (silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents), while soils at PN are Yolo silt loam (silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents). Soil temperature and volumetric water content (VWC) were monitored at 30 cm depth using TEROS 12 Advanced Soil Moisture, Temperature, and EC Sensor (METER Group, Inc.; <https://metergroup.com/products/teros-12/>), with two sensors installed in PN and nine in CH. Hourly measurements recorded on each sampling date were averaged to generate site-level estimates for each phenological stage (Supplemental Fig. 1). In both sites, soil temperature increased from bud break to veraison and declined thereafter, with the lowest values observed during dormancy. Over the same period, volumetric water content remained relatively consistent across phenological stages.

In addition to soil conditions, vineyard management practices were characterized for both sites. Both sites maintained cover crops consisting of sheep mix (1% Campeda Subclover, 2% Hykon Rose Clover, 2% Dwarf Essex Rape, 23% Austrian Winter Peas, 35% Winter Ryegrain, 35% Triticale) and oats. Drip irrigation was applied at 4 hours per week, with irrigation increased to 6 hours per week during the summer months. Fertigation was also applied at 5-10 gal ac⁻¹ during the summer months, with application rates adjusted based on foliar nutrient analysis. Herbicide applications were not utilized at either site, and weeds were removed through mechanical control methods.

2.2 Plant and soil sampling

From April 2023 to February 2024, soil and root samples (0-30 cm) were collected from both sites on the same date at the five grapevine phenological stages: bud break (April 2023), bloom (May 2023), veraison (August 2023), harvest (October 2023), and dormancy (February 2024).

Each site contained 18 plots arranged in three blocks, with each block comprising six plots. A plot was defined as four adjacent vine rows containing at least 60 vines per row. Within each block, five plots were selected for sampling, resulting in a total of 15 sampled plots per site at each phenological stage. Within each sampled plot, both soil and root samples were collected from a single vine at each phenological stage for a total of 15 vines sampled at each site and phenological stage (n=15). The same vine was not sampled across phenological stages. Instead, a different vine was randomly selected within each sampled plot at each time point.

Lateral roots were excavated from the surface soil (0-30 cm) adjacent to the rootstock crown. For each vine, fine root material was collected to fill approximately one-quarter of a standard sandwich-sized polyethylene bag (16.5 x 14.9 cm), providing sufficient tissue for microscopic quantification of AM fungal colonization and for DNA extraction for AM fungal community sequencing. Across both sites, a total of 30 root samples (15 plots x 2 sites) were collected at each phenological stage.

Soil samples (0 – 30 cm) were collected from three locations surrounding each sampled vine near the rootstock crown using a 2.2 cm diameter soil probe, yielding approximately 432 cm³ of soil per sampling plot. A total of 30 soil samples were collected per phenological stage (15 plots x 2 sites). Across the full study period, 150 soil and root samples were collected (n=150; 15 plots x 2 sites x 5 phenological stages). All soil and root samples were placed in polyethylene bags and stored in a 2 °C cold room until processing.

2.3 Analysis of AM fungal abundance and diversity in vine roots

After transport to the laboratory, fine roots were cleaned using a sequential rinsing process used to remove adhering soil particles. Initially, roots were rinsed under a stream of DI water to remove large, loosely attached soil particles. Soil tightly adhered to roots was then removed using forceps and a wash bottle. Cleaned roots were then divided into two subsamples: 1) stored in 70% ethanol at 4° C for root staining; 2) at -80° C for DNA analysis to assess AM fungal diversity. Samples preserved in ethanol were stored at 4 °C for approximately six

months prior to staining, and frozen subsamples were stored at -80 °C for approximately ten months before DNA extraction.

Grapevine roots preserved in 70% ethanol at 4°C were cleared and stained following Schreiner (2003). AM fungal colonization was quantified using a gridline intersect method at 40x magnification on a Leica DM1000 LED compound microscope. For each sample, stained roots were examined at 100 intersections. At each intersect, the presence or absence of AM fungal structures (i.e. hyphae, arbuscules, and vesicles) crossing the vertical crosshair was recorded. Percent colonization was expressed as the percentage of intersects with AM fungal structures, calculated as one minus the proportion of intersects with no detected AM fungal structure multiplied by 100.

Arbuscular mycorrhizal fungal DNA was extracted from roots using DNeasy Plant Pro Kits (Qiagen, Germantown, MD, United States). Amplicon sequencing targeting the internal transcribed spacer region (ITS-2) was performed at the University of California-Davis Host-Microbe Systems Biology Core Facility using the NexF-N3-5.8S_fun and nexR-N3-ITS2_fun primers of variable lengths (Taylor et al. 2016). Libraries were prepared using a two-step PCR analysis. The first PCR step involved an initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation, annealing, and extension, and final storage at 12°C. To incorporate molecular barcodes and Illumina adapters, a second PCR step was carried out, consisting of 8 additional cycles under similar thermal cycling conditions using dual-indexed Nextera XT primers. Amplicons were pooled, quantified, and submitted for paired-end sequencing on an Illumina MiSeq instrument (Illumina, San Diego, CA, United States).

After sequencing, raw FASTQ data were processed using the QIIME2 pipeline (version 2023.9; <http://qiime2.org/>). Taxonomic assignment was performed using the QIIME2 feature-classifier plugin trained on the UNITE ITS-2 reference database (Abarenkov et al. 2024). The resulting operational taxonomic unit (OTU) and taxonomy tables were imported into R statistical software and analyzed using the phyloseq package (McMurdie and Holmes 2013). All data were filtered to retain only taxa assigned to the phylum Glomeromycota, and downstream analyses were restricted to this group. Alpha diversity metrics, including species richness (observed and Chao 1), relative abundance, evenness, and the Shannon diversity index, were used to assess the diversity of AM fungal communities. Observed and Chao1 richness values were identical, so observed richness was reported. Analyses and interpretation were conducted at the genus level, with unassigned genera labeled as *gen. incertae*

sedis according to the classifier. These designations reflect varying levels of confidence placement, with some taxa placed to the family level (*Glomeraceae*, *Paraglomeraceae*, *Claroideoglomeraceae*), the order level (*Glomerales* and *Paraglomerales*), or the phylum level (*Glomeromycota*).

2.4 Soil analysis

After collection, soil samples were sieved to 2 mm and air-dried prior to analysis. Nitrate-N (NO_3^- -N) was determined by extracting 8 g of air-dried soil with potassium sulfate (K_2SO_4), following the method adapted from Drinkwater et al. (2015). The extract was then quantified colorimetrically using a modified protocol from Doane and Horwath (2003). Phosphate-P concentration (PO_4^{3-} -P) was determined by extracting 2.50 g air-dried soil with sodium bicarbonate (NaHCO_3), followed by colorimetric analysis of the filtered extract (Olsen et al. 1954). Both measurements were analyzed using a Biotek Synergy™ HTX multi-mode microplate reader (BioTek Instruments, Winooski, VT, USA).

Mineralizable C was measured by rewetting 10g of air-dried soil to 50% water-holding capacity with DI water, followed by a 24-hour incubation and quantification of CO_2 production ($\text{mg CO}_2\text{-C kg}^{-1}\text{ soil hr}^{-1}$) using a LI-COR LI-850 $\text{CO}_2/\text{H}_2\text{O}$ gas analyzer (Lincoln, NE, USA). Soil pH and EC were measured in a 2:1 (soil: water) slurry using a Mettler Toledo pH/conductivity meter (Mettler Toledo, Columbus, Ohio, USA).

2.5 Data analysis

All statistical analyses were performed in R version 4.4.0 (R Core Team 2024). Normality of soil, weather, and AM fungal variables was evaluated using the Shapiro-Wilk test, and homogeneity of variances across phenological stages and vineyards was assessed using Levene's test. When assumptions of normality and homoscedasticity were met, differences in soil and AM fungal variables were evaluated using one-way analysis of variance (ANOVA), with group comparisons conducted by phenological stage or vineyard, when applicable. When a significant effect was detected, post hoc pairwise comparisons were performed using Tukey's honestly significant difference (HSD) test. When assumptions were not met, data were transformed using square-root, reciprocal, logarithmic, reflection, or combined transformations. If transformations did not sufficiently meet model assumptions, nonparametric pairwise comparisons were performed using Wilcoxon rank-sum tests.

Principal Component Analysis (PCoA), a distance-based ordination that projects multivariate dissimilarities among samples into a reduced set of interpretable axes, was used to visualize differences in soils and AM fungal variables between vineyards and phenological stages. The `envfit` function in the `vegan` package (Oksanen et al. 2025) was used to identify the environmental variables most strongly associated with the ordination, ranking predictors by the strength of their correlations (R^2) with the ordination axes based on permutation tests.

Beta diversity analyses were conducted using the `vegan` package (v2.6.10) (Oksanen et al. 2025). Permutational multivariate analysis of variance (PERMANOVA) was applied to Bray–Curtis dissimilarities of AM fungal OTUs to test for differences in community composition among vineyards and phenological stages. Non-metric multidimensional scaling (NMDS), a rank-based ordination method that represents multivariate dissimilarities in a reduced-dimensional space, was performed on Bray-Curtis dissimilarities of AM fungal OTUs to visualize patterns in community structure. Environmental variables, including soil and weather factors, were fitted as vectors onto the NMDS ordination using permutation-based significance testing using the `envfit` function, which evaluates the strength of correlations (R^2) between each variable and the ordination axes to identify potential drivers of community patterns. To assess dissimilarity in AM fungal communities across phenological stages, beta diversity was quantified using the Sørensen dissimilarity index based on presence-absence transformed OTU data and partitioned into turnover and nestedness components using the *betapart* package (Baselga et al. 2025). SIMPER analysis was used to identify OTUs contributing most to observed dissimilarities in AM fungal community composition across phenological stages within vineyards.

Indicator species analysis was performed using the `indicspecies` package (v1.8.0) (De Cáceres and Legendre 2009) to identify AM fungal taxa significantly associated with specific phenological stages within vineyards. Threshold Indicator Taxa Analysis (TITAN) was performed using the `TITAN2` package (v2.4.3) (Baker et al. 2020) to determine directional responses of AM fungal OTUs to continuous gradients in soil properties.

3. Results

3.1. Variation in soil and weather properties across phenological stages

Soil and weather properties varied across phenological stages in the two sites studied. A PERMANOVA detected significant effects ($p = 0.001$) of phenological stage, site, and their interaction on measured properties.

These differences were reflected in a PCoA of measured soil, weather, and AM fungal variables, which showed distinct clustering by phenological stage (Fig 2.2). A pairwise PERMANOVA confirmed significant differences in measured properties across phenological stages ($p = 0.001$).

Variation among phenological stages was primarily structured along PCoA1, which was most strongly associated with weather variables (VWC, soil temperature, daily temperature, and monthly precipitation) and AM fungal colonization metrics (hyphae, vesicles, and percent colonization). In contrast, variation along PCoA2 was primarily explained by soil properties, particularly PO_4^{3-} and EC. These variables exhibited the largest vector loadings, highest r^2 values, and significant associations with the ordination axes (Supplementary Information, Table S2.1).

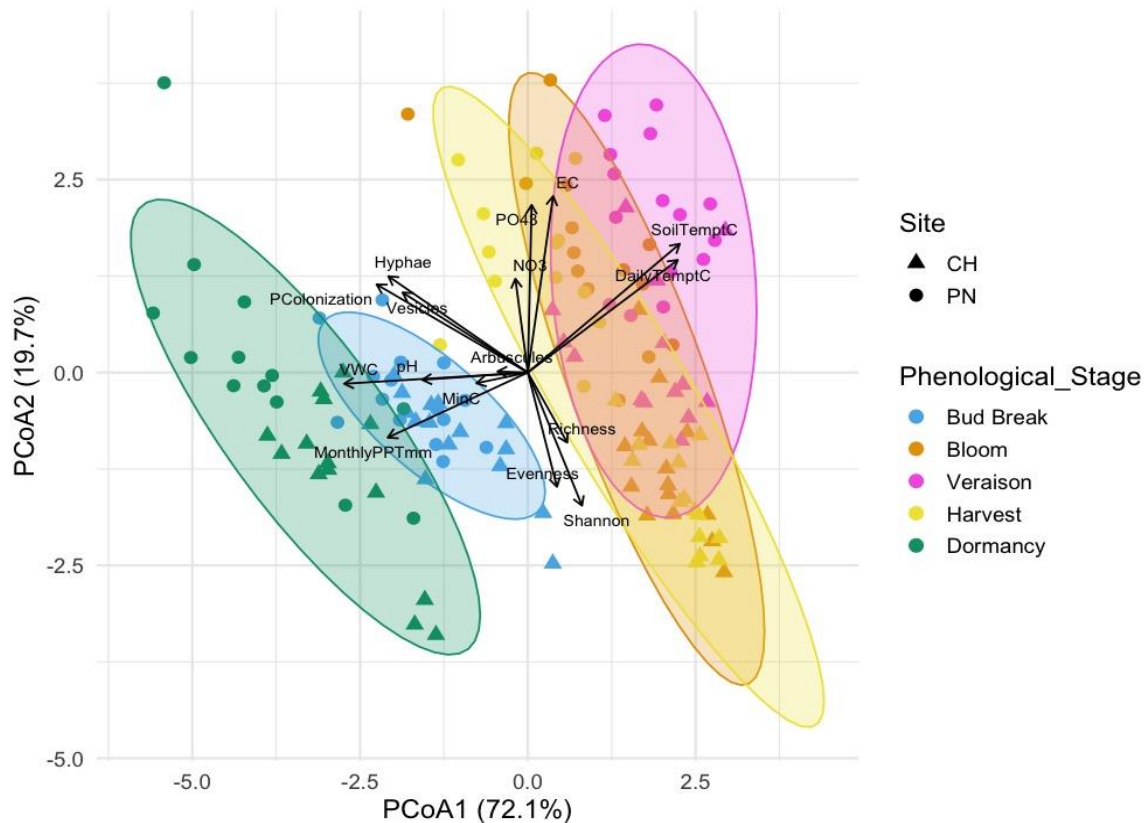


Fig 2.2 Principal Coordinate Analysis (PCoA) of measured soil, weather, and mycorrhizal variables in both sites, CH and PN, across phenological stages. Arrows depict all variables fitted to the ordination, where arrow length and direction reflect the strength and orientation of each variable with the PCoA axes. Variables included are volumetric water content (VWC), monthly precipitation (MonthlyPPTmm), soil temperature

(SoilTempC), daily temperature (DailyTempC), nitrate (NO₃), phosphate (PO₄), electrical conductivity (EC), mineralizable carbon (MinC), soil pH, percent colonization (PColonization), hyphae, vesicles, arbuscules, and AM fungal richness, evenness, and Shannon diversity. Ellipses represent 95% confidence intervals for each phenological stage. A pairwise PERMANOVA detected significant differences among phenological stages ($p = 0.001$)

Differences in soil chemical properties between sites are summarized in Supplementary Information, Table 2.2. Regardless of phenological stage, a significant effect of site was detected for PO₄³⁻ concentrations, with consistently higher PO₄³⁻ at PN compared to CH. EC also differed significantly between vineyards at all phenological stages except dormancy, with higher EC observed at PN than CH at bud break, bloom, veraison, and harvest. Significant site effects were additionally detected for soil pH in all phenological stages, with pH consistently higher at PN than at CH. In contrast, differences in mineralizable C were limited to early phenological stages, with significantly higher mineralizable C at CH compared to PN during bud break and bloom only. Given these vineyard-level differences in soil properties, seasonal trends in measured variables are presented separately for each site below.

In CH, soil properties changed between phenological stages (Fig 2.3a-e). Nitrate concentrations differed significantly between veraison and harvest, with higher concentrations observed at veraison. By comparison, PO₄³⁻ concentrations did not differ significantly across phenological stages. Soil pH was significantly lower at bloom relative to all other phenological stages. EC had a different seasonal pattern, with significantly lower values observed at bud break compared to subsequent phenological stages. Mineralizable C was significantly lower at harvest relative to all the other stages.

At PN, seasonal patterns in soil properties also varied across phenological stages (Fig 2.4a-e). Nitrate concentrations were significantly lower at harvest relative to bloom, veraison, and dormancy. In contrast, PO₄³⁻ concentrations were significantly higher at veraison than at bud break, harvest, and dormancy. Soil pH was significantly lower at bloom and veraison relative to bud break, harvest, and dormancy. Additionally, EC was

significantly higher during bloom, veraison, and harvest compared to bud break and dormancy. Mineralizable C was significantly lower at bloom than at all other phenological stages.

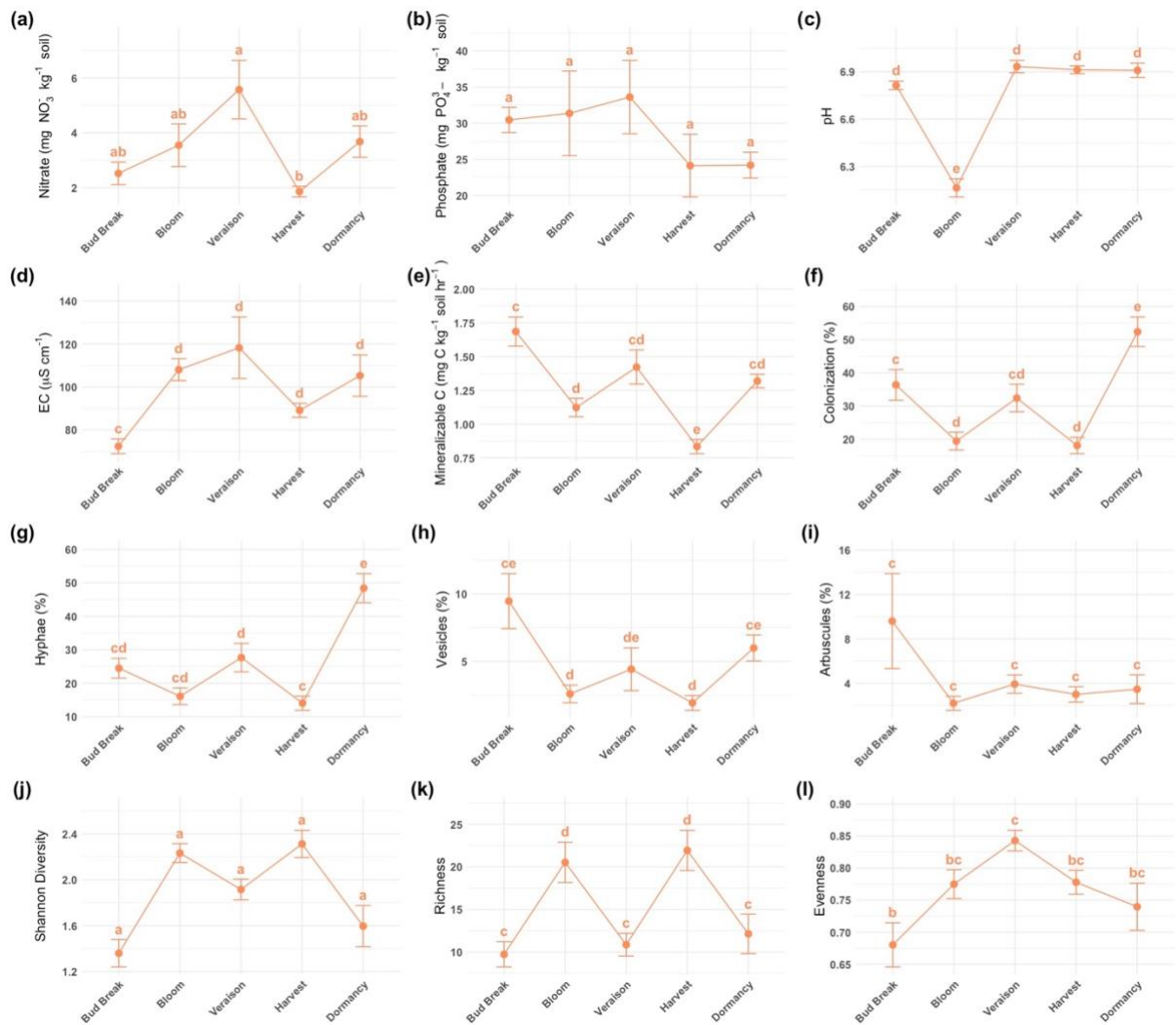


Fig 2.3 Seasonal dynamics of soil properties, AM fungal colonization, and diversity across phenological stages in Northern CA vineyard site, CH during the 2023-2024 growing season. Line plots show mean values ($\pm$ SE) each metric across five phenological stages: bud break, bloom, veraison, harvest, and dormancy. Panels shows (a) nitrate, (b) phosphate, (c) soil pH, (d) EC, (e) mineralizable C, (f) total AM fungal colonization, (g) hyphae, (h) vesicles, (i) arbuscules, (j) Shannon diversity, (k) richness, and (l) evenness. Different lowercase letters indicate significant differences across phenological stages within each site ($p < 0.05$). Significant differences were determined using one-way ANOVA with Tukey's HSD. When assumptions were not met, pairwise Wilcoxon tests were applied.

3.2 Variation in AM fungal colonization and alpha diversity across phenological stages

Phenological stage and site effects on AM fungal colonization and diversity metrics were further evaluated using two-way ANOVAs (Supplementary Information, Table S2.3). Phenological stage had a significant effect on all AM fungal colonization metrics (percent colonization, vesicles, arbuscules, and hyphae; $p < 0.001$), as well as Shannon diversity and richness ($p < 0.05$). However, phenological stage did not significantly affect evenness ($p = 0.42$). Site had a significant effect ($p < 0.01$) on all variables except arbuscules ($p = 0.64$) and richness ($p = 0.58$). Significant phenological stage x site interactions were detected for all colonization and diversity metrics except arbuscules ($p = 0.53$). Given consistent site and phenological stage x site interaction effects, patterns in AM fungal colonization and alpha diversity are presented separately for CH and PN.

Observed AM fungal richness differed significantly across phenological stages in CH (Figure 2.3k). Richness was significantly higher at bloom (20.5 ± 2.4) and harvest (21.9 ± 2.4) compared to bud break (9.7 ± 1.5), veraison (10.9 ± 1.3), and dormancy (12.1 ± 2.3). Evenness also varied across phenological seasons (Figure 3l), with bud break (0.7 ± 0.03) exhibiting significantly lower evenness than veraison (0.8 ± 0.02). Despite variation in richness and evenness across phenological stages, Shannon diversity did not differ significantly throughout the growing season (Figure 2.3j).

In addition to diversity metrics, AM fungal colonization and root-associated fungal structures varied across phenological stages in CH. AM fungal colonization was significantly higher at dormancy ($52.4 \pm 4.4\%$) than at other phenological stages (Figure 2.3f). Additionally, colonization at bloom ($19.5 \pm 2.7\%$) and harvest ($18.1 \pm 2.4\%$) was significantly lower than at bud break ($36.4 \pm 4.6\%$) and dormancy. Hyphal abundance exhibited a similar pattern, with significantly greater detection during dormancy ($48.4 \pm 4.3\%$) than other phenological stages (Figure 2.3g). Further, hyphal density was significantly lower at harvest ($14.0 \pm 2.4\%$) than at veraison ($27.6 \pm 4.4\%$) and dormancy, but did not differ significantly from bud break ($24.5 \pm 2.9\%$) or bloom ($16.1 \pm 2.5\%$). Vesicle abundance was significantly lower at bloom ($2.6 \pm 0.6\%$) and harvest ($1.9 \pm 0.5\%$) compared to bud break ($9.5 \pm 2.0\%$) and dormancy ($6.0 \pm 1.0\%$), but did not differ significantly from veraison ($4.4 \pm 1.6\%$) (Figure 2.3h). In contrast to other fungal structures, arbuscule abundance did not differ significantly across phenological stages (Figure 2.3i).

