Efecto del manejo del suelo en la salud del viñedo: un estudio a partir de las relaciones suelo-planta-microbioma

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Doctorado en Ciencias Agrarias

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a Remy, Robin, Phoebe, Negrito y Nieve,
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Resumen

La viticultura es una actividad de gran relevancia económica y cultural en Uruguay, que enfrenta crecientes desafíos asociados a la variabilidad climática y a la sanidad del cultivo. Entre las principales amenazas destaca la podredumbre gris del racimo (PGR), causada por *Botrytis cinerea*, cuyo manejo tradicional mediante fungicidas presenta eficacia limitada bajo condiciones favorables al patógeno y genera impactos ambientales negativos. En este marco, la rizósfera se reconoce como un componente clave de la salud vegetal al albergar comunidades microbianas capaces de modular la respuesta de la vid frente a estreses bióticos y abióticos. Este estudio evaluó los efectos del manejo del suelo bajo la fila y del estado hídrico sobre las comunidades microbianas de la rizósfera y la susceptibilidad a PGR en *Vitis vinifera* cv. Tannat, mediante ensayos a campo, en macetas y en viñedos comerciales durante las temporadas 2019-2020 y 2020-2021. Se compararon dos estrategias de manejo: cobertura vegetal permanente (CVP) y suelo desnudo mediante desmalezado químico (DH) o manual (DM). La diversidad y composición microbiana se determinaron mediante secuenciación de amplicones (16S rRNA e ITS2). En general, tanto el manejo del suelo como el estado hídrico modificaron la composición de las comunidades procarióticas y fúngicas. La CVP redujo de forma consistente la incidencia e intensidad de PGR a cosecha, asociada a un mayor espesor de la cutícula de las bayas, a cambios en la actividad antioxidante en hojas y bayas y a la abundancia diferencial de géneros como *Pseudomonas*, *Pantoea* y *Rahnella*, reportados como PGPR en vid. En los tratamientos de suelo desnudo predominaron taxones asociados a condiciones oligotróficas (p. ej., Acidobacteriota). Además, las predicciones funcionales indicaron que la CVP promovió vías microbianas relacionadas con defensas vegetales, como la biosíntesis de alcaloides isoquinolínicos y de ácido jasmónico. Estos hallazgos evidencian el potencial de integrar

coberturas vegetales y estrategias de manejo hídrico como herramientas para una viticultura más sostenible, al reducir el impacto de la PGR y favorecer interacciones microbianas beneficiosas.

Palabras clave: *Vitis vinifera,* podredumbre gris del racimo (*Botrytis cinerea*), rizósfera, microbioma, viticultura sostenible

Effect of soil management on vineyard health: a study from soil-plant-microbiome interaction

Summary

Viticulture is a major economic and cultural activity in Uruguay, yet it faces increasing challenges related to climate variability and crop health. Among the most significant threats is *Botrytis* bunch rot (BBR), caused by *Botrytis cinerea*, whose traditional control relies on fungicides with limited efficacy under pathogen-conducive conditions and undesirable environmental impacts. In this context, the rhizosphere emerges as a key component of plant health, hosting microbial communities that can modulate grapevine responses to both biotic and abiotic stresses.This thesis assessed the effects of under-vine soil management and water status on rhizosphere microbial communities and BBR susceptibility in *Vitis vinifera* cv. Tannat, through field trials, pot experiments, and commercial vineyards during the 2019/20 and 2020/21 seasons. Two soil management strategies were compared: permanent cover crop (PCC) and bare soil through herbicide weeding (HW) or manual weeding (MW). Microbial diversity and composition were determined by amplicon sequencing (16S rRNA and ITS2). Overall, both soil management and water status shaped prokaryotic and fungal community composition. PCC consistently reduced BBR incidence and severity at harvest, associated with thicker berry cuticles, changes in antioxidant activity in leaves and berries, and the differential abundance of genera such as *Pseudomonas*, *Pantoea*, and *Rahnella*, reported as grapevine PGPR. Bare soil treatments were dominated by taxa typically associated with oligotrophic environments, such as Acidobacteriota. Functional predictions highlighted that PCC favored microbial pathways related to plant defense, including isoquinoline alkaloid and jasmonic acid biosynthesis. These findings highlight the potential of integrating cover crops and water management strategies as

tools for sustainable viticulture, reducing disease impact while promoting beneficial plant–microbe interactions.

Keywords: *Vitis vinifera, Botrytis* bunch rot *(Botrytis cinerea),* rhizosphere, microbiome, sustainable viticulture

1. Introducción

La viticultura uruguaya enfrenta el desafío de equilibrar la producción de vinos de alta calidad con la sostenibilidad ambiental, económica y social, un objetivo que se ha visto reflejado en las últimas décadas en iniciativas y proyectos de investigación y extensión orientados a abordar estas complejas demandas por parte de instituciones como INAVI, INIA o Facultad de Agronomía en conjunto con organizaciones y cooperativas de productores. En este marco, un ejemplo claro lo constituye el Programa de Viticultura Sostenible impulsado por grupos de productores e INAVI, cuyo objetivo es promover prácticas de manejo respetuosas con el medioambiente, que garanticen la calidad e inocuidad de las uvas como materia prima para la elaboración de vinos. Este programa certificó en 2024 un total de 210 viñedos, correspondientes a 2.226 hectáreas y 37 bodegas, y se destaca por su enfoque en la trazabilidad de las uvas certificadas a lo largo de la cadena productiva (INAVI, 2025). Este esfuerzo refleja el compromiso del sector vitivinícola uruguayo con la sostenibilidad y la transparencia, en respuesta a un mercado cada vez más exigente en términos de responsabilidad ambiental y social. Además de apuntar a mejorar la calidad del producto final, estas iniciativas contribuyen a construir sistemas productivos resilientes, en línea con el análisis central de esta tesis sobre el impacto del manejo del viñedo en su salud.

En consonancia con estas transformaciones hacia una viticultura más sostenible, en el ámbito de la sanidad vegetal ha emergido un enfoque complementario y transformador: la protección vegetal agroecológica -PVA (Deguine et al., 2023), que propone un cambio de paradigma en el manejo de enfermedades y plagas, al pasar de una lógica de control basada en insumos externos a una estrategia de prevención y regulación ecológica de los

agroecosistemas. Esta visión se sustenta en dos pilares fundamentales: la biodiversidad funcional y la salud del suelo. A través de la promoción de comunidades biológicas diversas —tanto vegetales como animales— y del fortalecimiento de las interacciones entre la biodiversidad edáfica y aérea, la PVA busca garantizar el funcionamiento integral del agroecosistema y la provisión de servicios ecosistémicos clave como la regulación de poblaciones, la polinización, la fertilidad del suelo y la regulación climática. En este enfoque, el control de plagas y enfermedades se apoya en la conservación y el fomento de enemigos naturales, la reducción de prácticas agrícolas que los afectan negativamente y el rediseño del sistema productivo incorporando prácticas como el manejo del suelo, la sucesión de cultivos, la elección de portainjertos o el uso de cultivares menos susceptibles, las cuales contribuyen indirectamente a reducir la presión de plagas y enfermedades. En contraste con el modelo convencional, que simplifica el agroecosistema y deteriora su capacidad de autorregulación, la PVA promueve la resiliencia ecológica mediante la complejización de las redes tróficas y la sinergia entre prácticas agronómicas y procesos naturales y apunta a sistemas productivos más estables, sostenibles y funcionales (Deguine et al., 2023; Leoni, 2023).

En este contexto, surge la necesidad de profundizar en el entendimiento de las relaciones entre suelo, planta y microorganismos y cómo estas influyen en la salud del viñedo. Las técnicas genómicas ofrecen herramientas avanzadas para analizar los factores que inciden en el microbioma del suelo, entendido como el conjunto de microorganismos y sus genes (Berg et al., 2020). Los microorganismos del suelo (bacterias, arqueas, hongos y levaduras) asociados a los viñedos son esenciales para la salud y productividad de los agroecosistemas (Bettenfeld et al., 2022). El suelo actúa como un importante reservorio de microorganismos para la rizósfera (Berg y Smalla, 2009) y para

órganos aéreos como hojas, flores y bayas (Zarraonaindia et al., 2015). Sin embargo, las estrategias de manejo agronómico han prestado poca atención a la composición y funciones del microbioma.

Es ampliamente aceptado que las cualidades sensoriales de un vino producido en una región específica están determinadas por factores abióticos, como el clima y el suelo, factores bióticos, como la variedad o cultivar de vid y el portainjerto, así como por factores antropogénicos relacionados con el manejo vitícola y las prácticas de vinificación. Estos elementos en conjunto configuran el concepto de *terroir* (Belda et al., 2017). En este contexto, se ha propuesto que el microbioma del suelo y de la vid también desempeñaría un papel relevante en la calidad de la uva y del vino, lo que amplía la comprensión del *terroir* hacia una dimensión microbiana (Belda et al., 2017; Mocali et al., 2020).

Con los avances y la creciente accesibilidad de la tecnología de secuenciación de alto rendimiento, se dispone de mayor información a escala global sobre las comunidades microbianas asociadas a los suelos de viñedo y los factores clave que determinan su diversidad y estructura. Factores como la distancia espacial o ubicación geográfica (Burns et al., 2015; Gobbi et al., 2022; Yan et al., 2022), el clima (Gobbi et al., 2022) y el manejo agronómico (Chou et al., 2018; Hendgen et al., 2018; Longa et al., 2017) desempeñan un papel determinante en la diversidad del microbioma de los viñedos. No obstante, comprender cómo los diferentes manejos agronómicos afectan al microbioma del suelo y, en consecuencia, de la rizósfera, sigue siendo un desafío, al igual que entender su influencia en la salud del viñedo, por ejemplo, para favorecer la supresión de enfermedades.

En climas húmedos, la podredumbre gris del racimo (PGR) causada por *Botrytis cinerea* es una de las enfermedades más importantes que afecta a los viñedos (Elmer y Michailides, 2007). El uso de coberturas vivas o muertas ha

demostrado beneficios en la supresión de enfermedades fúngicas en sistemas vitícolas (Abad et al., 2021; Jacometti et al., 2007; Sharma et al., 2018). El uso de mulchs incrementa la actividad biológica del suelo, lo que favorece la degradación de los restos de poda de la vid, un sustrato clave para la supervivencia de *Botrytis cinerea* (Jacometti et al., 2007). El excesivo vigor de las vides, favorecido por la alta disponibilidad hídrica y de nutrientes, ocasiona un aumento de las podredumbres de racimos a través de la reducción de la aireación en la canopia (microclima húmedo) y del incremento de la compactación del racimo (bayas más grandes) (Garcia et al., 2018; Guilpart et al., 2017; Valdés-Gómez et al., 2008). Por su parte, Coniberti et al. (2018, 2023) reportaron diferencias significativas en la incidencia de PGR al comparar vides con riego sometidas a dos manejos de suelo en la fila: uso de herbicidas versus cobertura vegetal permanente. Las vides con cobertura vegetal permanente mostraron una menor incidencia de PGR, incluso cuando el desarrollo vegetativo, la compactación del racimo, el peso de las bayas y su madurez fueron similares a los observados en los tratamientos con herbicidas.

Diversos estudios han señalado que los cambios en la composición microbiana, ya sea como causa directa o consecuencia de factores de estrés, están estrechamente relacionados con distintos declives en la salud de las plantas (Bettenfeld et al., 2022). En el caso de las enfermedades de madera, se ha encontrado que la microbiota de vides sintomáticas presenta diferencias significativas en comparación con las de vides asintomáticas, especialmente en bacterias y hongos endófitos (Bruez et al., 2015; Darriaut et al., 2021, 2023; Fotios et al., 2021). Estas diferencias, observadas principalmente en la rizósfera, sugieren que una microbiota equilibrada podría desempeñar un papel protector frente a ciertos patógenos. No obstante, los mecanismos que conducen a estos desequilibrios siguen siendo en gran parte desconocidos, en

particular en relación con la influencia de factores ambientales y prácticas agronómicas sobre la dinámica del microbioma. En este sentido, estudios recientes como el de Carbone et al. (2021) han demostrado que condiciones de déficit hídrico pueden afectar significativamente la diversidad y composición del microbioma fúngico de las raíces y rizosfera de la vid, lo que promueve la proliferación de ciertos hongos micorrícicos arbusculares como *Funneliformis*, y reduce la abundancia de géneros asociados a enfermedades de raíz como *Dactylonectria* o *Thelonectria*. Estos hallazgos subrayan la relevancia de considerar el impacto del estrés hídrico y otras variables ambientales sobre el microbioma en estudios destinados a mejorar la sostenibilidad del viñedo y la prevención de enfermedades.

Considerando la relevancia del microbioma rizosférico en la salud de la vid y su capacidad para modular respuestas frente a estreses bióticos y abióticos (Berendsen et al., 2012; Raaijmakers et al., 2009), se plantea la hipótesis de que la presencia de cobertura vegetal permanente influye en las comunidades microbianas del suelo y la rizósfera, lo cual favorecería aquellas que contribuyen a la supresión de enfermedades aéreas en la vid. Con base en esta hipótesis, esta tesis tiene como objetivo analizar el impacto del manejo de la cobertura vegetal permanente del suelo bajo la fila sobre las comunidades microbianas del suelo y la rizósfera, empleando herramientas moleculares como la secuenciación de amplicones. En particular, se busca evaluar cómo estas prácticas afectan la diversidad de las comunidades de bacterias, arqueas y hongos, cómo estas se relacionan con la incidencia y severidad de la PGR causada por *Botrytis cinerea* y qué impactos tienen estos manejos en el rendimiento y calidad de las bayas. Para ello, se realizaron experimentos en macetas, en un viñedo experimental y en dos predios comerciales.

2. Marco teórico

2.1. Viticultura en Uruguay

La vitivinicultura uruguaya tiene sus raíces en el siglo XVII con la introducción de vides por los colonizadores españoles, pero se consolidó como actividad comercial durante el siglo XIX (Baptista, 2008). Desde entonces, ha evolucionado significativamente, hasta convertirse en una práctica enológica con tradición y reconocimiento internacional por la calidad de sus vinos (Baptista, 2008). En la actualidad, enfrenta importantes desafíos como la competencia en mercados globales, la sostenibilidad ambiental y la optimización de los recursos hídricos en un contexto de cambio climático, entre otros (Echevarría, 2017; Pereyra y Ferrer, 2023).

Uno de los principales retos ambientales es la alta dependencia de agroquímicos como herbicidas y fungicidas, lo que impacta negativamente en la salud del agroecosistema y la inocuidad de los productos (Fagnano et al., 2020; Nicolopoulou-Stamati et al., 2016; Rivas-Garcia et al., 2022). En este contexto, la PGR es una de las enfermedades más relevantes en viñedos, particularmente en climas húmedos y de temperaturas moderadas (González-Domínguez et al., 2015). Su control combina prácticas químicas y culturales, como la poda, el deshojado y una fertilización nitrogenada balanceada, lo que favorece el desarrollo de canopias más aireadas y menos conducentes a microclimas húmedos (Elmer y Michailides, 2007). Sin embargo, el uso de fungicidas sigue siendo la práctica preponderante para el control de la enfermedad, lo cual no representa una solución sostenible debido a su impacto ambiental, los riesgos para la salud humana y el desarrollo de resistencias en el patógeno (Aziz et al., 2016; Hobbelen et al., 2014).

Las coberturas vegetales (CV) se utilizan tradicionalmente en los viñedos en las entrefilas para prevenir la degradación y erosión del suelo (Chou y Heuvel, 2019). En la actualidad, esta práctica se fomenta en regiones vitícolas con limitaciones hídricas donde anteriormente era poco común (Abad et al., 2021). Además de conservar el suelo, las CV aportan servicios ecosistémicos clave como el fomento de la biodiversidad, la mejora de la fertilidad del suelo, el secuestro de carbono y el control de plagas (Jacometti et al., 2007; Kim et al., 2020; Vanden Heuvel y Centinari, 2021). Estos beneficios están vinculados a mejoras en las propiedades físicas, químicas y biológicas del suelo, fundamentales para la salud del agroecosistema y la expresión del *terroir* (Sharma et al., 2018). Sin embargo, la adopción de esta práctica sigue siendo limitada debido a posibles diservicios como la competencia por agua y nutrientes, especialmente en regiones áridas, y la falta de estrategias de manejo adecuadas (Kim et al., 2020; Sharma et al., 2018).

En Uruguay, la viticultura se ha desarrollado tradicionalmente como un sistema de secano. La estrategia más común de manejo del suelo consiste en permitir el crecimiento de vegetación espontánea en las entrefilas y aplicar herbicidas, principalmente glifosato y glufosinato de amonio, bajo la fila de las vides para reducir la competencia por agua y nutrientes (Coniberti et al., 2018). Sin embargo, el uso del glifosato es objeto de debate mundial y, aunque actualmente su aprobación en la Unión Europea se ha extendido hasta el 15 de diciembre de 2033, su aplicación está sujeta a diversas condiciones y restricciones (European Commission, 2025). Como alternativa, el uso de coberturas vegetales no solo permite reducir la dependencia de herbicidas, sino que también puede contribuir a regular el crecimiento vegetativo excesivo de la vid, lo que impacta en el desarrollo de enfermedades como la PGR y en la calidad de las bayas (Chou et al., 2018). No obstante, en condiciones de

sequía, la cobertura vegetal viva podría intensificar el estrés hídrico y afectar el rendimiento de la vid en cosechas futuras (Coniberti et al., 2018).

La estrategia de manejo de mantener el suelo cubierto bajo la vid, ya sea con mulchs o con coberturas vegetales vivas, ofrece una herramienta prometedora para promover la salud del agroecosistema más allá de su impacto en la regulación del vigor de las plantas. Estudios demuestran que el uso de coberturas vegetales puede disminuir la incidencia de *B. cinerea* al reducir el número de capas de hojas en la canopia, el porcentaje de racimos internos y la compactación de estos (Guilpart et al., 2017). Asimismo, se ha reportado que el uso de mulchs orgánicos y la presencia de cobertura vegetal bajo la fila mejora la actividad biológica del suelo, y promueve una descomposición más rápida de residuos de vid, donde el patógeno sobrevive (Jacometti et al., 2007; García et al., 2018). Estudios realizados en Uruguay encontraron menores niveles de PGR en vides con cobertura vegetal viva bajo la fila comparado con tratamientos con herbicidas, incluso en ausencia de diferencias notables en el desarrollo vegetativo, peso de bayas y madurez, lo que sugiere que otras causas más allá del vigor podrían estar explicando los resultados obervados con la CV permanente (CVP) (Coniberti et al., 2023; Coniberti et al., 2018).

2.2. Microbioma del suelo y rizósfera

Es importante destacar la distinción entre los términos *microbiota* y *microbioma*. Según Berg et al. (2020), la microbiota se refiere al conjunto de microorganismos vivos que residen en un entorno definido, como bacterias, arqueas, hongos y protistas. Por otro lado, el microbioma abarca no solo a estos microorganismos, sino también su «teatro de actividad», que incluye sus estructuras, metabolitos, elementos genéticos móviles (como transposones, fagos y virus) y el ADN relicto incrustado en las

condiciones ambientales del hábitat. En este contexto, la planta y su microbioma conforman un holobionte, es decir, una unidad biológica compuesta por el organismo hospedador y su comunidad microbiana asociada, que interactúan de manera dinámica y coevolutiva (Bettenfeld et al., 2022; Hassani et al., 2018; Vandenkoornhuyse et al., 2015).

Desde hace más de un siglo, el microbioma se considera una de las claves determinantes en la salud y productividad de las plantas (Berg et al., 2017). La diversidad microbiana es un componente esencial para la salud del suelo y, por ende, para sostener la productividad biológica, mantener la calidad ambiental y promover la salud de plantas, animales y personas (Berendsen et al., 2012; Saleem et al., 2019). Se estima que la diversidad de los microorganismos asociados a la rizósfera es enorme, del orden de decenas de miles de especies (Berendsen et al., 2012). Las plantas moldean su microbioma rizosférico mediante la secreción de exudados; las bacterias son especialmente receptivas a estos estímulos (Bakker et al., 2013; Berendsen et al., 2012). Las comunidades bacterianas de la rizósfera son reclutadas del reservorio de microorganismos del suelo, por lo que este juega un papel muy importante en la composición del microbioma rizosférico (Bakker et al., 2013). A través de la secreción activa de exudados por las raíces de las plantas, los microorganismos del suelo acceden a un ambiente auxiliar en la rizósfera y, a cambio, proveen de varios beneficios a las plantas como, por ejemplo, promoción del crecimiento, alivio del estrés y protección frente a patógenos (Sarma et al., 2015; Berendsen et al., 2012). En este último aspecto, el microbioma rizósferico juega un papel muy importante en la reprogramación de las respuestas de defensa de las plantas (Spence et al., 2014).

2.3. Mecanismos de defensa en la vid

Las plantas, como muchos otros organismos superiores, tienen la habilidad de defenderse de plagas y patógenos a través de diversos mecanismos (Flors et al., 2024). Las plantas han evolucionado para aumentar su resistencia basal luego de percibir un estimulo abiótico o biótico especifico, como por ejemplo ante la colonización de las raíces por rizobacterias benéficas o ante la percepción de patrones moleculares asociados a microorganismos (PAMP) (Berendsen et al., 2012; Pieterse et al., 2014). La resistencia inducida es un estado de capacidad defensiva mejorada en la planta cuando esta es apropiadamente estimulada (Bakker et al., 2007; Van Loon et al., 1998) y se expresa frecuentemente de forma sistémica en orgános que aún no han sido dañados. La resistencia sistémica inducida no solo depende de la activación directa de los mecanismos de defensa previo al ataque del patógeno, sino que también puede ser resultado de la sensibilización de los tejidos para expresar mecanismos de defensa basales de forma más rápida y fuerte luego de un ataque de un patógeno o de un herbívoro (Conrath et al., 2007). Esta sensibilización es lo que se conoce como *priming*. El *priming* y la inducción de defensas directas constituyen mecanismos de protección que permiten reducir la enfermedad o el daño (Flors et al., 2024).

La IR se divide generalmente en dos tipos: resistencia sistémica adquirida (SAR) y resistencia sistémica inducida (ISR) (Walters et al., 2013). El tipo de resistencia más estudiado es el SAR, que se expresa local y sistémicamente luego de una infección localizada por un patógeno necrotizante o como resultado de la aplicación de ciertos químicos (Pieterse et al., 2014). Se caracteriza por la acumulación de ácido salicílico (SA) y proteínas relacionadas a la patogénesis (PR) (Walters et al., 2013). La colonización de las raíces con microorganismos beneficiosos ya sea de forma endofítica o rizosférica

desencadena el tipo de resistencia conocido como ISR (Bakker et al., 2007; Verhagen et al., 2010). Los consorcios microbianos capaces de inducir el aumento de respuestas ISR en plantas tienen las ventajas de no solo suprimir los patógenos del suelo, sino también patógenos foliares localizados distanciadamente (Pieterse et al., 2014; Sarma et al., 2015). Este fenómeno se ha observado en varias especies como tomate, arroz, porotos, *Arabidopsis*, vid, entre otros, y las bacterias han sido los microorganismos más estudiados (Pieterse et al., 2014; Sarma et al., 2015). También se han reportado que algunas micorrizas arbusculares y algunos hongos del género *Trichoderma* son capaces de desencadenar ISR en ciertas especies (Fiorilli et al., 2024; Hermosa et al., 2012; Perazzolli et al., 2008; Salas-Marina et al., 2015). La capacidad de inducir ISR depende de la interacción planta-microorganismo, es decir, un microorganismo es capaz de inducir resistencia frente a cierto patógeno en una especie vegetal y no en otra (Emannuel Oliveira Vieira et al., 2024). A su vez, la ISR desencadenada por un microorganismo puede ser efectiva frente a ciertos patógenos, pero no para todos (Lee Díaz et al., 2021; Salwan et al., 2023).

El éxito de estos mecanismos depende del reconocimiento a tiempo de las señales o moléculas elicitoras generadas o liberadas durante el ataque del patógeno y de la rápida inducción de las reacciones de defensa apropiadas (Conrath et al., 2007). Se ha reportado que las respuestas ISR están directamente relacionadas con la reprogramación y movilización de las enzimas involucradas en la defensa del hospedero como las proteínas PR, PAL, peroxidasas (PO), superóxido dismutasa (SOD), y polifenol oxidasa (PPO) y la acumulación de fenoles y prolina (AbuQamar et al., 2017; Adrian et al., 2012; Aziz et al., 2006). Entre otras respuestas de defensa celulares y moleculares asociadas a la IR se mencionan rápidas e intensas transcripciones de genes relacionadas a la defensa, deposición de calosa, acumulación de especies

reactivas del oxígeno, formación de papilas, acumulación de fitoalexinas y acumulación de proteínas relacionadas a la patogénesis (PR), algunas de las cuales poseen propiedades antimicrobianas (Aziz et al., 2006, 2016; Verhagen et al., 2011; Verhagen et al., 2010).

En la vid, las reacciones de defensa inducibles durante la interacción con patógenos fúngicos más estudiadas son la acumulación de fitoalexinas y la síntesis de proteínas PR, como las quitinasas y las β-1,3-glucanasas. Las fitoalexinas más importantes, trans-resveratrol y la viniferina, son consideradas fungitóxicas a concentraciones fisiológicas contra *Botrytis cinerea* (Aziz et al., 2006). Por otro lado, el número y grosor de las capas de células de la epidermis e hipodermis del hollejo han sido positivamente correlacionadas con la resistencia a *B. cinerea* (Gabler et al., 2003). La resistencia física a la infección también depende de la cutícula y el contenido de cera así como de la composición y estructura de la pared celular en el hollejo (Deytieux-Belleau et al., 2009; Gabler et al., 2003). La presencia de diversos compuestos puede afectar la patogénesis fúngica: particularmente los taninos y compuestos fenólicos son conocidos por ser importantes en la resistencia a PGR (Deytieux-Belleau et al., 2009).

2.4. Hipótesis e objetivos

2.4.1. Hipótesis

El presente trabajo se desarrolló con el fin de responder a esta hipótesis: diferentes manejos de suelo (herbicidas, cobertura viva en la fila) presentan comunidades microbianas particulares capaces de inducir o desencadenar mecanismos de defensa en la vid frente a *Botrytis cinerea*.