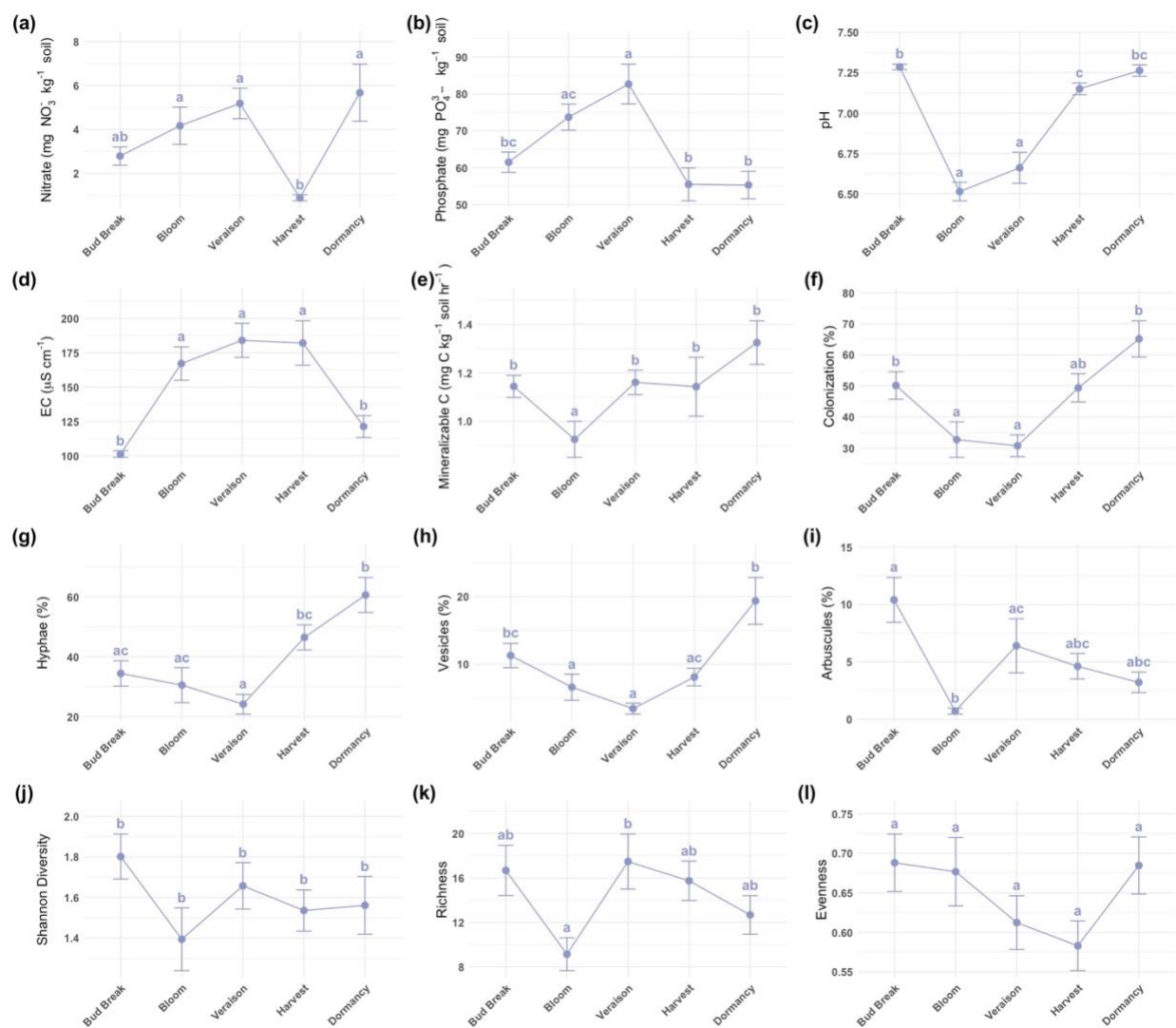


Fig 2.4 Seasonal dynamics of soil properties, AM fungal colonization, and diversity across phenological stages in Northern CA vineyard site, CH during the 2023-2024 growing season. Line plots show mean values ($\pm$ SE) each metric across five phenological stages: bud break, bloom, veraison, harvest, and dormancy. Panels shows (a) nitrate, (b) phosphate, (c) soil pH, (d) EC, (e) mineralizable C, (f) total AM fungal colonization, (g) hyphae, (h) vesicles, (i) arbuscules, (j) Shannon diversity, (k) richness, and (l) evenness. Different lowercase letters indicate significant differences across phenological stages within each site ($p < 0.05$). Significant differences were determined using one-way ANOVA with Tukey's HSD. When assumptions were not met, pairwise Wilcoxon tests were applied.

In PN, observed richness also differed significantly across phenological stages (Figure 2.4k). Richness was significantly higher at veraison (17.5 ± 2.5) than at bloom (9.1 ± 1.5), while no other phenological stages differed significantly from one another. Evenness (Figure 2.4l) and Shannon diversity (Figure 2.4j) did not differ significantly throughout the growing season.

AM fungal colonization and root-associated fungal structures also varied across phenological stages in PN. AM fungal colonization was significantly higher at dormancy ($65.1 \pm 6.0\%$) and bud break ($50.1 \pm 4.4\%$) than at bloom ($32.7 \pm 5.9\%$) and veraison ($30.7 \pm 49.4\%$) (Figure 2.4f). Hyphal abundance was significantly lower at veraison ($24.1 \pm 3.3\%$) than at harvest ($46.5 \pm 4.5\%$) and dormancy ($60.6 \pm 6.1\%$), but did not significantly differ from bud break ($34.4 \pm 4.2\%$) and bloom ($30.5 \pm 6.0\%$) (Figure 2.4g). Vesicle abundance at bloom ($6.6 \pm 2.0\%$) and veraison ($3.4 \pm 0.8\%$) was significantly lower than at bud break ($11.3 \pm 1.8\%$) and dormancy ($19.4 \pm 3.6\%$), but did not differ significantly from harvest ($8.1 \pm 1.4\%$) (Figure 2.4h). Arbuscule density was significantly higher at bud break ($10.4 \pm 1.9\%$) than at bloom ($0.7 \pm 0.3\%$), but did not differ significantly from the other phenological stages (Figure 2.4i).

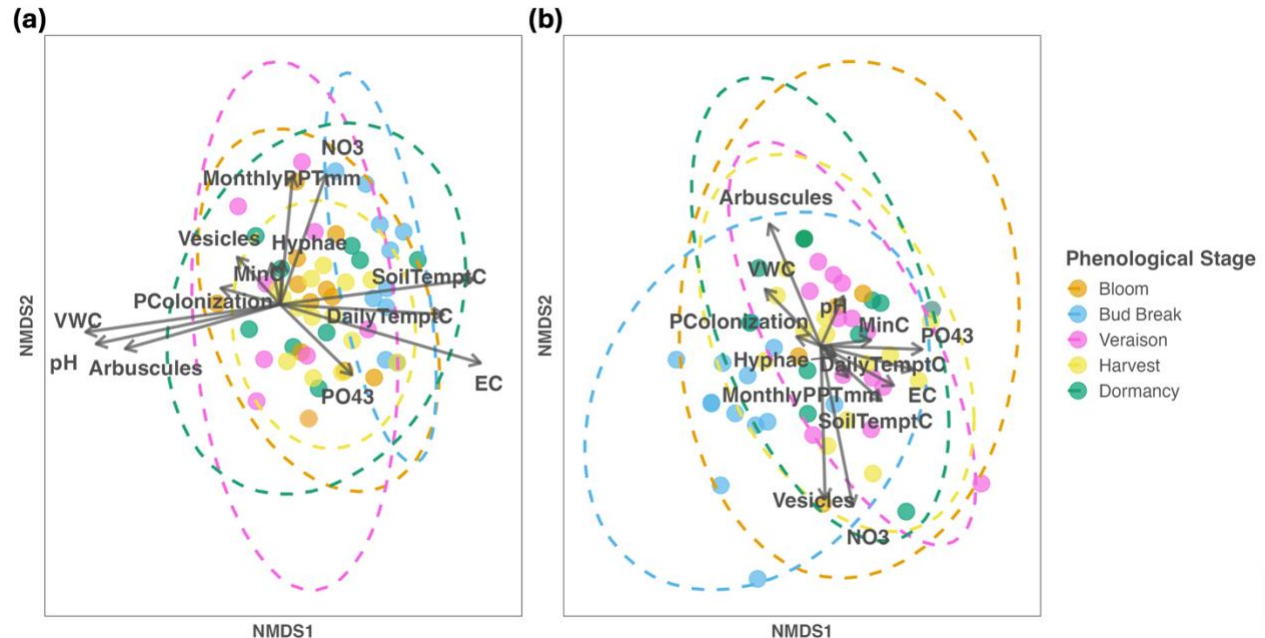


Fig 2.5 Non-metric multidimensional scaling (NMDS) ordination of AM fungi community composition based on

Bray-Curtis dissimilarity in (a) CH and (b) PN. Soil, weather, and mycorrhizal vectors were overlaid using the

envfit function, which fits variables to the ordination axes and assesses significance by permutation. Variables included are volumetric water content (VWC), monthly precipitation (MonthlyPPTmm), soil temperature (SoilTemptC), daily temperature (DailyTemptC), nitrate (NO3), phosphate (PO43), electrical conductivity (EC), mineralizable carbon (MinC), soil pH, percent colonization (PColonization), hyphae, vesicles, arbuscules, and AM fungal richness (GlomeroRichness), evenness (GlomeroEvenness), and Shannon diversity (GlomeroShannon). Arrow length and direction indicate the strength and orientation of correlations between variables and AM fungal community structure. Ellipses represent 95% confidence intervals for each region.

3.3 Variation in AM fungal community composition across phenological stages

Across all samples, 102 AM fungal OTUs spanning 16 genera were detected. Grapevine roots in CH hosted 78 OTUs across 14 genera, while roots collected from PN had 77 OTUs spanning 15 genera. Among these, a core group of AM fungal genera was detected at both sites, including *Glomeraceae gen incertae sedis* (21 OTUs), *Rhizophagus* (21), *Glomus* (12), *Paraglomus* (4), *Claroideoglomus* (3), *Diversispora* (3), *Funneliformis* (2), *Nanoglomus* (3), *Glomerales gen incertae sedis* (2), *Glomeromycota gen incertae sedis* (2), *Rhizoglomus* (1), and *Paraglomeraceae gen incertae sedis* (1). Despite this shared core community, the total number of taxa detected at each phenological stages differed considerably between CH and PN. In CH, taxa detection was lowest at bud break (8 species across 8 genera), peaked at bloom (13 species across 11 genera), and remained relatively stable through veraison (10 species across 8 genera), harvest (12 species across 11 genera), and dormancy (10 species across 8 genera). In contrast, PN showed a more variable pattern across the growing season, with detection declining from bud break (11 species across 8 genera) to bloom (7 species across 6 genera), peaking at veraison (12 species across 9 genera) and remaining high to harvest (11 species across 10 genera), before declining again at dormancy (8 species across 8 genera). Given these site-specific differences in taxa detection across phenological stages, variation in AM fungal community composition is presented separately for CH and PN.

In CH, phenological stage significantly influenced AM fungal community composition, as indicated by PERMANOVA based on Bray-Curtis dissimilarities of AM fungal OTUs ($p = 0.001$). Pairwise PERMANOVA comparisons showed significant differences between bud break and all other phenological stages ($p < 0.02$) as well

as between veraison and bloom ($p = 0.02$) and veraison and harvest ($p = 0.01$). NMDS ordination illustrated the separation of AM fungal OTUs across phenological stages (Figure 2.6a). Envfit analysis identified several soil and weather variables significantly correlated with variation in AM fungal community composition, including EC, soil temperature, and daily temperature ($p < 0.05$; Supplementary Information, Table S2.4). However, these variables explained only a relatively small proportion of community variation ($r^2 = 0.14$ - 0.16), indicating that their individual contributions to phenology-related community differences were modest.

Partitioning Sørensen dissimilarity revealed that phenological shifts in AM fungal community composition were primarily driven by nestedness rather than by turnover across stage transitions in CH (Figure 2.5a). The greatest total Sørensen dissimilarity occurred between bud break and bloom (0.78), where community differences were largely driven by turnover. This pattern reflects greater taxon replacement between these stages. From bloom to veraison, total dissimilarity declined (0.65), with contributions from turnover (0.25) and nestedness (0.30) being more evenly balanced. Community differences between veraison and harvest were dominated by nestedness (0.45) rather than turnover (0.05), resulting in a total dissimilarity of 0.51. Despite comparable total dissimilarity between harvest and dormancy (0.51), differences in community composition during this transition were driven primarily by nestedness (0.30) rather than turnover (0.21).

To identify taxa contributing to phenology-related differences in community composition, we examined patterns among AM fungal taxonomic groups in CH using SIMPER, TITAN2, and indicator species analysis (Supplementary Information, Table S2.6). Differences in the relative abundance of several taxonomic groups contributed to community dissimilarity across phenological stages in CH (Figure 2.7a). SIMPER results indicated *Rhizoglyphus* differed significantly between bud break and bloom, with higher detection at bud break. *Diversispora* also differed significantly between bud break and bloom and between bud break and dormancy, with higher detection at bud break for both contrasts. Differences in *Glomerales gen. incertae sedis* contributed significantly to community dissimilarities between bloom and veraison, with higher detection at bloom. TITAN2 analysis further indicated that *Glomerales gen. incertae sedis* was negatively associated with mineralizable C across phenological stages. *Glomeromycota gen. incertae sedis* contributed to community dissimilarities between bud break and dormancy, with higher detection during dormancy. Although *Rhizophagus* did not contribute to SIMPER-identified

community dissimilarities across phenological stages, TITAN2 analysis indicated a negative response of *Rhizophagus* to mineralizable C. Indicator species further showed a strong association of *Rhizophagus* with bloom and harvest.

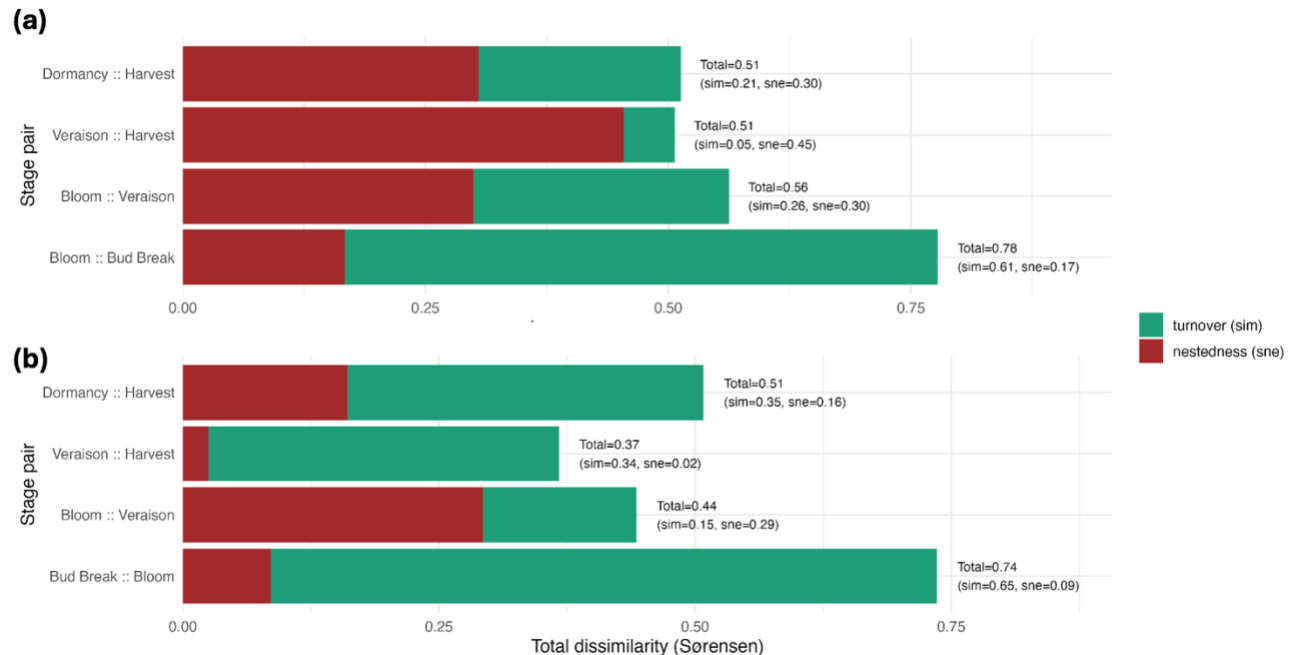


Fig 2.6 Proportional contributions of beta diversity components to total dissimilarity (Sørensen index) among phenological stage pairs of AM fungal communities in (a) CH and (b) PN. Stage pairs are ordered according to phenological progression. Total beta diversity is partitioned into turnover (green; sim) and nestedness (red; sne). Turnover reflects the replacement of taxa between stages, whereas nestedness reflects differences in community composition driven by taxon loss or gain. Horizontal bars depict the relative contributions of turnover and nestedness to each stage comparison, with total dissimilarity values and corresponding component estimates reported adjacent to each bar.

Similar to CH, a PERMANOVA based on Bray-Curtis dissimilarities of AM fungal OTUs showed that phenological stage significantly influenced AM fungal community composition in PN ($p = 0.001$). Pairwise PERMANOVA comparisons indicated significant differences between bud break and all other phenological stages ($p < 0.002$), as well as between dormancy and veraison ($p = 0.01$) and bloom and veraison ($p = 0.04$). NMDS ordination illustrated the separation of AM fungal OTUs across phenological stages (Figure 2.5b). Several soil and

weather variables significantly correlated with variation in AM fungal community composition, including pH, EC, arbuscule occurrence, VWC, soil temperature, and daily temperature ($p < 0.05$; Supplementary Information, Table S2.5). However, each variable accounted for only a small proportion of the observed community variation ($r^2 = 0.14-0.23$), indicating that their individual contributions to phenology-related community differences were modest.

Partitioning Sørensen dissimilarity indicated that phenological shifts in AM fungal community composition in PN were characterized by variable contributions of turnover and nestedness across stage transitions (Figure 2.6b). The greatest total Sørensen dissimilarity occurred between bud break and bloom (0.74), where community differences were largely driven by turnover (0.65). From bloom to veraison, total dissimilarity declined (0.44) and was primarily explained by nestedness (0.29) rather than turnover (0.15). Community differences were lowest (0.47) between veraison and harvest, with turnover (0.34) contributing more strongly than nestedness (0.02). Dissimilarity increased again between harvest and dormancy (0.51), where turnover (0.35) remained the dominant component rather than nestedness (0.16).

Differences in AM fungal community composition across phenological stages in PN were further examined using SIMPER, TITAN2, and indicator species analysis to evaluate patterns among taxonomic groups (Supplementary Information, Table S2.7). Variation in the relative abundance of multiple taxonomic groups contributed to community dissimilarity across the growing season (Figure 2.7b). SIMPER analysis indicated that *Glomus*, *Rhizophagus*, and *Glomerales gen incertae sedis* contributed to community dissimilarities between bud break and bloom and between bud break and harvest. *Rhizophagus* and *Glomerales gen incertae sedis* also contributed to dissimilarity between bud break and harvest, while *Glomerales gen incertae sedis* further contributed to differences between bud break and veraison. Across these contrasts, *Glomus*, *Rhizophagus*, and *Glomerales gen incertae sedis* were most abundant at bud break. Consistent with these patterns, indicator species analysis also identified *Rhizophagus* and *Glomerales gen incertae sedis* as strongly associated with bud break. TITAN2 analysis further indicated that *Rhizophagus* positively responded to pH and EC. *Glomeromycota gen incertae sedis* contributed to community dissimilarities between bud break and veraison and between veraison and dormancy, with higher detection during veraison. Indicator species analysis also identified it as strongly associated with veraison, while TITAN2 revealed negative associations with EC and positive associations with daily

temperature. *Glomeraceae gen incertae sedis* was identified as a strong indicator of harvest and veraison.

Although *Rhizoglossus* was not detected as a key contributor to phenological dissimilarities by SIMPER or indicator species analysis, TITAN2 indicated that both *Rhizoglossus* and *Glomeraceae gen incertae sedis* were negatively associated with EC and positively associated with daily temperature.

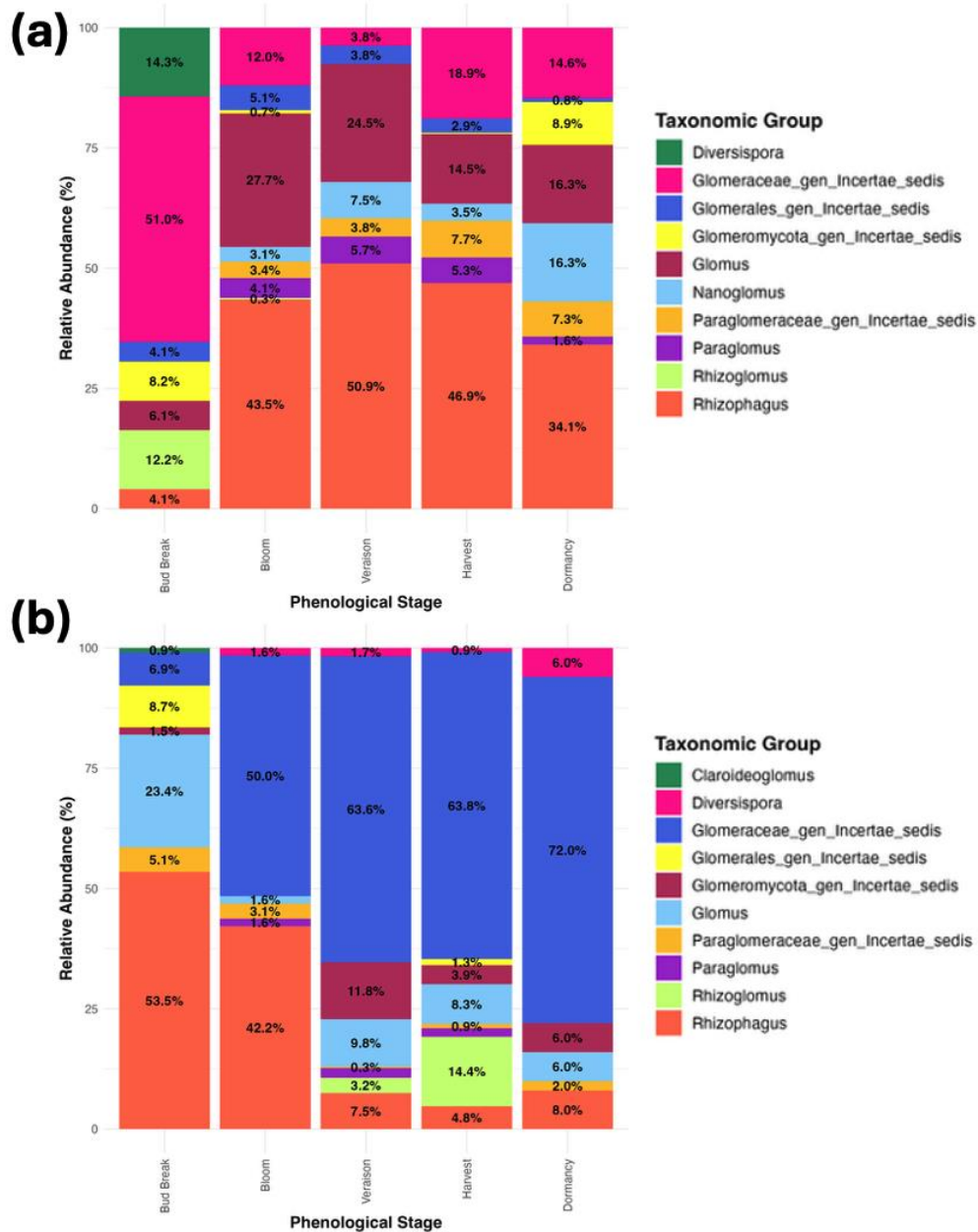


Fig 2.7 Relative abundance of dominant AM fungal taxonomic groups across phenological stages in (a) CH and (b)

PN. Stacked bar charts show the proportional contribution (%) of the ten most abundant AM fungal

taxonomic groups identified within each site. Relative abundance was calculated by summing sequence abundance for each taxonomic group within a stage and expressing them as a percentage of the total AM fungal community for the stage.