2.4.2. Objetivo general

Evaluar si las comunidades microbianas del suelo y la rizósfera, moduladas por manejos contrastantes del suelo bajo la fila (cobertura vegetal permanente y herbicidas), contribuyen a la reducción de la incidencia y severidad de la podredumbre gris de racimos (*Botrytis cinerea*) en vid, así como a la promoción de mecanismos de defensa en la planta.

2.4.3. Objetivos específicos

1. Conocer la diversidad microbiana presente en diferentes tipos de suelo con viñedos comerciales de cv. Tannat en Uruguay (capítulo 3).
2. Explorar las diferencias en diversidad de las poblaciones de bacterias, arqueas y hongos en el suelo y la rizósfera de vid con diferentes manejos de suelo bajo la fila; cobertura vegetal viva y suelo desnudo mediante herbicidas (capítulos 3, 4, 5 y 6).
3. Explorar los mecanismos de defensa involucrados en la supresión de la podredumbre gris del racimo y relacionarlos a la composición y estructura de las comunidades microbianas encontradas bajo los diferentes manejos de suelo (capítulos 5 y 6).

3. Influence of under-vine soil management on bulk soil microbiota and properties in three Tannat vineyards in Uruguay[1]

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3.1. Resumen

El microbioma del suelo desempeña un papel fundamental en la salud y productividad de los agroecosistemas influenciado por el manejo agronómico y los factores ambientales. Este estudio analizó la composición de las comunidades procariotas y fúngicas en tres viñedos de Tannat en Uruguay, todos sometidos al mismo manejo convencional del suelo bajo la vid (suelo desnudo mantenido con herbicidas: BS). Además, dentro de cada viñedo, se estableció una cobertura vegetal viva permanente (PLM) para evaluar los efectos de esta práctica sobre la microbiota del suelo. El uso continuado del suelo (vides del cv. Tannat) y el manejo uniforme del mismo (BS) durante al menos 10 años podrían haber homogeneizado la composición de las comunidades microbianas del suelo entre los viñedos, a pesar de las diferencias en tipo de suelo, altitud e historia de manejo. No obstante, se

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detectaron algunos taxones diferencialmente abundantes: en el viñedo 3, *Rubrobacter* fue menos abundante en comparación con los otros viñedos, mientras que en el viñedo 1 la clase Sordariomycetes y el género *Metarhizium* fueron más abundantes y el género *Boeremia* menos. El análisis de la composición de las comunidades procarióticas y fúngicas dentro de los viñedos reveló un impacto significativo del manejo bajo la vid únicamente en el Viñedo 2, donde el PLM fue implementado durante 10 años. El PLM mejoró propiedades del suelo como la respiración basal, el contenido de proteína del suelo, el carbono potencialmente oxidable y la densidad aparente. Además, la familia Latescibacteraceae y los géneros *Cladophialophora*, *Nigrospora* y *Pseudopithomyces* fueron más abundantes en el PLM. Nuestros resultados subrayan la necesidad de estudios a largo plazo para comprender mejor las respuestas microbianas al manejo del suelo. Estudios futuros que abarquen más sitios y estrategias de manejo podrían identificar diferencias más profundas, contribuyendo a la identificación de zonas vitícolas basadas en patrones microbianos.

Palabras clave: vid, mulch vivo permanente, herbicida, secuenciación de amplicones, microbioma del suelo, 16S rRNA, ITS2.

3.2. Summary

The vineyard soil microbiome plays a pivotal role in agroecosystem health and productivity, influenced by agricultural management and environmental factors. This study examined the composition of prokaryotic and fungal communities across three Tannat vineyards in Uruguay with the same conventional under-vine soil management (bare soil maintained with herbicides—BS). Additionally, within each vineyard, a permanent living mulch (PLM) was established to

explore the effects of this under-vine management on soil microbiota. The long-term cultivation of Tannat grapevines combined with consistent under-vine herbicide use may have contributed to a homogenization of soil microbial community composition across vineyards, despite differences in soil type, altitude, and management histories. A few differentially abundant taxa were detected: in Vineyard 3 *Rubrobacter* was less abundant compared to the other vineyards, while in Vineyard 1 the class Sordariomycetes and the genus *Metarhizium* were more abundant and the genus *Boeremia* was less. Prokaryotic and fungal communities' composition analyses within vineyards revealed a significant impact of under-vine management solely in Vineyard 2, the one with a 10-year implementation of PLM. PLM improved soil properties such as basal respiration, soil protein, potentially oxidizable carbon and bulk density. Also, the family Latescibacteraceae, and the genera Cladophialophora, Nigrospora, and Pseudopithomyces were more abundant in PLM. Our findings emphasize the need for long-term studies to capture microbial responses to soil management. Future studies involving more sites and managements could identify deeper differences, aiding in the identification of viticultural zones based on microbial patterns.

Keywords: grapevine, permanent living mulch, herbicide, amplicon sequencing, soil microbiome, 16S rRNA, ITS2.

3.3. Introduction

Soil microbiota (bacteria, archaea, fungi, protists, and viruses) associated with vineyards are essential for agroecosystem health and productivity [(1)]. Bulk soil microbiota is a major reservoir of microorganisms for the rhizosphere [(2)] and aerial organs as leaves, flowers and grapes [(3)]. It is widely recognized that the sensory qualities of a wine from a specific region are defined by abiotic (climate,

soil) and biotic (grape variety and rootstock and soil biology) factors along with anthropogenic ones (viticultural management and winemaking techniques), defining the concept of terroir [4]. In addition to these influences, recent studies emphasize the role of native vine microbiota in the winemaking process, imparting a unique character to the wines of each region [5]. As well, the potential influence of soil microbiota on grape and wine quality has also been suggested [4][6]. With the advances and increasing accessibility of high-throughput sequencing technology, more information is available worldwide about the microbial communities associated with vineyard soils and the key factors that drive their diversity and structure. Spatial distance or geographic location [7][8][9], climate [8], and agricultural management [10][11][12], are factors that have a determinant role in vineyard soil microbiota.

With regard to agricultural management, previous studies have shown that the use of cover crops and different soil management strategies under vines in vineyards influenced the composition and diversity of soil microbial communities. For example, Chou et al. [10] found that soil management under vines in vineyards altered the composition of soil microbial communities, although the microbiome of grapefruit was not affected. Similarly, Hendgen et al. [11] showed that different management regimes (integrated, organic, and biodynamic) shaped the composition of fungal community in vineyard soils, and that vegetation under vines increased the richness and activity of soil-borne fungi. In contrast, bacterial communities were more uniform across all treatments, although richness was reduced under integrated management. Longa et al. [12] also observed that green manure was the greatest source of soil microbial biodiversity and significantly changed microbial richness and community composition compared with other soils. Taken together, these studies support the idea that under-vine vegetation and cover crop strategies can modulate the

dynamics of microbial community dynamics in vineyard soils. Under-vine cover crops or living mulches in sustainable agricultural management are a promising strategy for simultaneously enhancing many ecosystem processes [13]. The use of cover crops or living mulches in vineyards serves multiples purposes, among them: 1- reducing plant vigour in regions with excessive precipitation, 2- diminishing cluster rot development through modifications in canopy microclimate, 3- minimizing herbicide use, and 4- fostering improvements in soil organic carbon (SOC), aggregate stability, soil respiration, and microbial diversity [14]. Despite these well-documented benefits, Uruguayan winegrowers primarily rely on herbicide application to maintain bare soil under the vine, particularly within non-irrigated vineyards [15].

The rhizosphere microbiota is the result of complex interactions in which both plant and soil characteristics play a key role in shaping its structure, stability, and succession [16]. Previous studies conducted in a Tannat/SO4 vineyard in Uruguay showed that under-vine soil management can influence both the sensory attributes of wine [17] and the composition of prokaryotic and fungal communities in the grapevine rhizosphere [18]. In particular, the use of an under-vine cover crop was associated with improved soil and vine health [17][18] and promoted the recruitment of microbial taxa with known beneficial traits [18]. However, the study by Bernaschina et al. [18] focused on a single vineyard and specifically on the rhizosphere compartment. In contrast, little is known about the diversity and composition of bulk soil microbiota—the broader reservoir from which rhizosphere communities are assembled—in Uruguayan soils, particularly those associated with vineyards.

To address this gap, the present study investigates whether there are significant differences in the composition of soil prokaryotic and fungal communities across three Tannat vineyards managed with herbicides under the

vines (conventional soil management) and evaluates, within each site, the effect of permanent living mulch (PLM) as an alternative management strategy. Amplicon sequencing of the 16S rRNA gene and ITS2 region was used to analyze bulk soil microbial diversity, as well as the shared and differentially abundant taxa among vineyards and under-vine soil management practices.

3.4. Materials and methods

3.4.1. Site characteristics

The study was conducted in Southern Uruguay which accounts for the 92% of the vineyards area in the country [19]. The three vineyards of Tannat cultivar were located in Cuatro Piedras-V1 (34º 62' S, 56º 28' W), Rincón del Colorado-V2 (34°44′ S, 56°13′ W) and Garzón-V3 (34.57' S, 54.63 W), geographically separated as follows: V1-V2 9 km, V1-V3 152 km and V2-V3 158 km (Figure S1, in Supplementary material). The vineyards exhibit differences in soil type, land use history and plant age (Table 1). According to the viticultural zoning of Uruguay based on bioclimatic indices (Heliothermal, Dryness, and Cool Night) [20], the three vineyards belong to the same climatic type IHA3 IFA2 ISA1, all of them influenced by the Atlantic Ocean and the estuary of Río de la Plata. The climatic type IHA3 IFA2 stands for temperate climate, moderate drought from bud break to harvest, and mild nights (14-18 °C) from veraison to harvest.

Table 1 Characterization of the three Uruguayan vineyards studied.

	Vineyard		
	Cuatro Piedras -V1	Rincón del Colorado - V2	Garzón - V3
Viticulture region[1]	RVR	RVR	RVSU
Soil type[2]	Thermic Pachic Argiudoll	Thermic Pachic Argiudoll	Thermic Entic Hapludoll
Texture[2]	Silt loam	Silt loam	Sandy loam
Proximity to large water bodies (km)	21	14	26
Elevation (m.a.s.l.)	38	29	136
Accumulated precipitation by phenological phase (BB-F/F-V/V-H) (mm)[3]	96/111/148	136/144/175	104/131/335
Potentially net available water (mm)[4]	129	110	57
Previous land use	Agriculture	Peaches	Grasslands
Vineyard age	20	19	12
Rootstock	3309	SO4	SO4
Irrigation system	No	Yes	Yes
Years with under-vine PLM[5]	1	10	1

Note: [1] Uruguayan viticulture region (RVR: Rioplatense, RVSU: Sierras del Uruguay).
[2] according to USDA Soil Taxonomy (https://www.nrcs.usda.gov/resources/guides-and-instructions/soil-taxonomy.

[3] BB-F (from Bud breaking to flowering), F-V (from flowering to veraison), V-H (from veraison to harvest).

[4] V1 and V3 according to Molfino 2009 [21]. V2 estimated by Coniberti et al. 2018a [17].

[5] PLM: permanent living mulch

Vines were trained to a vertical shoot positioning system with a planting density of approximately 1 m within rows and 2.5, 2.8 and 2 m between rows in V1, V2 and V3, respectively. Vineyard V1 was not irrigated, while in V2 and V3, the irrigation strategy aimed to avoid severe water stress until berries pea-sized stage (stage 31, [22]). From then on, deficit irrigation (70% ETc) was applied for all treatments once the plants reached -0.7 MPa mid-day stem water potential (Ψstem). To prevent Ψstem from becoming more negative than -1.1 MPa after a prolonged period of deficit irrigation, the amount of water the vines had consumed the previous week was occasionally applied at 100% ETc.

3.4.2. Under-vine soil management

The three vineyards implemented two under-vine soil management (U-VSM): bare soil by herbicide use (BS) and permanent living mulch (PLM). The treatments were imposed in alternating rows in each vineyard. Within each row with homogeneous management (BS or PLM), four plots with eight consecutive plants with similar plant vigour were defined. In the vineyard V1 and V3, PLM and BS were implemented in March 2020, while in V2 in 2011.

The BS management consists of a 1.0 m wide weed-free strip under the vine with a combination of herbicides: 3 applications of glyphosate (1L/ha) and one application of glufosinate-ammonium (3 L/ha) during grapevine growing season. Herbicide sprays were separated by at least one and a half months before the soil sampling date. PLM in vineyards V1 and V3 consisted of spontaneous vegetation characterized mainly by a mixture of gramineous species, while in V2 corresponds to a *Festuca arundinacea* (Schreb.) crop ("tall fescue") established in 2011 with a seeding rate of 60 kg/ha. In all cases, the

alleys between the rows consisted of permanent spontaneous vegetation mowed when necessary.

3.4.3. Soil sampling

Soil samples were collected at harvest in March 2021 (phenological stage 38 [22]). Eight composite bulk soil samples were collected per vineyard, four from PLM plots and four from BS plots, totaling 24 samples. Each composite sample consisted of 20 soil cores randomly collected with a soil core auger (2 cm diameter) at 0-15 cm depth, mixed and homogenized by sieving with a 2 mm mesh (roots and stones previously removed by hand) immediately after sampling. Then, the soil sample was split into subsamples for the different analyses.

3.4.4. Soil biological and physicochemical property analyses

Soil basal respiration (SR), autoclaved citrate-extractable soil protein (SP), and potentially oxidizable carbon (PoxC) were assessed in bulk soil samples (0–15 cm depth) [23]. SR and SP were determined in air-dried soil, while PoxC was measured in fresh soil stored at 4 °C until analysis. SR, used as a proxy for microbial activity, was measured by incubating 20 g of sieved soil for 4 days at 21 °C in sealed jars containing a 0.5 M KOH CO_2 trap, with CO_2 release estimated based on conductivity changes. SP, reflecting labile organic nitrogen, was extracted from 3 g of soil using sodium citrate buffer, autoclaved, and quantified via the BCA assay. PoxC, representing readily available organic carbon, was determined by oxidation with 0.2 M $KMnO_4$, and absorbance was measured at 550 nm to estimate carbon content. Air-dried bulk soil samples were analyzed at the Laboratorio de Suelos y Plantas – INIA La Estanzuela (http://www.inia.uy/productos-y-servicios/laboratorios/Laboratorio-de-Suelos-Plantas-y-Agua) for electrical conductivity (EC, mmhos/cm 25ºC), pH (H2O),

Total N (%), soil organic carbon - SOC (%), P Bray 1 (μg P/g), Ca (meq/100g), Mg (meq/100g), K (meq/100g), and Na (meq/100g). Soil bulk density (SBD) (2 - 7 cm depth) was determined by weighing the dry soil contained in a metal ring of known volume [24].

Soil under-vine management effects on soil physicochemical and biological properties were analyzed using linear models. In the case of the significance for Fisher test (Type III ANOVA), mean separation was performed by computing the estimated marginal means. Statistical analyses were performed using R v4.0.4, with the package's stats, lme4 [25], lmerTest [26], performance [27], emmeans [28], multcomp and multcompView [29].

3.4.5. Soil microbial community analysis

Total community-DNA (TC-DNA) was extracted from 500 mg of frozen bulk soil (wet weight) stored at – 20°C with the FastDNA SpinKit for Soil (MP Biomedicals, Santa Ana, CA, USA), using a FastPrep-24-bead-beating system, following the manufacturer's instructions. The integrity and concentration of TC-DNA were assessed by agarose gel electrophoresis and Nanodrop 2000 spectrophotometer (Invitrogen, USA), respectively.

From 24 bulk soil samples, the microbiota was determined by amplicon sequencing using Illumina MiSeq (2 x 300 bp, paired/end) at Macrogen Inc. (Korea). For the prokaryotic communities, the 16S rRNA gene (V3-V4 region) was selected and amplified with primers 341F 5'-CCTACGGGNGGCWGCAG-3' and 805R 5'-GACTACHVGGGTATCTAATCC-3' [30]. For the fungal communities, the ITS2 region was selected and amplified with primers 3F: 5'-GCATCGATGAAGAACGCAGC-3' and 4R: 5'-TCCTCCGCTTATTGATATGC-3' [31].

Raw sequence reads quality was evaluated using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and the

subsequent analyses were performed in R v4.0.4 environment. The DADA2 v1.24.0 package [32] was used to filter and trim the reads, correct by error learning, merge pairs and identify amplicon sequence variants (ASVs). Prokaryotic reads (16S) were trimmed after 250 bp while fungal (ITS2) reads were trimmed after 280 bp and 250 bp for forward and reverse, respectively. Also, 16S and ITS2 reads with expected errors higher than 3 for forward and reverse reads were discarded. The remaining filters were used by default. Reads were merged with a minimum overlap of 20 bp and a maxMismatch of 0. Chimeric sequences were identified and removed. For the quality control, we focused on filtering rare ASVs based on prevalence. First, we calculated the prevalence of each ASV across samples, added taxonomic information, and identified low-represented phyla that could be false positives, which were subsequently removed. Non-microbial taxa, such as chloroplasts and mitochondria, were also excluded. Taxa with a mean read count below a set threshold (1e-5) or observed in fewer than 10% of samples were filtered out. Samples with fewer than 1000 reads were also discarded, although no samples were removed in this case. Finally, a prevalence threshold of 5% was applied, keeping only ASVs present in at least 5% of the samples. ASVs taxonomic assignment was realized against reference training dataset SILVA v138.1 database [33] for prokaryotes and UNITE reference database (sh_general_release_dynamic_s_10.05.2021) for fungi [34]. Classification and subsequent analyses were done with Phyloseq v1.34.0 [35].

Alpha diversity indexes (Shannon, Pielou's evenness, Species Richness) were estimated using Microbiome v1.12.0 [36]. Permutational multivariate analysis of variance (Permanova) based on Bray-Curtis's distance was run with 10,000 permutations using Vegan v2.5.7 [37] to test the effect of the vineyards with conventional management and the effect of U-VSM within each vineyard on

the prokaryotic and fungal communities. Within the same package, a pairwise multilevel comparison using adonis2 was performed to test for differences among vineyards, and the analysis of multivariate homogeneity of group dispersions (variances) was done using betadisper. A principal coordinate analysis (PCoA) based on Bray-curtis dissimilarity as the distance method was performed to illustrate differences between the community's composition for prokaryotic and fungi separately. All data visualizations were produced using the ggplot2 package v3.4.4 [38].

To determine the core community across different vineyards for prokaryotes and fungi, the amp_venn function from the ampvis2 package was employed [39]. The analysis was grouped by the factor "vineyard" to investigate shared and unique prokaryotic and fungal taxa among vineyards. A minimum relative abundance threshold (cut_a) of 0.01% was set to ensure that only amplicon sequence variants (ASVs) present above this threshold were considered in the analysis. Additionally, a frequency cutoff (cut_f) of 50% was applied, meaning that an ASV must be present in at least 80% of the samples within each vineyard group to be included in the core microbiota.

Differential abundance (DA) analysis was performed using the analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC2) among vineyards and between U-VSM within V2 from package ANCOM-BC [40].

3.4.6. Accession numbers

Unassembled raw amplicon data were submitted to the NCBI Sequence Read Archive (SRA, https://www.ncbi.nlm.nih.gov/sra) and can be retrieved under BioProject accession number PRJNA1032922.

3.5. Results

3.5.1. Diversity and composition of vineyard microbiota managed with under-vine herbicide

A total of 1,487,685 and 1,547,636 reads were processed for the 16S and ITS2 datasets, respectively. Following quality filtering, 6,722 prokaryotic ASVs and 3,661 fungal ASVs were identified. The sequencing depth was enough to cover the microbial diversity, as a plateau was reached in the rarefaction curves (Figures S2 and S3, in Supplementary material).

The relative abundance profiling of prokaryotic communities was similar among vineyards with Actinobacteriota and Proteobacteria as the most abundant phyla (Figure S4A, in Supplementary material). The relative abundance profile of prokaryotic and fungal communities was similar among vineyards. The phyla Actinobacteriota and Proteobacteria were the most abundant prokaryotes (Figure S4A, in supplementary material), and Ascomycota, followed by Basidiomycota and Morteriellomycota (Figure S4B, in supplementary material) were the most abundant fungal phyla. (Figure S4B, in Supplementary material).

Alpha diversity indices (Observed ASVs and Shannon) revealed statistically significant differences among vineyards, but only for prokaryotic communities (Figure 1A and 1C). Vineyard V1 exhibited higher Observed ASVs and Shannon diversity values compared to V3, while V2 did not differ significantly from either V1 or V3. Vineyard identity accounted for 23% and 40% of the variation in beta diversity for prokaryotic ($R^2 = 0.23$, $p = 0.001$) and fungal communities ($R^2 = 0.40$, $p = 0.001$), respectively. However, no significant differences were found between the three vineyards for either prokaryotic or fungal communities in the multilevel pairwise comparisons (Table 2, Pr (>F) > 0.05). Variances for both prokaryotic and fungal communities were homogenous

(Pr (>F) for prokaryotes = 0.609; Pr (>F) for fungi = 0.154). PCoA visualizations showed that, for both communities, V1 and V2 clustered together and separated from V3 (Figure 1B and 1D).

The core microbiota -shared taxa- among vineyard soils was composed of 72 prokaryotic and 64 fungal ASVs (Figure S5A and S5B, in Supplementary material). The most represented taxa for prokaryotes were *Xanthobacteraceae* family (30 ASVs), from which the genus *Bradyrhizobium* accounted for 14 ASVs, while 16 ASVs could not be identified at genus level. The most represented families in the fungal core include *Nectriaceae* (8 ASVs), *Mortierellaceae* (6 ASVs), *Trichomeriaceae* (3 ASVs), *Didymellaceae* (3 ASVs), *Cucurbitariaceae* (2 ASVs), *Phaeosphaeriaceae* (2 ASVs), *Helotiales_fam_Incertae_sedis* (2 ASVs), and *Aspergillaceae* (2 ASVs). Additionally, 36 families were represented by a single ASV each. Several prokaryotic unique ASVs were identified for each vineyard (Figure S5 and Table S1, in Supplementary material). The most represented phylum for V1 were Actinobacteriota (132 ASVs), followed by Proteobacteria (39 ASVs) and Acidobacteriota (23 ASVs); for V2 Verrucomicrobiota (41 ASVs), followed by Actinobacteriota (36 ASVs), Proteobacteria (26 ASVs) and Firmicutes (11 ASVs); and for V3 Proteobacteria (40 ASVs), Actinobacteriota (30 ASVs) and Firmicutes (22 ASVs). Regarding unique fungal ASVs (Figure S5 and Table S2, in Supplementary material), the most represented phylum for V1 were: Ascomycota (113 ASVs), followed by Basidiomycota (35 ASVs), Chytridiomycota (11 ASVs) and Mortierellomycota (10 ASV); for V2 Ascomycota (132 ASVs), Basidiomycota (22 ASVs); and for V3 Ascomycota (223 ASVs), Basidiomycota (53 ASVs), Chytridiomycota (15 ASVs) Mortierellomycota (14 ASVs) and Rozellomycota (13 ASVs).

Table 2. Permutational multivariate analysis of variance (Permanova), pairwise multilevel comparison among vineyards and multivariate analysis of homogeneity of groups dispersion based on Bray-Curtis distance for prokaryotic and fungal communities. Vineyards: V1 (Cuatro Piedras), V2 (Rincón del Colorado) and V3 (Garzón), Uruguay.

Dataset	*Analysis*	*Factor*	*F. Model*	*R^2*	*Pr (> F)*
Prokaryotic	*Permanova*	*Vineyard*	*1.35*	*0.23*	*0.002***
	Pairwise Multilevel comparison	*pairs*	*F. Model*	*R^2*	*p.adjusted*
		V2 vs V3	*1.28*	*0.17*	*0.075*
		V2 vs V1	*1.23*	*0.17*	*0.117*
		V3 vs V1	*1.53*	*0.20*	*0.084*
	Homogeneity of groups dispersion	*groups*	*F value*		*Pr (> F)*
		Vineyards	*0.52*		*0.6085*
Dataset	*Analysis*	*Factor*	*F. Model*	*R^2*	*Pr (> F)*
Fungal	*Permanova*	*Vineyard*	*3.05*	*0.40*	*0.002***
	Pairwise Multilevel comparison	*pairs*	*F. Model*	*R^2*	*p.adjusted*
		V2 vs V3	*2.95*	*0.33*	*0.06*
		V2 vs V1	*2.61*	*0.30*	*0.06*
		V3 vs V1	*3.79*	*0.38*	*0.09*
	Homogeneity of groups dispersion	*groups*	*F value*		*Pr (> F)*
		Vineyards	*2.32*		*0.154*

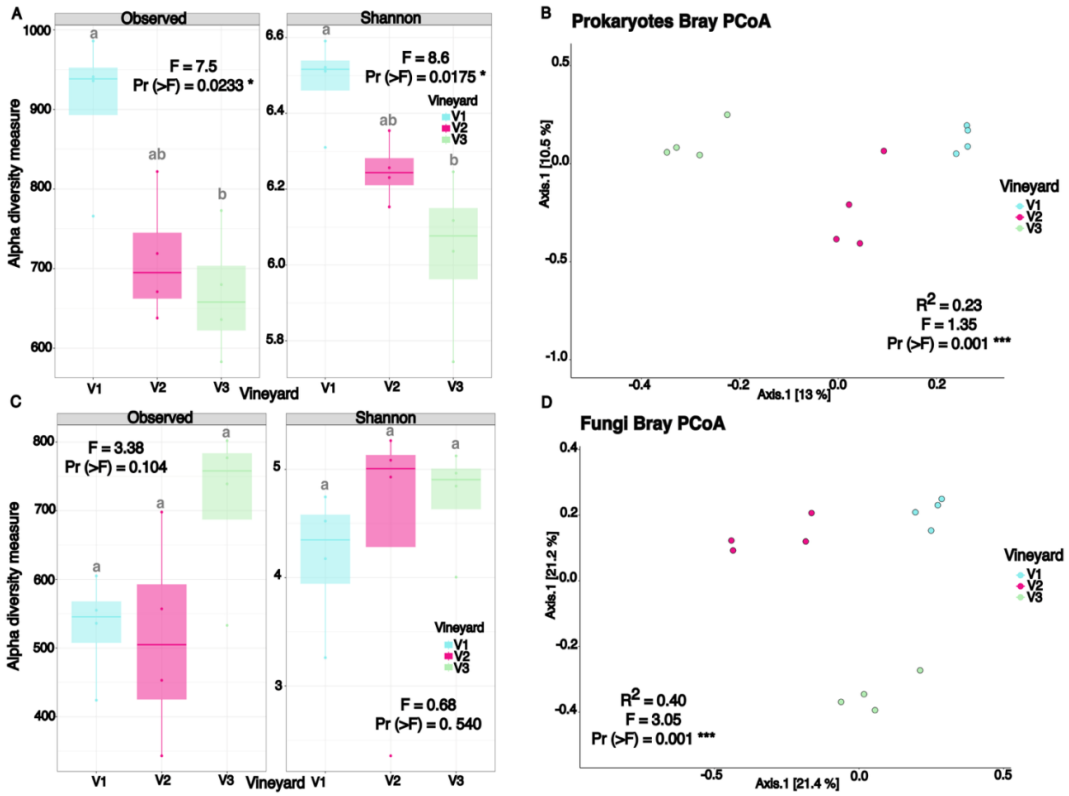

Figure 1. Alpha diversity indexes (Observed ASVs, Shannon) and Principal Coordinates Analysis based on Bray-Curtis distance of prokaryotic (A-B) and fungal communities (C-D) respectively. Vineyards: V1 (Cuatro Piedras), V2 (Rincón del Colorado) and V3 (Garzón), Uruguay.