4. Discussion

4.1. Seasonal dynamics of AM fungal colonization across grapevine phenological stages

This study examined how AM fungal colonization and community composition changed across grapevine phenological stages in two vineyard sites cultivating different scion-rootstock combinations. In both sites, AM fungal colonization and intraradical structure abundance were consistent with our hypotheses. As predicted, intraradical structure abundance and overall colonization rates varied significantly among phenological stages. The specific changes in the abundance of intraradical structures did not align with our hypotheses, as arbuscules did not peak at bloom, and vesicle abundance did not show a consistent dormancy-associated increase.

In contrast to previous grapevine studies that reported no temporal change in AM fungal colonization (Cesaro et al. 2021; Schreiner 2005), we observed differences in colonization rates between phenological stages. In both CH and PN, AM fungal colonization and vesicle abundance were significantly higher at bud break than at bloom. From harvest to dormancy, AM fungal colonization increased in both sites, a pattern driven primarily by higher vesicle detection at dormancy. These vesicle-driven patterns suggest a close link between fungal storage structures and shifts in C allocation associated with grapevine phenological patterns. In deciduous perennial systems, such as grapevines, permanent woody tissues are reservoirs of carbohydrates and mineral nutrients that support early-season growth following bud break, before canopy development and active photosynthesis are fully established (Giese et al. 2020; Mullins et al. 1992). Grapevines rely on these stored reserves to initiate bud break, sustain early shoot growth, and begin photosynthesis (Campos et al. 2016; Giese et al. 2020). Elevated vesicle abundance at bud break may therefore reflect fungal storage supported by host-derived C reserves available early in the growing season. Higher vesicle detection at bud break may also reflect storage structures formed during the preceding dormancy period that persist into the early phenological stages, as vesicles can remain detectable for several months following initial development (Brundrett 2009).

After harvest, removal of the fruit sink can increase C flow to the root system, which, together with seasonally higher soil moisture during the post-harvest period, can stimulate new root growth and support increased AM fungal colonization (Mahmud et al. 2023; Mullins et al. 1992; Schreiner 2005). Because colonization of fine roots by AM fungi is greatest where root density is highest (Schreiner 2005), post-harvest root flushes may create conditions that favor increased establishment in roots. In warmer viticultural regions, such as the sites examined here, grapevine leaves often remain photosynthetically active for several weeks to months after harvest, extending C assimilation well beyond fruit removal (Mahmud et al. 2023). This extended post-harvest photosynthetic activity may supply carbohydrates beyond immediate reserve replenishment (Mahmud et al. 2023), thereby supporting continued root growth, AM fungal colonization, and vesicle formation late into the season.

Vesicles, which typically develop at later stages of colonization and during periods of reduced plant acquisition, are therefore likely to become abundant during this phase (Abbot et al. 1984; Cera et al. 2021; Jakobsen et al. 2003). Consistent with this interpretation, studies in other systems have reported greater vesicular colonization during wetter periods, when AM fungi are thought to be retained for maintenance for storage rather than active nutrient exchange (Cera et al. 2021; Johnson 1993; Koyama et al. 2017).

4.2. Seasonal variation in AM fungal community composition and diversity

Arbuscular mycorrhizal fungal community composition varied among grapevine phenological stages, supporting our hypothesis of stage-associated community differences. However, patterns of turnover and nestedness in community composition differences were inconsistent between sites and only partially aligned with our hypothesis. For both CH and PN, we detected significant differences in community composition between bud break and all other phenological stages, highlighting distinct AM fungal-grapevine symbiosis at the onset of the grapevine growing season. Partitioning of Sørensen dissimilarity indicated that community dissimilarity between bud break and bloom was largely attributable to turnover in both sites, partially supporting our hypothesis. This turnover may have been driven by taxa more strongly associated with bud break than with later stages. In both sites, these included ruderal, fast-colonizing taxa such as *Glomus*, *Rhizophagus*, and *Glomerales gen incertae sedis* at PN and *Rhizoglomus* at CH. *Diversispora* was also more strongly associated with bud break in CH.

Although turnover dominated the bud break to bloom transition as predicted, the relative contributions of turnover and nestedness in subsequent stages were inconsistent between sites and did not align with our predictions. Community composition differed significantly between bloom and veraison in CH and PN, with nestedness rather than turnover driving this transition, contrary to our hypothesis. Taxa potentially contributing to these differences included *Glomerales gen incertae sedis*, which showed higher detection at bloom in CH, and *Glomeromycota gen incertae sedis* and *Glomeraceae gen incertae sedis*, which showed higher detection at veraison in PN. Patterns in nestedness and turnover from veraison to harvest and from harvest to dormancy similarly did not align with our predictions, with their relative contributions diverging across sites.

Relatively few studies have examined variation in AM fungal community composition across grapevine phenological stages (Cesaro et al. 2021; Massa et al. 2020). Cesaro et al. (2021) reported no detectable differences in AM fungal communities between bloom and early fruit development. However, their analysis was limited to two phenological stages and did not capture community dynamics across the full range of grapevine development. Studies in other herbaceous or annual cropping systems have documented phenology or seasonally associated shifts in AM fungal communities, which were attributed to host-mediated changes in plant C allocation and seasonal variation in abiotic conditions (Barnes et al. 2016; Bennett et al. 2013; Buade et al. 2021; Dumbrell et al. 2011; Escudero and Mendoza 2005; Goldmann et al. 2020; Pereira e Silva 2012).

Differences in AM fungal community composition at bud break relative to later phenological stages may reflect major shifts in grapevine C source and allocation during early seasonal development. Following dormancy, photosynthetic capacity is limited, and grapevines rely heavily on stored carbohydrate reserves within perennial woody tissues from the previous growing season to initiate bud break and begin shoot growth (Campos et al. 2016; Giese et al. 2020; Mullins et al. 1992). With increasing spring temperatures, grapevines enter a period of rapid vegetative growth during which shoot elongation accelerates, and C demand increases substantially, maintaining a strong reliance on carbohydrate reserves stored in the trunk, cordon, and roots (Giese et al. 2020). As canopy development increases photosynthetic C supply, grapevines shift from reliance on stored carbohydrate reserves to photosynthetic assimilates to support vegetative growth and fruit development (Mullins et al. 1992), potentially constraining C allocation to roots and associated symbionts. Overall, the distinct AM fungal communities observed

at bud break relative to later phenological stages suggest that shifts in host C source and allocation may lead to pronounced community reorganization at the onset of grapevine growth.

Ruderal, fast-colonizing taxa, such as *Glomus*, *Rhizophagus*, and *Rhizoglomus* (Chagnon et al. 2013; Hart and Reader 2002), may be well positioned to take advantage of stored carbohydrate reserves early in seasonal development. Their high C demands, associated with rapid hyphal turnover and intraradical structure formation (Chagnon et al. 2013; Staddon et al 2003), may be more readily met before plant C demands shift to rapid vegetative growth. In contrast, the presence of *Diversispora* at bud break in CH may be shaped by different factors. Although its life history remains poorly understood, *Diversispora* has been shown to thrive under lower input conditions, such as organic management (Moukarzel et al. 2024). Its strong association with bud break may therefore reflect a preference for the early season soil environment, when disturbance and management inputs have yet to intensify as they do later in the growing season. These taxon-specific associations suggest that multiple factors may shape AM fungal community composition at bud break. Beyond bud break, shifts in host C dynamics throughout grapevine physiological development may continue to drive community reorganization in subsequent phenological stages.

Building on these early-season shifts in host C dynamics, grapevines transitioning from bloom toward veraison experience additional changes in root-mediated hormone signaling that may further influence AM fungal community composition. From bloom to veraison, cytokinin signaling originating in root extremities declines as vegetative growth slows (Constantini and Bucelli 2014). At the same time, abscisic acid signaling increases in the roots and leaves. (Constantini and Bucelli 2014). These signaling changes reflect a shift in grapevine growth from vegetative expansion toward reproductive development (Cameron 2024; Giese et al. 2020). At veraison, this shift is expressed as berries becoming the dominant sinks for assimilated C (Mullins et al. 1992). Because AM fungi rely on host-derived C supplied through roots (Lee et al. 2013; Schreiner and Linderman 2005; Van Der Heijden et al. 2020; Vieira et al. 2020), this reorientation of C allocation may influence which AM fungal taxa are supported and persist within roots. This increased allocation to berry development coincides with reported declines in new root production after bloom (Cameron 2024; Freeman and Smart 1976; Mullins et al. 1992), potentially altering the root environment available to AM fungi for colonization. These shifts in C allocation and root environment may have

contributed to the higher detection of *Glomerales gen incertae sedis* at bloom in CH and *Glomeromycota gen incertae sedis* and *Glomeraceae gen incertae sedis* at veraison at PN. However, the uncertain taxonomic placement of these taxa limits more specific ecological interpretation. Together, these host-mediated phenological shifts provide important context for understanding patterns observed in AM fungal communities from bloom to veraison.

In addition to these host-mediated phenological shifts, variation in daily air temperature, soil temperature, and EC were associated with differences of community composition across phenological stages in both vineyards. Daily air temperature contributes to these patterns by regulating the timing of major phenological transitions, including bud break, bloom, and berry ripening, and by shaping seasonal variation in photosynthesis and transpiration (Cameron 2024; Dusenage et al. 2019; Macphie and Phillimore 2024; Mullins et al. 1992). These temperature-mediated shifts in physiological activity alter grapevine C assimilation and allocation between above-ground tissues and roots (Dusenage et al. 2019; Mullins et al. 1992). Seasonal variation in daily air temperature and soil temperature has been observed to influence patterns of root growth, with new root production peaking when soil temperatures reach approximately 15-30° C throughout the soil profile (Mahmud et al. 2023). This peak in root growth typically coincides with grapevine bloom, followed by a decline in new root production in the summer when soil temperatures are high, and soil water availability is low (Cameron 2024; Freeman and Smart 1976; Mullins et al. 1992). Consequently, shifts in root growth may influence grapevine-associated AM fungal communities by altering C supply and physical habitat availability within roots. Other soil properties, such as salinity, have also been reported to shape AM fungal symbiosis in vineyards (Almaliotis et al. 2008; Fors et al. 2023; Upreti et al. 2016), with elevated EC associated with reduced AM fungal spore abundance (Almaliotis et al. 2008). However, the influence of salinity on grapevine-associated AM fungal communities may vary with rootstock (Belew et al. 2016). Variations in rootstock salinity tolerance can alter how grapevines allocate resources from growth to stress maintenance (Downton 1977), potentially affecting C availability for AM fungal symbiosis. Collectively, the influence of abiotic variables on AM fungal community composition may operate largely through their effects on host physiology and C allocation, suggesting that grapevine-mediated regulation may represent a stronger driver of

community structure than environmental variables alone. The implications of rootstock-specific responses to environmental conditions for AM fungal symbiosis are explored further in the subsequent section.

Despite significant shifts in AM fungal community composition across the grapevine growing season in this study, our hypothesis that Shannon diversity would vary in response to shifts in host C allocation was not supported, as it remained stable across phenological stages in both sites. Similar stability in AM fungal diversity within a single growing season has been reported in grapevines (Schreiner and Mihara 2009). Schreiner and Mihara (2009) attributed this stability to the vine-row soil environment, which typically experiences minimal physical disturbance. Under these conditions, a relatively stable pool of AM fungal taxa may be maintained across the growing season, even as changes in host phenology alter the relative abundances of individual taxa.

Richness and evenness showed greater variation throughout the growing season, though neither metric corresponded to periods of increased C flow to the root system as predicted. Instead, patterns in both metrics differed between sites, with richness significantly higher at bloom and harvest in CH, while in PN only veraison and bloom differed significantly. Evenness varied significantly across phenological stages in CH but remained stable throughout the growing season in PN. These unexpected patterns suggest that different underlying drivers may shape richness and evenness. To our knowledge, variation in these metrics across phenological stages has not been examined. In a temperate grassland, AM fungal richness has been linked to shifts in host C availability and environmental conditions, such as root biomass, pH, and NH_4^+ concentration (Goldmann et al. 2020). Evenness has also been shown to fluctuate across developmental stages, with studies in soybean reporting that host root selection pressure resulted in colonization by fewer but more equally abundant taxa as the season progressed (Buade et al. 2021). Differences in root architecture and C allocation between scion-rootstock combinations may differentially mediate host carbon availability and root selection pressure across phenological stages, potentially contributing to the site-specific patterns observed. Underlying differences in soil properties between sites, such as PO_4^{3-} and EC, may have further compounded these effects.

4.3. Genotype and site-specific drivers of AM fungal symbiosis

Site strongly structured AM fungal colonization and diversity. Consistent with these patterns, site and its interaction with phenological stage were primary drivers of AM fungal community composition, rather than

phenological stage alone. These patterns likely reflected contributions from both scion-rootstock combination and site-specific soil conditions, partially supporting our hypothesis that phenological shifts in AM fungal symbiosis would manifest differently between scion-rootstock combinations. Across several phenological stages, AM fungal colonization was higher at PN than at CH, including at bud break, bloom, and harvest. Shannon diversity followed a similar pattern, with higher diversity at CH than at PN at these same three stages. The stage specific nature of these differences suggests that scion-rootstock combination may most strongly influence AM fungal symbiosis during major transitions in host C dynamics. Differences in scion specific berry demand may have further contributed to these patterns, particularly at harvest. These dynamics may be further influenced by underlying differences in soil properties between sites, including PO_4^{3-} availability and EC.

Differences in root system architecture of the two rootstocks may have contributed to the variation in AM fungal colonization and community diversity observed between sites. The 140R rootstock is a *V. berlandieri* x *V. rupestris* hybrid that develops a deep root system, facilitating access to water and nutrients from deeper soil layers (Chen et al. 2024). The high vigor and well-developed root systems characteristic of both parent species likely underlie its deep rooting pattern, making it well-suited to the dry, Mediterranean conditions of our sites (Yildirim et al. 2018). In contrast, the 101-14 rootstock is a *V. riparia* x *V. rupestris* hybrid that develops a shallower, less extensive root system suited to heavy, moist clay soils with limited tolerance to drought and salinity (Lambert et al. 2008; Pongracz 1983). The lower vigor and reduced root penetration of *V. riparia* likely underlie 101-14's rooting pattern, limiting access to water and nutrients from deeper soil layers (Pongracz 1983). At PN, the shallower root system and lower salinity tolerance of 101-14 likely increased host vulnerability to the elevated EC and water stress conditions of the site. This may have heightened physiological stress on the vine (Chen et al. 2024), limited C availability to symbionts (Salmeron-Santiago et al. 2022; Sapes et al. 2021), and potentially favored community shifts toward dominance by fewer AM fungal taxa. In contrast, the deeper root system of 140R at CH likely mitigated vine stress under dry, Mediterranean conditions, supporting more stable C allocation to AM fungal symbionts across the growing season (Chen et al. 2024; Salmeron-Santiago et al. 2022; Sapes et al. 2021).

These differences in C availability to AM fungal symbionts may have contributed to less stable plant-AM fungal associations at PN, increasing the sensitivity of AM fungal communities to seasonal changes in soil and

weather conditions. Consistent with this, phenology-related differences in AM fungal community composition at PN were associated with more environmental variables, including soil pH, EC, arbuscule occurrence, VWC, soil temperature, and daily temperature. In contrast, phenology-related community differences at CH were primarily structured by EC, soil temperature, and daily temperature.

Differences in the stability of plant-AM fungal associations may be further in patterns of community reorganization across sites. While PN showed variable contributions of turnover and nestedness across the season, compositional shifts in CH were primarily driven by nestedness. Patterns in taxa detection across the season may reflect these differences in community reorganization. At CH, both AM fungal genera and species detection were lower at bud break than at subsequent phenological stages. The progressive accumulation of taxa through the growing season is consistent with nestedness-dominated patterns, as community shifts were characterized by the gain of taxa rather than their replacement. In contrast, the considerable variation in taxa detection throughout phenological stages at PN may reflect the more variable contributions of turnover and nestedness observed at that site. Together, these differences in community reorganization suggest that AM fungal assemblages at CH were more stable throughout the season, whereas those at PN exhibited greater compositional fluctuation. This instability may have contributed to the differences in alpha diversity and colonization observed between sites.

In terms of alpha diversity, Shannon diversity was lower at CH than at PN at bud break but became significantly higher at bloom and harvest. Lower diversity observed at bud break in CH may reflect insufficient C availability to support a diverse AM fungal community in early stages of growth before photosynthesis is fully established. As canopy development increases vine photosynthetic capacity at bloom, excess photosynthate becomes available, and root growth peaks. The reduced vine stress conferred by the deeper root system of 140R may have supported more stable C allocation to symbionts at this stage, allowing a more diverse AM fungal community to be maintained. In contrast, 101-14's lower tolerance to drought and salinity may have increased physiological stress at PN, constraining C availability to symbionts at this stage and limiting AM fungal diversity. This constrained C availability may have intensified host root-selective pressure at PN, favoring higher colonization by a few AM fungal species (Buade et al. 2021). At harvest, differences in target berry ripeness between scions may have further contributed to site-specific Shannon diversity patterns. The greater sugar accumulation typically

required for Pinot Noir than Chardonnay at harvest may prolong vine C allocation toward berry development at PN, further constraining C availability to root symbionts. Although the extent to which scion-specific differences contributed to these differences remains unclear, scion identity has been shown to influence AM fungal community composition in grapevines, though generally to a lesser degree than rootstock (Lailheugue et al. 2024; Noceto et al. 2024).

Higher colonization observed at PN may reflect the dominance of *Glomeraceae gen incertae sedis* from bloom to dormancy, while *Rhizophagus* was more prominent at CH. Although both taxa share ruderal traits and belong to the Glomeraceae family, *Rhizophagus* has been observed to be more sensitive to C-limited and physiologically stressful conditions than other Glomeraceae taxa (Rodriguez et al., in preparation). Therefore, the physiologically stressful conditions at PN may have reduced *Rhizophagus*' competitiveness, favoring the dominance of *Glomeraceae incertae sedis* instead. Although *Glomeraceae gen incertae sedis* could not be classified beyond the family level, members of this family are known to allocate more resources to intraradical structures (Chagnon et al. 2013; Hart and Reader 2002). Taxa within this group may be highly colonizing and competitive under variable C conditions, favoring their dominance at PN over other AM fungal taxa. This competitive dominance may have contributed to the higher observed colonization and lower Shannon diversity in PN relative to CH.

Evidence from grapevine studies further indicates that rootstock identity mediates both salinity tolerance and mycorrhizal responses. In a greenhouse study examining salinity effects across multiple grapevine rootstocks, Belew et al. (2016) reported variation in AM fungal colonization, with rootstocks with lower salinity tolerance exhibiting greater mycorrhizal colonization under saline conditions. This pattern aligns with our field observations, in which PN (101-14) exhibited higher AM fungal colonization than CH (140R). Among environmental variables associated with differences in AM fungal community composition, EC, a measure of soil salinity, emerged as a consistent driver of AM fungal community shifts across grapevine growth stages in CH and PN. It was also identified as a key variable explaining differences in soil properties between the two sites. Together, these observations underscore the importance of considering soil salinity alongside rootstock identity when interpreting variation in AM fungal symbiosis in vineyards.

Phosphate concentration was also identified as a key soil property differentiating the two sites, with significantly higher PO_4^{3-} availability at PN throughout the growing season compared to CH. Grapevines are typically highly colonized by AM fungi under phosphorus (P)-limiting conditions and decline in P-rich soils, as reliance on AM fungi is reduced and direct nutrient uptake through roots becomes more effective (Schreiner 2006; Schreiner 2007; Schreiner and Scagel 2016). High plant P status may suppress AM fungal development by limiting the internal spread within colonized roots and reducing the formation of new infection sites, processes that can be linked to changes in C allocation to roots (Schreiner and Scagel 2016). However, this expected pattern was not observed in our study, as AM fungal colonization was consistently higher at PN than at CH across much of the growing season. Schreiner and Scagel (2016) similarly observed that under high plant P status, AM fungal colonization in grapevine roots was relatively insensitive to variation in C supply to roots. Instead, C-supply driven regulation was expressed most rapidly in arbuscule occurrence, which is particularly sensitive to photosynthate availability (Schreiner and Scagel 2016). In this case, seasonal variation in arbuscules was observed at PN alongside higher and more variable PO_4^{3-} availability, whereas arbuscule detection at CH remained comparatively stable under lower and more stable PO_4^{3-} concentrations. Despite these differences in arbuscule expression, PO_4^{3-} concentration was not identified as a significant variable associated with shifts in AM fungal community composition across phenological stages in either vineyard. Together, these results indicate that scion-rootstock interactions interact with site-specific soil conditions to shape seasonal dynamics of AM fungal symbiosis across phenological stages.

5. Conclusion

Our results show that AM fungal colonization and community composition varied across grapevine phenological stages in both sites, indicating that seasonal host development is associated with changes in AM fungal symbiosis. Notably, both scion-rootstock combinations exhibited higher colonization at bud break relative to bloom, as well as increased colonization from harvest to dormancy. These patterns coincided with higher vesicle detection at bud break and dormancy, which may reflect

greater investment in fungal storage supported by stored host C reserves early in the season and by increased C availability to roots following harvest.