A few differentially abundant taxa, named responders, were detected for prokaryotic and fungal communities. *Rubrobacter* was the only prokaryotic genus identified as differentially abundant among vineyards for prokaryotic communities (Figure 2A). The vineyard V3 had a lower abundance of *Rubrobacter* in comparison to V2 (q-value = 0.01), whilst among V1 and V2 no differences were found.

For fungal communities, 2 genera and 1 class were identified as differentially abundant between vineyards V3 and V1 and V2 (Figure 2B). The vineyard V3 showed a higher abundance of genus *Boeremia* (q-value = 0.01)

and a lower abundance of class *Sordariomycetes* (q-value = 0.02) and the genus *Metarhizium* (q-value = 0.03) in comparison to V2 (intercept).

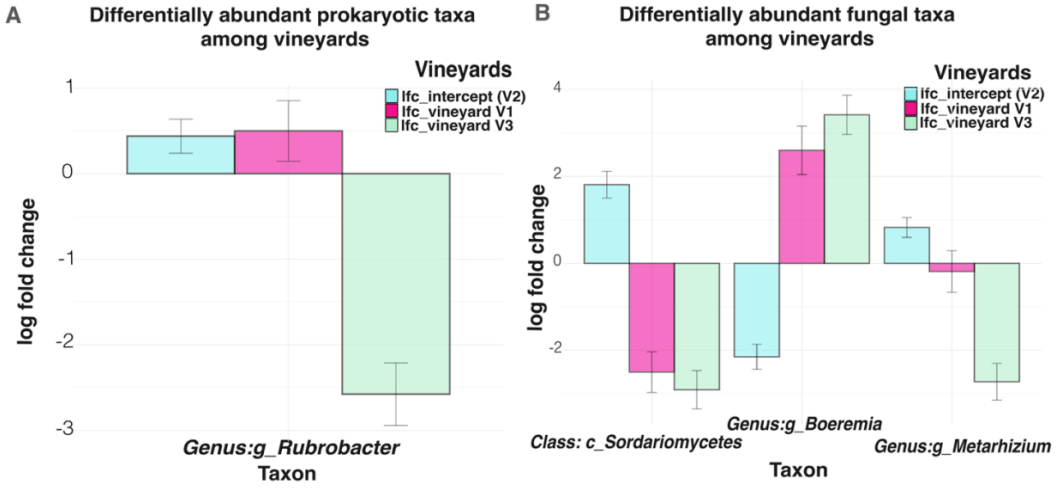

Figure 2. Differentially abundant taxa among vineyards: prokaryotic (A) and fungal (B). Vineyards: V1 (Cuatro Piedras), V2 (Rincón del Colorado) and V3 (Garzón), Uruguay.

3.5.2. Impact of under-vine soil management on soil microbiota and properties in vineyards

Analyses of the composition analyses of prokaryotic and fungal communities within vineyards revealed a significant impact of U-VSM solely in V2, the vineyard with a 10-year implementation of PLM (Figure 3). Permanova demonstrated a significant effect of U-VSM on both prokaryotic ($F = 1.23$, $R^2 = 0.15$, $Pr(>F) = 0.006$***) and fungal communities ($F = 2.7$, $R^2 = 0.27$, $Pr(>F) = 0.001$***). The homogeneity of dispersion tests ensured comparable variances within the prokaryotic ($F = 0.7$, $Pr(>F) = 0.444$) and fungal communities ($F = 3.5$, $Pr(>F) = 0.112$) across different U-VSM. PCoA visualization further supported these findings with more pronounced separation for fungal communities (Figure 3B and 3E).

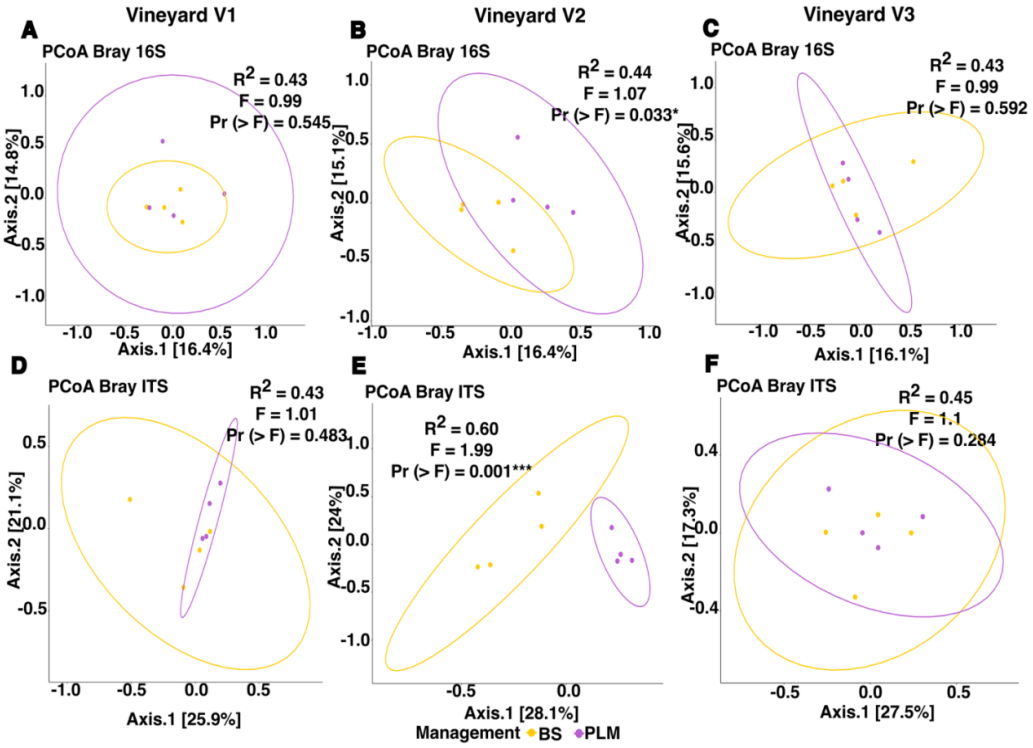

Figure 3. Principal Coordinates Analysis based on Bray-Curtis distance of prokaryotic (A-C) and fungal communities (D-F) from vineyard soils with two under-vine soil management: Bare soil (BS) and PLM (permanent living mulch). Vineyards: V1 (Cuatro Piedras), V2 (Rincón del Colorado) and V3 (Garzón), Uruguay.

In V2, three taxa were identified as key responders to soil management practices, for both prokaryotes and fungi. Among the prokaryotic responders, the class Alphaproteobacteria and the family *Micropepsaceae* were more abundant in BS, whereas the family *Latescibacteraceae* was prevalent in PLM. For fungi, the genera *Cladophialophora*, *Nigrospora*, and *Pseudopithomyces* were more abundant in PLM (Figure 4).

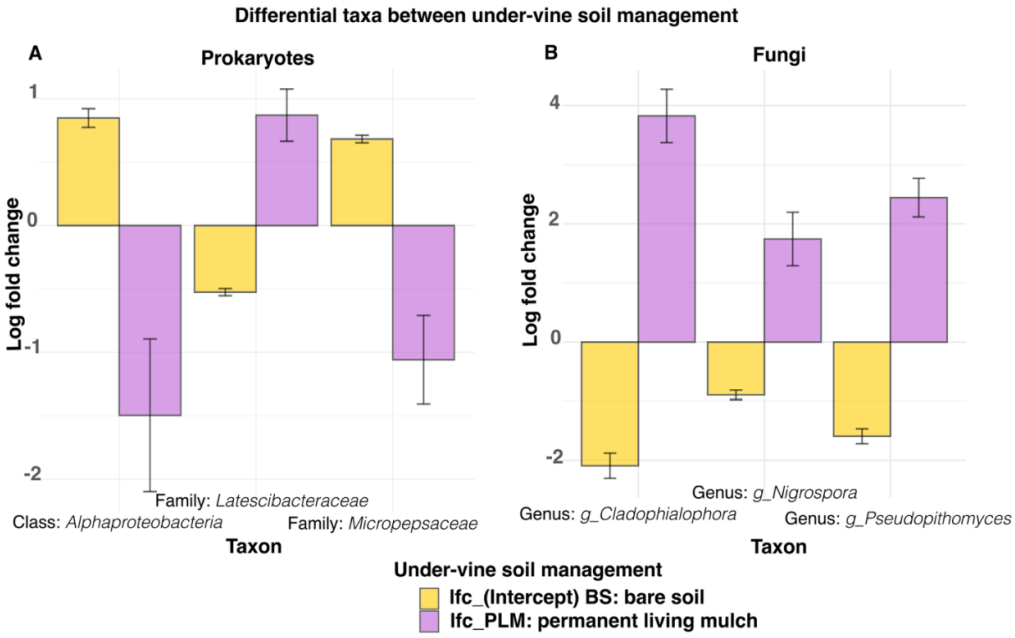

Figure 4. Differentially abundant taxa between under-vine soil management (Bare soil-BS and Permanent living mulch-PLM) in vineyard V2 for prokaryotic (A) and fungal (B) communities.

Regarding soil properties, soil respiration (SR), soil protein (SP), potentially oxidizable Carbon (PoxC) and soil bulk density (SBD) were significantly different only for U-VSM in V2. Mean values for SR, SP, PoxC were 47 %, 63 % and 36 % higher and SBD 20 % lower in PLM than in BS (Table 3). P Bray, Mg, and Na content were also affected by U-VSM, showing higher values under PLM in comparison to BS (Table 4), while texture variables were not affected (Table S3, in Supplementary material).

Table 3. Soil biological and physical properties from vineyard soils with two under-vine soil management: bare soil (BS) and permanent living mulch (PLM). Lower case letters in the columns indicate statistically significant differences (ANOVA, $p < 0.05$) between under-vine soil management within vineyards. V1 (Cuatro Piedras), V2 (Rincón del Colorado) and V3 (Garzón), Uruguay.

Vineyard	Under-vine soil management	Soil Respiration (mg CO_2/g dry weight/day)	Soil Protein (ACE Protein mg/g dry weight)	Potentially oxidizable Carbon (mol MnO_4 reduced kg^{-1} soil)	Soil Bulk Density (g/cm^3)
V1	BS	0.464 ± 0.05 a	6.16 ± 1.31 a	0.05 ± 0.01 a	1.19 ± 0.05 a
	PLM	0.556 ± 0.05 a	6.24 ± 1.31 a	0.05 ± 0.01 a	1.25 ± 0.05 a
V2	BS	0.374 ± 0.05 a	6.29 ± 1.31 a	0.07 ± 0.01 a	1.17 ± 0.05 b
	PLM	0.701 ± 0.05 b	16.73 ± 1.31 b	0.11 ± 0.01 b	0.94 ± 0.05 a
V3	BS	0.526 ± 0.05 a	15.73 ± 1.31 a	0.07 ± 0.01 a	1.29 ± 0.05 a
	PLM	0.533 ± 0.05 a	16.43 ± 1.31 a	0.08 ± 0.01 a	1.24 ± 0.05 a

Table 4. Soil chemical properties from vineyard soils with two under-vine soil management: bare soil (BS) and permanent living mulch (PLM). Lower case letters in the columns indicate statistically significant differences (ANOVA, $p < 0.05$) between under-vine soil management within vineyards. V1 (Cuatro Piedras), V2 (Rincón del Colorado) and V3 (Garzón), Uruguay.

Vineyard	Under-vine soil management	Soil Organic Carbon (%)	pH	N (%)	P Bray (mg P/g)	Ca (meq/100g)	Mg (meq/100g)	K (meq/100g)	Na (meq/100g)
V1	BS	1.53 ± 0.31 a	7.03 ± 0.17 a	0.12 ± 0.02 a	33.6 ± 6.66 a	14.85± 2.64 a	2.62 ± 0.32 a	0.39 ± 0.07 a	0.10 ± 0.06 a
	PLM	1.71 ± 0.31 a	6.95 ± 0.17 a	0.14 ± 0.02 a	35.2 ± 6.66 a	16.50± 2.64 a	3.08 ± 0.32 a	0.41 ± 0.07 a	0.14 ± 0.06 a
V2	BS	2.13 ± 0.31 a	7.88 ± 0.17 a	0.16 ± 0.02 a	21.6 ± 6.66 a	23.73± 2.64 a	3.10 ± 0.32 a	0.34 ± 0.07 a	0.87 ± 0.06 a
	PLM	2.6 ± 0.31 a	7.47 ± 0.17 a	0.21 ± 0.02 a	44.4 ± 6.66 b	20 ± 2.64 a	4.15 ± 0.32 b	0.48 ± 0.07 a	1.07 ± 0.06 b
V3	BS	2.45 ± 0.31 a	6.95 ± 0.17 a	0.17 ± 0.02 a	26.5 ± 6.66 a	6.08 ± 2.64 a	1.98 ± 0.32 a	0.34 ± 0.07 a	0.06 ± 0.06 a
	PLM	2.64 ± 0.31	6.8 ±0.17	0.21 ± 0.02	20.9 ± 6.66	4.83 ± 2.64	1.75 ± 0.32	0.39 ± 0.07	0.03 ± 0.06

		a	a	a	a	a	a	a	a

3.6. Discussion

3.6.1. Soil microbial community composition shows moderate differences among Tannat vineyards under conventional herbicide management

This work provides information on the impact of vineyard under-vine soil management on bulk soil microbiota. The composition of the bulk soil prokaryotic and fungal communities did not exhibit significant differences between the studied vineyards, despite notable variations in cropping history, irrigation practices, altitude and soil types (V3 vs V1 and V2). Although pairwise PERMANOVA comparisons between vineyards did not remain significant after FDR correction, the global analysis indicated a significant effect of vineyard for both prokaryotic and fungal communities. Furthermore, the Bray–Curtis PCoA visualization suggests that V3 is distinct from V1, and especially from V2, which could reflect the differences mentioned above. These observations highlight that, even with similar herbicide management under the vines, site-specific factors may contribute to shaping the composition of the microbial community. Although our study was conducted in a limited geographical area with vineyards sharing the same climatic classification (IHAS IFA2 ISA1) [20], previous research indicated that biogeographical soil patterns exert a strong influence on microbial diversity, which can vary between regions and between vineyards [4][41]. The same land-use (Tannat vines) and uniform soil management (bare soil with herbicides) over at least ten years is likely to have resulted in the homogenization of soil microbial communities. This phenomenon has been observed at global and regional scales, where monoculture-dominated landscapes reduce environmental heterogeneity, leading to homogenization of soil microbiome composition [42]. The differential abundance analysis confirms these findings, with only a few taxa being differentially abundant among

vineyards. Despite some differences were observed, such as lower relative abundance of *Rubrobacter* in V3 and higher abundance of *Sordariomycetes* and *Metarhizium* in V1, the ecological relevance of these taxa remains uncertain. These groups are commonly found in soil, and their differential abundance may reflect multiple factors beyond soil composition alone, such as microclimatic conditions or historical land use. While V3 differs in certain soil properties (e.g. texture) compared to V1 and V2, no direct correlation analysis was performed. Therefore, further research would be needed to clarify whether these taxa respond consistently to soil characteristics or hold functional importance in vineyard ecosystems.

Beta diversity revealed only subtle differences among vineyards, a small core of prokaryotic taxa was found, while fungal communities exhibited a larger core microbiota in terms of mean relative abundance. The relatively small core suggests a high microbial diversity among less abundant taxa, which may be attributed to substantial intra-vineyard variability. The intra-vineyard variability of soil properties has been observed in other Uruguayan vineyards within the same viticultural region as V1 and V2 (viticulture region: RVR). Particularly those variables related to soil moisture (e.g. electrical conductivity and clay content), nutrient availability (e.g. pH, calcium, magnesium, and potassium), and root development (e.g. sand content, sodium, and soil penetration resistance) have been identified as playing a critical role in the spatial differentiation of zones within a vineyard [(43)].

In the present study, bulk soil microbiota or some differentially abundant taxa may not yet be considered a reliable indicator to differentiate among vineyards. Nevertheless, further research involving a higher number of vineyards may reveal more profound distinctions and contribute to the definition of viticultural zones including information about microbial patterns. The potential

of using microbiome data to differentiate vineyards has recently been the subject of considerable interest, given the pivotal role played by the microbiota in shaping the terroir and influencing grape and wine quality [3][4].

3.6.2. Lasting Permanent Living Mulch shaped the soil microbial composition within the vineyard

The most substantial effect of U-VSM on bulk soil microbiota and properties was observed in vineyard V2, where PLM have been implemented for at least ten years. No differences in community composition and soil properties were observed between soil managements in either V1 or V3 vineyards, which had undergone a single year of implementation of PLM management. Our results suggest that soil microbiota and soil physical, chemical and biological properties need time to evolve in order to detect differences between managements.

The use of permanent living mulches or cover crops in vineyard soils (under the vine and between rows) is an alternative to the conventional management of spraying herbicides to avoid competition for water and nutrition [15]. Permanent living mulches provide several benefits, such as weed suppression, carbon sequestration, avoiding erosion and nutrient leaching and soil health promotion [14]. Little is known about how soil microbiota is impacted by PLM in vineyards and how many years are needed to observe significant changes, besides these changes may be different for prokaryotic and fungal communities. Soil microbiome is intricately linked with soil structure [44]. A stronger effect of geography has been suggested for bacterial community composition, while fungi seem more responsive to soil management or land use [45], especially when implementing tillage practices, probably due to the mechanical disruption of hyphal networks [46]. But also soil coverage strongly influences soil temperature and water dynamics [47], and soil porosity and pore

connectivity determine water availability and oxygen flux, modulating soil microbiota [46][48]. Furthermore, cover crops can influence microbial communities through diverse carbon inputs, which microorganisms then degrade or employ as substrate for production of soil-binding agents [44]. In our study, soil health indicators like SR, PoxC, SP and SBD suggested these potential impacts on soil microbiota.

PLM showed a major abundance of family *Latescibacteraceae*, while BS was characterized by a major abundance of Alphaproteobacteria and the family *Micropepsaceae*. In a previous study carried out in the same vineyard (V2) at three different phenological stages, U-VSM also affected grapevine rhizosphere microbiota, with several Alphaproteobacteria also identified as responders to herbicide use [18]. The prevalence of Alphaproteobacteria in soils managed with herbicides can be attributed to a multitude of ecological and environmental variables. The application of herbicides has the potential to disrupt plant-microbe interactions, reducing the availability of plant-derived carbon sources [50] and creating an environment conducive to the proliferation of microorganisms adapted to survive in low-nutrient environments [51], like Alphaproteobacteria. This Class is renowned for a multitude of members with a pivotal role in nitrogen cycling [52], which can be vital in soils where plant derived inputs, like root exudates or plant debris, are minimal.

The increased abundance of *Cladophialophora*, *Nigrospora*, and *Pseudopithomyces* in soil with PLM can be attributed to several factors. Vines managed with PLM, as opposed to bare soil, support more complex ecosystem-level interactions where soil microbiota are influenced not only by root exudates from the vines but also by the plant species that integrate the PLM [53]. In our study, root exudates from tall fescue, a perennial grass, provide a continuous and additional source of carbon and nutrients, promoting microbial growth and

creating favorable conditions for saprophytic fungi [53][54]. In addition to root exudates, the presence of plant residues offers a substrate for fungal species involved in decomposition processes [55]. Moreover, the enhanced soil structure (estimated by SBD) resulting from PLM is likely to provide a more conducive environment for fungal growth than the more compact and nutrient-poor conditions observed in bare soil.

Given the variability of factors that influence the composition of soil microbiota, it is crucial to determine whether the differentially abundant group for each management is consistent at different times (years of implementation, seasons, phenological stages) and soil/rhizosphere and plant compartments (roots, leaves, flowers and grapes) for the same vineyard and between vineyards with similar history of soil management implementation. Once the genera or groups that are consistently promoted by a particular management have been identified, further studies about their functional significance can be undertaken. By understanding the unique or differentially abundant microbial taxa associated with specific soils and managements, we may gain insights into how the soil microbiome contributes to the terroir concept, potentially impacting grape and wine quality, enriching our understanding of terroir beyond traditional climatic and geographic parameters.

3.7. Conclusions

Despite differences in crop history, irrigation practices, and soil types, continued herbicide use and bare soil maintenance might have contributed to reducing variability in bulk soil microbiota composition across vineyards. Vineyards with more than ten years of PLM exhibited distinct microbial communities and improved soil properties compared to BS, reinforcing the role of sustained management in shaping soil microbiota. Our findings emphasize

the need for long-term studies to capture microbial responses to soil management practices. Additional research incorporating a wider range of vineyards could refine microbial-based indicators for viticultural zoning, providing valuable insights into the microbial dimension of terroir and its implications for sustainable vineyard management.

Available data: The entire data set that supports the results of this study was published in the article itself.

3.8. References

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3.9. Supplementary material

Figure S1. Geographical location of the three vineyards: V1-Cuatro Piedras (34º 62' S, 56º 28' W), V2-Rincón del Colorado (34°44′ S, 56°13′ W) and V3-Garzón (34.57' S, 54.63 W), geographically separated as follows: V1-V2 9 km, V1-V3 152 km and V2-V3 158 km. Based on bioclimatic indices (Heliothermal, Dryness, and Cool Night), the three vineyards belong to the same climatic type IHA3 IFA2 ISA1, all of them influenced by the Atlantic Ocean and the estuary of Río de la Plata.

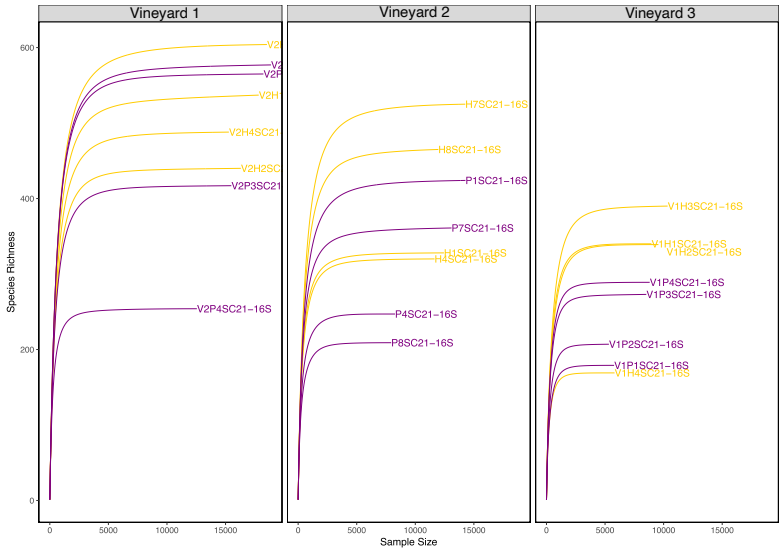

Figure S2. Rarefaction curves of 16S rRNA (V3-V4) samples facet by Vineyard. Under-vine soil management in colors: bare soil (BS): yellow, permanent living mulch (PLM): violet.

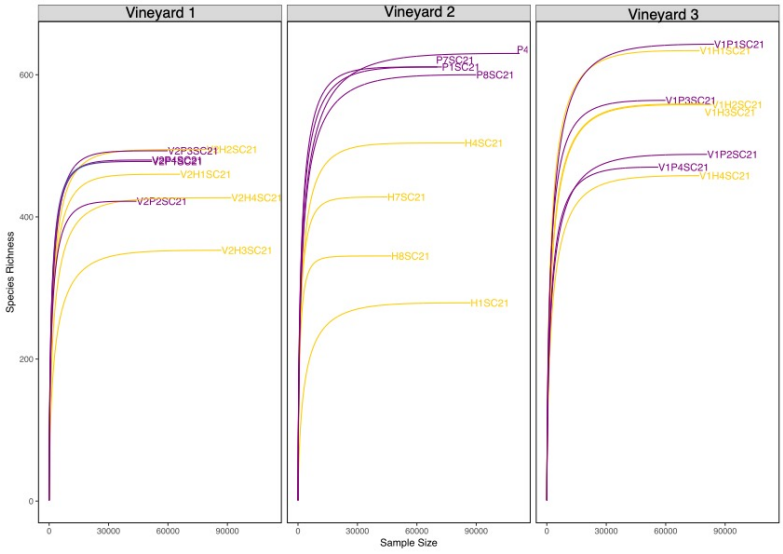

Figure S3. Rarefaction curves of ITS2 rRNA samples facet by Vineyard. Under-vine soil management in colors: bare soil (BS): yellow, permanent living mulch (PLM): violet.

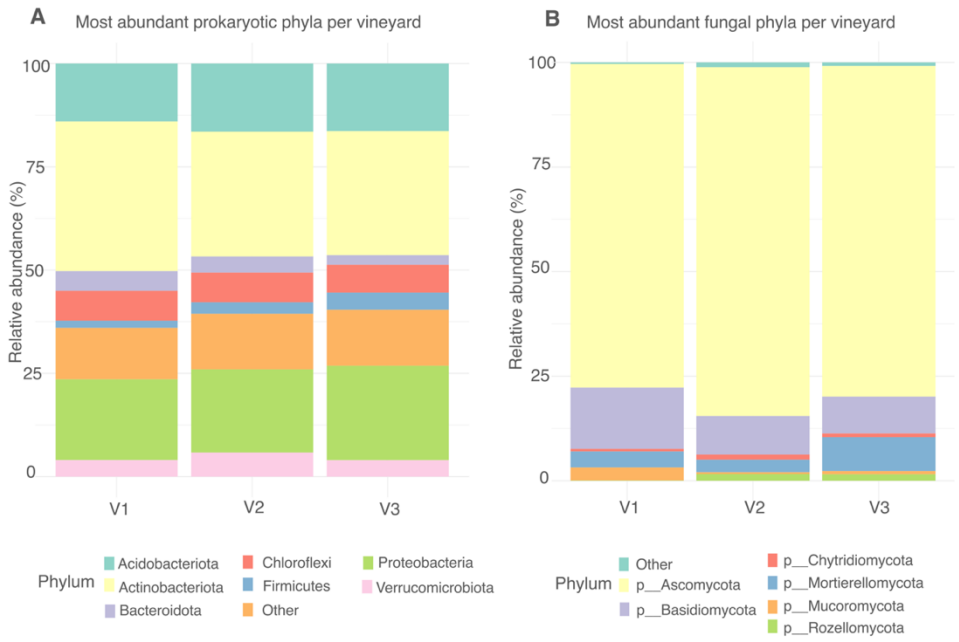

Figure S4. Relative abundance (%) of most abundant phyla per vineyard: A- prokaryotic, B- Fungal. Vineyards: V1-Cuatro Piedras, V2- Rincón del Colorado and V3-Garzón, Uruguay.

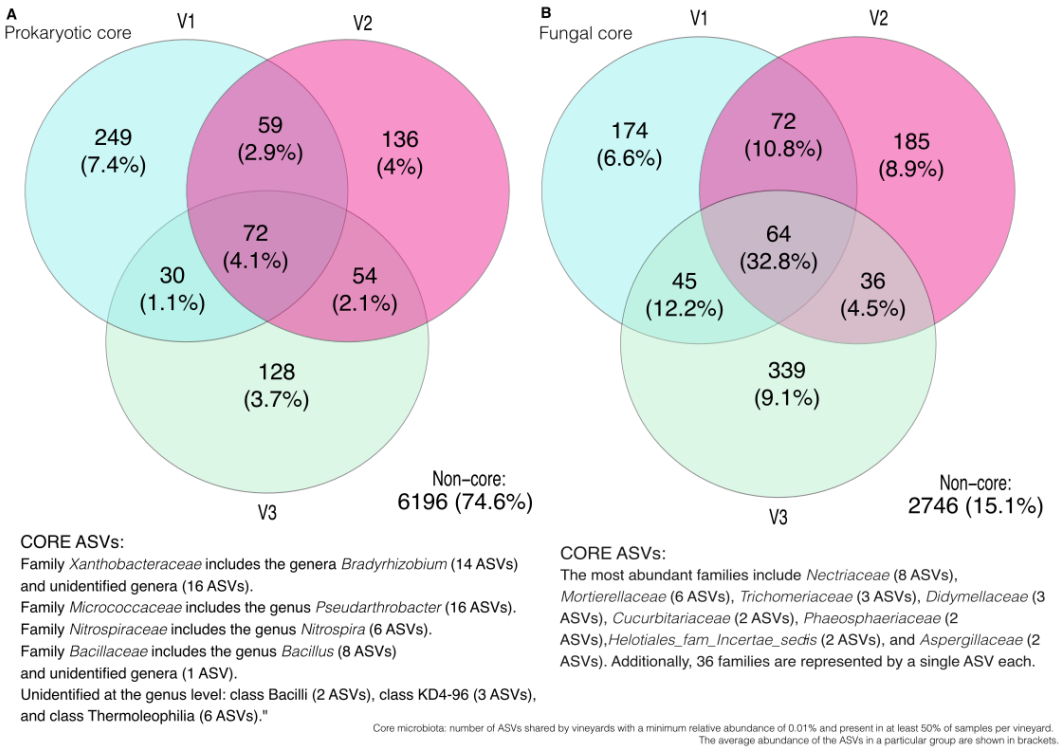

Figure S5. Venn Diagram showing the core microbiota and unique ASVs of soil prokaryotic (A) and fungal communities (B). Average abundance of the ASVs in a particular group are shown in brackets. Vineyards: V1 (Cuatro Piedras), V2 (Rincón del Colorado) and V3 (Garzón). Uruguay.

Table S1. Unique Prokaryotic ASVs classified by genus (maximum level reached in the taxonomic assignment) and phylum by Vineyard. Vineyards: V1-Cuatro Piedras, V2- Rincón del Colorado and V3-Garzón, Uruguay, South America. NA means not classified at genus level.

Vineyard	Species	Phylum
V1	NA, Microlunatus, Lapillicoccus, Kribbella, Ilumatobacter, Haliangium, Pseudonocardia, Gaiella, RB41, Knoellia, Skermanella, Microvirga, Ellin6067, Rubrobacter, Nocardioides, Bacillus, Dongia, Streptomyces, Povalibacter, Archangium, Phaselicystis, Mycobacterium, Sphingomonas, Reyranella, Steroidobacter, Pseudolabrys, Virgisporangium, Aeromicrobium, Tychonema CCAP 1459-11B, Psychroglaciecola, Dactylosporangium, Labrys, Methylosinus, Geodermatophilus, Microcoleus SAG 1449-1a, Aridibacter, Candidatus Udaeobacter, Flavisolibacter, Blastococcus, Actinocorallia, Rhodanobacter, Marmoricola, Solirubrobacter, Parafilimonas, Gemmatimonas, Methylobacterium-Methylorubrum, Trichocoleus SAG 26,92, Umezawaea, Ellin6055, Lysobacter, mle1-7, Bryobacter	Actinobacteriota, Gemmatimonadota, Methylomirabilota, Chloroflexi, Acidobacteriota, Myxococcota, Proteobacteria, Firmicutes, Cyanobacteria, Verrucomicrobiota, Latescibacterota, Bacteroidota, Planctomycetota
V2	NA, Bryobacter, Sphingomonas, Pseudolabrys, Gaiella, Reyranella, Virgisporangium, Acidibacter, Kribbella, Pirellula, Dongia, Lysobacter, Acidothermus, Pseudaminobacter, Bacillus, Candidatus Udaeobacter, Nocardioides, MND1, Mycobacterium, Novosphingobium, Steroidobacter, Rhodomicrobium, Pedomicrobium, Candidatus Protochlamydia	Chloroflexi, Actinobacteriota, Acidobacteriota, Proteobacteria, Methylomirabilota, Planctomycetota, Bacteroidota, Firmicutes, Verrucomicrobiota, Entotheonellaeota, Latescibacterota
V3	NA, Ilumatobacter, Nocardioides, Streptomyces, Steroidobacter, Gaiella, Dongia, Sphingomonas, Mycobacterium, Pedomicrobium, Blastococcus, Pir4 lineage, Candidatus Udaeobacter, Bacillus, Candidatus Xiphinematobacter, Nitrospira, Pseudarthrobacter	Chloroflexi, Actinobacteriota, RCP2-54, Bacteroidota, Acidobacteriota, Proteobacteria, Firmicutes, Planctomycetota, Verrucomicrobiota, Nitrospirota

Table S2. Unique Fungal ASVs classified by genus, species (maximum level reached in the taxonomic assignment) and phylum by Vineyard. Vineyards: V1-Cuatro Piedras, V2- Rincón del Colorado and V3-Garzón, Uruguay, South America. NA means not classified at genus level.

Vineyard	Species	Phylum
V1	g__Calyptella s__capula, g__Mucor s__genevensis, g__Coniella, NA, g__Iodophanus, g__Articulospora s__proliferata, g__Thanatephorus s__cucumeris, g__Mycenella, g__Vishniacozyma s__victoriae, g__Talaromyces, g__Gibberella s__tricincta, g__Cadophora s__luteo-olivacea, g__Mortierella s__elongata, g__Flagelloscypha, g__Fusarium s__asiaticum, g__Clitopilus, g__Schizothecium, g__Neodevriesia s__capensis, g__Arxiella, g__Penicillium, g__Knufia s__tsunedae, g__Leohumicola, g__Alternaria, g__Tetracladium s__marchalianum, g__Penicillium s__miczynskii, g__Setophoma s__terrestris, g__Lophiotrema s__rubi, g__Gibberella s__nygamai, g__Terfezia, g__Paraphaeosphaeria s__angularis, g__Calvatia, g__Podospora s__decidua, g__Schizothecium s__glutinans, g__Diplodia, g__Sclerostagonospora, g__Byssonectria s__fusispora, g__Trichoderma s__brevicompactum, g__Lycoperdon s__pratense, g__Neosetophoma, g__Mortierella s__gamsii, g__Metarhizium s__marquandii, g__Efibulobasidium s__albescens, g__Rhizophlyctis s__rosea, g__Pyrenochaetopsis s__leptospora, g__Trichoderma s__virens, g__Vishniacozyma s__foliicola, g__Pseudallescheria s__fusoidea, g__Phaeosphaeria s__acaciae, g__Mortierella, g__Lipomyces s__tetrasporus, g__Leucoagaricus s__leucothites, g__Coprinellus s__domesticus, g__Ceratobasidium, g__Arthrocladium, g__Fusarium s__poae, g__Exophiala, g__Phaeosphaeria, g__Peniophora, g__Atractiella s__solani, g__Knufia, g__Scedosporium s__dehoogii, g__Hydropisphaera s__bambusicola, g__Ciliophora, g__Setosphaeria s__pedicellata, g__Spizellomyces s__dolichospermus, g__Ochroconis, g__Pyrenochaeta s__inflorescentiae, g__Cystofilobasidium s__macerans, g__Ascobolus, g__Triscelophorus, g__Nigrospora s__musae, g__Chaetomium s__afropilosum, g__Cyphellophora, g__Pseudallescheria s__boydii, g__Ochroconis s__robusta, g__Monographella s__nivalis, g__Aspergillus s__muricatus, g__Gibberella, g__Udeniomyces s__pyricola, g__Mortierella s__alpina, g__Keissleriella s__rosarum, g__Ascobolus s__crenulatus, g__Plenodomus s__biglobosus, g__Neoascochyta s__paspali, g__Curvularia, g__Tetracladium s__furcatum, g__Niesslia s__indica, g__Chaetomium, g__Entoloma s__calongei, g__Vermispora s__spermatophaga, g__Oliveonia, g__Spizellomyces, g__Lamprospora, g__Occultifur, g__Dactylella, g__Preussia s__terricola, g__Ramicandelaber, g__Itersonilia s__perplexans, g__Vishniacozyma s__tephrensis, g__Thelonectria s__mammoidea, g__Metarhizium s__carneum	p__Basidiomycota, p__Mucoromycota, p__Ascomycota, p__Mortierellomycota, p__Chytridiomycota, p__Rozellomycota, p__Kickxellomycota

V2	g__Alternaria s__betae-kenyensis, g__Fusarium s__oxysporum, g__Arthroderma s__curreyi, NA, g__Schizothecium s__dakotense, g__Ruhlandiella, g__Fusarium, g__Aspergillus, g__Trichocladium s__pyriforme, g__Clitopilus, g__Oedocephalum, g__Dendrosporium s__lobatum, g__Arthrobotrys, g__Descomyces, g__Staphylotrichum s__boninense, g__Lipomyces s__tetrasporus, g__Chaetomium s__angustispirale, g__Sigarispora s__scrophulariae, g__Bartalinia, g__Crocicreas, g__Coniochaeta, g__Acrophialophora, g__Tomentella s__pilosa, g__Clohesyomyces s__aquaticus, g__Phialophora s__livistonae, g__Aspergillus s__terreus, g__Chaetomium, g__Clavaria, g__Fusarium s__solani, g__Periconia, g__Idriella, g__Schizothecium, g__Rhizophlyctis s__rosea, g__Paraphoma s__radicina, g__Leptodiscella s__africana, g__Paracladophialophora s__carceris, g__Mortierella s__capitata, g__Clonostachys, g__Mucor s__gigasporus, g__Veronaea, g__Neophaeosphaeria, g__Spiromastix, g__Acremonium s__persicinum, g__Aspergillus s__carneus, g__Oliveonia s__pauxilla, g__Ochroconis, g__Devriesia s__pseudoamericana, g__Phaeoacremonium s__iranianum, g__Dichotomopilus, g__Dactylellina, g__Daldinia, g__Glomus s__microcarpum, g__Delitschia s__chaetomioides, g__Poaceascoma s__helicoides, g__Didymosphaeria, g__Phylliscum, g__Psathyrella s__candolleana, g__Mortierella, g__Pseudogymnoascus s__appendiculatus, g__Lecanicillium s__saksenae, g__Talaromyces s__yelensis, g__Tomentella, g__Glutinoagger s__fibulatus, g__Ustilago s__xerochloae, g__Talaromyces s__ucrainicus, g__Terfezia, g__Geniculospora s__grandis, g__Circinella s__lacrymispora, g__Magnaporthiopsis, g__Exophiala s__cancerae, g__Fusidium s__griseum, g__Trichoderma s__lixii, g__Aspergillus s__sparsus, g__Hanseniaspora s__uvarum, g__Botryosphaeria, g__Ciliophora, g__Devriesia s__americana, g__Fusarium s__algeriense, g__Cladosporium s__sphaerospermum, g__Neobulgaria s__pura, g__Microdochiella s__fusarioides, g__Septoglomus, g__Achroiostachys s__betulicola, g__Lophiostoma, g__Entoloma, g__Exserohilum s__rostratum, g__Magnaporthe, g__Neodactylaria, g__Metarhizium, g__Phaeodothis, g__Spiromastix s__warcupii, g__Agaricus s__deserticola, g__Mortierella s__beljakovae, g__Abortiporus s__biennis, g__Nectria s__diminuta, g__Schizothecium s__fimbriatum, g__Tetrapisispora s__fleetii, g__Spizellomyces s__dolichospermus, g__Sclerogaster, g__Curvularia, g__Ustilago s__nunavutica, g__Orbilia, g__Kochiomyces, g__Cunninghamella s__blakesleeana, g__Vaginatispora s__nypae, g__Aspergillus s__heterocaryoticus	p__Ascomycota, p__Basidiomycota, p__Rozellomycota, p__Chytridiomycota, p__Mortierellomycota, p__Mucoromycota, p__Glomeromycota, p__Blastocladiomycota, p__Kickxellomycota

V3	g__Truncatella s__angustata, NA, g__Talaromyces s__flavus, g__Papiliotrema s__terrestris, g__Fibulochlamys s__chilensis, g__Knufia s__perforans, g__Articulospora s__proliferata, g__Fusarium s__biseptatum, g__Paracladophialophora, g__Bartalinia, g__Mortierella, g__Setophaeosphaeria s__hemerocallidis, g__Dactylonectria s__ecuadoriensis, g__Keissleriella, g__Antennariella s__placitae, g__Trichoderma, g__Mortierella s__minutissima, g__Ramophialophora s__humicola, g__Chloridium s__aseptatum, g__Minimedusa s__polyspora, g__Pichia s__terricola, g__Agrocybe, g__Entoloma, g__Cylindrocarpon, g__Lectera s__longa, g__Geastrum s__lageniforme, g__Saitozyma s__podzolica, g__Gongronella, g__Mortierella s__elongata, g__Stachybotrys s__limonispora, g__Gongronella s__butleri, g__Acremonium s__spinosum, g__Thanatephorus s__cucumeris, g__Torula s__hollandica, g__Tulostoma, g__Coniochaeta, g__Teichospora s__thailandica, g__Mortierella s__fluviae, g__Aspergillus s__awamori, g__Fusicolla s__violacea, g__Alternaria, g__Chaetosphaeria, g__Pyxidiophora s__microspora, g__Pyrenochaeta, g__Pseudeurotium, g__Serendipita, g__Mortierella s__amoeboidea, g__Apseudocercosporella s__trigonotidis, g__Preussia, g__Trichoderma s__brevicompactum, g__Polyschema, g__Rhizophlyctis s__rosea, g__Pestalotiopsis s__trachicarpicola, g__Fusarium, g__Diaporthe, g__Tetracladium, g__Samsoniella s__hepiali, g__Dichotomopilus, g__Absidia s__koreana, g__Ilyonectria s__mors-panacis, g__Cercophora s__coronata, g__Leucosporidium s__fragarium, g__Myrothecium s__cinctum, g__Clarireedia s__bennettii, g__Holocotylon s__brandegeeanum, g__Knufia, g__Cunninghamella, g__Ochroconis s__tshawytschae, g__Psathyrella s__luteopallida, g__Pyrenochaeta s__inflorescentiae, g__Chaetomium, g__Clitopilus, g__Lepiota s__helveola, g__Polyschema s__sclerotigenum, g__Tetracladium s__marchalianum, g__Veronaeopsis s__simplex, g__Calycina s__vulgaris, g__Ramophialophora s__vesiculosa, g__Acrocalymma s__fici, g__Pseudocoleophoma s__bauhiniae, g__Dendryphion s__fluminicola, g__Curvularia, g__Diplodia s__pseudoseriata, g__Acremonium s__furcatum, g__Absidia, g__Gliomastix s__roseogrisea, g__Chrysosporium s__lobatum, g__Preussia s__persica, g__Solicoccozyma, g__Mortierella s__alpina, g__Fusarium s__delphinoides, g__Schizothecium, g__Oidiodendron, g__Talaromyces s__luteus, g__Coniochaeta s__verticillata, g__Cyphellophora s__oxyspora, g__Xylaria, g__Penicillium s__sacculum, g__Preussia s__tenerifae, g__Angustimassarina s__premilcurensis, g__Cyphellophora s__fusarioides, g__Periconia s__lateralis, g__Tomentella, g__Pteridiospora, g__Colletotrichum s__circinans, g__Monocillium s__mucidum, g__Lecanicillium s__saksenae, g__Humicola s__sardiniae, g__Melanoleuca s__griseobrunnea, g__Mortierella s__beljakovae, g__Lipomyces s__starkeyi, g__Exophiala, g__Thysanorea, g__Entrophospora s__infrequens, g__Heterophoma, g__Mariannaea, g__Aspergillus s__inflatus, g__Chaetomium s__strumarium, g__Leucoagaricus s__rubroconfusus, g__Scolecobasidium s__constrictum, g__Phialemoniopsis, g__Olpidium s__brassicae, g__Talaromyces, g__Metarhizium s__carneum, g__Rhizoctonia s__bicornis, g__Trichocladium s__pyriforme, g__Scytalidium s__aurantiacum, g__Papiliotrema s__laurentii, g__Bullera s__unica, g__Ganoderma, g__Bovista, g__Pithomyces s__valparadisiacus, g__Mycoleptodiscus, g__Byssonectria, g__Clathrus s__archeri, g__Pyxidiophora s__arvernensis, g__Entorrhiza, g__Phaeomoniella, g__Claroideoglomus s__claroideum, g__Xylaria s__oligotoma, g__Spizellomyces, g__Penicillium s__osmophilum, g__Cercophora s__thailandica, g__Scytalidium, g__Paracladophialophora s__carceris, g__Geastrum, g__Kalmusia s__variispora, g__Allophaeosphaeria s__cytisi, g__Coniochaeta s__canina, g__Sakaguchia s__meli, g__Cladophialophora, g__Paracylindrocarpon s__aloicola, g__Corynascus s__citrinus, g__Hannaella s__oryzae, g__Myrothecium s__inundatum, g__Ramicandelaber, g__Veronaea, g__Metapochonia s__goniodes, g__Eutypella s__citricola, g__Exophiala s__brunnea, g__Operculomyces s__laminatus, g__Epicoccum, g__Pyrenophora s__chaetomioides, g__Lasiobolidium s__spirale, g__Fusicolla s__acetilerea, g__Thermomyces s__lanuginosus, g__Clonostachys, g__Cistella, g__Nigrospora, g__Paracremonium, g__Vishniacozyma s__dimennae, g__Hirsutella, g__Helicosporium s__aquaticum, g__Arcuadendron s__ovatum, g__Conocybe s__fuscimarginata, g__Ciliophora, g__Ochroconis s__bacilliformis, g__Holtermanniella s__takashimae, g__Phaeomoniella s__chlamydospora, g__Hypholoma s__fasciculare, g__Myrmecridium, g__Dictyosporella, g__Pezicula s__melanigena, g__Metarhizium s__marquandii, g__Spizellomyces s__dolichospermus, g__Acrocalymma, g__Conlarium, g__Trichoderma s__aurantioeffusum, g__Parasola s__lilatincta	p__Ascomycota, p__Basidiomycota, p__Mortierellomycota, p__Rozellomycota, p__Mucoromycota, p__Chytridiomycota, p__Kickxellomycota, p__Glomeromycota, p__Olpidiomycota, p__Entorrhizomycota

Table S3. Soil physical properties for the two under-vine soil management within vineyards. Soil under-vine management: bare soil (BS) and permanent living mulch (PLM). Lower case letters in the columns indicate statistically significant differences (ANOVA, $p < 0.05$) between under-vine soil management within vineyards. V1 (Cuatro Piedras), V2 (Rincón del Colorado) and V3 (Garzón).

Vineyard	Under-vine soil management	Clay (%)	Silt (%)	Sand-coarse (%)	Sand-fine (%)
V1	BS	16.13 ± 4.20 a	67.1 ± 5.48 a	3.98 ± 1.7 a	12.8 ± 1.09 a
	PLM	21.39 ± 4.20 a	62.5 ± 5.48 a	5.31 ± 1.7 a	10.8 ± 1.09 a
V2	BS	9.06 ± 2.14 a*	64.2 ± 5.50 a*	8.61± 4.2 a*	18.1±0.90 a*
	PLM	12.40 ± 1.66 a	60.7 ± 4.26 a	6.74 ± 3.3 a	20.2 ± 0.70 a
V3	BS	3.00 ± 1.09 a	24.1 ± 1.75 a	58.46±1.4 a	14.4 ± 0.35 a
	PLM	1.53 ± 1.09 a	27.1 ± 1.75 a	55.7 ± 1.4 a	15.7 ± 0.35 a

*The values for clay, silt, and sand fractions for BS in V2 are based on measurements from three samples instead of four, as one sample showed significantly divergent values, likely due to an analytical error.

4. Permanent cover crop as a strategy to promote soil health and vineyard performance*

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4.1. Resumen

La viticultura convencional implica un elevado uso de insumos que repercuten negativamente en los servicios ecosistémicos y la biodiversidad. Tradicionalmente, los viñedos son sistemas de secano y la vegetación se elimina o se rocía con herbicidas para evitar una competencia excesiva por el agua y los nutrientes. La introducción de cultivos de cobertura puede promover positivamente varios servicios ecosistémicos y, en particular, la salud del suelo y de los cultivos y la biodiversidad. Para evaluar el impacto del manejo del suelo bajo la fila (cobertura vegetal permanente - CVP vs desherbado con herbicidas - H) en un viñedo con riego de Tannat/SO4, se estudió la microbiota de la rizosfera de la vid, la salud del suelo y el rendimiento de la planta a lo largo de la temporada 2019/2020. Se determinó la diversidad y composición de la microbiota de la rizosfera (procariótica y fúngica) en tres etapas fenológicas diferentes (floración, envero, cosecha) mediante métodos dependientes e independientes del cultivo y se exploraron las propiedades físicas, químicas y biológicas del suelo y el rendimiento de las plantas. La microbiota de la rizosfera difirió entre los distintos manejos y estadios fenológicos. Varios taxones responden a la CVP, entre ellos *Pseudomonas*, *Pantoea*, *Butiaxella*, *Enterobacter*, *Trichoderma* y *Penicillium*. La CVP, en comparación con el H, mejoró la densidad aparente del suelo, la tasa de respiración del suelo, el índice

de proteína del suelo y el carbono potencialmente oxidable, y mostró una mayor proporción de agregados medios, así como mayores niveles de pH, carbono orgánico del suelo y nitrógeno. También se observó una menor incidencia de infecciones latentes de *Botrytis cinerea* bajo CVP. El rendimiento de la vid, la composición del mosto y el estado nutricional no se vieron afectados. En este contexto, la CVP aparece como una práctica agrícola sostenible para viñedos que promueve la biodiversidad y la salud del suelo y de las plantas. Se necesitan más estudios para evaluar cómo los cultivos de cobertura promueven los microbios beneficiosos, en particular los implicados en el crecimiento de las plantas y las respuestas de defensa.

Palabras claves: microbioma, vid, rizósfera, manejo del suelo, secuenciación de amplicones.

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4.2. Summary

Conventional viticulture involves a high use of inputs that negatively impact ecosystem services and biodiversity. Traditionally, vineyards are rainfed systems and vegetation is removed or sprayed with herbicides to avoid excessive competition for water and nutrients. Introducing cover crops can positively promote several ecosystem services and particularly soil and crop health and biodiversity. To assess the impact of under trellis soil management (permanent cover crop - PCC vs herbicide weeding - HW) in an irrigated vineyard of Tannat/SO4, grapevine rhizosphere microbiota, soil health and plant performance were studied along 2019/2020 season. Rhizosphere microbiota (prokaryotic and fungal) diversity and composition at three different phenological stages (flowering, veraison, harvest) was determined by culture dependent and independent methods and soil physical, chemical, and biological properties and plant performance was explored. Rhizosphere microbiota differed between managements and phenological stages. Several taxa respond to PCC, among them *Pseudomonas*, *Pantoea*, *Butiaxella*, *Enterobacter*, *Trichoderma* and *Penicillium*. PCC compared to HW improved bulk soil density, soil respiration rate, soil protein index and potentially oxidizable Carbon and showed greater proportion of medium aggregates, as well as increased levels of pH, soil organic carbon and nitrogen. Also, less incidence of Botrytis cinerea latent infections was observed under PCC. Vine yield, grape must composition and nutritional status were not affected. In this context, PCC appears as a sustainable agricultural practice for vineyards to promote biodiversity and soil and plant health. More studies are needed to assess how cover crops promote beneficial microbes, particularly those involved in plant growth and defense responses.