Patterns in AM fungal community composition also varied across phenological stages. In both vineyard sites, communities at bud break differed from those at later phenological stages, potentially reflecting shifts in host C source and allocation during early seasonal development. Community differences between bloom and veraison may similarly reflect host-mediated shifts in C allocation from vegetative to reproductive growth. In addition to host-mediated shifts, seasonal variation in daily air temperature, soil temperature, and EC emerged as important drivers of AM fungal community differences across phenological stages in both vineyards. Despite these compositional shifts, Shannon diversity remained relatively stable across the growing season, indicating that changes in community structure were not accompanied by large shifts in overall diversity.

Site identity also played a key role in structuring patterns of AM fungal colonization, diversity, and community composition across the growing season. These site-level patterns suggest that scion-rootstock associated differences in root architecture due to breeding parentage may contribute to variation in AM fungal community structure. Differences in nestedness and turnover dynamics between vineyards may further reflect differences in the stability of C allocation to symbionts conferred by the distinct root architectures of the 140R and 101-14 rootstocks. Underlying differences in soil properties between site, such as EC and PO_4^{3-} concentrations, may further interact with rootstock-specific traits to shape seasonal patterns in AM fungal symbiosis. Scion may have further influenced AM fungal communities due to differences in target berry ripeness between scions, influencing the duration of vine C allocation toward berry development.

Overall, this study highlights the importance of examining AM fungal symbiosis across the growing season to better understand grapevine-mycorrhizal interactions. Accounting for seasonal timing

and rootstock identity may help growers more effectively harness AM fungal symbiosis across phenological stages.

6. Acknowledgements

This research was funded by the Foundation for Food & Agriculture Research (Grant number CA21-SS-0000000193) and the California Department of Food and Agriculture (Grant number 21-0433-021-SF). The authors are grateful to the personnel of Jackson Family Vineyards for their help in establishing and maintaining this field trial.

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8. Supplementary Information

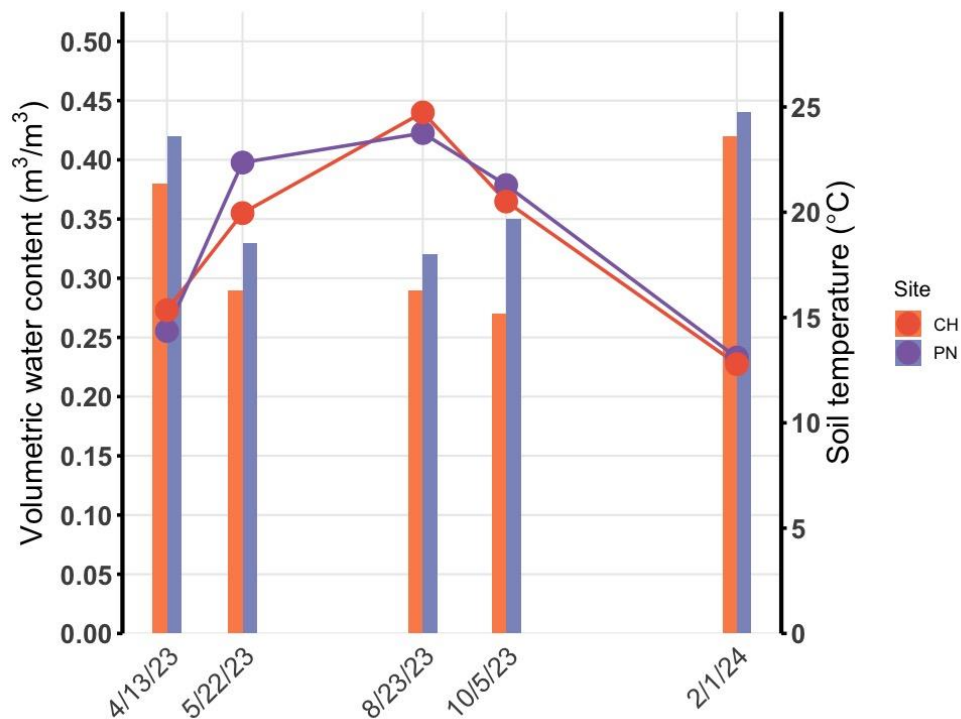


Fig S2.1 Soil volumetric water content (bars, $\text{m}^3 \text{ m}^{-3}$; left axis) and soil temperature (connected points, $^{\circ}\text{C}$; right axis) measured in two vineyard sites (CH and PN) across five sampling dates corresponding to bud break, bloom, veraison, harvest, and dormancy during the 2023-2024 growing season. Measurements were obtained using TEROS 12 sensors installed at 30 cm depth, with two sensors installed in PN and nine in CH. Values represent 24-hr averages calculated from all sensor readings at each vineyard for each sampling date.

Table S2.1 Vector loadings, coefficients of determination (r^2), and significance values for climate, soil, and AM fungal variables fitted to the first two principal coordinate axes (PCoA1 and PCoA2) from Fig 2.2. Loadings represent the direction and strength of correlations between each variable and ordination axes. The r^2 values indicate the proportion of variance in the ordination explained by each variable, while p-values reflect the significance of these relationships based on permutation testing. All loadings, r^2 values, and p-values were generated using the envfit function with 999 permutations.

	PCoA1	PCoA2	r^2	p-value
<u>Climate</u>				
Temperature (°C)	0.84	0.55	0.79	0.001
Precipitation (mm)	-0.93	-0.37	0.56	0.001
<u>Soil Measurements</u>				
Temperature (°C)	0.81	0.59	0.88	0.001
Volumetric Water Content ($\text{m}^3 \text{m}^{-3}$)	-0.99	-0.05	0.83	0.001
Nitrate ($\text{mg NO}_3^- \text{kg}^{-1} \text{soil}$)	-0.15	0.99	0.17	0.001
Phosphate ($\text{mg PO}_4^{3-} \text{kg}^{-1} \text{soil}$)	0.03	1.00	0.52	0.001
pH	-1.00	-0.06	0.28	0.001
EC ($\mu\text{S cm}^{-1}$)	0.16	-0.99	0.60	0.001
Mineralizable C ($\text{mg C kg}^{-1} \text{soil hr}^{-1}$)	-0.98	-0.18	0.07	0.008
<u>AM Fungal Colonization</u>				
Colonization (%)	-0.89	-0.45	0.71	0.001
Hyphae (%)	-0.86	0.51	0.65	0.001
Vesicle (%)	-0.87	0.49	0.51	0.001
Arbuscule (%)	-1.00	0.03	0.02	0.211
<u>AM Fungal Diversity</u>				
Shannon Diversity	0.42	-0.91	0.40	0.001
Richness	0.54	-0.84	0.13	0.001
Evenness	0.28	-0.96	0.26	0.001

Table S2.2 Mean values with standard errors shown in parentheses for soil, root, and fungal variables measured across grapevine phenological stages in NCA-CH and NCA-PN vineyards. Significant differences between vineyards at each phenological stage were tested using one-way ANOVA. When assumptions were not met, the Welch's one-way test were applied and are marked with "†." Different letters indicate significant differences ($p < 0.05$). Soil temperature and volumetric water content were obtained from TEROS 12 sensors installed at a 30 cm depth. Values represent the average readings from all sensors on each sampling date. Soil chemical properties, including nitrate and phosphate concentrations, pH, electrical conductivity (EC), and mineralizable carbon, were measured in 0–30 cm soil samples collected near the vine trunk. Arbuscular mycorrhizal (AM) fungal colonization and structures (hyphae, vesicles, and arbuscules) were quantified from cleaned and trypan blue–stained lateral roots using the crosshair-intersection method. AM fungal diversity indices were calculated from operational taxonomic units (OTUs) assigned to the Glomeromycota phylum.

	Bud Break		Bloom		Veraison		Harvest		Dormancy	
	NCA-CH	NCA-PN	NCA-CH	NCA-PN	NCA-CH	NCA-PN	NCA-CH	NCA-PN	NCA-CH	NCA-PN
<u>Soil Measurements</u>										
Temperature (°C)	15.3	14.3	20.0	22.4	24.7	23.8	20.5	21.3	12.8	13.1
Volumetric Water Content (m ³ m ⁻³)	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4
Nitrate (mg NO ₃ ⁻ kg ⁻¹ soil)	2.5 (0.4) ^a	2.8 (0.4) ^a	3.5 (0.8) ^a	4.2 (0.8) ^a	5.6 (1.1) ^a	5.2 (0.7) ^a	1.9 (0.2) ^a	0.9 (0.1) ^b	3.7 (0.6) ^a	5.7 (1.3) ^a
Phosphate (mg PO ₄ ³⁻ kg ⁻¹ soil)	30.4 (1.7) ^a	61.5 (2.7) ^b	31.4 (5.8) ^a	73.7 (3.5) ^b	33.6 (5.1) ^a	82.6 (5.4) ^b	24.1 (4.3) ^a	55.5 (4.5) ^b	24.2 (1.8) ^a	55.3 (3.7) ^b
pH	6.8 (0.03) ^a	7.3 (0.01) ^b	6.2 (0.06) ^a	6.5 (0.06) ^b	†6.9 (0.04) ^a	†6.7 (0.10) ^b	6.9 (0.02) ^a	7.1 (0.03) ^b	6.9 (0.05) ^a	7.3 (0.03) ^b
EC (μS cm ⁻¹)	72.4 (3.4) ^a	101.2 (2.4) ^b	†108.1 (5.1) ^a	†167.1 (12.1) ^b	118.3 (14.3) ^a	184.0 (12.4) ^b	†89.1 (3.2) ^a	†182.1 (16.2) ^b	105.3 (9.6) ^a	121.3 (8.0) ^a
Mineralizable C (mg C kg ⁻¹ soil hr ⁻¹)	1.7 (0.11) ^a	1.1 (0.05) ^b	1.1 (0.07) ^a	0.9 (0.07) ^b	†1.4 (0.13) ^a	†1.2 (0.05) ^a	†0.8 (0.05) ^a	†1.1 (0.12) ^a	†1.32 (0.05) ^a	†1.32 (0.09) ^a
<u>AM Fungal Colonization</u>										
Colonization (%)	36.4 (4.6) ^a	50.1 (4.4) ^b	19.5 (2.7) ^a	32.7 (5.9) ^b	32.4 (4.3) ^a	30.7 (3.5) ^a	18.1 (2.4) ^a	49.4 (4.9) ^b	52.4 (4.4) ^a	65.1 (6.0) ^a
Hyphae	24.5 (2.9) ^a	34.4 (4.2) ^a	16.1 (2.5) ^a	30.5 (6.0) ^a	27.6 (4.4) ^a	24.1 (3.3) ^a	14.0 (2.1) ^a	46.5 (4.5) ^b	48.4 (4.3) ^a	60.6 (6.1) ^a
Vesicle	9.5 (2.0) ^a	11.3 (1.8) ^a	2.6 (0.6) ^a	6.6 (2.0) ^a	4.4 (1.6) ^a	3.4 (0.8) ^a	1.9 (0.5) ^a	8.1 (1.4) ^b	6.0 (1.0) ^a	19.4 (3.6) ^b
Arbuscule	9.6 (4.3) ^a	10.4 (1.9) ^a	†2.2 (0.6) ^a	†0.7 (0.3) ^a	3.9 (0.8) ^a	6.4 (2.3) ^a	3.00 (0.7) ^a	4.6 (1.2) ^a	3.5 (1.3) ^a	3.2 (0.9) ^a
<u>AM Fungal Diversity</u>										
Shannon Diversity	1.4 (0.1) ^a	1.8 (0.1) ^b	2.2 (0.1) ^a	1.4 (0.1) ^b	1.9 (0.1) ^a	1.7 (0.1) ^a	2.3 (0.1) ^a	1.5 (0.1) ^b	†1.6 (0.2) ^a	†1.6 (0.1) ^a
Richness	9.7 (1.5) ^a	16.7 (2.3) ^b	20.5 (2.4) ^a	9.1 (1.5) ^b	10.9 (1.3) ^a	17.5 (2.5) ^b	21.9 (2.4) ^a	15.7 (1.8) ^b	12.1 (2.3) ^a	12.7 (1.7) ^a
Evenness	0.7 (0.03) ^a	0.7 (0.04) ^a	†0.8 (0.02) ^a	†0.7 (0.04) ^b	†0.8 (0.02) ^a	†0.6 (0.03) ^b	0.8 (0.02) ^a	0.6 (0.03) ^b	0.7 (0.04) ^a	0.7 (0.04) ^a

Table S2.3 Summary of two-way ANOVA p-values testing the effects of phenological stage, site, and their interaction on AM fungal colonization and diversity metrics.

Response Variable	P-Value		
	<u>Phenological Stage</u>	<u>Site</u>	<u>Interaction</u>
<u>AM Fungal Colonization</u>			
Percent Colonization	< 0.001	< 0.001	0.010
Vesicles	< 0.001	< 0.001	0.021
Arbuscules	< 0.001	0.641	0.531
Hyphae	< 0.001	<0.001	<0.001
<u>AM Fungal Diversity</u>			
Shannon Diversity	0.021	0.003	< 0.001
Richness	0.017	0.577	< 0.001
Evenness	0.415	< 0.001	0.001

Table S2.4 Vector loadings, coefficients of determination (r^2), and significance values for climate, soil, and AM fungal variables fitted to the first two non-metric multidimensional axes (NMDS1 and NMDS2) from Figure 5a. Loadings represent the direction and strength of correlations between each variable and ordination axes. The r^2 values indicate the proportion of variance in the ordination explained by each variable, while p-values reflect the significance of these relationships based on permutation testing. All loadings, r^2 values, and p-values were generated using the envfit function with 999 permutations.

Variable	NMDS1	NMDS2	r^2	p-value
NO ₃ ⁻	-0.33	0.94	0.02	0.56
PO ₄ ³⁻	-0.81	0.59	0.04	0.30
pH	-0.97	0.22	0.01	0.87
EC	-0.31	0.95	0.16	0.01
Min C	0.08	0.62	0.00	0.92
Hyphae	0.07	0.99	0.00	0.92
Arbuscules	0.43	-0.90	0.03	0.39
Vesicles	0.97	-0.25	0.06	0.19
P Colonization	0.99	-0.10	0.00	0.93
VWC	0.96	-0.28	0.08	0.10
Soil Tempt (°C)	-0.86	0.51	0.14	0.02
Daily Tempt (°C)	-0.88	0.48	0.15	0.02
Monthly PPT (mm)	0.12	0.99	0.01	0.70

Table S2.5 Vector loadings, coefficients of determination (r^2), and significance values for climate, soil, and AM fungal variables fitted to the first two non-metric multidimensional axes (NMDS1 and NMDS2) from Figure 5b. Loadings represent the direction and strength of correlations between each variable and ordination axes. The r^2 values indicate the proportion of variance in the ordination explained by each variable, while p-values reflect the significance of these relationships based on permutation testing. All loadings, r^2 values, and p-values were generated using the envfit function with 999 permutations.

Variable	NMDS1	NMDS2	r^2	p-value
NO ₃ ⁻	0.43	0.90	0.06	0.14
PO ₄ ³⁻	0.81	-0.59	0.04	0.29
pH	-0.99	-0.16	0.19	0.003
EC	0.98	-0.21	0.23	0.002
Min C	-0.27	0.96	0.01	0.88
Hyphae	0.06	1.00	0.01	0.83
Arbuscules	-0.98	-0.21	0.14	0.01
Vesicles	-0.77	0.63	0.02	0.61
P Colonization	-0.98	0.20	0.02	0.56
VWC	-0.99	-0.10	0.21	0.002
Soil Tempt (°C)	0.99	0.10	0.21	0.003
Daily Tempt (°C)	0.99	-0.05	0.15	0.01
Monthly PPT (mm)	0.13	0.99	0.05	0.23

Table S2.6 Summary for SIMPER analysis results showing arbuscular mycorrhizal (AM) fungal taxonomic groups detected in the NCA-CH vineyard that contribute significantly to differences in community composition across phenological stages. Listed is the phenological stage where each taxonomic showed higher relative abundance, the contrasts in which those differences were significant, and their relative contribution (%) to total Bray-Curtis dissimilarity. Diss/SD indicates the consistency of each taxonomic group's contribution across sample comparisons, with higher values reflecting more uniform patterns of difference. Environmental variables associated with negative and positive responders are shown, where negative responders correspond to variables correlated with lower taxon abundance and positive responders correspond to variables correlated with higher taxon abundance. Indicator associations by phenological stage are also provided.

Taxonomic Group	Phenological Stage with greatest abundance	Significant Contrasts	Contribution (%)	Diss/SD	Environmental variables (negative responders)	Environmental variables (positive responders)	Indicator Association (phenological stage)
<i>Glomerales gen incertae sedis</i>	Bloom	BL – V**	15.5	0.2	Min C	---	---
<i>Rhizoglopus</i>	Bud Break	BB – BL*	5.3	0.1	---	---	---
<i>Diversispora</i>	Bud Break	BB – D*; BB – BL*	2.0 - 2.2	~0.05	---	---	---
<i>Glomeromycota gen incertae sedis</i>	Dormancy	BB – D**	9.2	0.1	---	---	---
<i>Rhizophagus</i>	---	---	---	---	Min C	---	Bloom, Harvest

Significance levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Regional contrasts: BB – D = Bud Break vs Dormancy; BB – BL = Bud Break vs Bloom; BL – V = Bloom vs Veraison

Min C = mineralizable C; VWC = volumetric water content; Monthly PPT = monthly precipitation

“---” indicates that information was available for this parameter

Table S2.7 Summary for SIMPER analysis results showing arbuscular mycorrhizal (AM) fungal taxonomic groups detected in the NCA-PN vineyard that contribute significantly to differences in community composition across phenological stages. Listed is the phenological stage where each taxonomic showed higher relative abundance, the contrasts in which those differences were significant, and their relative contribution (%) to total Bray-Curtis dissimilarity. Diss/SD indicates the consistency of each taxonomic group's contribution across sample comparisons, with higher values reflecting more uniform patterns of difference. Environmental variables associated with negative and positive responders are shown, where negative responders correspond to variables correlated with lower taxon abundance and positive responders correspond to variables correlated with higher taxon abundance. Indicator associations by phenological stage are also provided.

Taxonomic Group	Phenological Stage with greatest abundance	Significant Contrasts	Contribution (%)	Diss/SD	Environmental variables (negative responders)	Environmental variables (positive responders)	Indicator Association (phenological stage)
<i>Glomus</i>	Bud Break	BB – D*, BB – BL*	14.9 – 16.1	0.2	---	---	---
<i>Rhizophagus</i>	Bud Break	BB – D***, BB – BL***, BB – V*, BB – H**	6.7 – 27.7	~0.2	---	pH, EC	Bud Break
<i>Glomerales gen incertae sedis</i>	Bud Break	BB – D***, BB – BL*, BB – H**	4.4 – 5.9	0.1	---	---	Bud Break
<i>Glomeromycota gen incertae sedis</i>	Veraison	BB – V**, V – D*	6.7 – 9.7	0.1	EC	Daily Tempt	Veraison
<i>Glomeraceae gen incertae sedis</i>	---	---	---	---	EC	Daily Tempt	Harvest, Veraison
<i>Rhizoglomus</i>	---	---	---	---	EC	Daily Tempt	---

Significance levels: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

Regional contrasts: BB – D = Bud Break vs Dormancy; BB – BL = Bud Brak vs Bloom; BB – V = Bud Break vs Veraison; BB – H = Bud Break Harvest; V – D = Veraison vs. Dormancy

EC = electrical conductivity; Daily Tempt = daily temperature (°C); Soil Tempt = soil temperature (°C); VWC = volumetric water content; Monthly PPT = monthly precipitation (mm)

“---” indicates that information was available for this parameter

Chapter 3: Assessing the Short-Term Influence of Regenerative Management Practices on Arbuscular Mycorrhizal Fungal Communities in Northern California (USA) Vineyards

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Abstract

Grapevine is among the most economically important perennial fruit crops, yet vineyard productivity increasingly faces pressure from climate change and management practices that weaken soil health and reduce biodiversity. Regenerative agriculture (RA) proposes the stacking of soil conservation practices, including no-till, compost application, and livestock grazing, to enhance soil health and improve crop productivity while promoting beneficial soil organisms, such as arbuscular mycorrhizal (AM) fungi. While individual RA practices have been shown to influence AM fungal communities, their combined effects in vineyards remain poorly understood. Our study evaluated the short-term effects of RA practices on AM fungal colonization, diversity, and community composition in two adjacent vineyard sites, PN and CH, cultivating different grapevine scion-rootstock combinations (Pinot Noir x 101-14 and Chardonnay x 140R, respectively). Six treatments representing combinations of no-till versus alternate tillage, compost application, and sheep grazing were applied across both sites. AM fungal colonization, assessed through microscopy, and community diversity, obtained via ITS-2 amplicon sequencing, were examined alongside soil physicochemical properties across all treatments and scion-rootstock combinations. Site was a stronger driver of AM fungal colonization, diversity, and community composition than treatment, suggesting that differences in soil physicochemical properties and scion-rootstock identity modulate AM fungal communities more than the practices themselves. While 16 genera, including *Rhizophagus*, *Glomus*, and *Claroideoglomus*, were shared between both sites, *Dominikia* and *Archeospora* were uniquely detected in treatments with alternate till

and compost application, while no treatment-specific genera were found at CH. Treatment effects on AM fungal symbiosis also differed between sites, with no till, compost, and grazing driving significantly higher colonization rates at CH, and alternate tillage, compost, and grazing supporting greater Shannon diversity at PN. Together, these findings underscore the importance of site characteristics and scion-rootstock identity in shaping AM fungal community responses to regenerative management practices in vineyards.

1. Introduction

Grapevines are among the most important perennial fruit crops, with California generating \$5.64 billion in revenue from grape production in 2024 (U.S. Department of Agriculture, Economic Research Service 2025). Vineyard productivity, however, faces growing pressure from climate change, as seasonal droughts and extreme weather events increasingly threaten vine health and yield (Parker et al. 2020). Conventional management practices, such as intensive tillage and reliance on synthetic fertilizers, compound these challenges by weakening soil structure, depleting organic matter, and reducing soil biodiversity (Gomiero et al. 2011; Naval et al. 2024; Ortega et al. 2024). In response to these challenges, regenerative agriculture (RA) practices are gaining attention as a strategy to enhance soil health and improve crop quality, yield, and profitability (Lazcano et al. 2020). Supporting biodiversity is a core principle of RA, as fostering diverse communities of organisms facilitates enhanced nutrient cycling and soil health while increasing ecosystem resilience to disturbances and productivity (Jansson et al. 2023; Khangura et al. 2023). One approach to promoting agricultural biodiversity is to leverage the symbiotic relationship between grapevines and arbuscular mycorrhizal (AM) fungi.