Keywords: microbiota, grapevine, rhizosphere, soil management, amplicon sequencing

4.3. Introduction

Vitis vinifera L. grapes are one of the most popular fruit crops worldwide, located mostly between latitudes 30 and 50 in the southern and northern hemispheres (Venkitasamy et al., 2019). There are 7.3 mha of vineyards with a production of 85 million tons of grapes in 2020 (OIV, 2021), where 50-75% are used for wine production (Venkitasamy et al., 2019). Vineyards are traditionally rainfed systems, and to avoid excessive competition for water and nutrients under trellis vegetation is removed or spayed with herbicides while some systems maintain spontaneous vegetation in the alleys between rows. Introducing vine under-trellis cover crop, can positively promote several ecosystem services and particularly soil and crop health and biodiversity (Coniberti et al., 2018b; Kim et al., 2020; Reganold, 2005; Sharma et al., 2018; vanden Heuvel & Centinari, 2021). Soil health understood as the “capacity of soil to function as a vital living system to sustain biological productivity, maintain environmental quality, and promote plant, animal, and human health” (Larkin, 2015).

In areas with fertile soils and high precipitation rates, under trellis vegetation can reduce excessive plant vigor by competition resulting in a reduced canopy density and pruning weight (vanden Heuvel & Centinari, 2021), but also excessive competition during dry seasons can result in a significant reduction of grape yield (Coniberti et al., 2018b; Garcia et al., 2018; Guilpart et al., 2017). Therefore, the incorporation of this agronomic practice requires a balance between the services and disservices. Among the benefits of under trellis vegetation, a moderate competition can improve sensory attributes associated to reduced vegetative growth and canopy size like increased fruit Brix and anthocyanin concentration in Tannat grapes and wines as well as

increased fruity and overall aroma intensity in wine (Coniberti et al., 2018a). Also reduced vegetative growth, more aerated canopies and less compacted bunches are associated with lower incidence of *Botrytis* bunch rot- BBR (Jacometti et al., 2007; Valdés-Gómez et al., 2008). Interestingly, reduced incidence of *Botrytis* bunch rot disease has been observed in grapevine with under trellis permanent cover crop (PCC) in comparison to herbicide weeding (HW), even when plants showed similar levels of vegetative development, berry size and fruit maturation, suggesting that some other factor can be explaining the differences in disease expression (Coniberti et al., 2018b). Moreover, under trellis vegetation allows a decrease in herbicide use, with its associated ecological and economic impact for the environment and viticulturists.

The effects of plant associated soil and rhizosphere microorganisms and its diversity on plant fitness and health have been widely investigated (Bakker et al., 2012; Berg et al., 2017; Mendes et al., 2013; Mohanty et al., 2021). These effects can be beneficial, deleterious or neutral from the plant response perspective (Mendes et al., 2013). Among the beneficial ones, the most reported are related to nutrient acquisition, tolerance to abiotic stresses, immune response, and protection against pathogens, like rhizobacteria belonging to *Pseudomonas* and *Bacillus* genera and *Trichoderma* and *Gliocladium* fungi. Those with detrimental effects on plant health belong to soil-borne plant pathogens, either fungi, oomycetes, or nematodes. Finally, some microorganisms have neutral effects on plants but serious harmful effects on human health as described for some Enterobacteriaceae and Pseudomonas aeruginosa (Mendes et al., 2013).

Grapevine associated microbiota has been recently characterized in several studies using culture-independent techniques, especially at fungal and bacteria communities level. These studies assessed the effect of numerous

factors; space/geographic (Aguilar et al., 2020; Berlanas et al., 2019; Burns et al., 2015; Liu & Howell, 2021; Manici et al., 2017; Zarraonaindia et al., 2015), vine developmental stage (Berlanas et al., 2019; Liu & Howell, 2021; Novello et al., 2017; Vink et al., 2021; Zarraonaindia et al., 2015), edaphic parameters (Burns et al., 2015), environmental stresses (Carbone et al., 2021), cultivar genotype (Aguilar et al., 2020; Vink et al., 2021; Zarraonaindia et al., 2015), rootstock genotype (Berlanas et al., 2019; Dries et al., 2021; Marasco et al., 2018), soil-plant compartments (Liu & Howell, 2021; Martínez-Díaz et al., 2019; Novello et al., 2017), plant age (Berlanas et al., 2019; Manici et al., 2017), and agricultural management (Chou et al., 2018; Schmid et al., 2011; Vega-Avila et al., 2015; Vink et al., 2021) on the rhizosphere, soil, and/or vine organs microbiota.

Soil and rhizosphere grapevine microbiota composition and diversity is highly influenced by agricultural practices. Vega-Avila et al. (2015) compared conventional versus organic management of two Syrah vineyards and found a clear difference in the bacterial community structure. Manici et al. (2017) observed that main differences in rhizosphere bacterial microbiota were due to geographic area while fungal community were mostly influenced by vegetative ground cover management and the cultivar-rootstock genotype. Hendgen et al. (2018) were able to determine that the fungal community composition in under trellis vineyards soils differed between an integrated, organic and biodynamic management, but species richness was not affected. The bacterial community composition was similar in all treatments, but species richness was significantly reduced in the soil under integrated management. Also, they observed a positive impact of alley vegetation on soil-borne fungi. Different irrigation regimes and their effect on the root endosphere, rhizosphere and bulk soil fungal communities were analyzed by Carbone et al. (2021) in Tannat/SO4 grapevine.

They found that under severe water deficit, fungal communities showed a lower diversity in all the compartments and an enrichment of *Funneliformis* (Arbuscular Mycorrhiza Fungi) in the rhizosphere in comparison with an absence of water deficit condition. Vink et al. (2021) showed that soil bacterial communities in grapevines were shaped by plant-associated effects (cultivar and phenological stage), soil management (tillage and no-tillage, irrigated and no-irrigated) and physio-chemical properties. Bacterial communities under no-tillage treatment (naturally growing vegetation) varied less among the different phenological stages in comparison to those from tillage treatment (bare soil), suggesting a buffering effect of the cover crop in the bacterial microbiota dynamics. Chou et al. (2018) did not find changes in the microbial composition of grapes among different managements (herbicides, cultivated bare soil and natural vegetation) despite the fungal and bacterial composition of soil microbiota differed between natural vegetation and bare soil.

The present study aims to assess the impact of soil under trellis management (permanent cover crop vs herbicide weeding) on grapevine rhizosphere microbiota along with soil health and plant performance, in a 17 years-old vineyard Tannat/SO4. Particularly, rhizosphere microbiota diversity and composition at three different phenological stages (flowering, veraison, harvest) was determined by culture dependent and independent techniques and the relationships with soil physical, chemical and biological properties and plant performance was explored.

4.4. Material and methods

4.4.1. Experimental site and vineyard management

The experiment was conducted in 2019 in an experimental vineyard (17 years old) of Tannat grapevines grafted onto SO4 rootstock, located in Las

Brujas, Canelones, in the Southern Uruguay (34°44′ S, 56°13′ W) at an altitude of 29 m.a.s.l.

The mean annual temperature is 16.8 ºC, and the climate is classified as humid subtropical - Cfa according to the Köppen-Geiger climate classification, with a mean annual precipitation of 1276 mm (Castaño et al., 2011). Weather data were registered during the experiment with an automated weather station located in the area (http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico). Soil type is a Typic Argiudoll, with a silty clay texture.

The experiment was arranged in a randomized complete block design with four replications. Two soil under trellis managements were evaluated: herbicide weeding (HW) and permanent living cover crop (PCC). The HW management consists of a 1.0 m wide weed-free strip under the vine with a combination of herbicides (mainly glyphosate and glufosinate ammonium) to maintain a bare soil. The PCC management corresponds to a *Festuca arundinacea* (Schreb.) crop (“tall fescue”), established in 2011 with a seeding rate of 60 kg/ha. In all cases the alleys between rows were maintained with spontaneous vegetation and mowed as necessary.

Vines were trained to a vertical shoot positioning system (VSP) in N-S oriented rows, with a planting density of 1.1 m between vines and 2.8 m between rows. Cordon-trained vines were pruned to seven two-bud spurs per meter during dormancy. Irrigation water was applied with drip emitters (4 L/min) located directly under the vines and distributed 0.3 m apart. The irrigation criteria had the objective to avoid moderate and/or severe water stress and it was adjusted by weekly monitoring of water stress in leaves. The vineyard canopy, phytosanitary control of fungal diseases (except for Botrytis Bunch Rot) and fertilization were managed following the standards for the integrated production of Wine Grapes (DGSA, 2022).

4.4.2. Bulk soil and rhizosphere sampling

Bulk soil and grapevine rhizosphere samples were collected at three different phenological stages: flowering, veraison and harvest, corresponding to stages 23, 35 and 38 of the Eichhorn & Lorenz (1977) scale, respectively. Bulk soil samples were taken from the plots of the two under-trellis soil managements. For chemical and microbial analysis, 20 soil core samples per replicate (four per management) were randomly collected between grapevines with a soil core auger (2 cm diameter) at 0–15 cm depth, mixed and homogenized by sieving with 5 mm mesh size (roots and stones were removed) immediately after sampling. For microbial analysis, samples were stored at –20ºC until total community DNA (TC-DNA) extraction. Simultaneously and following the same methodology, 0-5 cm bulk soil samples were taken and stored at 5ºC until the assessment of potentially oxidizable Carbon. For determination of aggregate size distribution, an undisturbed soil core (20×20×20 cm) was taken from each replicate. Samples for rhizosphere microbiota analysis were taken from the vine roots found in the sampled soil (0-15 cm depth). Vine roots were gently brushed to remove loosely adhering soil and pooled to a composite sample. Rhizosphere was extracted from 5 g of roots by Stomacher treatment followed by centrifugation according to Schreiter et al. (2014). Rhizosphere pellets were stored at –20ºC until TC-DNA extraction.

4.4.3. Culture-dependent rhizosphere communities quantification

Total bacteria and fungi, *Trichoderma* spp., actinomycetes, *Bacillus* spp. and *Pseudomonas fluorescens* CFU per gram were determined by suspending 1 ml of rhizosphere extract in 9 ml of saline solution 1.5% and mixed vigorously. Several dilution series (*Trichoderma*: 1/10 and 1/100; *Bacillus* and total fungi: 1/100, 1/1000 and 1/10000; Actinomycetes, total bacteria and *Pseudomonas*

fluorescens: 1/100000, 1/1000000 and 1/10000000) of the rhizosphere soil suspension was made with sterile saline solution 1.5% and100 µl aliquots of each dilution were spread onto three replicate plates containing THSM (Williams et al., 2003), Starch-Casein Agar (Leoni & Ghini, 2003), LB and King B media (Hiddink et al., 2005) for *Trichoderma* spp., actinomycetes, *Bacillus* spp. and *Pseudomonas fluorescens* respectively. For *Bacillus* spp. quantification, prior to dilution, the 1/10 suspension was heated for 4 minutes at 80ºC. CFUs were counted following 5-7 days of incubation at 25°C in the dark for *Trichoderma* spp., total fungi and actinomycetes and 2-3 days for *Bacillus* spp., total bacteria, and *Pseudomonas fluorescens*.

4.4.4. Total community-DNA extraction and amplicon sequencing

Total community DNA (TC-DNA) was extracted from 500 mg of frozen bulk soil and from 500 mg of frozen rhizosphere pellet (wet weight) by the FastDNA SpinKit for Soil (MP Biomedicals, Santa Ana, CA, USA), using a FastPrep-24-bead-beating system, following the manufacturer's instructions. The integrity and concentration of TC-DNA were acceded by agarose gel electrophoresis and Nanodrop 2000 spectrophotometer (Invitrogen, USA) respectively. Bulk soil (at veraison only) and rhizosphere prokaryotic community (flowering, veraison and harvest) were characterized. Prokaryotic community by sequencing amplicons of the 16S rRNA gene (V3-V4 region) using Illumina MiSeq [2 x 300 bp, paired/end] at Macrogen Inc. (Korea) with primers 341F 5'-CCTACGGGNGGCWGCAG-3' and 805R 5'-GACTACHVGGGTATCTAATCC-3' (Takahashi et al., 2014). Fungal community by sequencing amplicons of the ITS2 rRNA gene using Illumina MiSeq [2 x 300 bp, paired/end] at Macrogen Inc. (Korea) with primers 3F: 5'-GCATCGATGAAGAACGCAGC-3' and 4R: 5'-TCCTCCGCTTATTGATATGC-3' (Schmidt et al., 2013).

4.4.5. Bioinformatic and statistical analysis

Raw sequence reads quality were evaluated using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and results summarized into a single report using MultiQC (Ewels et al., 2016). The DADA2 v1.24.0 package (Callahan et al., 2016) was used to filter and trim the reads, correct by error learning, merge pairs and identify amplicon sequence variants (ASVs). Prokaryotic reads (16S) were trimmed after 299 and 279 bp for forward and reverse respectively. Fungi (ITS2) reads were trimmed after 245 nucleotides only for reverse reads. Also, 16S and ITS2 reads with expected errors higher than 5 for forward and reverse reads were discarded. The remaining filters were used by default. Reads were merged with a minimum overlap of 20 bp and a maxMismatch of 0. Chimeric sequences were identified and removed. ASVs taxonomic assignment was realized against reference training dataset SILVA SSU rel. 132 database (Quast et al., 2013) for prokaryotes and UNITE reference database (sh_general_release_dynamic_s_10.05.2021) for fungi (Nilsson et al., 2019). Sequences classified as chloroplasts, mitochondria and eukaryotes were discarded. Classification and general data manipulation was done using phyloseq v1.34.0 (McMurdie & Holmes, 2013).

Sequence data was analyzed under the framework of R software 4.0.4 (https://www.r-project.org/) and MicrobiomeAnlayst (Chong et al., 2020). Alpha diversity indexes (Shannon, Pielou's evenness, Species Richness and Phylogenetic Diversity) were estimated for rhizosphere samples using microbiome v1.12.0 (Lahti et al, 2017). To test the effect of the soil under vine management on the prokaryotic and fungal communities, a non-parametric multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity index was run with 10000 permutations using the vegan v2.5.7 (Oksanen et al., 2020). A pairwise PERMANOVA was performed to test for

differences between managements. The analysis of multivariate homogeneity of group dispersions (variances) was done using betadisper from vegan package. A principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity index as distance method was performed to illustrate the difference between the communities' composition for prokaryotic and for fungi separately in the three phenological stages (flowering, veraison and harvest).

The core vine rhizosphere microbiota, defined as the ASVs shared between managements, was visualized by Venn diagrams using the VennDiagram v1.6.20 (Chen, 2018), using those ASVs with a prevalence equal to or greater than 75%. Unique ASVs for each management were identified as those ASVs that are present in only one of the soil managements in all replicates.

To assess ASVs relative abundance differences between managements in the rhizosphere, a likelihood ratio test under negative binomial distribution and generalized linear models with data normalized according to developer's recommendations was performed using edgeR v3.34.1 (FDR-corrected $p < 0.05$ and $p < 0.01$ for fungi and prokaryotic communities respectively) and MicrobiomeAnlayst (Chong et al., 2020) was used to run a Linear Discriminant Analysis (LDA) Effect Size (LEfSe) to identify the taxa with the greater effect size to discriminate.

Soil under-trellis management effects on vine productive variables, grape must composition, soil physicochemical and biological properties, plant nutritional status, BBR incidence, total bacteria, total fungi, *Trichoderma* spp., actinomycetes, *Bacillus* spp. and *Pseudomonas fluorescens* culture-dependent quantification, and alpha-diversity indices (Species Richness, Phylogenetic Diversity, Pielou's evenness, Shannon) were evaluated by one-way ANOVA using agricolae v1.3.5 (de Mendiburu, 2020). The block effect was treated as a

random effect and considered in the model. When the effect of management was significant ($p < 0.05$), means were compared by LSD Fisher test, with the p-value corrected by Bonferroni.

The relationship between soil physical, chemical and biological properties and rhizosphere microbial communities was assessed by a Constrained Analysis of Principal Coordinates (CAP) (Anderson & Willis, 2003) using with Bray–Curtis distance from vegan v2.5.7 (Oksanen et al., 2020). The analysis was performed for prokaryotic and fungal communities separately. Significance of the model parameters was determined with permutational multivariate analysis of variance with 999 permutations.

4.4.6. Soil health assessment

Soil respiration (SR) and autoclaved-citrate extractable soil protein (SP) were assessed in air-dried bulk soil samples (0-15 cm depth) and potentially oxidizable Carbon (PoxC) in fresh-bulk soil samples (0 -5 cm depth). These determinations were done following the Comprehensive Assessment of Soil Health (Moebius-Clune et al., 2016).

Air-dried bulk soil samples were analyzed in the Soil, Plant and Water Analysis Laboratory-INIA (http://www.inia.uy/productos-y-servicios/laboratorios/Laboratorio-de-Suelos-Plantas-y-Agua) for electric conductivity (EC, mmhos/cm 25ºC), pH (H2O), N (%), Soil Organic Carbon-SOC (%), Bray 1 (μg P/g), Ca (meq/100g), Mg (meq/100g), K (meq/100g) and Na (meq/100g). Soil bulk density (SBD) was assessed according to McKenzie et al. (2004) and Total Porosity (TP) was estimated as TP (%) = 1 – (SBD/2.65) according to USDA (USDA, 1999). Aggregate size distribution was determined based on Kemper & Chepil (1965).

4.4.7. Nutritional and sanitary plant status

Several leaves opposite to bunches were sampled at flowering and veraison in each replication and sent to the Soil, Plant and Water Analysis Laboratory -INIA for determination of N (%), C (%), % (Ca), Mg (%), K (%) and P (mg/g).

To assess the effect of the under trellis soil management on the vineyard sanitary condition, the percentage of bunches (incidence) showing visible symptoms of Botrytis bunch rot (BBR) as well as the percentage of each bunch that was infected (severity) was determined at harvest by visual inspection using a seven-point scale (0, 1, 5, 25, 50, 75 and 100%) at harvest. BBR severity index (SI) was calculated as follows: $SI = \Sigma (ni * si)/N$, where ni is the number of bunches in each category, si the severity value of the class and N the total number of evaluated bunches. Latent infections of *B. cinerea* at veraison and harvest were assessed in 60 grapes per treatment and plot by freezing berries for 2 hours, previous skin disinfection, and incubated at 22º C for 15 days (Sanzani et al., 2012). Incidence of latent infections was expressed as the percentage of grapes infected/total grapes.

4.4.8. Harvest, yield, and berry must quality

PCC and HW plots (4 replications each) were harvested at the same date. Total fruit yield and clusters per vine were determined, as well as mean cluster weight and berry weights. Berry must quality was determined on a must obtained from 10 kg of berries, and pH, total soluble solids (TSS) by refractometry, and titratable acidity (TA) by titration with NAOH 0.1 N and expressed as tartaric acid equivalents (w/w), were evaluated.

4.5. Results

4.5.1. Effect of under-trellis management on rhizosphere prokaryotic and fungal communities

Culture-dependent quantification of *Trichoderma* spp. and *Bacillus* spp. in the grapevine rhizosphere showed a higher abundance in PCC than HW at all phenological stages. Actinomycetes were more abundant in PCC at harvest, while total fungi were more abundant in HW at veraison. Total bacteria and *Pseudomonas fluorescens* abundance were not influenced by under trellis soil management at any of the phenological stages (Table 1).

Regarding culture-independent studies of rhizosphere microbiota, a total number of 447,908 and 1,775,004 reads were processed for 16S and ITS2 datasets, respectively. A total of 2233 prokaryotic ASVs and 1135 fungal ASVs were obtained after removal of singletons and sequences classified as non-target plant sequences (chloroplasts, mitochondrial and unclassified ASVs at domain level). Sequencing depth was enough to cover the microbial diversity as shown in the rarefaction curves (Supplementary information, Fig. S1). The most abundant prokaryotic taxa were Proteobacteria (60%), followed by Actinobacteriota (20%), Acidobacteriota (6%), Verrucomicrobiota (5%), Bacteroidota (3%) and Firmicutes (3%). Three fungal taxa were dominant: Ascomycota (51%), Rozellomycota (35%) and Basidiomycota (11%) (Supplementary information, Fig. S2).

Table 1 Culture-dependent (CFU/g rhizosphere soil) quantification of total bacteria, total fungi, *Trichoderma*, Actinomycetes, *Bacillus* and *Pseudomonas fluorescens* from grapevine rhizosphere

	Flowering		Veraison		Harvest	
	PCC	HW	PCC	HW	PCC	HW
Total bacteria	8.9±0.06 a	8.9± 0.06 a	8.5±0.07 a	8.7±0.07 a	8.7± 0.04 a	8.8± 0.04 b
Total fungi	ND	ND	**4.12±0.06 a**	**4.38±0.06 b**	4.2 ± 0.05 a	4.2± 0.05 a
Trichoderma spp.	**2.9±0.03 b**	**2.7± 0.03 a**	**2.96±0.03 b**	**2.6 ± 0.03 a**	**3.08 ± 0.05 b**	**2.85±0.05 a**
Actinomycetes	8.1±0.09 a	8.0± 0.09 a	8.3 ± 0.06 a	8.1 ± 0.06 a	**8.9 ± 0.06 b**	**8.6 ± 0.06 a**
Bacillus spp.	**6.5±0.06 b**	**6.1± 0.06 a**	**5.81±0.07 b**	**5.59±0.07 a**	**5.73 ± 0.06 b**	**5.45±0.06 a**
Pseudomonas fluorescens	9.1±0.07 a	8.8± 0.07 a	8.9 ± 0.31 a	8.2 ± 0.31 a	6.72 ± 0.82 a	6.18±0.82 a

No significant differences between PCC and HW were observed for Shannon indexes at any of the phenological stages evaluated, with average values between 5.8 and 6.1, and 2.5 and 3.7 for prokaryotic and fungal communities respectively (Fig. 1 and Supplementary information, Tables S1 and S2). For Species Richness no significant differences were observed for prokaryotic communities (values between 571 and 671), but PCC fungal communities showed higher richness at flowering and veraison compared to HW (values between 212 and 324). This trend was also observed for Phylogenetic Diversity with values between 5334 and 6011 for prokaryotic, and between 9089 and 12559 for fungal communities. Finally, Pielou's evenness varied between 0.91 and 0.95 for prokaryotic communities and between 0.47 and 0.66 for fungal communities (Supplementary information, Table S1 and S2).

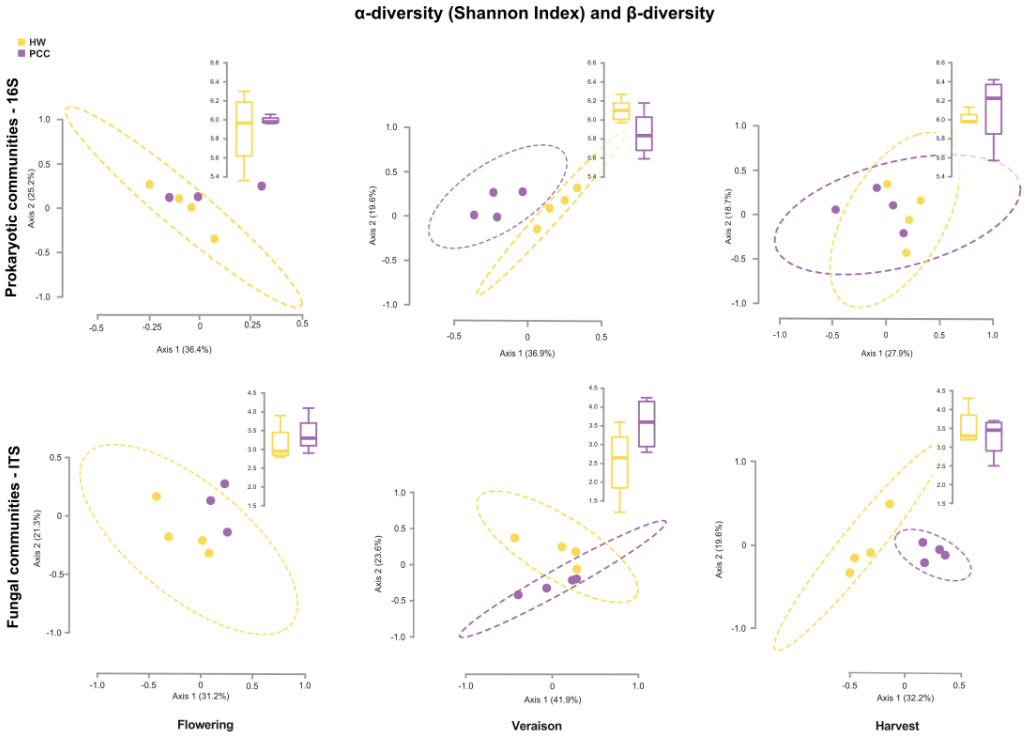


Fig. 1 Alpha (Shannon Index-boxplots) and Beta diversity (Principal Coordinates Analysis-PCoA based on Bray-Curtis dissimilarity) evolution for prokaryotic and fungal rhizosphere communities with different under trellis soil management in grapevine: PCC (permanent cover crop) and HW (herbicide weeding)

Table 2 PERMANOVA results of category effect on rhizosphere prokaryotic and fungal Bray-Curtis distance matrix

Data	Dataset	Factor	F	R^2	*p*-value
Prokaryotic	Rhizosphere: whole	Management	2.03	0.09	0.006
		Sample time	1.59	0.13	0.021
		Management*Sample time	1.16	0.09	0.208
	Rhizosphere: flowering	Management	1.11	0.18	0.361
	Rhizosphere: veraison	Management	3.09	0.34	0.026
	Rhizosphere: harvest	Management	1.15	0.16	0.221
Fungal	Rhizosphere: whole	Management	2.40	0.10	0.014
		Sample time	1.70	0.14	0.02
		Management*Sample time	0.76	0.06	0.826
	Rhizosphere: flowering	Management	1.57	0.24	0.361
	Rhizosphere: veraison	Management	2.07	0.26	0.026
	Rhizosphere: harvest	Management	2.71	0.31	0.026

Fifteen prokaryotic and 17 fungal ASVs differentially abundant for soil managements were detected by LEfSe analysis. Six prokaryotic ASVs were identified with PCC (genus *Pseudomonas*, *Paenarthrobacter*, *Pantoea*, *Buttiauxella*, *Enterobacter* and *Rahnella*) and nine ASVs were characteristic of HW (genus *Aquicella*, *Bauldia*, *Reyranella*, *Nocardioides*, *Streptomyces*,

Bradyrhizobium, *Acidibacter*, *Mycobacterium* and *Steroidobacter*) (**Fig. 2a**). For fungal ASVs, among the 17 taxa identified, 10 were associated with PCC (*Pyrenochaetopsis*, *Dichotomopilus, Nectriopsis, Chloridium*, *Trichoderma, Penicillium*, *Purpureocillium* and two *NA)* and seven with HW (*Aspergillus*, *Penicillium*, *Lipomyces*, *Gongronella* and *Talaromyces*) (**Fig. 2b**). Similarly, through edgeR analysis it was possible to identify specific taxa for each management. Eighteen prokaryotic ASVs were responders for soil management with eight associated with PCC belonging to *Pseudomonas* and *Buttiauxella* genus, and 10 with HW belonging to *Streptomyces*, *Nocardioides*, *Novosphingium* and *Aquicella* genus (**Supplementary information, Table S3**). For the fungal communities, 37 ASVs were identified, six associated with HW and 31 with PCC. Some PCC - ASVs were identified at species level like *Penicillium atrosanguineum*, *Monocillium griseo-ochraceum*, *Nectriopsis fulginicola*, *Chloridium aseptatum*, *Umbelopsis westeae*, *Metarhizium marquandii*, *Xilaria apiculata* and *Trichoderma lixii* (**Supplementary information, Table S4**).

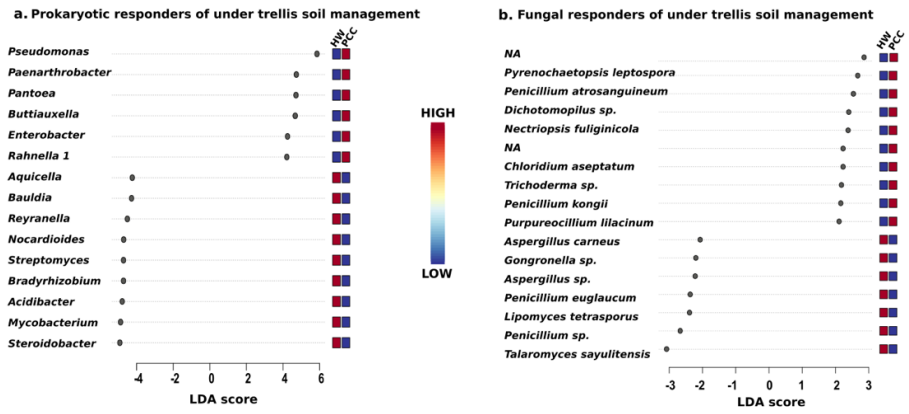


Fig. 2 Differential abundance taxa of the grapevine rhizosphere microbiota with different under trellis soil management (PCC: permanent cover crop, HW: herbicide weeding) determined with Linear Discriminant Analysis (LDA) Effect

Size (LEfSe) of Total Sum Scaling (TSS) normalized counts. a. Prokaryotic. b. Fungal

4.5.2. Core rhizosphere microbiota of grapevine

Regardless of under trellis soil management, a core grapevine rhizosphere microbiota was identified, composed of 139 prokaryotic and 49 fungal ASVs with a prevalence equal or greater than 0.75 in all samples. Three and four phyla represent the prokaryotic and fungal core microbiota of grapevine rhizosphere: Proteobacteria, Actinomycetota and Firmicutes for prokaryotes, and Ascomycota, Basidiomycota, Mortierellomycota and Rozellomycota for fungi. Most of the core prokaryotic ASVs belonged to the genus *Pseudomonas* (30%), *Bradyrhizobium* (19%), *Mycobacterium* (16%), *Nordella* (14%) and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (9%). Regarding the core fungal ASVs, most could not be identified at the genus level (16%), but when possible, the genus more represented were *Mortiriella* (6%), *Aspergillus* (6%), *Pseudeurotium* (6%), *Lipomyces* (4%) and *Purpureocillium* (4%) (**Fig. 3**).

In the PCC management, 61 prokaryotic and 41 fungal unique ASVs were identified. The prokaryotic ones belonged to the genus *Pseudomonas* (51%), *Pantoea* (15%), *Paenarthrobacter* (3%), *Bauldia* (1.6%), *Buttiauxella* (1.6%), *Enterobacillus* (1.6%), *Pseudarthrobacter* (1.6%) and not identified (24.6 %). The most abundant fungal unique ASVs belonged to the genus *Penicillium* (12%), *Vishniacozyma* (10%), *Arhtropsis* (5%), *Exophiala* (5%), *Talaromyces* (5%) and *Trichoderma* (5%) among others.

In the HW management, 105 and 23 ASVs were unique for prokaryotic and fungal communities respectively. The prokaryotic ASVs belonged to *Bradyrhizobium* (22%), *Acidibacter* (12%), *Mycobacterium* (12%) *Steroidobacter* (11%), *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (11%), not identified (10%), *Nordella* (9%), *Candidatus udaeobacter* (3.8%), *Sphingomonas*

(1.9%), *Streptomyces* (1.9%), *Devosia* (1%), *Inquilinus* (1%), *Novosphingobium* (1%), and *Pseudarthrobacter* (1%). For fungal ASVs, predominant genus were *Aspergillus* (17%), *Penicillium* (13%), *Fusarium* (9%), *Lipomyces* (9%) and *Pseudeurotium* (9%) among others.

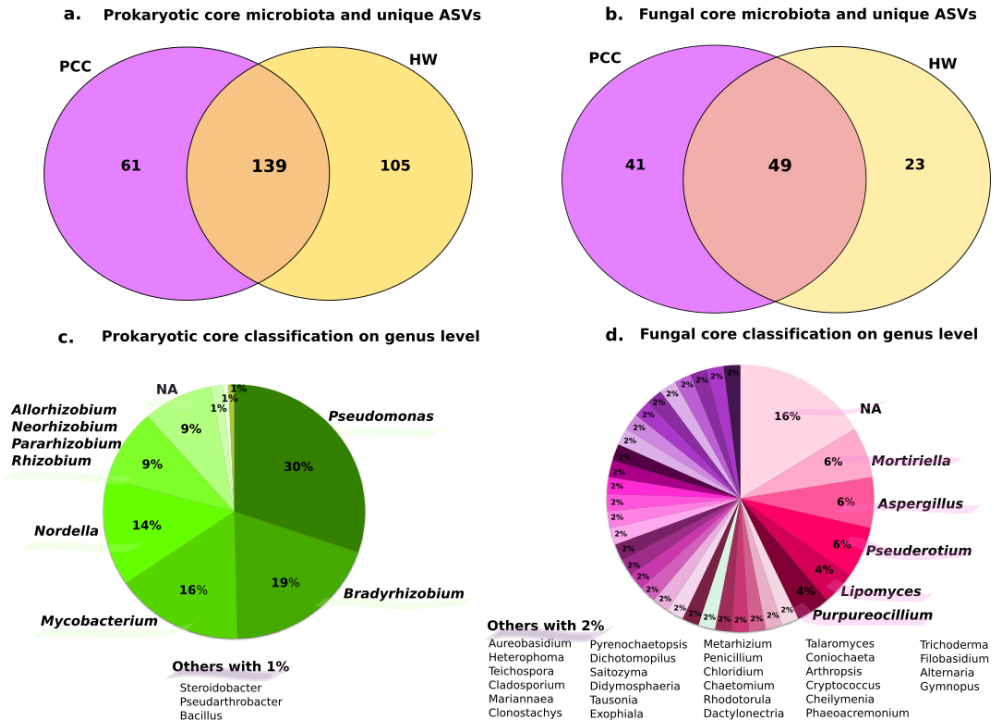


Fig. 3 Venn diagram showing the common and exclusive prokaryotic (a) and fungal (b) ASVs between grapevine rhizosphere with different under trellis soil management (PCC: permanent cover crop, HW: herbicide weeding). Taxonomic classification at genus level of the 139-core prokaryotic (c) ASVs and 49-core fungal (d) ASVs present in grapevine rhizosphere

4.5.3. Effect of soil under-trellis management on soil health

Bulk soil health indicators showed a long-term effect of under trellis management. Soil biological indicators -Potentially Oxidizable Carbon (PoxC), Soil Protein (SP), Soil Respiration (SR) - significantly increased in PCC compared to HW at flowering, veraison and harvest with average values for

PoxC of 1473 and 692 mg PoxC/Kg dry soil, SP of 12 and 5.4 mg soil protein/mg dry soil and SR of 0.93 and 0.54 mg CO_2/g dry soil for PCC and HW respectively (**Fig. 4)**.

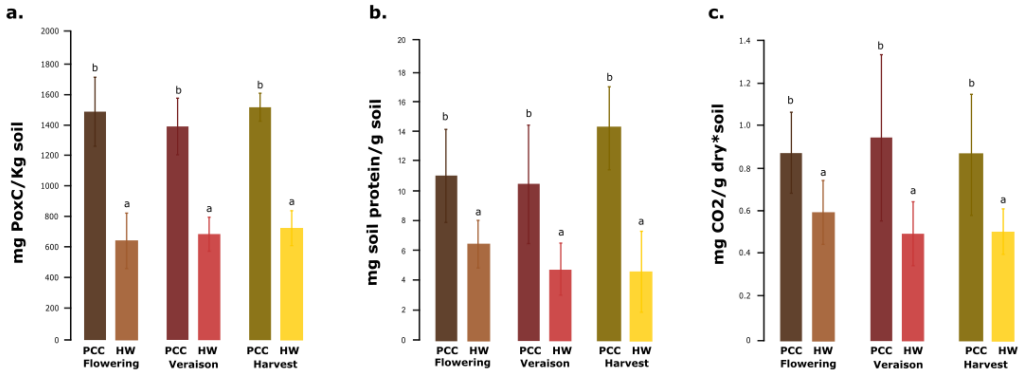


Fig. 4 Soil health (biological) indicators. a- Potentially Oxidizable Carbon of different under trellis soil managements at three phenological stages: flowering, veraison and harvest. Soil depth 0-5 cm. b- Soil protein of different under trellis soil managements at three phenological stages: flowering, veraison and harvest. Soil depth 0-15 cm. c- Soil respiration of different under trellis soil managements at three phenological stages: flowering, veraison and harvest. Soil depth 0-15 cm. Error bars show standard error. Common letters are not significantly different according to the Bonferroni test ($p > 0,05$)

Also, physical parameters -Soil Bulk Density (SBD) and total porosity (TP %) - were significantly different between managements, with SBD values of 0.94 g/cm^2 and 1.1 g/cm^2, and TP values of 64.5 and 55.8 % for PCC and HW respectively. Soil aggregate size distribution showed no large (> 2 mm) nor medium-large (1 – 2 mm) aggregates in any soil management. PCC showed a higher proportion (33.63% vs 20.62% in HW) of medium aggregates (0.5 – 1 mm) while the proportion of small aggregates (0.1 - 0.25 mm) was higher in HW (35.35% vs 18.28% in PCC). No differences were detected in small-medium

aggregates (0.25 – 0.5 mm) proportion between managements (PCC: 46.59%, HW: 42.12%) **(Table 3)**.

Soil chemical parameters - pH, N (%), SOC (%), and Na (meq/100g) - were influenced by under trellis management, showing higher levels in PCC (pH: 7.14 vs 6.75 in HW, N: 0.26 % vs 0.22 % in HW, SOC: 2.67 % vs 2.13 % in HW, Na: 1.33 meq/100 g vs 0.93 meq/100 g in HW), while EC, Ca, Mg, K and P were similar between treatments **(Table 3)**.

Table 3 Physical and chemical parameters in bulk soil. Data represents average and standard error of four replicates. Significant differences among soil under- trellis managements are indicated by different capital letters, according to ANOVA-Bonferroni test (p-value < 0.05)

		PCC	HW
Soil physical parameters	Aggregate size distribution (%)		
	0.1 - 0.25 mm (small)	**18.28 ± 1.5 B**	**35.35 ± 2.11 A**
	0.25 - 0.5 mm (small-medium)	46.59 ± 2.41 A	42.12 ± 2.29 A
	0.5 - 1 mm (medium)	**33.63 ± 2.05 A**	**20.62 ± 1.60 B**
	1 - 2 mm (medium-large)	0.0	0.0
	> 2 mm (large)	0.0	0.0
	Soil Bulk Density (g/cm3)	**0.94 ± 0.05 B**	**1.17 ± 0.05 A**
	Total Porosity (%)	**64.5 ± 1.91 A**	**55.8 ± 1.91 B**
Soil chemical parameters	EC[1] (mmhos/cm, 25ºC)	0.66 ± 0.03 A	0.55 ± 0.03 A
	pH	**7.14 ± 0.07 B**	**6.75 ± 0.07 A**
	N (%)	**0.26 ± 0.01 B**	**0.22 ± 0.01 A**
	SOC[2] (%)	**2.67 ± 0.09 B**	**2.13 ± 0.09 A**
	Ca (meq/100g)	19.51 ± 0.56 A	18.99 ± 0.56 A
	Mg (meq/100g)	3.83 ± 0.1 A	3.51 ± 0.1 A
	K (meq/100g)	0.4 ± 0.03 B	0.34 ± 0.03 B
	Na (meq/100g)	**1.33 ± 0.07 B**	**0.93 ± 0.07 A**
	P (μg P/g)	54.24 ± 4.94 A	51.12 ± 4.94 A

[1] Electric conductivity

[2] Soil Organic Carbon

CAP analysis to assess the relationship between soil variables and prokaryotic and fungal communities of soil under-trellis managements explained the 39 and 45 % of the total variance, respectively. For prokaryotic communities, the CAP1 axis explained 22% of the data set (p – value = 0.091) and was associated with pH (r = 0.56), N (r = 0.50), medium aggregates (0.5 -1 mm) (r = 0.93), small aggregates (0.1 – 0.25 mm) (r = - 0.75), SR (r = 0.81) and BSD (r = -0.80). The CAP2 was associated with pH (r = - 0.53), N (r = - 0.40), SR (r = - 0.22) and BSD (r = 0.22). For fungal communities, the CAP1 axis explained 37% of the data set (p – value = 0.005) and was associated with pH (r = 0.85), N (r = 0.41), medium aggregates (0.5 -1 mm) (r = 0.52), small aggregates (0.1 – 0.25 mm) (r = - 0.35), SR (r = 0.58) and BSD (r = -0.45). The CAP2 was associated with pH (r = 0.28), N (r = 0.52), medium aggregates (0.5 -1 mm) (r = 0.76), small aggregates (0.1 – 0.25 mm) (r = - 0.67), SR (r = 0.80) and BSD (r = - 0.65). (**Supplementary information, Fig. S3**).

4.5.4. Effect of soil under-trellis management on vineyard performance and health

Despite no significant (p>0.05) differences were found between treatments, PCC values were slightly reduced for vine yield (PCC = 3.7 Kg/m, HW = 3.9 kg/m), berry weight (PCC = 1.7 g, HW = 1.76 g) and pruning weight (PCC = 0.36 Kg/m, HW = 0.43 kg/m. In addition, no differences were found for cluster weight (PCC: 284 g, HW: 290 g) and grape must composition at harvest for TSS (PCC: 23.2, HW: 21.1 Brix), TA (PCC: 7.5, HW: 7.1 g/L), and pH (PCC: 3.21, HW: 3.22) (**Supplementary information, Table S5**).

No visual nutrient deficiency symptoms were detected during the season nor statistically significant differences (p > 0.05) between managements were detected in leaf N (PCC: 2.44% and 1.67%, HW: 2.45% and 1.85%), C (PCC: 45.43% and 43.54%, HW: 44.92% and 43.61%), Ca (PCC: 1.93% and 3.23%,

HW: 1.89% and 3.11%), Mg (PCC: 0.16% and 0.17%, HW: 0.17% and 0.21%), K (PCC: 1.1% and 0.97%, HW: 1.05% and 0.93%) and P (PCC: 2.84 mg/g and 2.88 mg/g, HW: 3.19 mg/g and 2.99 mg/g) at flowering and veraison respectively (**Supplementary information, Table S6**).

BBR incidence (visible symptoms) was extremely low, independent of the under-trellis soil management (HW: Incidence = 1.13% and Severity index = 0.08, PCC: Incidence = 0.14 % and Severity index = 0.001, severity data not shown) and no significant differences were detected between managements. The incidence of latent infections was significantly lower under PCC treatment (veraison = 1.9%, harvest = 2.5%) in comparison to HW (veraison = 6.2%, harvest = 7.1%) (**Supplementary information, Table S7**).

4.6. Discussion

4.6.1. Permanent Cover Crop shape rhizosphere microbial diversity and composition

Microbial diversity in grapevine rhizosphere and vineyard soils is shaped by geographic area (Manici et al., 2017), genotype (Dries, Bussotti, et al., 2021; Marasco et al., 2018; Vink et al., 2021), plant age (Berlanas et al., 2019; Manici et al., 2017), phenological stage (Berlanas et al., 2019; Liu & Howell, 2021; Novello et al., 2017; Vink et al., 2021; Zarraonaindia et al., 2015) and management (Carbone et al., 2021; Chou et al., 2018; Hendgen et al., 2018; Longa et al., 2017; Manici et al., 2017; Vega-Avila et al., 2015; Vink et al., 2021). But few studies have assessed the effect of complete permanent cover crop either in soils or on grapevine rhizosphere microbiota (Baumgartner et al., 2005; Chou et al., 2018; Longa et al., 2017; Vink et al., 2021).

In our study, rhizosphere α-diversity of prokaryotic communities was not affected by management at any phenological stage, while rhizosphere fungal

communities from the PCC showed higher values of Species Richness (R) and Phylogenetic Diversity (PD) at flowering and veraison. In terms of β-diversity, we observed that under trellis soil management had an impact on prokaryotic and fungal communities at veraison (grapes start to ripening), while at harvest only on fungal ones. Veraison was reported as a key stage in terms of diversity changes in grapevine-associated fungi (Liu & Howell, 2021). From veraison onwards, dramatic physiological and biochemical changes take place, being anthocyanin synthesis and sugar accumulation in berries one of the most relevant processes. In grapevine, rhizodeposition changes may be significant as well, since quality and quantity of roots compounds exudates differs among phenological stages (Bettenfeld et al., 2022b). In addition, the susceptibility of berries to *Botrytis cinerea* increases from veraison to ripe (harvest) (Deytieux-Belleau et al., 2009), so these phenological stages can be an important stimulus for microbes' selection by the grapevine.

We found that grapevine rhizospheric prokaryotic microbiota was mainly composed by Proteobacteria and Actinobacteria, both accounting for 80% of the relative abundance. Proteobacteria has been reported as the predominant bacteria phylum in grapevine rhizosphere and soils (Berlanas et al., 2019; Dries et al., 2021; Hendgen et al., 2018; Köberl et al., 2020; Marasco et al., 2018; Morgan et al., 2017; Vega-Avila et al., 2015; Zarraonaindia et al., 2015).

Plants select microorganisms from the surrounding soil through root exudation to form its rhizosphere microbiome, and these microorganisms help plants with essential functions, such as promote stress resistance, stimulate growth, nutrient acquisition and disease suppression (Berg et al., 2014; Chaparro et al., 2014). We identified several prokaryotic taxa as management responders, some known by having members with beneficial traits (**Fig. 2**). *Pseudomonas* and *Enterobacteriaceae* family members (*Pantoea*, *Buttiauxella*

and *Enterobacter*) were highly associated with PCC, not only its abundance was higher in grapevine rhizosphere but also unique ASVs were found. Among *Pseudomonas*, several strains and its secondary metabolites are known to enhance plant growth (Plant Growth Promoting Rhizobacteria-PGPR) and reduce disease severity, including grapevines diseases like BBR and Eutypa dieback (Compant et al., 2013; Ganeshan & Kumar, 2005; B. W. M. Verhagen et al., 2010). High relative abundance of *Pseudomonas* in grapevine endosphere has been associated with canker-free tissues in a study conducted in Australia suggesting a plausible antagonistic role of *Pseudomonas* in inhibiting symptoms of grapevine trunk diseases (Niem et al., 2020). *Pantoea*, *Buttiauxella* and *Enterobacter* genus involves members with known PGP features, bioremediation potential and biocontrol capabilities through antagonism of pathogens and/or the induction of plant systemic defenses (Almasia et al., 2020; Aziz et al., 2016; Bell et al., 1995; Walterson & Stavrinides, 2015; Wu et al., 2018). Also, *Rahnella* genus (orden Enterobacterales, family *Yersiniaceae*) have some species or strains reported as PGPR and biological controllers in several crops, including grapevine (Bell et al., 1995; Chen et al., 2007; El-Hendawy et al., 2005). Nevertheless, among these genera there are also plant and animal pathogens (Walterson & Stavrinides, 2015), being *Enterobacter* the most known, as it belongs to ESKAPE antibiotic resistant group (Davin-Regli et al., 2019).

Among the HW prokaryotic responders, several Alphaproteobacteria (*Bauldia*, *Reyranella*, *Bradyrhizobium*), Gammaproteobacteria (*Aquicella*, *Acidibacter* and *Steroidobacter*) and Actinobacteria (*Nocardioides*, *Streptomyces*, *Mycobacterium*) were identified. Those responders have the ability to inhabit HW-soil, which was slightly acid and nutritionally poorer (less N and SOC) than PCC, and with higher soil temperatures (data not shown). *Acidibacter* can reduce ferric iron (Falagán & Johnson, 2014), *Steroidobacter*

and *Bradyrhizobium* have members involved in nitrate reduction (Fahrbach et al., 2008) and nitrogen fixation (Ormeño-Orrillo & Martínez-Romero, 2019), and *Bauldia* can use several carbon sources including mono- and disaccharides and organic acids (Yee et al., 2010). *Aquicella* genus includes species that can grow in temperatures ranging from 30 °C to 43 °C (Santos et al., 2003), the range of soil temperatures found in bare soils in summer. Among the Actinobacteria, three genera were associated with HW: *Nocardioides*, *Streptomyces* and *Mycobacterium*. The long history of under trellis herbicide application may promoted the selection of these microorganisms in the grapevine rhizosphere with HW, as these microorganisms are known by its role on nitrogen fixation and pesticides degradation and by its ability to decompose the more resistant and indecomposable organic matter in soils (Bhatti et al., 2017). A *Nocardioides* isolate from atrazine-treated agricultural soils has been reported to degrade a variety of s-triazine herbicides (Topp et al., 2000). Beyond the actinomycetes role in biodegradation and nutrient cycling, a great biotechnological potential has been reported for this group, for instance the *Streptomyces* ability to produce antimicrobial compounds and inhibits several plant pathogens (Abatneh, 2021; Bhatti et al., 2017).

The major fungal phyla found in our study was Ascomycota (51 %) in accordance with previous works (Berlanas et al., 2019; Carbone et al., 2021; Liu & Howell, 2021; Martínez-Díaz et al., 2019; Morgan et al., 2017), followed by Rozellomycota with 35 % of the relative abundance. Seventeen fungal ASVs were identified as responders of under trellis soil management (**Fig. 2b**), in PCC several had beneficial traits while detrimental ones were prevalent in HW. *Pyrenochaetopsis leptospora* a soil borne fungi associated with gramineous plants, found in grapevine rhizosphere under PCC could be related with the *F. arundinacea* cover crop, (de Gruyter et al., 2010). *Purpureocillium lilacinum* a

widely distributed soil-habituated fungi with biocontrol ability against plant parasitic nematodes and insects (Chen & Hu, 2022) was also in PCC. Interestingly, *Trichoderma* was identified as a PCC responder either by LEfSe analysis, edgeR and the culture-independent quantification. *Trichoderma* is well known for its ability to promote plant growth and disease suppression by antagonism and mycoparasitic activity, and through elicitation of localized or systemic resistance responses, including *Botrytis cinerea* and *Plasmopara viticola* in grapevines (Estrada-Rivera et al., 2019; Harman et al., 2004; Hermosa et al., 2012; O'Neill et al., 1996; Perazzolli et al., 2008, 2011; Salas-Marina et al., 2011, 2015; Shoresh et al., 2010). Four *Penicillium* were identified as responders for soil management, *P. atrosanguineum* and *P. kongii* were more abundant in PCC while *P. euglaucum* and *Penicillium* sp. in HW along with *Talaromyces sayulitensis* which is closely related with *Penicllium*. Also, two *Aspergillus* were responders of HW. Both *Penicillium* and *Aspergillus* are potentially food-borne, can produce mycotoxins and induce allergenic responses (Bennett, 2007; Perrone & Susca, 2017; Yilmaz et al., 2014) while *Aspergillus carneus* can degrade Trifluralin, a selective pre-emergence herbicide (Zayed et al., 1983).

4.6.2. Permanent Cover Crop promotes soil health

Soil health improved in PCC compared with HW as showed by soil respiration rate (↑ 69%), soil protein content (↑ 131%) and the potentially oxidizable carbon content (↑ 113%). Also, soil N (↑ 15%) and SOC (↑ 25%) were improved in PCC-soil. Similar trends in soil respiration, soil N and SOC changes with cover crops in relation to bare soils were observed by previous studies (Chou & Heuvel, 2019; Gattullo et al., 2020; Karl et al., 2016; Tarricone et al., 2020). Carbon availability (SOC and PoxC) in PCC-soil favored a higher microbial activity than HW-soil, reflected by the increased soil respiration rate at

all phenological stages. Microbial communities play a major role in C cycling and its activity mediate the most relevant biogeochemical processes (Bhattacharyya et al., 2022; Kuzyakov & Blagodatskaya, 2015). Inputs of labile C in soils produces microbial hotspots, being the rhizosphere one of the most relevant due to the plant rhizodeposits (Kuzyakov & Blagodatskaya, 2015; Mohanty et al., 2021). PCC-soil not only was influenced by rhizodeposits of grapevine but also the cover crop, which due to its perennial condition provides a contribution of above- and belowground plant biomass and rhizodeposits for soil microbiota growth in a steady basis. Soil microbiota has an important role on soil aggregation, where bacteria contribute strongly to both macro- and microaggregates while fungi strongly affect macro-aggregation (Lehmann 2017). In our study, the absence of medium-large and large aggregates -independent of under trellis soil management- is explained by its long history of intensive and conventional agricultural practices. Nevertheless, a positive effect on soil aggregation size distribution, SBD (↓ 20%), and TP (↑ 16%) was observed in PCC-soil. The formation of aggregates is stimulated by microbiological bonding agents such as extracellular polysaccharides, fungal mycelia, and glycoproteins (Bhattacharyya et al., 2022). For instance, *Pseudomonas* - a taxon identified as a PCC responder- is known by its exopolysaccharides production, an important factor affecting soil aggregation (Sandhya & Ali, 2015). Also, *Pseudomonas* spread rate in soils decreases with increased BSD, highlighting the influence of soil structure on bacterial activity (Juyal et al., 2021).