Arbuscular mycorrhizal fungi are a well-known example of microbial symbiosis in agriculture, establishing mutualistic associations with plant roots to enhance nutrient uptake and water use efficiency in exchange for plant-derived carbohydrates and lipids necessary for their survival and reproduction (Lee et al. 2013; Schreiner and Linderman 2005; Van der Heijden et al. 1998; Vieira et al. 2020). AM fungi colonize host roots by attaching to and penetrating root cells with their fungal hyphae, within which they form specialized intraradical structures (Tikhonovich and Provorov 2007). Within root cells, fungi develop arbuscules, which are highly branched structures that facilitate nutrient exchange between the fungus and the plant (Tikhonovich and Provorov 2007), as well as vesicles that primarily function as nutrient storage structures (Souza 2005). Plant species exhibiting low root

density, short root hairs, and relatively large diameter fine roots, such as grapevines, tend to benefit most from AM fungal symbiosis for nutrient uptake (Schreiner and Linderman 2005; Vieira et al. 2020). In wine grape production particularly, AM fungal symbiosis supports optimal vine health and fruit development by enhancing nutrient acquisition to maintain a balanced nutrient supply while avoiding excessive canopy growth that can compromise fruit quality (Khan et al. 2020; Schreiner and Linderman 2005; Torres et al. 2016; Trouvelot et al. 2015). The nature of this symbiosis, however, varies with soil type and grapevine scion-rootstock combination, as differences in root architecture, morphology, and rhizodeposition influence both vine performance and AM fungal colonization, diversity, and community composition (Rodriguez et al., *in preparation a and b*; see also Chapter 2 and 3).

In most commercial vineyards worldwide, growers graft a grapevine scion onto a rootstock, which comprises the root system and serves as the link between the soil environment and the vine (Ollat et al. 2016). Rootstock identity strongly influences vine performance, as the species from which each rootstock is bred shapes its root architecture, drought tolerance, and nutrient acquisition strategies (Chen et al. 2024; Koepke and Dhingra 2013; Moukarzel et al. 2021; Moukarzel et al. 2022; Ollat et al. 2016; Warschefsky et al. 2016). For example, the 140R rootstock is a *V. berlandieri* x *V. rupestris* hybrid characterized by a deeply developed root system that facilitates access to water and nutrients from deeper soil layers, making it well-suited to the dry, Mediterranean-like conditions (Chen et al. 2024). Both parent species have been observed to confer high scion vigor and enhanced macro- and micronutrient acquisition to the scion relative to other rootstocks with *V. riparia* parentage (Gautier et al. 2020). The 101-14 rootstock, a *V. riparia* x *V. rupestris* hybrid, exemplifies this pattern through its characteristically lower scion vigor (Gautier et al. 2020; Yildirim et al. 2018). This rootstock develops a shallower and less extensive root system than 140R, a trait linked to the reduced root penetration of *V. riparia*, making it better suited to heavy, moist clay soils (Lambert et al. 2008; Pongracz 1983). As a species with limited drought tolerance, its *V. riparia* parentage further influences 101-14 performance under dry conditions (Yildirim et al. 2018). These differences in root system architecture and vigor among rootstocks, as well as variation in the amount and composition of root exudates, can modify the soil-root environment and influence AM fungal colonization and the recruitment of specific taxa (Lailheugue et al. 2024; Moukarzel et al. 2021; Noceto et al. 2024;

Philippot et al. 2013; Warschefsky et al. 2016). Scion genotype can also influence AM fungal communities by shaping the quantity and composition of photosynthates and signaling compounds delivered to the root system, though these effects are generally modest relative to those of the rootstock in grafted vines (Lailheugue et al. 2024; Noceto et al. 2024).

While the grapevine scion-rootstock combination strongly influences AM fungal symbiosis, agricultural management practices also shape AM fungal community composition and colonization in vineyards (Cavagnaro 2014; Cavagnaro 2015). Guided by fundamental agroecological principles, RA prioritizes minimizing disturbance, enhancing biodiversity, integrating livestock, and reducing reliance on synthetic inputs, such as herbicides and fertilizers (Khangura et al. 2023; Schreefel et al. 2020). To implement these principles, practices such as no-till, compost application, and livestock grazing are increasingly being adopted in viticulture. These practices have been shown to influence AM fungal symbiosis, as their immediate and long-term effects can alter soil physicochemical properties and fungal colonization dynamics (Cavagnaro 2014; Cavagnaro 2015; Kabir 2005; Yang et al. 2020). Tillage immediately disrupts extraradical hyphal networks and reduces spore density, which limits the ability of AM fungi to colonize host roots (Kabir 2005). Over time, this disturbance reduces AM fungal species richness and diversity by differentially affecting taxa depending on their colonization strategies, promoting disturbance-tolerant taxa (Brito et al. 2012; Oehl and Koch 2018). In contrast, no-till preserves intact hyphal networks and supports greater spore densities, facilitating greater root colonization (Cornejo et al., 2009; Kabir 2005). Over successive growing seasons, reduced disturbance from no till supports more diverse AM fungal communities and distinct community composition relative to tilled treatments (Brito et al. 2012; Oehl and Koch 2018).

Compost application and livestock grazing can further influence AM fungal communities through organic matter (OM) inputs and modifications to soil nutrient availability. Unlike synthetic fertilizers, which can suppress AM fungal colonization by rapidly elevating soil nutrient concentrations, compost application has been observed to support or maintain AM fungal colonization through the slow release of nutrients (Cavagnaro 2014; Cavagnaro 2015). Such conditions are particularly favorable to AM fungal symbiosis, as high nutrient concentrations reduce plant dependence on AM fungi by increasing aboveground allocation and reducing host investment in AM fungal colonization (Johnson 2010; Johnson et al. 2013; Schreiner 2005; Smith and Read 1997). Over time, compost also

increases soil organic matter and stimulates microbial activity, fostering conditions conducive to greater AM fungal diversity and sporulation (Freitas et al. 2011; Montiel-Rozas et al. 2017). The effects of grazing on AM fungal communities, however, are less characterized than those of compost, as most research has focused on grazed grassland plants rather than perennial, woody plant species, such as grapevines. Nevertheless, livestock grazing may influence AM fungal communities through animal excrement inputs that provide organic nutrients and stimulate microbial nutrient cycling (Brewer et al. 2023; Cui et al. 2026). These grazing benefits, however, may be counteracted by livestock trampling, which increases soil bulk density and reduces aeration that is necessary for AM fungal abundance and colonization (Arvidsson 1999; Yang et al. 2018).

Although these RA practices have typically been studied in isolation, they are rarely implemented that way, and their stacked application may produce cumulative or synergistic effects on AM fungal communities that extend beyond what any single practice alone would predict. For example, combined no-till and organic fertilization has been observed to result in greater AM fungal diversity than either practice alone, with their interaction significantly shaping AM fungal community composition (Liu et al. 2022). While the combined effects of grazing and tillage on AM fungal communities have not been directly studied, no-till combined with grazing has been shown to promote surface soil carbon (C) stabilization (Quiroga et al. 2009), which may support greater AM fungal activity and colonization. However, these interactive effects have been documented over five or more years, suggesting that synergistic effects on AM fungal communities may require longer management periods to fully develop. Further, the combined effects of regenerative management practices in vineyards remain poorly understood, limiting our understanding of how AM fungal communities and grapevine symbiosis are shaped under real-world management conditions.

To address these gaps, this study evaluated the short-term effects of RA practices on AM fungal colonization, diversity, and community composition across two grapevine genotypes (PN x 101-14 and CH x 140R) in the Russian River Valley, Sonoma County, California. Our objectives were to 1) evaluate the short-term effects of stacked soil conservation practices (sheep grazing, compost application, and no-till) on soil physicochemical conditions and AM fungal colonization, diversity, and community composition; and 2) assess the influence of grapevine-soil interactions on AM fungal communities and vine performance across two scion-rootstock

combinations. We hypothesize that the combination of no-till, compost, and grazing, representing the most intensive stacking of regenerative practices, may enhance AM fungal colonization relative to fewer or no stacked soil conservation practices. This is expected to be reflected in greater hyphal, vesicle, and arbuscule occurrence within roots. We further hypothesize that AM fungal diversity, including richness, evenness, and Shannon diversity, will be greatest in treatments with compost and grazing. We lastly hypothesize that grapevine-soil interactions will shape AM fungal community composition, colonization responses to soil management practices, and vine nutrient status. Specifically, scion-rootstock combination and soil type are expected to drive differential responses between sites in AM fungal metrics, leaf N, P, and K concentrations, and yield.

2. Materials and Methods

2.1. Study Design

A controlled, replicated trial was established in 2022 within a commercial vineyard in the Russian River Valley, Sonoma County, California (USA). The trial consisted of two adjacent sites, CH and PN, located within ~500 ft of one another and distinguished by their cultivated grapevine scion-rootstock combination (PN; Pinot Noir x 101-14 and CH; Chardonnay x 140-R, respectively) (Fig 3.1a). Vine spacing and planting density differed between sites, with PN planted at 5 x 4 ft spacing (5,382 vines ha⁻¹) and CH planted at 6 x 5 ft spacing (3,588 vines ha⁻¹). According to the NRCS Web Soil Survey, soils at CH are classified as Yolo clay loam (silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents), while soils at PN are classified as Yolo silt loam (silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents). Soil texture at CH was clay loam (31.1% sand, 28.7% silt, 40.2% clay), while PN soils were silty clay loam (15.9% sand, 29.2% silt, 54.9% clay).

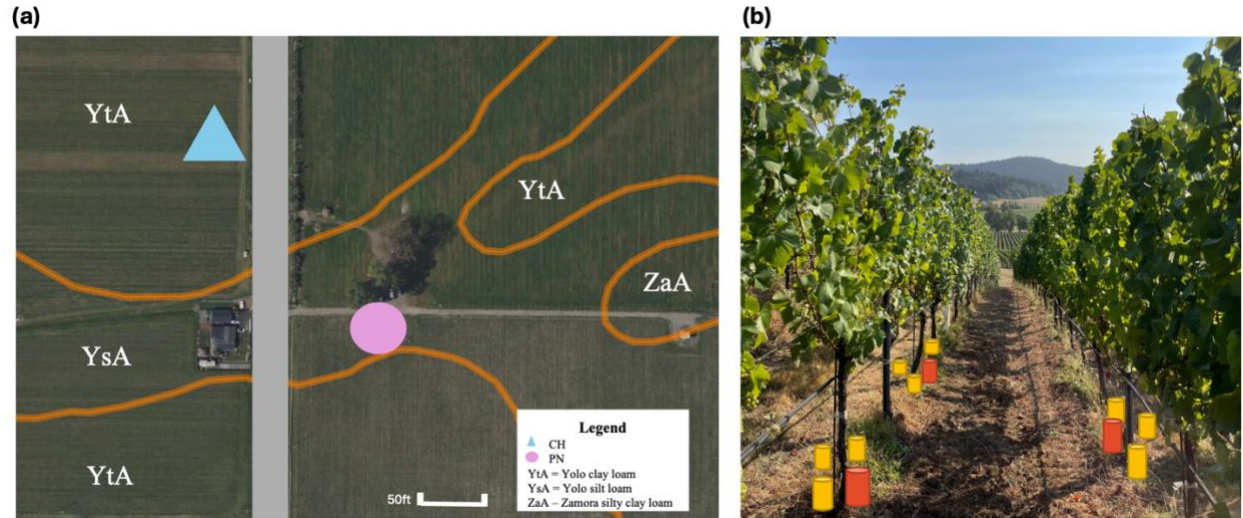


Fig 3.1 Locations of the two experimental vineyard sites in the Russian River Valley, Sonoma County, California (a) and soil and root sampling design (b). An aerial map shows the CH (blue triangle) and PN (pink circle) sites, each cultivating different grapevine scion-rootstock combinations (Chardonnay x 140R and Pinot Noir x 101-14, respectively). NRCS soil classifications are overlaid, including Yolo clay loam (YtA), Yolo silt loam (YsA), and Zamora silty clay loam (ZaA). A representative treatment row illustrates the sampling design, where yellow cylinders mark soil sampling locations and red cylinders indicate root sampling locations adjacent to the rootstock crown

Each site featured six treatments representing a range of stacked soil conservation strategies consistent with RA principles: 1) no-till, compost application (NT, CA); 2) no-till, no compost application (NT, NC); 3) alternate till, compost application (AT, CA); 4) alternate till, no compost application (AT, NC); 5) no-till, compost application, and sheep grazing (NT, CA, G); 6) alternate till, compost application, and sheep grazing (AT, CA, G). Of the six treatments, Treatment 5 (NT, CA, G) is considered the fully regenerative treatment, while Treatment 4 (AT, NC) is considered the conventional treatment in this study. Treatments were arranged in a randomized complete block design (RCBD) across three blocks. Each block contained one replicate per treatment, resulting in 18 sampling plots per site. Each sampling plot encompassed four adjacent vine rows and three tractor rows comprising 60 vines per row. The sampling area was located within the middle tractor row, while the adjacent tractor rows served as buffer zones.

Baseline vineyard management practices included drip fertigation and tractor row cover cropping seeded at a rate of 112 kg ha⁻¹ using sheep mix (1% Campeda Subclover, 2% Hykon Rose Clover, 2% Dwarf Essex Rape, 23% Austrian Winter Peas, 35% Winter Ryegrain, 35% Triticale) and oats. No-till plots remained undisturbed throughout the experiment. Alternate till plots followed standard vineyard practices, involving one to four tractor passes in spring and fall, which were applied alternately to tractor rows each year. Narrower row spacing at PN limited the number of tillage passes per year compared to CH. Compost with a carbon (C): nitrogen (N) ratio of approximately 12 was broadcast annually to designated plots at a rate of 9.0 Mg ha⁻¹ (fresh weight). Grazing plots underwent high-intensity, short-duration sheep grazing twice per year during vine dormancy (December through April).

2.2 Plant and soil sampling

Soil and plant samples were collected two years after trial establishment. From July to August 2024, root and soil samples were collected from three vines per treatment (Fig 1b). Sampling coincided with grapevine veraison, a stage marking the onset of berry ripening and color change, when grapevines have absorbed the majority of essential soil nutrients.

Lateral roots were excavated from the surface soil (0-30 cm) adjacent to the rootstock crown (Fig 1b). For each vine, fine root material was collected to fill approximately one-quarter of a standard sandwich-sized polyethylene bag (16.5 x 14.9 cm) to obtain sufficient tissue for microscopic quantification of AM fungal colonization and for DNA extraction for AM fungal community sequencing. A total of 54 root samples (3 vines x 6 treatments x 3 blocks) were collected per site.

Soil samples (0 - 30 cm) were collected from three locations surrounding each sampled vine near the rootstock crown using a 2.2 cm diameter soil probe, yielding approximately 432 cm³ of soil per treatment (Fig 1b). Soil collected from the three locations was composited into a single sample per vine. A total of 54 soil samples (3 vines x 6 treatments x 3 blocks) were collected per site. Samples were not composited across vines within a plot, yielding three separate samples per plot. In total, 108 soil and root samples were collected across this study (n = 108; 3 vines x 6 treatments x 3 blocks x 2 sites). All soil and root samples were placed in polyethylene bags and stored in a 2 °C cold room until processing.

To assess vine nutrient status, leaf samples were collected at veraison from the same node as a fruit cluster and from the upper segment of the shoot. From each treatment, 50 leaf blades were collected from 10 selected vines and composited into a single sample per plot (Schreiner and Skinkis 2019), resulting in 18 composite leaf samples per site (6 treatments x 3 blocks) and 36 composite leaf samples across the study (6 treatments x 3 blocks x 2 sites). Leaf samples were then dried at 55 °C and sent to the UC Davis Analytical Lab for analysis of N, P, and potassium (K) contents.

Harvest was conducted jointly by research and vineyard staff when fruit reached 23-24 °Brix for Pinot Noir and 22-23 °Brix for Chardonnay. Yield was determined from 10 selected vines within each treatment by averaging fruit cluster numbers and cluster weight (kg vine⁻¹) across all selected vines and multiplying these means to obtain average yield (kg vine⁻¹) per treatment.

2.3 Analysis of AM fungal abundance and diversity in vine roots

After transport to the laboratory, fine roots were cleaned using a sequential rinsing process to remove adhering soil particles. Initially, roots were rinsed under a stream of DI water to remove large, loosely attached soil particles. Soil tightly adhered to roots was then removed using forceps and a wash bottle. Cleaned roots were then divided into two subsamples: 1) stored in 70% ethanol at 4° C for root staining; 2) at -80° C for DNA analysis to assess AM fungal diversity. Samples preserved in ethanol were stored at 4 °C for approximately six months prior to staining, and frozen subsamples were stored at -80 °C for approximately ten months before DNA extraction.

Grapevine roots preserved in 70% ethanol at 4°C were cleared and stained following Schreiner (2003). AM fungal colonization was quantified using a gridline intersect method at 40x magnification on a Leica DM1000 LED compound microscope. For each sample, stained roots were examined at 100 intersections. At each intersect, the presence or absence of AM fungal structures (i.e. hyphae, arbuscules, and vesicles) crossing the vertical crosshair was recorded. Percent colonization was expressed as the percentage of intersects with AM fungal structures, calculated as one minus the proportion of intersects with no detected AM fungal structure multiplied by 100.

Arbuscular mycorrhizal fungal DNA was extracted from roots using DNeasy Plant Pro Kits (Qiagen, Germantown, MD, United States). Amplicon sequencing targeting the internal transcribed spacer region (ITS-2) was performed at the University of California-Davis Host-Microbe Systems Biology Core Facility using the NexF-N3-

5.8S_fun and nexR-N3-ITS2_fun primers of variable lengths (Taylor et al. 2016). Libraries were prepared using a two-step PCR analysis. The first PCR step involved an initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation, annealing, and extension, and final storage at 12°C. To incorporate molecular barcodes and Illumina adapters, a second PCR step was carried out, consisting of 8 additional cycles under similar thermal cycling conditions using dual-indexed Nextera XT primers. Amplicons were pooled and quantified for paired-end sequencing on an Illumina MiSeq instrument (Illumina, San Diego, CA, United States).

After sequencing, raw FASTQ data were processed using the QIIME2 pipeline (version 2023.9; <http://qiime2.org/>). Taxonomic assignment was performed using the QIIME2 feature-classifier plugin trained on the UNITE ITS-2 reference database (Abarenkov et al. 2024). The resulting operational taxonomic unit (OTU) and taxonomy tables were imported into R statistical software (R Core Team 2024) and analyzed using the phyloseq package (McMurdie and Holmes 2013). All data were filtered to retain only taxa assigned to the phylum Glomeromycota, and downstream analyses were restricted to this group. Alpha diversity metrics, including species richness (observed and Chao 1), relative abundance, evenness, and the Shannon diversity index, were used to assess the diversity of AM fungal communities. Observed and Chao1 richness values were identical, so observed richness was reported. Analyses and interpretation were conducted at the genus level, with taxa of uncertain phylogenetic placement labeled as *gen. incertae sedis* according to the classifier. These designations reflect varying levels of confident placement, with some taxa placed to the family level (*Glomeraceae*, *Paraglomeraceae*, *Pervetustaceae*, *Archaeosporaceae*, and *Claroideoglomeraceae*), the order level (*Glomerales* and *Paraglomerales*), or the phylum level (*Glomeromycota*).

2.4 Soil analysis

After collection, soil samples were sieved to 2 mm and air-dried prior to analysis. Nitrate-N (NO_3^- -N) was determined by extracting 8 g of air-dried soil with potassium sulfate (K_2SO_4) adapted from Drinkwater et al. (2015). The extract was then quantified colorimetrically using a modified protocol from Doane and Horwath (2003). Phosphate-P (PO_4^{3-} P) concentration was determined by extracting 2.50 g air-dried soil with sodium bicarbonate (NaHCO_3), followed by colorimetric analysis of the filtered extract (Olsen et al. 1954). Both measurements were analyzed using a Biotek SynergyTM HTX multi-mode microplate reader (BioTek Instruments, Winooski, VT, USA).

Available water holding capacity (WHC) was determined by saturating 10 g of air-dried soil with deionized water and allowing it to drain for 24 hours at 4 °C. Available water holding capacity was calculated as the difference between the saturated and oven-dried (105 °C) soil weights (Nelson et al. 2024). Mineralizable C was measured by rewetting 10g of air-dried soil to 50% water-holding capacity with DI water, followed by a 24-hour incubation and quantification of CO₂ production (mg CO₂-C kg⁻¹ soil hr⁻¹) using a LI-COR LI-850 CO₂/H₂O gas analyzer (Lincoln, NE, USA). Soil pH and EC were measured in a 2:1 (soil: water) slurry using a Mettler Toledo pH/conductivity meter (Mettler Toledo, Columbus, Ohio, USA).

2.5 Data analysis

All statistical analyses were performed in R version 4.4.0 (R Core Team 2024). As three vine and soil samples were collected per plot, individual measurements were treated as subsamples. To account for pseudoreplication, block was included as a nested random effect or blocking factor across all analyses. Normality of soil, plant, and AM fungal variables was evaluated using the Shapiro-Wilk test, and homogeneity of variances was assessed using Levene's test. When assumptions of normality and homoscedasticity were met, differences in soil and AM fungal variables were evaluated using one-way or two-way analysis of variance (ANOVA), with group comparisons conducted by treatment or site, when applicable. Welch's one-way ANOVA and ART ANOVA were applied for one-way and two-way comparisons, respectively, when model assumptions were violated. When a significant effect was detected, post hoc pairwise comparisons were performed using Tukey's honestly significant difference (HSD) or Games-Howell tests, depending on variance homogeneity. When assumptions were not met, data were transformed using square-root, reciprocal, or logarithmic transformations. If transformations did not sufficiently meet model assumptions, Kruskal-Wallis tests were performed, followed by pairwise Wilcoxon ranksum tests for post hoc comparisons.

Principal Coordinates Analysis (PCoA), a distance-based ordination that projects multivariate dissimilarities among samples into a reduced set of interpretable axes, was used to visualize differences in soil, plant, and AM fungal variables between sites and treatments using Euclidean distances of standardized variables. Permutational multivariate analysis of variance (PERMANOVA) was performed to determine significant differences in soil, plant, and AM fungal variables among treatments and sites. Multivariate homogeneity of group dispersions

was evaluated using the `betadisper` function in the `vegan` package (v2.6.10) (Oksanen et al. 2025) to assess whether significant PERMANOVA results were influenced by differences in group dispersion rather than composition. The `envfit` function was used to identify the environmental variables most strongly associated with the ordination, ranking predictors by the strength of their correlations (R^2) with the ordination axes based on permutation tests. Correlations among environmental variables, plant traits, and AM fungal metrics were evaluated using Pearson correlation coefficients and visualized using the `corrplot` package (v0.95) (Wei and Simko 2024).

Beta diversity analyses were conducted using the `vegan` package (v2.6.10). A PERMANOVA was applied to Bray-Curtis dissimilarities of AM fungal OTUs to test for differences in community composition among sites and treatments. To assess whether significant PERMANOVA results were influenced by differences in group dispersion rather than composition, multivariate homogeneity of group dispersions was evaluated using the `betadisper` function. Non-metric multidimensional scaling (NMDS), a rank-based ordination method that represents multivariate dissimilarities in a reduced-dimensional space, was performed on Bray-Curtis dissimilarities of AM fungal OTUs to visualize patterns in community structure. Environmental variables, including soil, plant, and AM fungal metrics, were fitted as vectors onto the NMDS ordination using the `envfit` function, which evaluates the strength of correlations (R^2) between each variable and the ordination axes based on permutation tests to identify potential drivers of community patterns.

3. Results

3.1 Variation in soil, plant, and AM fungal variables across treatments

Soil, plant, and AM fungal properties varied across treatments in the two vineyard sites studied. A two-way PERMANOVA detected a significant treatment and site interaction ($p = 0.001$; Fig 3.2). It is important to note that the significant treatment x site interaction may be influenced by significant differences (`betadisper`, $p = 0.007$) in dispersion between treatments across both sites, as no till and compost application was observed to be significantly different from all treatments except alternate till, compost application, and grazing. Thus, its effect should be interpreted cautiously. In contrast, dispersion between sites was homogenous (`betadisper`, $p = 0.197$). Variation between sites was primarily structured along PCoA1, which was strongly associated with soil properties

(EC, PO_4^{3-} , WHC) and yield. In contrast, variation along PCoA2 was primarily explained by AM fungal colonization metrics, particularly the overall colonization rate and the abundance of vesicles and hyphae. These variables exhibited the largest vector loadings, highest r^2 values, and significant associations with the ordination axes (Supplementary Information, Table S3.1).

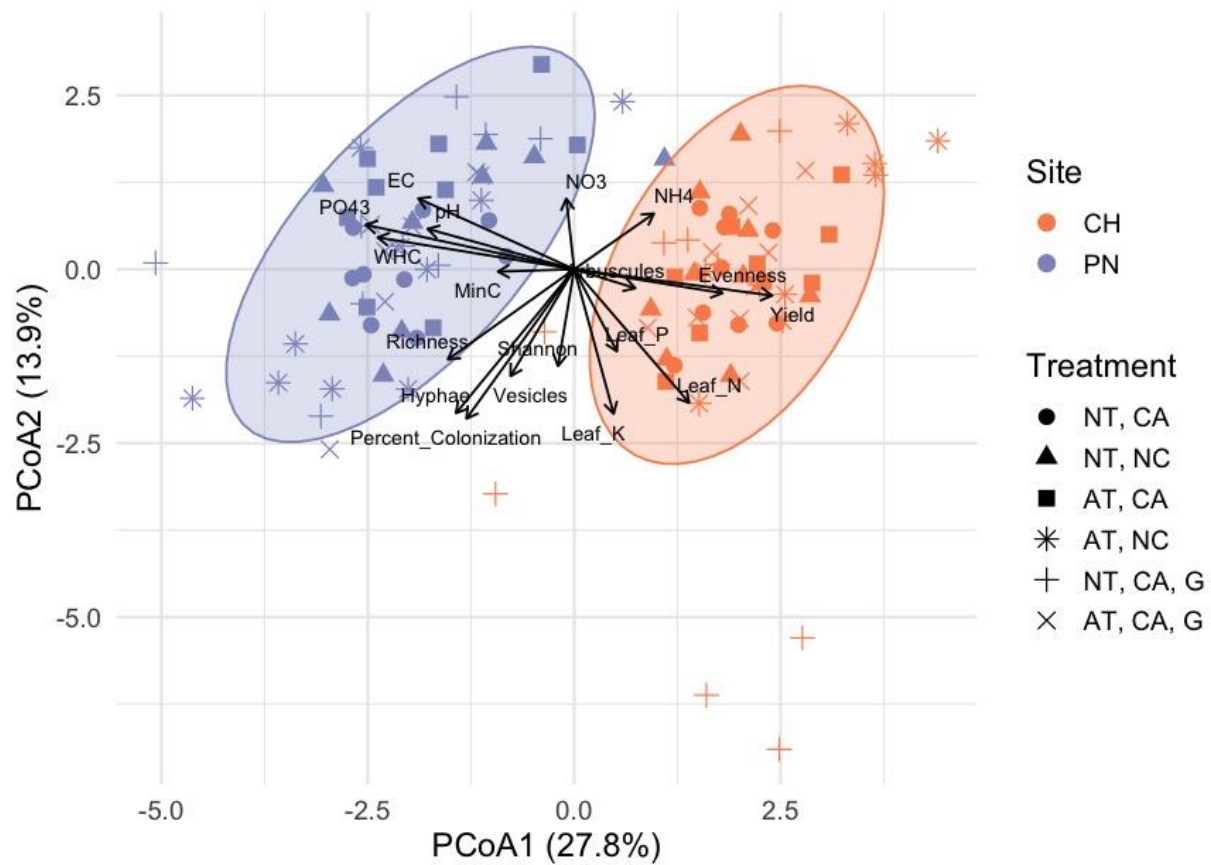


Fig 3.2 Principal Coordinates Analysis (PCoA) of soil, plant, and AM fungal variables across treatments across both sites, CH and PN, in the Russian River Valley, Sonoma County, California. Treatments represent combinations of stacked soil conservation strategies: (1) no-till (NT), compost application (CA); (2) NT, no compost (NC); (3) alternate till (AT), CA; (4) AT, NC; (5) NT, CA, sheep grazing (G); and (6) AT, CA, G. Soil variables included electrical conductivity (EC), soil pH, phosphate (PO_4^{3-}), nitrate (NO_3), ammonium (NH_4), mineralizable carbon (MinC), and water holding capacity (WHC). Plant variables included yield and leaf N, P,

and K. AM fungal variables included percent colonization and hyphae, vesicles, and arbuscule abundance, as well as richness, evenness, and Shannon diversity (Shannon). Arrows depict environmental vectors fitted to the ordination, where arrow length and direction reflect the strength of associations and orientation of each variable with the PCoA axes. Ellipses represent 95% confidence intervals for each site

Two-way ANOVAs confirmed that site had a significant effect on the majority of soil, plant, and AM fungal variables (Supplementary Information, Table S3.2). Variables significantly influenced by site included soil (EC, pH, PO_4^{3-} , mineralizable C) and AM fungal (colonization rate, arbuscule and hyphae abundance, and evenness) variables. Significant treatment x site interactions were detected for soil (pH, NO_3^- , NH_4^+ , WHC) and plant (yield and leaf N, P, K) variables, as well as AM fungal richness. Given these differences between CH and PN, the effect of treatment on individual variables was examined separately for each site.

Table 3.1 One-way ANOVA p-values testing the effect of soil magement on soil, plant, and AM fungal metrics for CH and PN, individually. “+” denotes variables that used the Kruskal-Wallis test with post hoc Wilcox test due to violations of normality or homoscedasticity assumptions.

	<u>CH</u>	<u>PN</u>
<i>Soil Properties</i>		
EC ($\mu\text{S cm}^{-1}$)	0.240	0.784
pH	< 0.001	0.478 ⁺
Phosphate ($\text{mg PO}_4^{3-} \text{ kg}^{-1} \text{ soil}$)	0.800	0.964
Nitrate ($\text{mg NO}_3^- \text{ kg}^{-1} \text{ soil}$)	0.006	0.118
Ammonium ($\text{mg NH}_4^+ \text{ kg}^{-1} \text{ soil}$)	0.238	0.126
Mineralizable Carbon ($\text{mg C kg}^{-1} \text{ soil hr}^{-1}$)	0.681	0.620 ⁺
Water Holding Capacity (WHC) ($\text{g water g}^{-1} \text{ soil}$)	0.057	0.068

<i>Plant Properties</i>		
Yield (kg vine ⁻¹)	0.019	0.041
Leaf N (mg N kg ⁻¹ leaf)	0.015 [†]	< 0.001
Leaf P (mg P kg ⁻¹ leaf)	0.290 [†]	< 0.001 [†]
Leaf K (mg K kg ⁻¹ leaf)	0.103 [†]	0.003
<u>AM Fungal Colonization</u>		
Colonization (%)	0.009	0.721
Vesicles (%)	0.018	0.353
Arbuscules (%)	0.993 [†]	0.273 [†]
Hyphae (%)	0.012	0.721
<u>AM Fungal Diversity</u>		
Shannon Diversity	0.360	0.002
Richness	0.201	0.175
Evenness	0.614	0.591

In CH, several soil and plant properties were significantly different across treatments (Table 3.1; Table 3.2). Among soil properties, pH and NO₃⁻ varied significantly across treatments (p = 0.002 and 0.015, respectively). Soil pH was significantly higher (7.1 ± 0.03) in no till and compost treatments than in alternate till, no compost (7.0 ± 0.04). Additionally, soil pH in the alternate till, compost application, and grazing treatment was significantly higher (7.1 ± 0.03) than both the alternate till and compost (7.0 ± 0.03) and alternate till and no compost treatments (7.0 ± 0.04). Regarding NO₃⁻ concentration, it was significantly lower in the alternate till and compost treatment (1.6 ± 0.04 mg NO₃⁻ kg⁻¹ soil) than the no till and no compost treatment and the alternate till and no compost treatment (4.0 ± 0.8 and 3.6 ± 0.4 mg NO₃⁻ kg⁻¹ soil, respectively). Among plant properties, yield and leaf N were significantly different across treatments (p = 0.019 and 0.015, respectively). Yield was significantly lower in no till, compost application, grazing and alternate till, compost application, and grazing treatments (101 ± 8.2 and 97 ± 3.9 kg vine⁻¹, respectively) than in no till and compost. Leaf N was significantly higher in alternate till and compost (21500 ± 362 mg N kg⁻¹ leaf) than in no till and compost (20433 ± 167 mg N kg⁻¹ leaf), no till and no compost (19617 ± 173 mg N kg⁻¹ leaf), and alternate till and compost (18733 ± 815 mg N kg⁻¹ leaf).

Table 3.2 Treatment differences in soil, plant, and AM fungal properties in CH. Values represent means, with standard errors shown in parentheses. Treatments represent combinations of stacked soil conservation strategies: (1) no-till (NT), compost application (CA); (2) NT, no compost (NC); (3) alternate till (AT), CA; (4) AT, NC; (5) NT, CA, sheep grazing (G); and (6) AT, CA, G. Different lowercase letters indicate significant differences across treatments ($p < 0.005$). Significant differences were determined using one-way ANOVA with Tukey's HSD. When assumptions were not met, the Kruskal-Wallis test was followed by post hoc pairwise Wilcoxon tests

	Treatment					
	NT,CA	NT, NC	AT, CA	AT, NC	NT, CA, G	AT, CA, G
Soil Properties						
EC ($\mu\text{S cm}^{-1}$)	115 (3.7) ^a	116 (5.8) ^a	105 (3.6) ^a	125 (7.1) ^a	120 (5.8) ^a	118 (6.7) ^a
pH	7.1 (0.04) ^a	7.1 (0.04) ^{abc}	7.0 (0.04) ^b	7.0 (0.04) ^{bd}	7.1 (0.04) ^{abc}	7.1 (0.04) ^{ad}
Phosphate (mg PO_4^{3-} kg ⁻¹ soil)	23.3 (1.3) ^a	23.1 (2.8) ^a	20.7 (2.1) ^a	20.8 (2.1) ^a	19.7 (1.6) ^a	25.1 (4.4) ^a
Nitrate (mg NO_3^{2-} kg ⁻¹ soil)	3.2 (0.2) ^{ab}	4.0 (0.8) ^a	1.6 (0.4) ^b	3.6 (0.4) ^a	3.6 (0.8) ^a	3.5 (0.7) ^{ab}
Ammonium (mg NH_4^+ kg ⁻¹ soil)	0.14 (0.05) ^a	0.13 (0.04) ^a	0.10 (0.01) ^a	0.55 (0.29) ^a	0.27 (0.16) ^a	0.39 (0.21) ^a
Mineralizable Carbon (mg C kg ⁻¹ soil hr ⁻¹)	2.4 (0.06) ^a	2.4 (0.15) ^a	2.4 (0.14) ^a	2.5 (0.09) ^a	2.3 (0.13) ^a	2.3 (0.10) ^a
Water Holding Capacity (WHC) (g water g ⁻¹ soil)	0.30 (0.005) ^a	0.30 (0.007) ^a	0.31 (0.006) ^a	0.29 (0.006) ^a	0.32 (0.003) ^a	0.31 (0.005) ^a
Plant Properties						
Yield (kg vine ⁻¹)	139 (7.8) ^a	132 (16.3) ^{ab}	94 (12.0) ^{ab}	135 (10.4) ^{ab}	101 (8.2) ^b	97 (3.9) ^b
Leaf N (mg N kg ⁻¹ leaf)	20433 (167) ^a	19617 (173) ^a	18733 (815) ^a	21500 (362) ^b	24233 (3370) ^{ab}	21550 (1026) ^{ab}

Leaf P (mg P kg ⁻¹ leaf)	1920 (7.6) ^a	1860 (28.9) ^a	2253 (186.8) ^a	2043 (72.2) ^a	2056 (206.4) ^a	2541 (332.5) ^a
Leaf K (mg K kg ⁻¹ leaf)	7633 (60.1) ^a	6933 (88.2) ^a	7033 (142) ^a	8067 (586.2) ^a	14300 (4175.2) ^a	8600 (614.4) ^a
AM Fungal Structures						
Hyphae (%)	28 (3.8) ^{ab}	25 (4.0) ^{ab}	21 (5.4) ^{ab}	16 (4.1) ^a	38 (4.5) ^b	22 (3.2) ^{ab}
Arbuscules (%)	2.2 (0.7) ^a	2.8 (0.9) ^a	3.4 (1.4) ^a	2.9 (0.9) ^a	2.3 (0.6) ^a	2.2 (0.9) ^a
Vesicles (%)	4.3 (1.2) ^{ab}	3.1 (0.9) ^a	2.7 (1.0) ^a	4.1 (1.0) ^{ab}	10.1 (2.9) ^b	3.4 (0.9) ^{ab}
Colonization (%)	32 (3.4) ^{ab}	30 (4.9) ^{ab}	25 (4.9) ^a	21 (4.2) ^a	44 (4.1) ^b	25 (3.8) ^a
AM Fungal Diversity						
Shannon Diversity	1.8 (0.2) ^a	2.1 (0.2) ^a	1.9 (0.2) ^a	1.7 (0.2) ^a	2.1 (0.2) ^a	1.9 (0.1) ^a
Richness	10.8 (1.9) ^a	17.0 (4.2) ^a	10.6 (1.6) ^a	9.4 (1.5) ^a	17.5 (3.7) ^a	11.0 (1.8) ^a
Evenness	0.8 (0.03) ^a	0.8 (0.04) ^a	0.9 (0.03) ^a	0.8 (0.02) ^a	0.8 (0.02) ^a	0.8 (0.03) ^a

Treatment also significantly influenced several AM fungal metrics in CH (Fig 3.3; Table 3.1; Table 3.2). Hyphae and vesicle abundance ($p = 0.013$ and 0.030 , respectively), as well as colonization rate ($p = 0.009$), were significantly affected by treatment. The abundance of hyphae was significantly higher in no till, compost, and grazing ($38 \pm 4.5\%$) than in alternate till and compost ($16 \pm 4.1\%$) (Fig 3.3a). The abundance of vesicles were also significantly higher in no till, compost, and grazing ($10.1 \pm 2.9\%$) than in no till and no compost ($3.1 \pm 4.9\%$) and alternate till and compost ($2.7 \pm 1.0\%$) (Fig 3.3c). No till, compost, and grazing also exhibited significantly higher colonization rate ($44 \pm 4.1\%$) than alternate till and compost ($25 \pm 4.9\%$), alternate till and no compost ($21 \pm 4.2\%$), and alternate till, compost, and grazing ($25 \pm 3.8\%$) (Fig 3d). Despite the significant effect of treatment on AM fungal colonization, AM fungal diversity did not vary significantly across treatments.

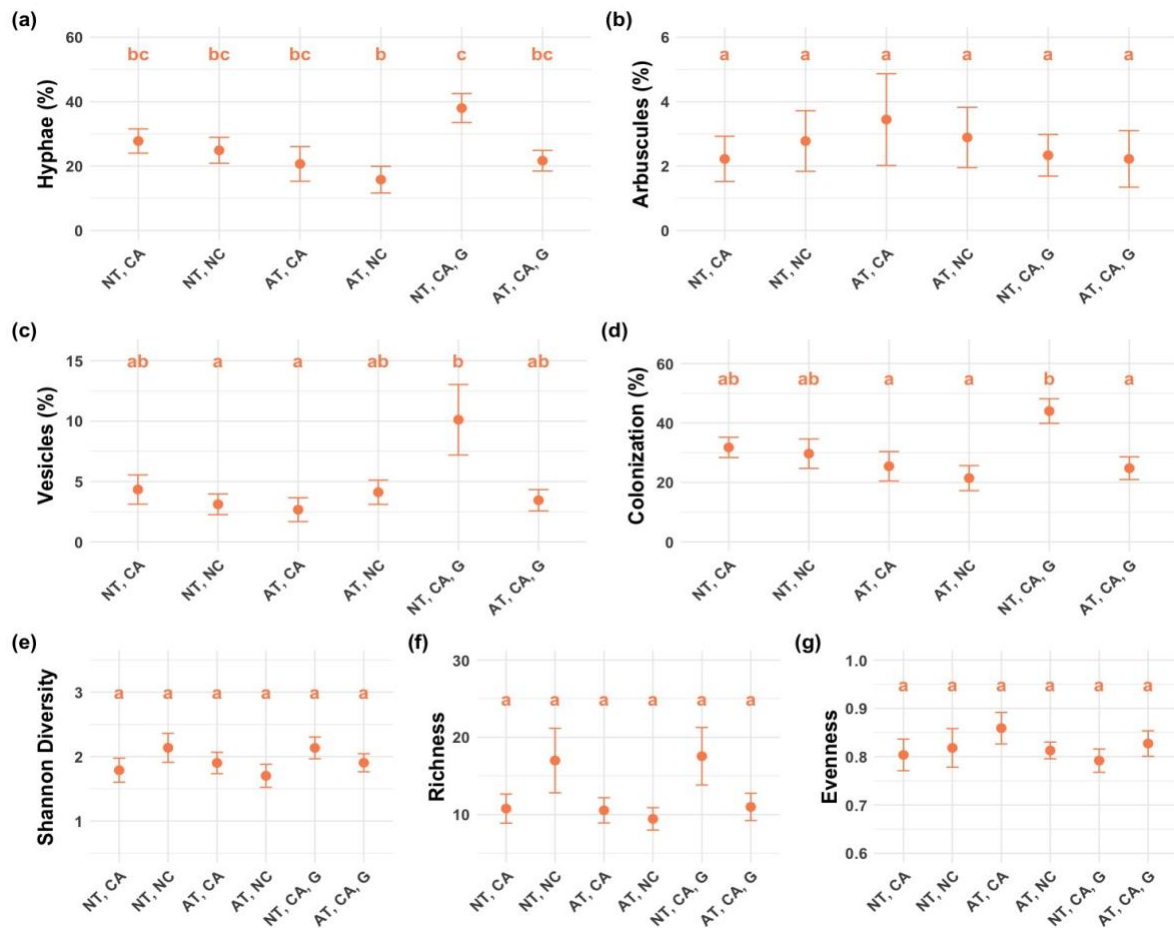


Fig 3.3 AM fungal colonization and diversity across treatments at CH, cultivating grapevine scion-rootstock

combination Chardonnay x 140R in Northern California. Treatments represent combinations of stacked soil conservation strategies: (1) no-till (NT), compost application (CA); (2) NT, no compost (NC); (3) alternate till (AT), CA; (4) AT, NC; (5) NT, CA, sheep grazing (G); and (6) AT, CA, G. Line plots show mean values (± SE) of (a) hyphae (%), (b) arbuscule (%), (c) vesicles (%), (d) colonization (%), (e) Shannon diversity, (f) richness, and (g) evenness. Different lowercase letters indicate significant differences across treatments ($p < 0.05$). Significant differences were determined using one-way ANOVA with Tukey's HSD. When assumptions were not met, the Kruskal-Wallis test was followed by post hoc pairwise Wilcoxon tests

In PN, soil properties did not vary significantly across treatments (Table 3.1; Table 3.3). However, treatment significantly influenced ($p < 0.04$) leaf N, P, and K concentration. Leaf N was significantly lower in no till, compost, grazing (14633 ± 317 mg N kg⁻¹ leaf) and alternate till, compost, grazing (5433 ± 332 mg N kg⁻¹ leaf) than

in no till and no compost (17283 ± 167 mg N kg^{-1} leaf), alternate till and compost (17333 ± 335 mg N kg^{-1} leaf), and alternate till and no compost treatments (17233 ± 240 mg N kg^{-1} leaf). Leaf P was significantly lower in alternate till and compost (1853 ± 28.0 mg P kg^{-1} leaf) than in the no till and no compost treatment (2008 ± 36.1 mg P kg^{-1} leaf) and the alternate till and no compost treatment (2063 ± 34.7 mg P kg^{-1} leaf). Additionally, leaf P was significantly lower in the alternate till, compost, and grazing treatment (1853 ± 48.1 mg P kg^{-1} leaf) than in alternate till and no compost. Leaf P was also significantly lower in no till, compost, and grazing (1730 ± 39.7 mg P kg^{-1} leaf) than in no till and compost (1936 ± 40.5 mg P kg^{-1} leaf), no till and no compost (2008 ± 36.1 mg P kg^{-1} leaf), and alternate till and no compost treatments (2063 ± 34.7 mg P kg^{-1} leaf). Regarding leaf K, alternate till and compost (6367 ± 72.6 mg K kg^{-1} leaf) was significantly lower than the no till and no compost (7183 ± 82.1 mg K kg^{-1} leaf) and alternate till, compost, and grazing treatments (7366 ± 231.5 mg K kg^{-1} leaf).