4.6.3. Permanent Cover Crop maintains performance and improves vineyard health

The presence of *F. arundinacea* as an under-trellis cover crop did not show differences in nutritional plant status, vine yield nor grape must composition in our study. The irrigation was crucial for the PCC grapevine management to

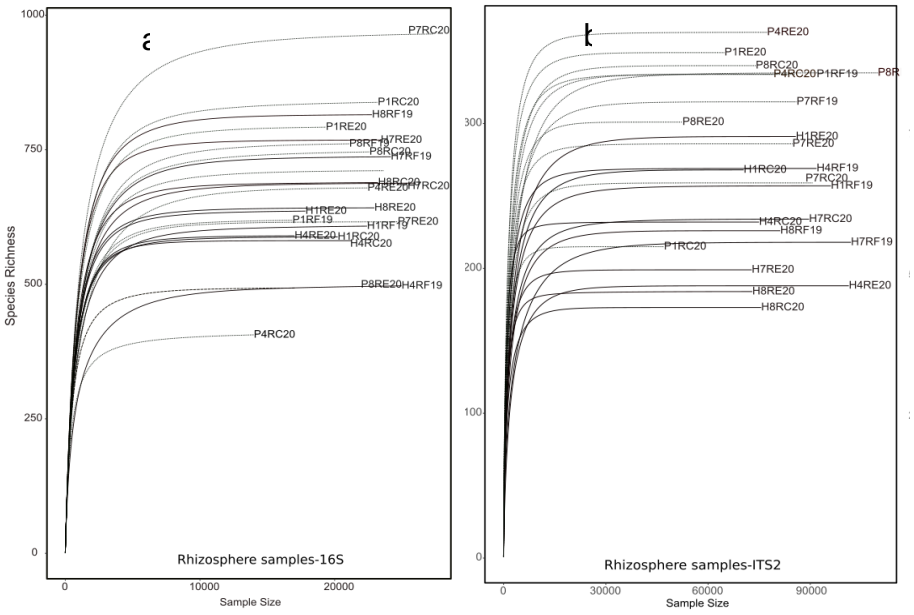
achieve a similar productive performance to HW, as precipitation were very scarce during the growing season (2019/20: 441 mm, historical: 784 mm). The competition for water between the grapevine and the under-trellis cover crop during the maximum growth period can be detrimental for vine yield (Coniberti et al., 2018b; Gattullo et al., 2020). In line with our results, vineyards managed with under trellis cover crops, either for table grapes or wine, found no differences in productivity and quality (Chou & Heuvel, 2019; Gattullo et al., 2020).

Despite of soil management, a very low incidence of BBR at harvest was observed, explained primarily by the agroclimatic conditions -low precipitation- registered during the growing season. However, a reduction in latent infections of *B. cinerea* at veraison and harvest was detected with PCC (**Supplementary information, Table S7**). In previous studies, BBR low incidence was observed in an irrigated Tannat/SO4 vineyard with PCC and the hypothesis beyond was that seasonal variations in water status (drought stress imposed by the complete cover crop) triggers molecular processes resulting in an improved immune response (Coniberti, Ferrari, Disegna, García Petillo, et al., 2018 b). Since the irrigation criteria in our study was to avoid water stress and no differences in vine yield, plant vigor and nutritional status were detected, the reduction in *B. cinerea* latent infections with PCC cannot be explained by water stress. Therefore, we set up a new hypothesis: the lower incidence of BBR in grapevine with PCC is the result of defense response mechanisms facilitated by the rhizosphere microbiome.

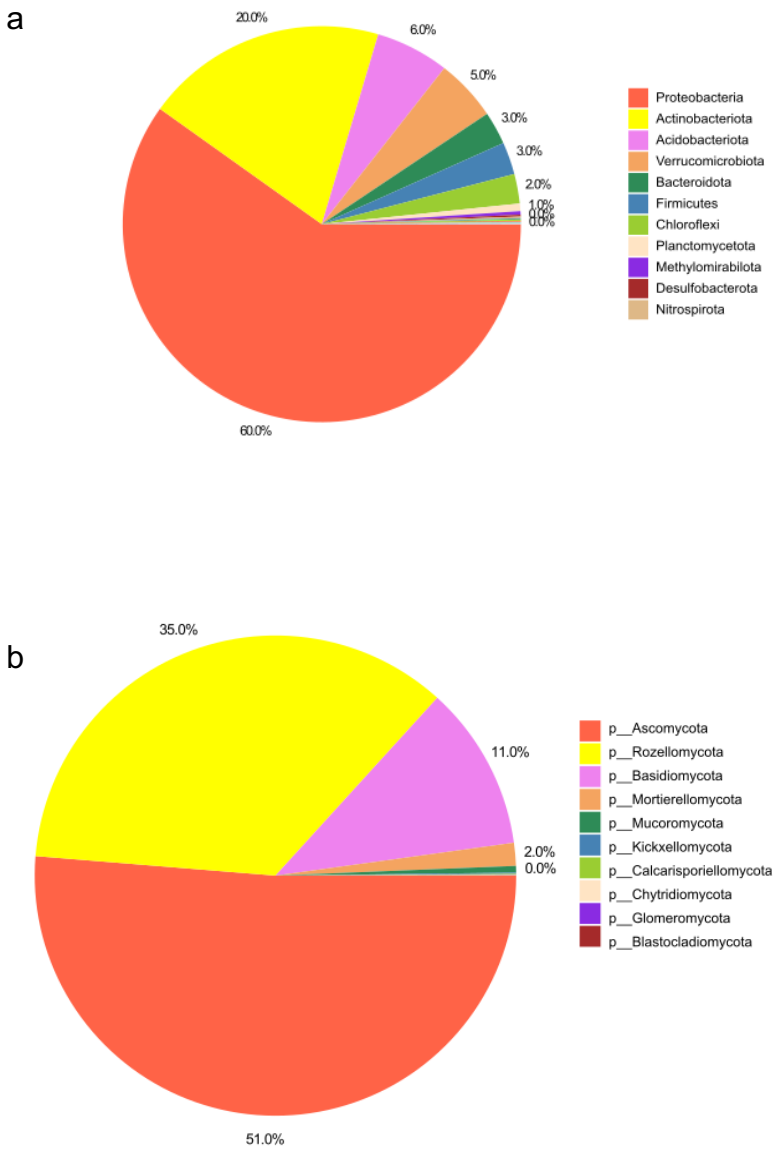
Plant-associated microorganisms and their theatre of activity - defined as plant microbiome (Berg, Rybakova, Fischer, Cernava, Vergès, Charles, Chen, Cocolin, Eversole, Corral, Kazou, Kinkel, Lange, Lima, Loy, Macklin, Maguin, Mauchline, McClure, Mitter, Ryan, Sarand, Smidt, Schelkle, Roume, Kiran, Selvin, Souza, van Overbeek, et al., 2020) - has a profound impact on plant

development, productivity and health (Mendes et al., 2013). As well as beneficial microbes - commonly isolated from the rhizosphere and soil - are inoculated in agriculture for disease management, cover crops used as intercrops, can also promote pathogen suppression by encouraging the proliferation of beneficial microbes (Bender et al., 2016a; Vukicevich et al., 2016). The enrichment of beneficial microorganisms on the grapevine roots is not a random but a targeted process, therefore the agronomical practices that promote healthy vineyards aided by soil microbial communities must be locally studied and encouraged. This idea has been embraced by a new paradigm in plant protection, the Agroecological Crop Protection (ACP) (J.-P. Deguine et al., 2021). ACP aims to promote the ecological health of agroecosystems based on the management of two main pillars: biodiversity (both aerial and edaphic) and soil health. The strategy is to promote interactions between living (plant, animal, microbial) communities both below and above the ground to make agroecosystems less susceptible to biotic stresses and encourages practices which directly or indirectly enhance ecosystem services, such as pest regulation. In this context, PCC (in combination with irrigation) appears as a sustainable agricultural practice for vineyards systems to promote biodiversity and soil health. However, more studies are needed to achieve a long-term assessment of taxa responders associated with PCC management and its functional meaning in relation with grapevine defense responses to support its viability as a successful practice.

4.7. Supporting information



Supplementary information, Fig. S1 Rarefaction curves of rhizosphere samples a- prokaryotic. b- fungal



Supplementary information, Figure S2 Grapevine rhizosphere relative abundances of a. prokaryotic phyla b. fungal phyla

Supplementary information, Figure S3 2D representation of Constrained Analysis of Principal coordinates (CAP) based on Bray–Curtis distance of prokaryotic and fungal communities. BSD: bulk soil density, Agg_0.1: small aggregates (0.1 - 0.25 mm), Agg_0.5: medium aggregates (0.5 – 1 mm), N: soil Nitrogen, Respiration: soil respiration and pH: soil pH

Supplementary information, Table S1 Alpha diversity of prokaryotic communities

Time	Alpha diversity Index	PCC	HW
Flowering	Shannon diversity	5.79 ± 0.09	5.89 ± 0.07
	Species richness	571.1 ± 15	597.8 ± 12
	Pielou's evenness	0.91 ± 0.01	0.92 ± 0.01
	Phylogenetic diversity	5524.4 ± 390	5404.3 ± 302.3
Veraison	Shannon diversity	5.88 ± 0.09	6.1 ± 0.09
	Species richness	579.3 ± 39	604.5 ± 39
	Pielou's evenness	0.92 ± 0.01	0.95 ± 0.01
	Phylogenetic diversity	5830.4 ± 483	5537.5 ± 483
Harvest	Shannon diversity	6.07 ± 0.14	6.03 ± 0.14
	Species richness	671. 3 ± 63	587.8 ± 63
	Pielou's evenness	0.94 ± 0.01	0.95 ± 0.01
	Phylogenetic diversity	6010.8 ± 407	5333.7 ± 407
No statistical differences were detected between treatments *LSD Fisher (Alfa=0.05)* *Procedimiento de corrección de p-valores: Bonferroni*			

Supplementary information, Table S2 Alpha diversity of fungal communities

Time	Alpha diversity Index	PCC	HW
Flowering	Shannon diversity	3.65 ± 0.3 A	3.11 ± 0.23 A
	Species richness	324 ± 6.97 A	232.8 ± 5.4 B
	Pielou's evennes	0.63 ± 0.05 A	0.57 ± 0.04 A
	Phylogenetic diversity	12050.6 ± 372 A	9487.3 ± 288 B
Veraison	Shannon diversity	3.53 ± 0.57 A	2.5 ± 0.57 A
	Species richness	311 ± 17.3 A	212 ± 17.3 B
	Pielou's evennes	0.61 ± 0.1 A	0.47 ± 0.1 A
	Phylogenetic diversity	12558.7 ± 487 A	9110.5 ± 487 B
Harvest	Shannon diversity	3.23 ± 0.18 A	3.52 ± 0.18 A
	Species richness	275.5 ± 32.03 A	217.5 ± 32.03 A
	Pielou's evennes	0.58 ± 0.03 A	0.66 ± 0.03 A
	Phylogenetic diversity	11335.1 ± 1245.6 A	9089.4 ± 1245.6 A
LSD Fisher (Alfa=0.05) *Procedimiento de corrección de p-valores: Bonferroni*			

Supplementary information, Table S3 edgeR: bacterial ASVs biomarkers FDR adjusted < 0.1

ASV responders	LogFC	Genus
PCC vs HW		
ASV868	7.13965947	*Pseudomonas*
ASV1036	6.93375432	*Pseudomonas*
ASV1191	7.30868197	*Buttiauxella*
ASV462	-7.88736741	*Streptomyces*
ASV1297	6.55267831	*Pseudomonas*
ASV396	-6.85248946	*Nocardioides*
ASV322	-8.00953235	*Streptomyces*
ASV1612	6.19678917	*Pseudomonas*
ASV345	-7.7378906	*Streptomyces*
ASV265	5.70560378	*Pseudomonas*
ASV573	-7.1999674	*Novosphingobium*
ASV1681	6.79301231	*Buttiauxella*
ASV1868	6.47429778	*Pseudomonas*
ASV876	-6.1233141	*Novosphingobium*
ASV267	-7.36158549	*Streptomyces*
ASV938	-6.79306257	*Novosphingobium*
ASV1039	-7.25324069	*Aquicella*
ASV613	-6.4558085	*Novosphingobium*

Supplementary information, Table S4 edgeR: fungal ASVs biomarkers FDR adjusted < 0.05

ASV responders	LogFC	Genus	Species
PCC vs HW			
ASV45	12.6	*g__Penicillium*	*s__atrosanguineum*
ASV21	13.6	*NA*	*NA*
ASV95	10.6	*g__Talaromyces*	*NA*
ASV123	10.6	*g__Monocillium*	*s__griseo-ochraceum*
ASV160	-9.4	*g__Idriella*	*NA*
ASV155	9.9	*NA*	*NA*
ASV234	9.7	*g__Penicillium*	*NA*
ASV107	-7.8	*g__Aspergillus*	*s__carneus*
ASV294	8.8	*NA*	*NA*
ASV209	9.9	*g__Trechispora*	*NA*
ASV238	7.3	*NA*	*NA*
ASV51	5.2	*g__Nectriopsis*	*s__fuliginicola*
ASV159	10.6	*g__Chloridium*	*s__aseptatum*
ASV265	9.1	*NA*	*NA*
ASV464	7.6	*g__Arthrographis*	*NA*
ASV353	8.4	*g__Umbelopsis*	*s__westeae*
ASV322	8.0	*g__Metarhizium*	*s__marquandii*
ASV56	-11.6	*g__Macrophomina*	*s__pseudophaseolina*
ASV247	9.5	*NA*	*NA*
ASV42	4.0	*g__Dichotomopilus*	*NA*
ASV199	9.7	*g__Xylaria*	*s__apiculata*
ASV225	5.1	*g__Talaromyces*	*s__atricola*
ASV11	-4.6	*g__Talaromyces*	*s__sayulitensis*
ASV12	-4.9	*g__Aspergillus*	*s__terreus*
ASV240	9.0	*NA*	*NA*

ASV347	8.5	*NA*	*NA*
ASV374	7.8	*NA*	*NA*
ASV14	5.8	*g__Trichoderma*	*s__lixii*
ASV41	-4.4	*g__Penicillium*	*s__euglaucum*
ASV431	7.9	*NA*	*NA*
ASV254	9.2	*g__Pseudogymnoascus*	*s__appendiculatus*
ASV740	6.6	*g__Cladophialophora*	*NA*
ASV62	3.5	*g__Trichoderma*	*NA*
ASV227	6.9	*g__Geomyces*	*s__asperulatus*
ASV364	7.8	*NA*	*NA*
ASV328	6.1	*g__Phaeosphaeria*	*NA*
ASV372	6.7	*g__Lycoperdon*	*s__pratense*

Supplementary information, Table S5 Productive variables and grape must composition of Tannat grapevines and berries under two soil managements: PCC (permanent cover crop) and HW (herbicide weeding). Data represents average and standard error of four replicates. Significant differences among soil under vine managements are indicated by different capital letters, according to ANOVA-Bonferroni test (Alfa= 0.05)

	PCC	HW
Productive variables		
Cluster weight (g)	0.28 ± 0.02	0.29 ± 0.02
Berry weight (g)	1.7 ± 0.02	1.76 ± 0.02
Vine yield (kg/m)	3.71 ± 0.32	3.91 ± 0.32
Prunning weight (kg/m)	0.36 ± 0.04	0.43 ± 0.04
Grape must composition		
Soluble solids (Brix)	23.25 ± 1.26	21.1 ± 1.26
Tritatable acidity (g/L)	7.5 ± 0.03	7.1 ± 0.03
pH	3.21 ± 0.03	3.22 ± 0.03
No statistically significant differences were detected between treatments		

Supplementary information, Table S6 Nutrient status of Tannat grapevines (leaves) under two soil managements: PCC (permanent cover crop) and HW (herbicide weeding) at flowering and harvest

Phenological state	Flowering		Veraison	
Management	PCC	HW	PCC	HW
N (%)	2.44 ± 0.05	2.45 ± 0.05	1.67 ± 0.08	1.85 ± 0.08
C (%)	45.43 ± 0.14	44.92 ± 0.14	43.54 ± 0.36	43.61 ± 0.36
Ca (%)	1.93 ± 0.08	1.89 ± 0.08	3.23 ± 0.17	3.11 ± 0.17
Mg (%)	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.21 ± 0.01
K (%)	1.1 ± 0.05	1.05 ± 0.05	0.97 ± 0.05	0.93 ± 0.05
P (mg/g)	2.84 ± 0.19	3.19 ± 0.19	2.88 ± 0.3	2.99 ± 0.3
No statically differences were detected between treatments				

Supplementary information, Table S7 Botrytis bunch rot incidence of visible symptoms and latent infections in bunches and berries respectively under two soil managements: PCC (permanent cover crop) and HW (herbicide weeding) at veraison and harvest. Data represents average and standard error of four replicates. Significant differences among soil under vine managements are indicated by different capital letters, according to ANOVA-Bonferroni test (Alfa= 0.05)

BBR incidence	Veraison		Harvest	
	PCC	HW	PCC	HW
Visible symptoms in bunches (%)	-	-	0.14 ± 0.58 A	1.13 ± 0.58 A
Latent infections in berries (%)	1.88 ± 0.86 A	6.25 ± 0.86 B	2.5 ± 0.53 A	7.08 ± 0.5 B

4.8. References

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5. Permanent cover crop reduces *Botrytis* bunch rot associated to changes in berry skin anatomy, antioxidant contents and rhizosphere bacteria[2]

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5.1. Resumen

Los cultivos de cobertura permanentes ofrecen múltiples beneficios, incluyendo la mejora de la salud del suelo y de las plantas, así como la reducción en el uso de agroquímicos. Este estudio examinó el impacto del manejo del suelo bajo la fila de la vid—desmalezado con herbicida (HW) vs. cultivo de cobertura permanente (PCC) con *Festuca arundinacea*—sobre la Podredumbre Gris del Racimo (BBR) causada por *Botrytis cinerea*, y los posibles mecanismos de defensa vegetal involucrados. El experimento se llevó a cabo en un viñedo experimental irrigado de Tannat injertado sobre SO4, donde HW y PCC se mantuvieron durante más de 10 años. Durante la temporada 2020/2021 se evaluaron la incidencia e intensidad de BBR, la progresión de la enfermedad en hojas inoculadas, los niveles de fitoalexinas y la actividad antioxidante en hojas y bayas, la anatomía de la piel de las bayas y las comunidades microbianas de la rizósfera. PCC se asoció con menor incidencia ($p = 0.001$) e intensidad ($p < 0.001$) de BBR para síntomas visibles e infecciones latentes ($p = 0.002$), y con una progresión reducida de la enfermedad en hojas inoculadas ($p < 0.001$). Las bayas de PCC presentaron una cutícula más gruesa ($p < 0.001$) y epidermis más gruesa ($p = 0.008$), mayor contenido total de polifenoles en hojas ($p = 0.01$) y mayor actividad de ascorbato peroxidasa en la piel de las bayas ($p < 0.001$). Se observó una composición diferencial de la comunidad microbiana en la cosecha, con taxones diferenciales identificados como indicadores de cada manejo del suelo, con mayor abundancia de bacterias potencialmente beneficiosas en PCC. La respuesta mejorada de las vides a BBR bajo manejo con PCC constituye una respuesta compleja explicada por el efecto combinado de varios factores.

Palabras clave: manejo del suelo, mecanismos de defensa vegetal, *Botrytis cinerea*.

5.2. Summary

Permanent cover crops offer multiple benefits, including improved soil and plant health and reduced use of agrochemicals. This study examined the impact of under-vine soil management—herbicide weeding (HW) vs. permanent cover crop (PCC) with *Festuca arundinacea*—on Botrytis Bunch Rot (BBR) caused by *Botrytis cinerea*, and potential plant defense mechanisms involved. The experiment was conducted on an irrigated experimental vineyard of Tannat grafted on SO4 where HW and PCC were imposed for more than 10 years. During the 2020/2021 season, BBR incidence and intensity, disease progression in inoculated leaves, levels of phytoalexins and antioxidant activity in leaves and berries, berry skin anatomy and rhizosphere microbial communities were evaluated. PCC was associated with lower BBR incidence ($p = 0.001$) and intensity ($p < 0.001$) for visible symptoms and latent infections ($p = 0.002$), and reduced disease progression in inoculated leaves ($p < 0.001$). Berries from PCC exhibited thicker cuticle ($p < 0.001$) and epidermis (p-value = 0.008), higher total polyphenol content in leaves ($p = 0.01$) and increased ascorbate peroxidase activity in berry skin ($p < 0.001$). Distinct microbial community composition was observed at harvest, with differential taxa identified as indicators for each soil management, with a higher abundance of potentially beneficial bacteria in PCC. The enhanced response of the grapevines to BBR when managed with PCC is a complex response explained by the stacked effect of several factors.

Keywords: soil management, plant-defense mechanisms, *Botrytis cinerea*.

5.3. Introduction

Botrytis bunch rot (BBR) or gray mold, caused by *Botrytis cinerea* (Pers.: Fr), is one of the most important diseases of grapevines worldwide, particularly in temperate and humid climates where dense canopies are conducive for the

disease (Elmer & Michailides, 2007). Conventional BBR management relies on fungicide applications, canopy management, and vigor reduction through targeted cultural practices (Jacometti et al., 2010). However, fungicide resistance, environmental concerns, and rising production costs pose limitations to chemical control strategies, making integrated and non-chemical alternatives increasingly important (Hobbelen et al., 2014; Shtienberg, 2007).

With a mean annual precipitation of 1276 mm (Castaño et al., 2011), 90% of Uruguayan vineyards rely on rainfed conditions (Pereyra & Ferrer, 2023). Therefore, soil management focuses on maintaining bare soil under the vine through herbicide application to minimize competition between adjacent vegetation and grapevines during water deficit periods (Coniberti et al. 2018b). However, when excessive rainfall happens, the absence of under-vine vegetation can result in excessive vine growth and vigor, along with conducive conditions for disease development, including BBR, and negative impacts on grape quality (Coniberti, Ferrari, Disegna, García Petillo, et al., 2018; Vanden Heuvel & Centinari, 2021). Additionally, environmental and regulatory concerns regarding herbicide use have intensified the search for alternative soil managements (European Commission, 2025; Rivas-Garcia et al., 2022).

Among sustainable vineyard soil managements, cover crops have gained attention, either with sown or spontaneous vegetation, single or mixed crops (typically grasses and legumes), annual or permanent, and complete or partial ground cover with different termination methods (e.g., mowing, tilling, herbicides) (Abad et al., 2021). While inter-row cover crops are commonly used to prevent soil erosion and improve soil structure, their implementation under the vine row (under-vine cover crops -UVCC) has been less studied in its effects on vine health and disease susceptibility (Vanden Heuvel & Centinari, 2021). UVCC offers a valuable strategy for managing excessive precipitation, by reducing vine

vigor and enhancing canopy aeration, and indirectly impact on disease development (Vanden Heuvel & Centinari, 2021). However, the direct effect of UVCC on promoting plant health remains nuclear.

UVCC may influence anatomical and biochemical traits of grape berries, such as cuticle thickness, epidermis structure, and the accumulation of antioxidant compounds, which could improve physical and chemical barriers against different pathogens, among them *B. cinerea* (Guilpart et al., 2017; Jacometti et al., 2010; Valdés-Gómez et al., 2008). Previous studies have linked UVCC to reductions in BBR incidence (Bernaschina et al., 2023; Coniberti, Ferrari, Disegna, García Petillo, et al., 2018; Jacometti et al., 2007), but the underlying physiological and biochemical responses in the grapevine, such as the production of phytoalexins, antioxidant activity, and structural changes in berry skin, are not well understood. Understanding these defense responses is crucial for optimizing UVCC as a tool for disease management.

UVCC also influences bulk soil (Chou et al., 2018) and rhizosphere microbiota (Dries et al. 2021), emerging as a promising approach to modulate grapevine-associated microbiota and promoting plant health (Dries et al. 2021). The composition and structure of the grapevine microbiota are influenced by multiple factors, including compartmentalization within the plant (grapes, flowers, leaves, canes, trunk, roots), soil properties, pedoclimatic conditions, genetic and intra-vineyard diversity, and vineyard management (Bettenfeld et al., 2022a). Among these factors, the use of cover crops influences the abundance, activity, and composition of bulk soil and rhizosphere microbiota, fostering beneficial microorganisms (Bernaschina et al., 2023; Kim et al., 2020; Lumini et al., 2010; Sharma et al., 2018; Vukicevich et al., 2016). Several fungal and bacterial strains isolated from soils have demonstrated pathogen suppression via direct antagonism or indirect plant-mediated mechanisms when reintroduced into the

soil (Aziz et al., 2016; Berg et al., 2017, 2021; B. Verhagen et al., 2011; B. W. M. Verhagen et al., 2010).

This study aims to evaluate the impact of contrasting soil management practices under the vine -herbicide weeding (HW) versus a *Festuca arundinacea* permanent cover crop (PCC) - on the incidence and severity of BBR, grapevine performance (vigor and must composition) and microbial communities' composition in the Tannat grapevine rhizosphere. Additionally, we explore potential plant defense mechanisms that may explain differences in disease susceptibility between treatments, including changes in berry skin anatomy, phytoalexin levels and enzymatic and non-enzymatic antioxidant activity. By focusing on these defense responses, this study provides new insights into soil management as a tool for disease management and contributes to the development of sustainable viticulture. We hypothesized that UVCC, particularly PCC with *F. arundinacea*, contributes to BBR management through changes in rhizosphere microbiota and associated defense responses.