Table 3.3 Treatment differences in soil, plant, and AM fungal properties in PN. Values represent means, with standard errors shown in parentheses. Treatments represent combinations of stacked soil conservation strategies: (1) no-till (NT), compost application (CA); (2) NT, no compost (NC); (3) alternate till (AT), CA; (4) AT, NC; (5) NT, CA, sheep grazing (G); and (6) AT, CA, G. Different lowercase letters indicate significant differences across treatments ($p < 0.005$). Significant differences were determined using one-way ANOVA with Tukey's HSD. When assumptions were not met, the Kruskal-Wallis test was followed by post hoc pairwise Wilcoxon tests

	Treatment					
	NT,CA	NT, NC	AT, CA	AT, NC	NT, CA, G	AT, CA, G
Soil Properties						
EC ($\mu\text{S cm}^{-1}$)	146 (6.1) ^a	153 (9.1) ^a	158 (9.5) ^a	154 (8.9) ^a	147 (7.7) ^a	144 (7.1) ^a
pH	7.2 (0.03) ^a	7.2 (0.04) ^a	7.2 (0.04) ^a	7.3 (0.03) ^a	7.4 (0.07) ^a	7.3 (0.03) ^a
Phosphate (mg PO_4^{3-} kg^{-1} soil)	50.0 (3.5) ^a	49.8 (3.7) ^a	50.6 (2.7) ^a	51.8 (4.2) ^a	53.5 (3.6) ^a	49.8 (2.4) ^a
Nitrate (mg NO_3^{2-} kg^{-1} soil)	3.0 (0.4) ^a	3.7 (0.8) ^a	5.3 (1.2) ^b	3.4 (0.3) ^a	2.7 (0.8) ^a	3.0 (0.6) ^a

Ammonium (mg NH ₄ ⁺ kg ⁻¹ soil)	0.10 (0.03) ^a	0.07 (0.02) ^a	0.13 (0.03) ^a	0.05 (0.01) ^a	0.11 (0.04) ^a	0.04 (0.01) ^a
Mineralizable Carbon (mg C kg ⁻¹ soil hr ⁻¹)	2.5 (0.18) ^a	2.5 (0.10) ^a	2.5 (0.21) ^a	2.9 (0.21) ^a	2.5 (0.11) ^a	2.5 (0.10) ^a
Water Holding Capacity (g water g ⁻¹ soil)	0.34 (0.003) ^a	0.34 (0.004) ^a	0.34 (0.006) ^a	0.35 (0.003) ^a	0.33 (0.004) ^a	0.35 (0.004) ^a
Plant Properties						
Yield (kg vine ⁻¹)	30.7 (2.3) ^a	36.3 (2.6) ^a	35.3 (1.5) ^a	35.6 (2.2) ^a	26.9 (2.9) ^a	30.5 (1.4) ^a
Leaf N (mg N kg ⁻¹ leaf)	16033 (573) ^{ab}	17283 (167) ^b	17333 (335) ^b	17233 (240) ^b	14633 (317) ^a	5433 (332) ^a
Leaf P (mg P kg ⁻¹ leaf)	1936 (40.5) ^{abe}	2008 (36.1) ^{ae}	1853 (28.0) ^{bd}	2063 (34.7) ^e	1730 (39.7) ^{cd}	1853 (48.1) ^{ad}
Leaf K (mg K kg ⁻¹ leaf)	6800 (229.1) ^{ab}	7183 (82.1) ^a	6367 (72.6) ^b	7033 (130.2) ^{ab}	7066 (187.8) ^{ab}	7366 (231.5) ^a
AM Fungal Structures						
Hyphae (%)	43 (3.9) ^a	40 (9.0) ^a	31 (6.8) ^a	43 (10) ^a	38 (7.5) ^a	34 (5.8) ^a
Arbuscules (%)	0.7 (0.3) ^a	1.1 (0.6) ^a	0.8 (0.3) ^a	2.3 (1.0) ^a	2.0 (0.8) ^a	0.7 (0.3) ^a
Vesicles (%)	3.8 (0.9) ^a	3.8 (1.4) ^a	5.1 (1.6) ^a	9.0 (2.3) ^a	6.7 (1.7) ^a	6.2 (2.6) ^a
Colonization (%)	44 (4.0) ^a	40 (8.2) ^a	34 (6.7) ^a	46 (9.8) ^a	40 (7.5) ^a	36 (5.7) ^a
AM Fungal Diversity						
Shannon Diversity	2.1 (0.1) ^{ab}	1.6 (0.1) ^c	1.7 (0.1) ^{ac}	2.0 (0.1) ^{abc}	1.8 (0.1) ^{abc}	2.2 (0.1) ^b
Richness	24.0 (3.2) ^a	15.0 (2.3) ^a	18.6 (2.6) ^a	23.6 (4.1) ^a	20.3 (2.7) ^a	23.8 (2.6) ^a
Evenness	0.7 (0.03) ^a	0.6 (0.05) ^a	0.6 (0.07) ^a	0.7 (0.05) ^a	0.7 (0.05) ^a	0.7 (0.03) ^a

Unlike CH, treatment effects on AM fungal metrics were less pronounced in PN (Fig 3.4; Table 3.3). Only Shannon diversity ($p = 0.002$) differed significantly across treatments, with no till and no compost (1.6 ± 0.1) and alternate till and compost treatments (1.7 ± 0.1) exhibiting significantly lower diversity than alternate till, compost, and grazing (2.2 ± 0.1) (Fig 4e). No till and no compost treatments were also significantly lower than no till and compost treatments (2.1 ± 0.1). Despite the significant effect of treatment on AM fungal diversity, AM fungal colonization did not vary significantly across treatments.

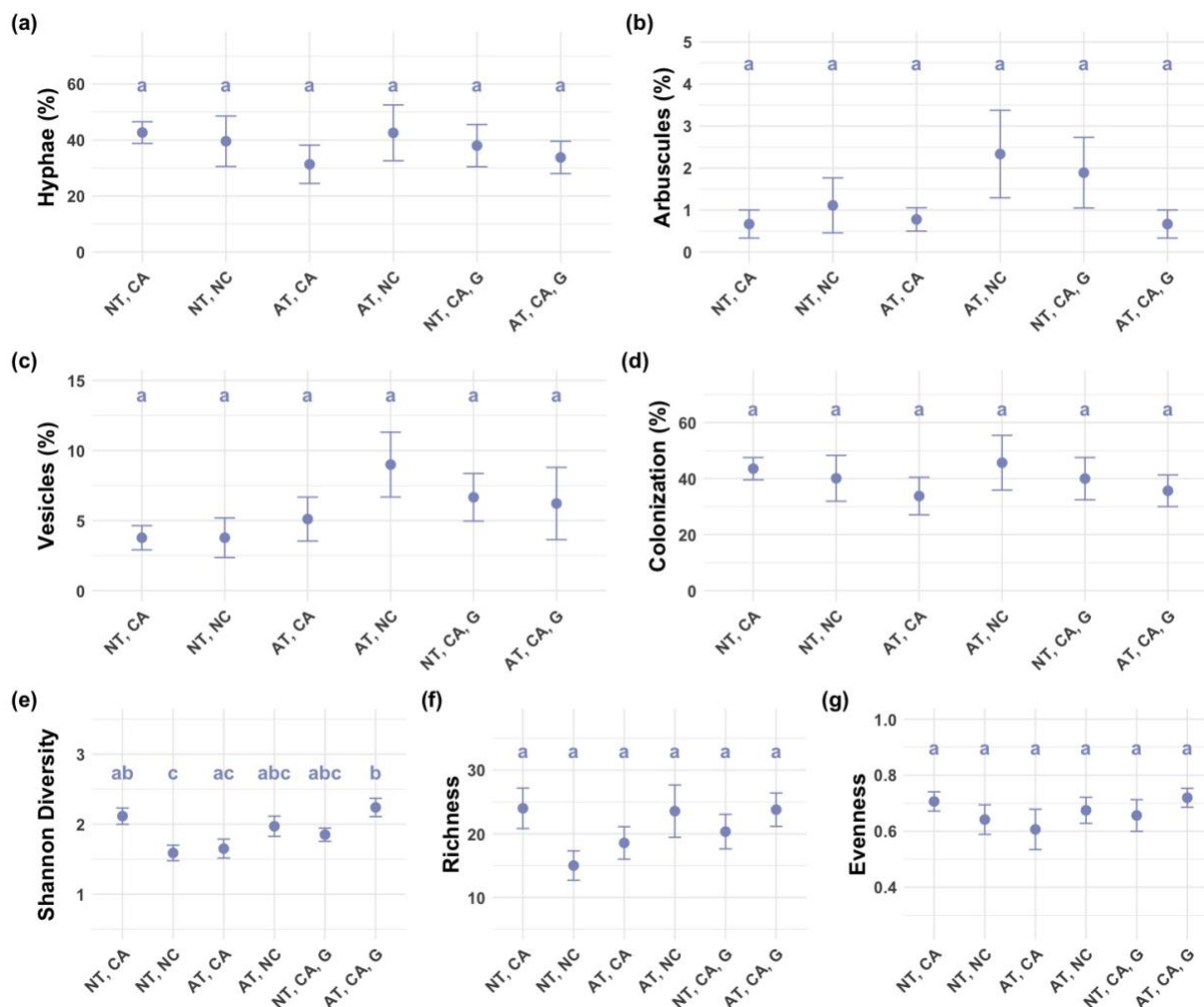


Fig 3.4 AM fungal colonization and diversity across treatments at PN, cultivating grapevine scion-rootstock

combination Pinot Noir x 101-14 in Northern California. Treatments represent combinations of stacked soil conservation strategies: (1) no-till (NT), compost application (CA); (2) NT, no compost (NC); (3) alternate till (AT), CA; (4) AT, NC; (5) NT, CA, sheep grazing (G); and (6) AT, CA, G. Line plots show mean values (± SE) of (a) hyphae (%), (b) arbuscule (%), (c) vesicles (%), (d) colonization (%), (e) Shannon diversity, (f) richness, and (g) evenness. Different lowercase letters indicate significant differences across treatments (p < 0.05). Significant differences were determined using one-way ANOVA with Tukey's HSD. When assumptions were not met, the Kruskal-Wallis test was followed by post hoc pairwise Wilcoxon tests

3.2 Variation in AM fungal community composition across treatments

Across all samples, 155 AM fungal OTUs spanning 19 genera were detected. Grapevine roots in CH hosted 106 OTUs across 17 genera, while roots collected from PN had 134 OTUs spanning 18 genera. Of these, 85 OTUs were shared between sites, representing a core group of AM fungal taxon present in both sites (Fig 3.5). Spanning 16 genera, these included *Glomeraceae gen incertae sedis* (16 OTUs), *Rhizophagus* (14), *Glomus* (10), *Claroideoglomus* (5), *Paraglomus* (5), *Nanoglomus* (3), *Septoglomus* (3), *Diversispora* (2), *Glomerales gen incertae sedis* (2), *Rhizoglomus* (2), *Archeospora* (1), *Archeosporales gen incertae sedis* (1), *Dominikia* (1), *Glomeromycota gen incertae sedis* (1), *Paraglomeraceae gen incertae sedis* (1), and *Paraglomerales gen incertae sedis* (1). An additional 17 shared OTUs could not be resolved to a known taxon.

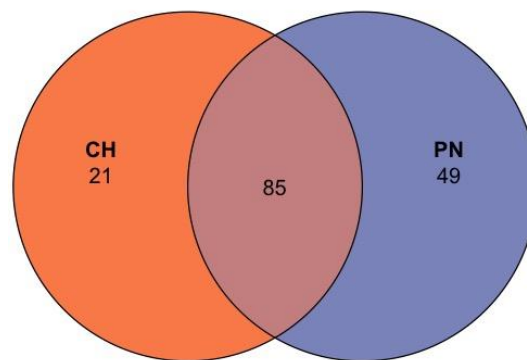


Fig 3.5 Venn diagram illustrating the distribution of AM fungal OTUs across two vineyard sites, CH and PN, cultivating grapevine scion-rootstock combinations Chardonnay x 140R and Pinot Noir x 101-14, respectively. Numbers represent AM fungal OTUs unique to each site and those shared between sites.

The distribution of these genera, however, varied across treatments within each site. Within CH, the number of genera detected per treatment ranged from 11 to 16, with no till, compost and grazing treatments hosting the highest number of genera (16), followed by no till and compost and no till and no compost treatments (14 each); then alternate till, compost, and grazing (12); and lastly, alternate till and compost and alternate till and

no compost treatments (11 each). Nine genera were shared across all treatments, including *Claroideoglossum*, *Glomeraceae gen incertae sedis*, *Glomerales gen incertae sedis*, *Glomus*, *Nanoglossum*, *Paraglomeraceae gen incertae sedis*, *Paraglossum*, *Rhizoglossum*, and *Rhizophagus*. Beyond this core group of AM fungal genera, no treatment-specific genera were detected in CH.

In PN, the number of genera detected per treatment ranged from 13 to 15, with alternate till and compost hosting the highest number of genera (15), followed by no till and compost; alternate till and no compost; and no till, compost, and grazing (14 each). The no till and no compost and the alternate till, compost, and grazing treatments hosted the lowest number of genera (13 each). Ten genera were shared across all treatments, including *Claroideoglossum*, *Diversispora*, *Glomeraceae gen incertae sedis*, *Glomerales gen incertae sedis*, *Glomeromycota gen incertae sedis*, *Glomus*, *Paraglomeraceae gen incertae sedis*, *Paraglossum*, *Rhizoglossum*, and *Rhizophagus*. Notably, *Dominikia* and *Archaeospora* were exclusively associated with the alternate till and compost treatment, representing the only treatment-specific genera detected in PN.

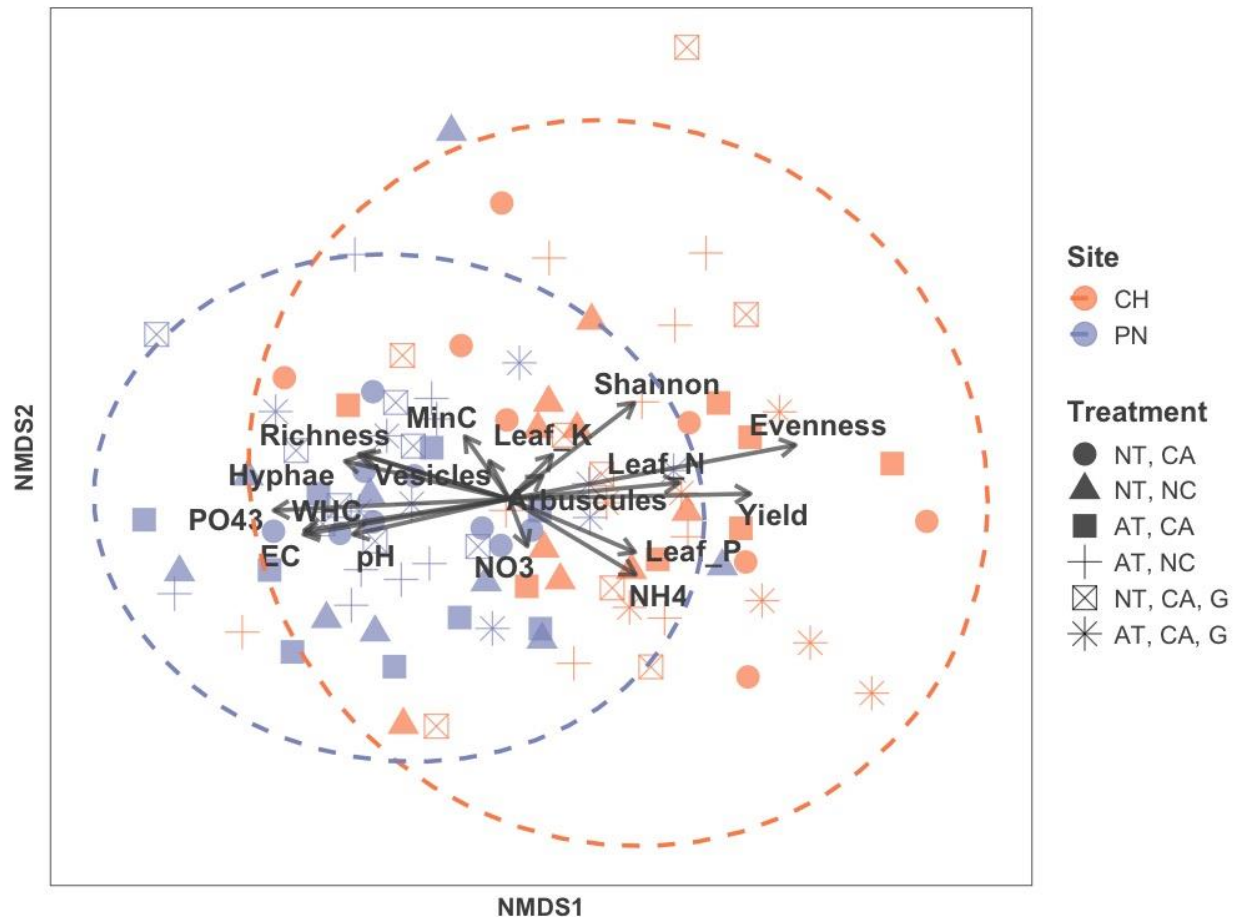


Fig 3.6 Non-metric Multidimensional Scaling (NMDS) ordination based on Bray-Curtis dissimilarity of AM fungal OTUs across treatments and sites (CH and PN). Treatments represent combinations of stacked soil conservation strategies: (1) no-till (NT), compost application (CA); (2) NT, no compost (NC); (3) alternate till (AT), CA; (4) AT, NC; (5) NT, CA, sheep grazing (G); and (6) AT, CA, G. Arrows represent environmental vectors fitted to the ordination using the envfit function, where arrow length and direction reflect the strength of association and orientation of each variable with the NMDS axes. Soil variables included electrical conductivity (EC), soil pH, phosphate (PO43), nitrate (NO3), ammonium (NH4), mineralizable carbon (MinC), and water holding capacity (WHC). Plant variables included yield and leaf N, P, and K. AM fungal variables included percent colonization and hyphae, vesicles, and arbuscule abundance, as well as richness, evenness, and Shannon diversity (Shannon). Ellipses represent 95% confidence intervals for each site.

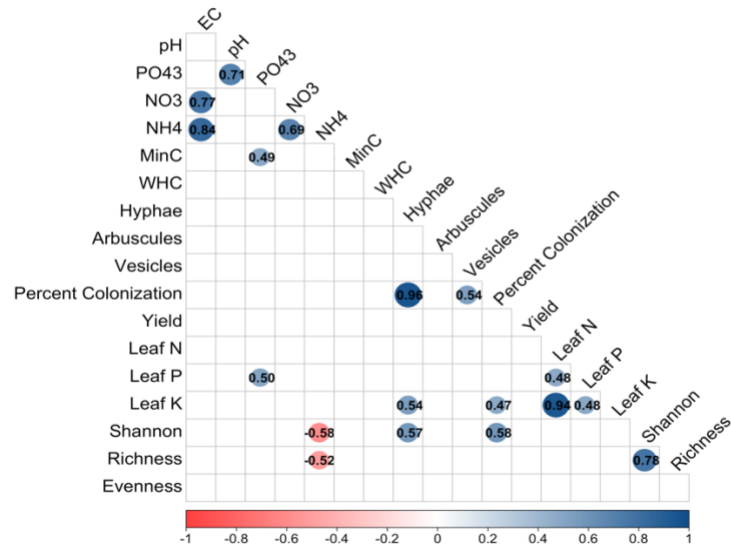
To evaluate whether AM fungal community composition varied between treatments and sites, differences in community structure were assessed using PERMANOVA based on Bray-Curtis dissimilarity of AM fungal OTUs. Results indicated that site, treatment, and their interaction did not significantly ($p > 0.05$) influence AM fungal community composition (Fig. 3.6). However, envfit analysis identified several soil, plant, and AM fungal variables significantly correlated with variation in AM fungal community composition across sites, including yield, PO_4^{3-} , EC, WHC, evenness, and Shannon diversity ($p = 0.001$; Supplementary Information, Table 3.3). However, these variables explained only a relatively small proportion of community variation ($r^2 = 0.13\text{-}0.37$).

3.3 Relationships between AM fungal metrics and soil and plant properties

Given the strong differences among sites in soil, plant, and AM fungal variables, Pearson correlation matrices were constructed for CH and PN independently to examine relationships between AM fungal colonization and diversity metrics and soil and plant variables (Fig 3.7). In CH, hyphal abundance and colonization rate were positively correlated with leaf K concentration (Fig. 3.7a). In contrast, AM fungal colonization and structure occurrence in PN were not significantly correlated with any soil and plant variables (Fig 3.7b).

Regarding diversity metrics, correlations with soil and plant variables differed between sites. In CH, Shannon diversity and richness were negatively correlated with soil NH_4^+ concentration. A greater number of significant correlations were detected in PN, as Shannon diversity and evenness were positively correlated with EC. Richness was negatively correlated with soil NO_3^- and NH_4^+ concentration.

(a)



(b)

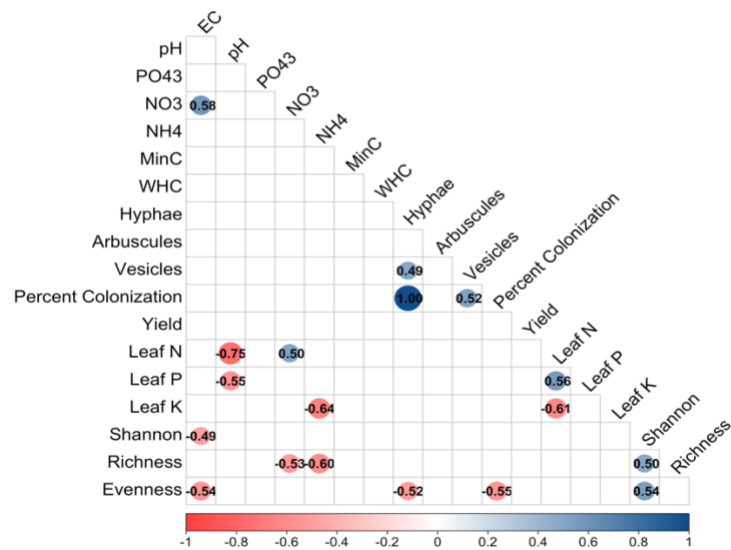


Fig 3.7 Pearson correlation matrices of soil, plant, and AM fungal variables in (a) CH and (b) PN. Soil variables included electrical conductivity (EC), soil pH, phosphate (PO43), nitrate (NO3), ammonium (NH4),

mineralizable carbon (MinC), and water holding capacity (WHC). Plant variables included yield and leaf N, P, and K. AM fungal variables included percent colonization and hyphae, vesicles, and arbuscule abundance, as well as richness, evenness, and Shannon diversity (Shannon). Circle size and color intensity reflect the strength of the correlation, with blue indicating positive and red indicating negative relationships. Only statistically significant correlations are shown ($p < 0.05$). Blank cells indicate non-significant correlations

4. Discussion

4.1 Influence of stacked soil conservation practices on AM fungal root colonization

This study evaluated the short-term effects of stacked soil conservation practices on AM fungal colonization, diversity, and community composition in two Northern California (USA) vineyard sites, that varied in soils characteristics and grapevine scion-rootstock combinations (Chardonnay x 140-R and Pinot Noir x 101-14). As grape growers show growing interest in soil biodiversity and its role in supporting vine health and production goals (Gonzalez-Maldonado et al. 2024), AM fungal symbiosis is of particular importance given its well-established role in supporting grapevine nutrient uptake, drought tolerance, and pathogen resistance (Trouvelot et al. 2015). Overall colonization rates observed in this study (21-46%) were consistent with values previously observed at these same vineyard sites (Rodriguez et al., *in preparation b*). These rates, however, fall generally at the lower range of those reported in other vineyard studies, which range from 26-94% (Betancur-Agudelo; Biasi et al. 2023; Likar et al. 2013; Schreiner 2020; Schreiner et al. 2007).

In this study, our hypothesis that greater stacking of soil conservation practices would enhance AM fungal colonization was partially supported, as the fully regenerative treatment (no till, compost, and grazing) exhibited the highest colonization rate, but only in the Chardonnay vineyard. No till, compost, and grazing treatments exhibited significantly higher colonization relative to tilled treatments, suggesting that tillage disturbance may have been a primary driver of colonization differences in CH, with compost application and grazing potentially compounding these effects. Studies conducted across a range of agroecosystems have consistently documented lower colonization rates under tillage practices (Alguacil et al. 2008; Curaqueo et al. 2011; Kabir 2005; Schalamuk and Cabello 2010; Verzeaux et al. 2017). This relationship is likely mediated by the physical disruption of extraradical hyphal network and reduction of spore density under tillage, which reduces propagule availability and

limits the ability of AM fungi to colonize host roots (Alguacil et al. 2008; Kabir 2005; Verzeaux et al. 2017). In contrast, the absence of soil disturbance under no-till preserves intact hyphal networks and supports greater spore densities, facilitating greater initial colonization of plant roots by AM fungi (Corejo et al. 2009; Kabir 2005; Schalamuk and Cabello 2010).

Compost application and grazing may have further supported AM fungal root colonization by providing slow-release organic nutrient inputs that maintain low to moderate plant nutrient availability (Cavagnaro 2014; Li et al. 2021). While high rates of compost application (50 Mg ha^{-1}) have been observed to reduce AM fungal colonization by releasing elevated amounts of mineralized P, the compost application rate in our study (9 Mg ha^{-1}) was well below rates observed to suppress colonization (Cavagnaro 2014). Rather, compost application may have provided slow-release nutrient inputs conducive to colonization (Cavagnaro 2015). Similarly, sheep dung inputs from grazing may have contributed organic nutrients that stimulated microbial nutrient cycling without elevating plant-available nutrient concentrations to levels that would suppress AM fungal colonization (Cui et al. 2026; Brewer et al. 2023). Although the expected inverse relationship between nutrient availability and colonization was not captured in our correlation analysis, this may reflect the consistently low and narrow range of soil nutrient concentrations across treatments, leaving little variation to detect. Moreover, the concentrations at which nutrient availability begins to suppress colonization are not well defined, making it difficult to interpret whether our values were within a range conducive to colonization. Nonetheless, differences in intraradical structure abundance across treatments may provide further context for interpreting colonization patterns.

Our correlation analysis showed that the higher colonization rates in the no till, compost, and grazing treatment were driven primarily by the higher density of hyphal and vesicle structures within grapevine roots. These results supported our hypothesis that hyphal and vesicle occurrence would increase with greater stacking of soil conservation practices, although this trend was observed only in CH. Under high nutrient availability, plants reduce carbohydrate allocation to AM fungal symbionts and the fungi become increasingly C-limited (Read 1991). This may constrain intraradical hyphae development, as hyphae rely on plant-derived hexoses as an essential C source to facilitate root colonization and nutrient and water transfer to host roots (Ramos et al. 2008; Souza 2025). As vesicles originate from intraradical hyphae (Souza 2025), their occurrence may similarly reflect the level of host

C allocation to AM fungal symbionts. Overall, the concurrent elevation of both structures under the no till, compost, and grazing treatment suggests that stacking soil conservation practices may have promoted host carbon allocation toward AM fungal symbiosis in CH.

Our prediction that arbuscule frequency would increase with greater practice stacking was not supported, as arbuscule frequency did not differ significantly across treatments in either site. Arbuscules tend to be most abundant during periods of high plant nutrient demand, when rates of nutrient exchange between fungi and host plants are elevated (Allen 1983; Mullen and Schmidt 1993). However, as ephemeral structures with a lifespan of only 2-3 days (Kobae and Hata 2010), arbuscule turnover may have been too rapid to detect treatment-driven differences.

The absence of significant colonization differences in PN may reflect site-specific factors such as grapevine scion-rootstock combination or soil conditions (e.g. soil texture) that may have modulated colonization responses to more stacked soil conservation practices. Differences in tillage frequency between sites may have further contributed to this pattern, as the narrower row spacing at PN resulted in fewer tillage passes than at CH. As a result, tillage-driven differences in AM fungal colonization may have been less pronounced at PN than at CH. Additionally, differences in colonization rates and intraradical structure occurrence between sites may also reflect differences in AM fungal community composition, as genera are known to colonize root tissue more intensively than others (Johansen et al. 2016).

4.2 Influence of stacked soil conservation practices on AM fungal diversity

In addition to colonization, we examined whether stacked soil conservation practices would influence AM fungal diversity, including Shannon diversity, richness, and evenness. The influence of vineyard management practices on AM fungal diversity has important implications for grapevine performance, as more diverse communities may increase the likelihood of functionally beneficial host-fungal combinations that enhance nutrient acquisition and resilience (Piotrowski and Rilling 2008). Shannon diversity values in this study (1.6 – 2.2) were consistent with values previously observed at these same vineyard sites (Rodriguez et al., *in preparation b*) and with those reported in other vineyard studies, which have ranged from 0.25 to 2.8 (Balestrini et al. 2010; Cesaro et al. 2021).

Our hypothesis that AM fungal diversity would be greatest in treatments with compost and grazing was partially supported. While diversity did not vary significantly across treatments in the Chardonnay trial, Shannon diversity did vary significantly across treatments in PN, though not all treatments with compost and grazing showed significantly higher diversity than those without. Specifically, no-till treatments without compost exhibited significantly lower Shannon diversity than those receiving compost application, indicating that its application plays a key role in supporting AM fungal diversity. Compost application increases soil organic matter, which has been associated with greater AM fungal diversity, potentially through increased sporulation (Freitas et al. 2011; Montiel-Rozas et al. 2017). Compost application may further influence AM fungal diversity by modifying host root exudates through increased organic compound production, which may potentially enhance AM fungal recruitment and diversity (Kumar et al. 2024). These soil improvements are also known to foster greater microbial activity, which has been positively correlated with AM fungal diversity in agricultural soils (Alguacil et al. 2014; Freitas et al. 2011). Collectively, these mechanisms suggest that compost application may foster a healthier, more biologically active soil environment capable of supporting a greater variety of AM fungal taxa.

In PN, alternate till, compost, and grazing treatments exhibited significantly higher Shannon diversity than the no till and no compost and alternate till and compost application treatments. Alternate tillage treatments maintaining comparable or higher AM fungal diversity relative to no-till are surprising, given that less disturbed environments have been shown to create more stable conditions that foster greater AM fungal diversity (Brito et al. 2012; Oehl and Koch 2018; Visconti et al. 2024). Soil disturbance has been shown to reduce AM fungal richness by promoting disturbance-tolerant taxa while impairing others (Brito et al. 2012; Douds Jr et al. 1995). However, compost application may buffer these effects on AM fungal diversity, as Sommer et al. (2011) found that tillage with compost promoted a more uniform distribution of soil organic matter throughout the profile. This distribution may extend high organic matter conditions deeper in the soil profile to support greater AM fungal diversity than surface organic matter accumulation under no-till systems. Grazing may further compound these effects, as Brewer et al. (2023) observed that the combination of tillage and grazing contributed to deeper carbon distribution in the soil profile.

Despite significant differences in Shannon diversity across treatments in PN, no corresponding differences in richness or evenness were detected in either site. The absence of treatment effects on Shannon diversity in CH may reflect differences in the AM fungal communities inhabiting each site, which may respond differently to applied soil conservation practices. Collectively, these patterns suggest that site-specific soil and grapevine genotype characteristics may modulate the degree to which AM fungal communities respond to soil conservation practices.

4.3 Role of grapevine genotype-soil interactions in shaping AM fungal communities and vine performance

Across all analyses, site consistently emerged as a stronger driver of AM fungal colonization, diversity, and community composition than treatment. While the RA treatments were established within the same commercial vineyard and initially distinguished by grapevine genotype, some soil properties, including EC, pH, PO_4^{3-} , NH_4^+ , mineralizable C, WHC, and soil texture, also differed significantly between sites. This suggests that observed differences in AM fungal community responses and vine performance may reflect grapevine genotype-soil interactions, supporting our hypothesis that these interactions play an important role in shaping these outcomes.

Rootstock identity shapes vine nutrient status directly through differences in root system architecture, resulting in significant variation in leaf macro- and micro-nutrient concentrations between rootstocks even under shared conditions (Lambert et al. 2008 Moukarzel et al 2022). Breeding parentage underlies these architectural differences, as the *Vitis* species from which each rootstock is derived shapes rooting depth, scion vigor, and nutrient acquisition strategy (Chen et al. 2024; Koepke and Dhingra 2013; Moukarzel et al. 2021; Moukarzel et al. 2022; Ollat et al. 2016; Warschefsky et al. 2016). For example, the 140R rootstock cultivated at CH is a *V. berlandieri* x *V. rupestris* hybrid that derives its high vigor and a deeply developed root system from both parent species (Chen et al. 2024). Both parent species have also been observed to confer enhanced macro- and micronutrient acquisition to the scion relative to rootstocks with *V. riparia* parentage (Gautier et al. 2020). Consistent with this, higher leaf N, P, and K concentrations were observed at CH relative to PN. The 101-14 rootstock, cultivated at PN, is a *V. riparia* x *V. rupestris* hybrid, whose *V. riparia* parentage is associated with lower scion vigor and reduced root penetration (Gautier et al. 2020; Yildirim et al. 2018). Reflecting these traits, 101-14

develops a shallower, less extensive root system than 140R that is suited to heavy, moist clay soils and less tolerant of the drought and saline conditions present at PN (Lambert et al. 2008; Pongracz 1983). The shallower root system of 101-14 increases its reliance on surface soil layers for nutrient acquisition, which may heighten its sensitivity to treatment-induced changes in soil nutrient availability and contribute to the more pronounced leaf nutrient response at PN than CH. Differences in leaf nutrient concentrations may further reflect differences in AM fungal communities harbored by each rootstock, as the interaction between rootstock identity and AM fungal community composition has been shown to influence leaf nutrient status significantly (Moukarzel et al. 2022).

Rootstock identity is a well-documented driver of AM fungal community composition, with distinct assemblages observed across rootstocks (Chen et al. 2024; Moukarzel et al. 2021; Moukarzel et al. 2022; Rodriguez et al., *in preparation a*). Consistent with this, 140R and 101-14 cultivated at the same Northern California vineyard supported distinct AM fungal assemblages when examined at a single timepoint and across the full growing season (Rodriguez et al., *in preparation ab*). Differences in rhizodeposition among rootstocks, including the amount and composition of root exudates, have been linked to differences in AM fungal colonization and taxa recruitment (Lailheugue et al. 2024; Philippot et al. 2013). However, rootstock identity is not the sole determinant of AM fungal community composition, as soil properties, agricultural management practices, and scion have been shown to interact with rootstock in determining which taxa colonize grapevine roots (Belew et al. 2026; Catalani et al. 2024; Fors et al. 2023; Moukarzel et al. 2024; Noceto et al. 2024). The complexity of these interactions was evident across a broader edaphoclimatic gradient, where variation in AM fungal communities was best explained by the shared effects of soil and plant variables rather than by either group alone (Rodriguez et al., *in preparation a*). Given the contrasting soil environments across vineyard sites, the AM fungal communities associated with each rootstock may have differed in their responsiveness to management treatments applied at each site.

Beyond rootstock identity, the distinct physicochemical environments at each site likely acted as selective filters on AM fungal community composition, with properties such as EC, pH, PO_4^{3-} , NH_4^+ , and WHC known to drive shifts in community structure and taxa abundance. The higher pH and EC at PN relative to CH may have jointly contributed to a distinct AM fungal community composition, with pH broadly supporting greater diversity and elevated EC selectively favoring salinity-tolerant taxa (Belew et al. 2016; Fors et al. 2023; Qin et al. 2015). The role

of EC as a driver of community composition is further corroborated by related studies, where it was among the soil properties significantly associated with variation in AM fungal community composition (Rodriguez et al., *in preparation a and b*). Higher PO_4^{3-} at PN may have reduced plant carbon allocation to AM fungi and promoted the dominance of certain taxa over others, which is consistent with the lower AM fungal evenness observed at PN relative to CH (Johnson 1993; Johnson et al. 2015). The influence of NH_4^+ on AM fungal community composition likely differed between sites, as the effects of N availability on AMF communities are mediated by soil P fertility levels, which were higher at PN than CH (Johnson et al. 2003; Treseder 2004). At PN, this may have translated into further reductions in plant dependence on AM fungi, while N availability at CH may have indirectly supported AM fungal diversity by stimulating microbial P cycling (Treseder 2004). Higher WHC at PN may have also shaped community composition between sites, as AM fungi differ in their moisture preferences, with certain species better adapted to tolerate drier conditions (Piotrowski and Rilling 2008; Ruiz-Lozano 1999). Soil texture may have also contributed to distinct AM fungal communities between sites, as CH soils were classified as clay loam while PN soils were silty clay loam. Increased porosity in coarser, sandier soils facilitates root elongation and hyphal growth, generally supporting greater AM fungal colonization, while clay-rich soils tend to show the opposite pattern due to reduced aeration and mechanical stress on roots (Aker et al. 2022). Contrary to this expected pattern, colonization was higher at PN despite its greater clay content (54.9%) relative to CH (40.2%). Rather, differences in AM fungal community composition between sites may instead explain this pattern, as some genera are characterized by more extensive root colonization than others (Johansen et al. 2016). Collectively, these physicochemical differences between sites likely created distinct environments that fostered unique AM fungal communities, contributing to the differences observed between PN and CH.

In addition to rootstock-soil interactions, scion differences between sites may be another source of variability in both AM fungal community composition and vine nutrient status. While scion has been observed to exert less influence on AM fungal community composition relative to rootstock in grafted grapevines, scion-specific differences in nutrient metabolism can affect rootstock performance and leaf nutrient concentrations (Christensen 1984; Lailheugue et al. 2024; Noceto et al. 2024; Virgona et al. 2003). Together, these findings suggest

that grapevine genotype and site-specific soil conditions interact to shape both AM fungal symbiosis and vine performance, modulating vineyard responses to soil conservation practices.

5. Conclusion

Our results show that AM fungal colonization, diversity, and community composition were largely modulated by site-specific factors, such as soil properties and cultivated scion-rootstock combination, rather than by the stacked soil conservation practices themselves. Reflecting these differences, the two sites differed in which aspects of AM fungal symbiosis responded to treatment.

At CH, colonization was highest under the fully regenerative treatment, incorporating no till, compost, and grazing. This response was likely driven by reduced tillage, which preserves hyphal networks, while compost and grazing may have further supported colonization through the addition of slow-release organic inputs. At PN, treatment effects were instead expressed in diversity. Shannon diversity was greatest under compost and grazing combined with alternate tillage, suggesting that organic inputs may offset the disruptive effects of tillage on AM fungal communities.

These findings demonstrate that the effects of stacked soil conservation practices on grapevine-AM fungal symbiosis are strongly mediated by the soil environment and scion-rootstock combination in which they are implemented. As regenerative agriculture becomes increasingly adopted in viticulture, accounting for these site-specific interactions will be critical for leveraging AM fungal symbiosis to support vine health and productivity.

Future studies should extend beyond two years, as synergistic interactions among no-till, compost, and grazing may require multiple growing seasons to fully manifest. Additionally, comparative studies across a broader range of rootstock-scion combinations and soil types would further clarify how site-specific genotype-soil interactions govern AM fungal responses to regenerative management in vineyards.

6. Acknowledgements

This research was funded by the Foundation for Food & Agriculture Research (Grant number CA21-SS-0000000193) and the California Department of Food and Agriculture (Grant number 21-0433-021-SF). The authors are grateful to the personnel of Jackson Family Vineyards for their help in establishing and maintaining this field trial.

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8. Supplemental Information

Table S3.1 Vector loadings, coefficients of determination (r^2), and significance values for soil, plant, and AM fungal variables fitted to the first two principal coordinate axes (PCoA1 and PCoA2) from Figure 2. Loadings represent the direction and strength of correlations between each variable and ordination axes. The r^2 values indicate the proportion of variance in the ordination explained by each variable, while p-values reflect the significance of these relationships based on permutation testing. All loadings, r^2 values, and p-values were generated using the envfit function with 999 permutations.

	PCoA1	PCoA2	r^2	p-value
Soil Properties				
EC ($\mu\text{S cm}^{-1}$)	-0.88	0.47	0.51	0.001
pH	-0.95	0.31	0.39	0.001
Phosphate ($\text{mg PO}_4^{3-} \text{ kg}^{-1} \text{ soil}$)	-0.97	0.24	0.75	0.001
Nitrate ($\text{mg NO}_3^- \text{ kg}^{-1} \text{ soil}$)	-0.09	1.00	0.11	0.003
Ammonium ($\text{mg NH}_4^+ \text{ kg}^{-1} \text{ soil}$)	0.77	0.64	0.17	0.002
Mineralizable Carbon ($\text{mg C kg}^{-1} \text{ soil hr}^{-1}$)	-1.00	-0.04	0.09	0.007
Water Holding Capacity (WHC) ($\text{g water g}^{-1} \text{ soil}$)	-0.98	0.19	0.65	0.001
Plant Properties				
Yield (kg vine^{-1})	0.99	-0.17	0.65	0.001
Leaf N ($\text{mg N kg}^{-1} \text{ leaf}$)	0.59	-0.81	0.63	0.001
Leaf P ($\text{mg P kg}^{-1} \text{ leaf}$)	0.40	-0.92	0.18	0.003
Leaf K ($\text{mg K kg}^{-1} \text{ leaf}$)	0.22	-0.97	0.50	0.001
AM Fungal Colonization				
Colonization (%)	-0.52	-0.85	0.70	0.001
Vesicles (%)	-0.45	-0.89	0.33	0.001
Arbuscules (%)	0.94	-0.35	0.07	0.026

Hyphae (%)	-0.57	-0.82	0.70	0.001
<u>AM Fungal Diversity</u>				
Shannon Diversity	-0.14	-0.99	0.22	0.001
Richness	-0.76	-0.65	0.45	0.001
Evenness	0.98	-0.19	0.37	0.001

Table S3.2 Two-way ANOVA p-values for the effect testing of treatment, site, and their interaction on soil, plant, and AM fungal metrics. “†” denotes variables that used ART ANOVA due to violations of normality or homoscedasticity assumptions.

Response Variable	P-Value		
	<u>Treatment</u>	<u>Site</u>	<u>Interaction</u>
<i>Soil Properties</i>			
EC ($\mu\text{S cm}^{-1}$)	0.778	< 0.001	0.303
pH	< 0.001	0.01	0.006
Phosphate ($\text{mg PO}_4^{3-} \text{ kg}^{-1} \text{ soil}$)	0.995	< 0.001	0.697
Nitrate ($\text{mg NO}_3^- \text{ kg}^{-1} \text{ soil}$)	0.600	0.711	0.003
†Ammonium ($\text{mg NH}_4^+ \text{ kg}^{-1} \text{ soil}$)	0.282	0.18	0.003
†Mineralizable Carbon ($\text{mg C kg}^{-1} \text{ soil hr}^{-1}$)	0.123	0.229	0.998
Water Holding Capacity (WHC) ($\text{g water g}^{-1} \text{ soil}$)	0.560	< 0.001	0.004
<i>Plant Properties</i>			
Yield (kg vine^{-1})	0.001	< 0.001	0.010
†Leaf N ($\text{mg N kg}^{-1} \text{ leaf}$)	0.073	< 0.001	< 0.001
†Leaf P ($\text{mg P kg}^{-1} \text{ leaf}$)	0.057	0.038	0.012
†Leaf K ($\text{mg K kg}^{-1} \text{ leaf}$)	< 0.001	< 0.001	< 0.001
<u>AM Fungal Colonization</u>			
Colonization (%)	0.163	0.298	0.198
Vesicles (%)	0.032	0.541	0.499

Arbuscules (%)	0.496	0.070	0.869
Hyphae (%)	0.090	0.237	0.397
<u>AM Fungal Diversity</u>			
Shannon Diversity	0.367	0.868	0.008
Richness	0.689	0.050	0.015
Evenness	0.834	0.017	0.428

Table S3.3 Vector loadings, coefficients of determination (r^2), and significance values for soil, plant, and AM fungal variables fitted to the first two axes (NMDS1 and NMDS2) from Figure 6. Loadings represent the direction and strength of correlations between each variable and ordination axes. The r^2 values indicate the proportion of variance in the ordination explained by each variable, while p-values reflect the significance of these relationships based on permutation testing. All loadings, r^2 values, and p-values were generated using the envfit function with 999 permutations.

	NMDS1	NMDS2	r^2	p-value
<i>Soil Properties</i>				
EC ($\mu\text{S cm}^{-1}$)	-0.98	-0.21	0.19	0.001
pH	-0.96	-0.28	0.11	0.003
Phosphate ($\text{mg PO}_4^{3-} \text{ kg}^{-1} \text{ soil}$)	-1.00	-0.06	0.24	0.001
Nitrate ($\text{mg NO}_3^- \text{ kg}^{-1} \text{ soil}$)	0.29	-0.96	0.02	0.445
Ammonium ($\text{mg NH}_4^+ \text{ kg}^{-1} \text{ soil}$)	0.79	-0.61	0.11	0.006
Mineralizable Carbon ($\text{mg C kg}^{-1} \text{ soil hr}^{-1}$)	-0.48	0.88	0.04	0.157
Water Holding Capacity (WHC) ($\text{g water g}^{-1} \text{ soil}$)	-0.98	-0.19	0.19	0.001
<i>Plant Properties</i>				
Yield (kg vine^{-1})	1.00	0.03	0.25	0.001
Leaf N ($\text{mg N kg}^{-1} \text{ leaf}$)	0.99	0.12	0.13	0.003
Leaf P ($\text{mg P kg}^{-1} \text{ leaf}$)	0.88	-0.48	0.09	0.012
Leaf K ($\text{mg K kg}^{-1} \text{ leaf}$)	0.61	0.79	0.02	0.354
<u>AM Fungal Colonization</u>				
Colonization (%)	-0.93	0.36	0.10	0.003
Vesicles (%)	-0.37	0.93	0.01	0.556

Arbuscules (%)	0.74	0.67	0.01	0.649
Hyphae (%)	-0.96	0.28	0.13	0.002
<u>AM Fungal Diversity</u>				
Shannon Diversity	0.71	0.70	0.13	0.001
Richness	-0.93	0.36	0.11	0.005
Evenness	0.97	0.23	0.37	0.001

Conclusion

This dissertation examined how scion-rootstock combination and site-specific edaphoclimatic properties interact to shape grapevine-AM fungal symbiosis across phenological stages and under varying management practices. Understanding how these factors shape AM fungal symbiosis in commercial vineyards is critical for growers seeking to leverage this relationship to support vine health and productivity.

When evaluating AM fungal communities across a broad edaphoclimatic gradient, our findings suggest that these communities are structured at multiple scales, with broad environmental gradients establishing baseline differences in community composition and plant-level traits refining taxon abundances. Scion-rootstock identity shaped AM fungal symbiosis throughout the growing season, as AM fungal colonization and community composition varied across phenological stages. These patterns differed between scion-rootstock combinations, likely reflecting host physiological responses to site conditions and phenological development. When examining the effects of stacked regenerative management practices, site characteristics, including soil physicochemical properties and scion-rootstock identity, were stronger drivers of AM fungal communities than the practices themselves. The effects of these practices, however, varied between sites, further underscoring the importance of local soil conditions in shaping AM fungal symbiosis.

Together, these studies advance our understanding of the factors shaping grapevine-AM fungal symbiosis in commercial vineyards, providing growers with a framework for leveraging this relationship to support vine health and productivity. Future studies examining a broader range of scion-rootstock combinations and soil types will further clarify how these factors interact to shape AM fungal communities. Ultimately, this knowledge will enable growers to make more informed decisions about rootstock selection and management practices to promote AM fungal symbiosis in their vineyards.