5.4. Materials and methods

5.4.1. Experimental site and vineyard management

The study was conducted in an 18 years-old experimental vineyard of Tannat grafted onto SO4 during 2020-2021 season, at the experimental station of the Instituto Nacional de Investigación Agropecuaria, Canelones, Southern Uruguay (34°44′ S, 56°13′ W). The vineyard is established on a typical Argiudoll soil, with a silty clay texture. The humid subtropical climate, Cfa according to Koppen-Geiger classification, has mean annual temperature of 16.8 ºC and mean annual precipitation of 1276 mm (Castaño et al. 2011). During the experiment, effective precipitation (mm), relative humidity (%) and degree days (> 10°C) were recorded by an automatic meteorological station located 150 m

from the experimental vineyard (http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico).

Two under-vine soil management strategies were implemented: Herbicide Weeding (HW) and Permanent Cover Crop (PCC) (Fig. 1). HW consisted of a weed-free zone of 1.0 meter wide maintaining bare soil through a combination of glyphosate and glufosinate ammonium herbicides. PCC consisted of a permanent living ground covered with 'tall fescue' (*Festuca arundinacea* Schreb.), established in 2011, seed rate of 60 kg/ha. For both HW and PCC treatments, the spaces between vine rows (alleys) were allowed to develop spontaneous vegetation and was mowed as needed to allow vineyard management. The experimental vineyard was arranged in a randomized plot design with four replications.

Vines were trained to a vertical shoot positioning, with rows-oriented north-south. Planting density was 1.1 m between vines within the rows and 2.8 m between rows. During the dormant season, the cordon-trained vines underwent pruning to leave seven, two-bud spurs per meter. To maintain comparable nitrogen levels across treatments, urea was applied at a rate of 25 kg N ha^{-1} when shoots reached approximately 40 cm in length, followed by a second application of 50 kg N ha^{-1} immediately after harvest. Drip irrigation was established with emitters delivering 4 L min^{-1} positioned beneath the vines at 0.3-meter intervals, and irrigation decisions were based on preventing moderate to severe water stress, -1.2 to -1.6 MPa (Deloire et al., 2020), monitored bi-weekly through using a leaf pressure chamber (Soil Moistue Equipment Corp. Santa Barbara, CA). All other vine managements - canopy, phytosanitary (excluding treatments for BBR), and fertilization – adhered to the standards for the national integrated wine grape production (DGSA, 2022).

Fig. 1 Experimental vineyard of Tannat grafted onto SO4 with two under-vine soil management. (A) General view; planting density: 2.8 m between rows and 1.1 m within the rows. (B) Herbicide weeding (HW). (C) Permanent cover

crop (PCC, *Festuca arundinacea*, tall fescue'). Location: Rincón del Colorado, Canelones, Uruguay.

5.4.2. Assessment of vegetative growth, canopy characteristics, and grape must composition

In winter, pruning weight was recorded and averaged per plot. Shoot elongation rate was assessed weekly from bud break to end of vegetative elongation by repeated measurements of shoot length on two shoots from two representative vines per plot. At harvest, on the same date for PCC and HW plots, total fruit yield and the number of clusters per vine were recorded, and mean cluster weight and berry weight were calculated. A composite sample of 10 kg berries was used to obtain berry must, to analyze free amino nitrogen (FAN), total soluble solids (TSS), titratable acidity (TA) and pH. FAN was determined after adding 38% formol solution adjusted to pH 8 and titrated with NaOH 0.1N. TSS was measured with a digital refractometer (Atago DBX-55). TA was quantified by titration with 0.1 N NaOH and expressed as tartaric acid equivalents (w/w).

5.4.3. Disease assessment and challenge inoculation

To evaluate the presence of BBR at harvest, bunches were visually inspected and classified based on symptom expression. The assessment included both disease incidence (percentage of symptomatic bunches) and severity (affected area per bunch), using a six-grade ordinal scale: 0 (0%), 1 (<5%), 2 (5–25%), 3 (25–50%), 4 (50–75%), and 5 (>75%). Disease intensity, an index which includes both healthy and diseased bunches, was calculated with the formula: $SI = \Sigma (ni \times si)/N$, where 'ni' corresponds to the number of bunches in each severity category, si' is the numerical value of the respective class, and 'N' is the total number of bunches assessed. To detect latent *B.*

cinerea infections at both veraison and harvest, 60 berries per treatment and plot were sampled. Berries were subjected to a two-hour freezing period, followed by surface disinfection, and then incubated at 22°C for 15 days following the protocol described by Sanzani et al. (2012). The incidence of latent infections was expressed as the proportion of infected berries.

Challenge inoculation was performed as described by Aziz et al. (2016). Briefly, 20 young fully expanded leaves per plot were excised from the shoots at flowering, veraison and harvest, and immediately placed into cooled and moistened bags and taken to the laboratory. Leaves were then washed with sterile distilled water and placed on sterile wet absorbing paper in Petri dishes. One needle-prick wound was applied on the middle of the abaxial surface of each leaf, and the fresh wounds were covered with 10-µl drops of a conidial suspension of *B. cinerea* (1×10^6 conidia per ml). The Petri dishes were then placed at 22 °C with a 16 h light photoperiod. Disease incidence (proportion of infected leaves) and severity (percentage of leaf area affected) was assessed at 4, 7 and 12 days pos-inoculation. The area under the disease progress curve (AUDPC) was calculated from severity data at each phenological stage to quantify disease development over time.

5.4.4. Defense responses

5.4.4.1. Enzymatic and non-enzymatic antioxidant activity

Twenty berries and leaves per plot and treatment were collected at flowering, veraison, and harvest, immediately frozen in liquid nitrogen, and stored at –80 °C until enzymatic analysis. Crude enzyme extracts were obtained from 100 mg of powdered berry skin or leaf tissue using an ice-cold phosphate buffer (pH 7.0) (10% glycerol, 0.2% Triton X-100, 2 mM EDTA, 2 mM ascorbic acid, 1 mM phenylmethanesulfonyl fluoride, 1 mM β-mercaptoethanol, and a pinch of polyviniylpyrrolidone). Samples were homogenized in buffer and

centrifuged at 10,000 × g for 15 minutes at 4 °C, then the supernatants were collected and stored on ice for immediate enzymatic assays (Signorelli et al. 2013). Enzymatic activities were determined according to Conde-Innamorato et al. (2024) and normalized to total soluble protein. Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined based on its ability to inhibit the photochemical reduction of nitroblue tetrazolium and expressed as units per minute per milligram of protein. Catalase (CAT, EC 1.11.1.6) activity was assessed by monitoring the decomposition of hydrogen peroxide at 240 nm, using a molar extinction coefficient of $\varepsilon = 39.4\ mM^{-1}\ cm^{-1}$. Peroxidase (POD, EC 1.11.1) activity was quantified by the oxidation of a chromogenic substrate in the presence of H_2O_2, measured at 460 nm. Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined by the decrease in absorbance at 290 nm due to ascorbate oxidation, using a molar extinction coefficient of $\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$. The total phenolic contents (TPC) were determined by the Folin–Ciocalteu method at 725 nm (Sánchez-Rangel et al., 2013). and expressed as mg gallic acid equivalents per gram of extract (mg GAE/g dry weight, FW).

5.4.4.2. Phytoalexin quantification in leaves and berry skin

Stilbenes were extracted from lyophilized leaves and berry skins (1 g) using a pestle and mortar in an ethyl acetate: methanol (50:50, v/v) buffer solution at a ratio of 10 mL of buffer per gram of tissue. The extraction flasks were incubated at room temperature (~25 °C) under continuous agitation using an orbital shaker at ~100-150 rpm for 24 h and then centrifuged at 10,000 g for 10 min (Liu et al., 2013). The supernatants were evaporated using a rotary vacuum evaporator at 40 °C and 100 rpm, and the dried residues were resuspended in 1 mL of 100% methanol. The extracts were stored in dark vials at -20 °C until HPLC analysis (Shimadzu Liquid Chromatograph LC-20AT; Column Oven CTO-20A; Diode Array Detector SPD-M20A; Fluorescence

Detector RF-10AXL). All extraction steps were performed under subdued light conditions to prevent compound degradation. Before HPLC analysis, the extracts were clarified by filtration through a Millex-GN 0.22-µm filter (Millipore, St-Quentin en Yvelines, France). Samples (20 µL) were injected onto a Lichrocart C-18 reversed-phase column (250 × 4 mm, 5 µm, Waters, St-Quentin en Yvelines, France), equilibrated with a 90:10 (v/v) H_2O/acetonitrile mobile phase. Phytoalexins were eluted using a linear gradient from 10% to 85% acetonitrile at a flow rate of 1 mL/min. Detection was performed using a photodiode array detector coupled to a fluorometer (λex = 330 nm, λem = 374 nm). Quantification of *trans*-resveratrol and *ε*-viniferin was based on standard calibration curves (*trans*-resveratrol: y = 2E+08x + 217198, R^2 = 0.9913; *ε*-viniferin: y = 6E+07x + 45543, R^2 = 0.996). Calibration curves were constructed for concentrations ranging from 1 to 25 µg/mL for trans-resveratrol and 1 to 30 µg/mL for ε-viniferin. Three independent extractions were performed per sample. Reference standards used were ε-viniferin (CAS #62218-08-0, phyproof®, Phytolab, Germany) and trans-resveratrol (CAS #501-36-0, Merck KGaA, Germany).

5.4.4.3. Anatomical studies in berry skin

Five berries per plot with the same maturity level and size were sampled at harvest. The equatorial face of the berry was cut and immersed in FAA fixative (5 mL 38% formaldehyde, 5 mL glacial acetic acid, and 90 mL 70% ethanol) for then processed by the paraffin embedding technique (Li, 1978). The dehydration of the berry samples and their paraffin embedding was carried out in a MTP carousel tissue processor (SLEE medical GmbH, Germany), using a series of ethanol of ascending gradation (from 70 to 100%) and Tertiary butyl alcohol (TBA) as an intermediate for paraffin infiltration. The inclusion of paraffin-embedded samples in paraffin cubes was performed in a Tissue Embedding

Machine (MedGroup). The berry skin sections were obtained in a precision rotary microtome (SLEE, Germany). At least 30 serial sections were performed for each berry sample (7 μm). Subsequently, the serial sections were stained with Safranin-Fast green double on a YIDI automatic staining equipment (model YABO700) and mounted with Canada balsam.

The samples were observed using an optic microscope (Axio Imager, Zeiss, Germany). Six fields of view were selected, and the following anatomical parameters of the berries were measured using Image J software (Schneider et al., 2012). The pericarp was divided into the cuticle (wax coating on the berry skin), epidermal cells (outermost two cell layers), sub-epidermal cells or hypodermis (the seven to nine cell layers immediately below the epidermis) and mesocarp (polygonal flesh cells from the outside to the core) (Battista et al., 2015). The thickness was measured for the first three regions, and the number of cell layers was counted for epidermis and hypodermis.

5.4.5. Rhizosphere sampling

Sampling was performed at flowering 2020 and harvest 2021, phenological stages 23 and 38 respectively from modified Eichorn and Lorenz scale (Coombe, 1995). To obtain vine roots, four composite soil samples were collected per treatment (HW, PCC) and phenological stage. Each soil sample consisted of 8 soil cores collected with a soil core auger, 2 cm diameter at 0-15 cm depth along the vine. Vine roots were gently brushed to remove loosely adhering soil and pooled to a composite sample. Rhizosphere was extracted from 5 g of roots suspended in saline solution 0.3 % by Stomacher treatment followed by centrifugation according to Schreiter et al. (2014). Rhizosphere pellets were stored at –20ºC until DNA extraction.

5.4.6. DNA extraction and amplicon sequencing

Rhizosphere total community DNA was extracted from 500 mg of frozen rhizosphere pellet (wet weight) by the FastDNA SpinKit for Soil (MP Biomedicals, Santa Ana, CA, USA), using a FastPrep-24-bead-beating system, according to manufacturer's instructions. The integrity and concentration of DNA were acceded by agarose gel electrophoresis and Nanodrop 2000 spectrophotometer (Invitrogen, USA) respectively. Prokaryotic community was characterized by sequencing amplicons of the 16S rRNA gene (V3-V4 region) with primers 341F 5'-CCTACGGGNGGCWGCAG-3' and 805R 5'-GACTACHVGGGTATCTAATCC-3' (Takahashi et al., 2014) and fungal community with primers 3F: 5'-GCATCGATGAAGAACGCAGC-3' and 4R: 5'-TCCTCCGCTTATTGATATGC-3' (Schmidt et al., 2013). of the ITS2 rRNA gene, using Illumina MiSeq [2 x 300 bp, paired/end] at Macrogen Inc. (Korea)

5.4.7. Bioinformatic and statistical analysis

Raw sequence reads quality were evaluated using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The DADA2 v1.24.0 package (Callahan et al., 2016) was used to filter and trim the reads, correct by error learning, merge pairs and identify amplicon sequence variants (ASVs). Prokaryotic reads (16S) were trimmed after 280 and 250 bp for forward and reverse, respectively. Fungi (ITS2) reads were trimmed after 245 nucleotides only for reverse reads. Also, 16S and ITS2 reads with expected errors higher than 3 for forward and reverse reads were discarded. Reads were merged with a minimum overlap of 20 bp and a maxMismatch of 0. Chimeric sequences were identified and removed. ASVs taxonomic assignment was realized against reference dataset SILVA SSU rel. 138.1 database (Quast et al., 2013) for prokaryotes and UNITE reference database

(sh_general_release_dynamic_04.04.2024) for fungi (Nilsson et al., 2019). Classification and general data analysis was done using phyloseq v1.34.0 (McMurdie & Holmes, 2013).

Sequence data was analyzed under the framework of R software (version 4.0.4) (https://www.r-project.org/). Before performing beta diversity analyses, the phyloseq object was rarefied to an equal sequencing depth across all samples to minimize biases associated with differences in library size. To test the effect of the soil under vine management on the prokaryotic and fungal communities, a non-parametric multivariate analysis of variance (PERMANOVA) based on weighted Unifrac dissimilarity index for prokaryotic communities and Bray Curtis for fungal, was run with 10000 permutations using the vegan v2.5.7 (Oksanen et al., 2020). A pairwise PERMANOVA was performed to test for differences between treatments. The analysis of multivariate homogeneity of group dispersions (variances) was done using betadisper from vegan package.

To identify differentially abundant taxa between HW and PCC, we employed the ALDEx2 package (Fernandes et al., 2014) in R. The analysis was performed on the phyloseq object for fungal and prokaryotic communities at harvest, using the aldex() function with 500 Monte Carlo samples and the Welch's t-test. P-values were adjusted for multiple testing using the False Discovery Rate (FDR) method. Volcano plots were generated using the ggplot2 (Wickham, 2016) package to visualize effect sizes and significance levels, with taxa below FDR-adjusted thresholds highlighted.

5.4.8. Statistical analysis

Statistical analyses were performed in R software (version 4.3.0) using linear mixed models (LMM) or generalized linear mixed models (GLMMs) implemented in the lme4 (Bates et al., 2015) or glmmTMB (Brooks et al., 2017) packages, respectively. Response variables were modeled as functions of

under-vine soil management, with block included as a random intercept. Model diagnostics were conducted using DHARMa, and fixed effects were evaluated using Type II Wald chi-square tests. Marginal means were estimated for pairwise comparisons. Disease incidence was analyzed using beta-binomial GLMMs with a logit link. Disease intensity was analyzed using a zero-inflated beta regression, where severity categories were converted to their midpoints to obtain continuous proportions for parametric modeling (Chiang & Bock, 2022). AUDPC was estimated from disease severity using agricolae package. The model included soil management and phenological stage as fixed effects, with block as a random intercept, and was fitted using a Gamma distribution with a log link. Vine water status, vegetative growth, canopy characteristics, grape must composition, anatomical traits, phytoalexin quantification and enzymatic and non-enzymatic antioxidant activity were modeled with the most appropriate family distribution selected based on goodness-of-fit measures and residual analysis.

A principal component analysis (PCA) using the singular value decomposition approach was carried out with “factoextra” package using all disease assessments and defense response variables at harvest (Supporting Information, Table S1) to determine which properties accounted for most of the variability between HW and PCC. The overall significance of the PCA, of each PC axis, and of the contributions of each observed variable to the significant axes were assessed based on permutation-based statistical tests with “arleyc/PCAtest” package (Camargo, 2022).

A distance-based redundancy analysis (dbRDA) was conducted separately for prokaryotic and fungal communities to assess the influence of plant variables on community microbial composition. The explanatory variables included in the model—cuticle thickness, incidence of visible symptoms, APX activity in berry

skin, and total phenolic content in leaves—were selected based on prior statistical analyses of plant defense, biochemical, and anatomical responses. For prokaryotic communities, the analysis was based on a weighted UniFrac distance matrix, obtained through the phyloseq package (McMurdie & Holmes, 2013). For fungal communities, a Bray–Curtis dissimilarity matrix was calculated from ASV abundances using the vegan package (Oksanen et al., 2020).

5.5. Results

5.5.1. Agroclimatic data

The 2020/2021 season had significantly lower accumulated precipitation compared to historical averages (Supporting information, Fig. S1A), with 455 mm of total rainfall, falling short of the historical average of 784 mm (Coniberti, Ferrari, Disegna, Dellacassa, et al., 2018). From budbreak to flowering (70 days after budbreak - DAB), rainfall amounted to 136 mm, well below the historical average of 227 mm. Similarly, from full bloom to veraison (70-130 DAB) precipitation reached 144 mm compared to the historical average of 291 mm, and from veraison to harvest (130-180 DAB) 175 mm versus the historical average of 266 mm.

Accumulated Growing Degree Days (base 10°C GDD) during the season was 1632 GDD, also under historical average for the region (1700 GDD) (Supporting information, Fig. S1B). Relative humidity remained below 80% on average throughout the season. It averaged 74% from budbreak to full bloom, dipped to 65% from full bloom to veraison, and then rose back to 74% on average from veraison to harvest (Supporting information, Fig. S1C).

5.5.2. Plant stem water potential, vegetative growth, canopy characteristics and grape must composition

Despite irrigation was applied, between 95 and 114 DAB, grapevine with PCC showed significant lower values of Ψstem than HW (Chisq = 17.69, $p < 0.001$), coinciding with phenological stages 31 (berries pea-sized) and 32 (beginning of bunch closure) of modified Eichhorn-Lorenz scale (Supporting information, Fig. S2A). Shoot elongation rate varied significantly over time ($F = 38.09$, $p < 0.001$) with the highest rates observed between 67 and 83 DAB, from stage 19 (beginning of flowering) to 26 (complete caps fall) of modified Eichhorn-Lorenz scale (Supporting information, Fig. S2B). However, under-vine soil management did not affect shoot elongation rate ($F = 0.0007$, $p = 0.979$) or any of the following variables: yield per plant ($F = 3.8$, $p = 0.144$), pruning weight ($F = 2.5$, $p = 0.208$), bunch weight ($F = 0.7$, $p = 0.456$), berry weight ($F = 0.5$, $p = 0.521$), TSS ($F = 1.04$, $p = 0.382$), pH ($F = 1.5$, $p = 0.311$), TA ($F = 6.2$, $p = 0.09$) or FAN ($F = 1.64$, $p = 0.200$) (Table 1).

Table 1. Canopy characteristics and grape must composition of Tannat grapevines under herbicide weeding (HW) and permanent cover crop (PCC) at harvest 2021. Values represent means ± SE. No significant differences were observed between soil management (Tukey's test, $p < 0.05$)

	HW	PCC
Canopy characteristics		
Yield per plant (kg/m)	3.5 ± 0.6	3.1± 0.6
Prunning weight (kg/m)	0.5 ± 0.1	0.4 ± 0.1
Bunch weight (g)	250 ± 0.0	237 ± 0.0
Berry weight (g)	1.9 ± 0.1	1.9 ± 0.1
Grape must composition		
Total Soluble Solids (°Brix)	23.6 ± 0.6	24.5 ± 0.6
pH	3.5 ± 0.0	3.5 ± 0.0
Titratable acidity (g/L)	4.1 ± 0.1	4.6 ± 0.1
Free Amino Nitrogen (g/L)	70.9 ± 6.2	73.6 ± 6.2

5.5.3. Disease assessment

At harvest disease incidence of visible symptoms in Tannat bunches ranged from 5% to 11% for HW and from 0% to 6% for PCC (Fig. 2A). Disease incidence was significantly different among treatments, with mean values of 8% in HW and 1% in PCC (Chisq = 10.1, p = 0.001). Also, disease intensity was significantly different among treatments (Chisq = 13.66, p = 0.0002) despite the low values of affected area observed: 0.63% for HW and 0.04% for PCC (Fig. 2B).

The incidence of latent infections in berries at veraison was low and ranged from 1% to 5% for HW and from 0.05% to 3% for PCC (Fig. 2C), and no significant differences were found between treatments (Chisq = 2.79, p = 0.09). At harvest, latent infections increased, ranging from 13% to 17% for HW and

from 5% to 8% for PCC, and differences were detected for average values (HW: 14.5% and PCC 6.7%; Chisq = 9.66, p = 0.002) (Fig. 2D).

In inoculated leaves, AUDPC of *Botrytis* lesions differed significantly between phenological stages (Chisq = 17.8, $p < 0.001$) and under-vine soil management (Chisq = 11.44, p = 0.003). On average, AUDPC was higher at flowering (PCC: 24.1, HW: 64.3) compared to veraison (PCC: 4.3, HW: 13.2) and harvest (PCC: 10.8, HW: 15.2) (Fig. 2E, 2F and 2G). Only at flowering, AUDPC was significantly different between HW and PCC.

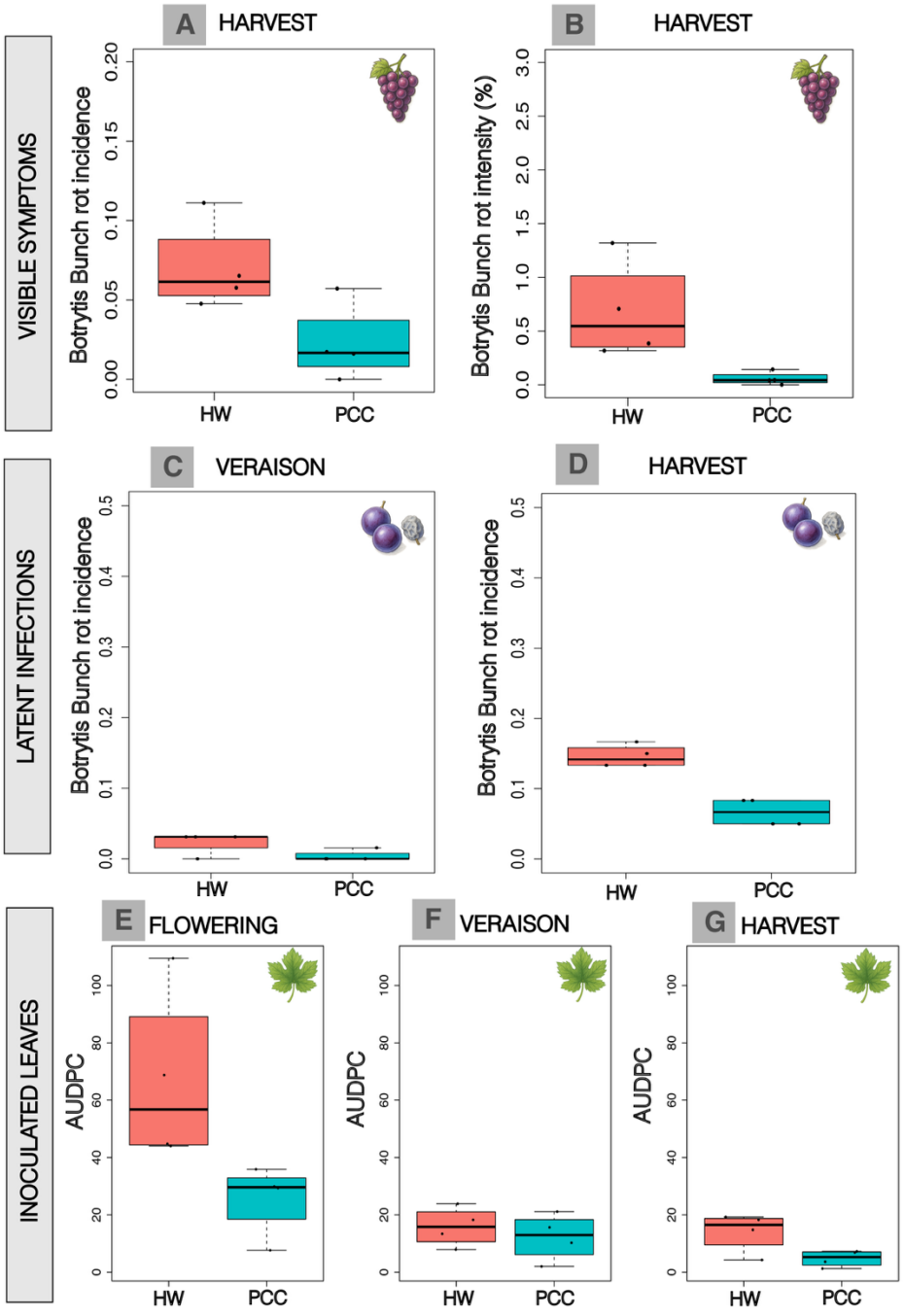

Fig. 2 Assessment of *Botrytis cinerea* infections in Tannat berries and leaves under herbicide weeding (HW) and permanent cover crop (PCC) soil

management. Visible symptoms of *Botrytis* Bunch Rot incidence (A) and intensity (B) at harvest. Latent infections of BBR incidence at veraison (C) and harvest (D). Area under the disease progress curve (AUDPC) in inoculated leaves at flowering (E), veraison (F) and harvest (G).

5.5.4. Biochemical and anatomical defense responses

Antioxidant activity showed variable responses to soil management. SOD (Chisq = 0.95, p = 0.330), POD (Chisq = 0.106, p = 0.744), and CAT (Chisq = 1.11, p = 0.291) activity exhibited no significant differences between soil management, regardless of the organ at any phenological stage (Table 2). APX activity in berry skins at harvest was significantly higher in PCC compared to HW (Chisq = 36.88, $p < 0.001$). TPC in leaves at harvest from plants under PCC were higher and significantly different from HW (Chisq = 5.79, p = 0.01) (Table 2).

The content of the phytoalexins ε-viniferin and trans-resveratrol quantified in leaves and berry skin under HW and PCC showed no significant differences between them ($p > 0.05$). However, differences were found among phenological stages, only for *trans*-resveratrol in leaves (F = 5.28, p = 0.02), with lower values observed at flowering. *ε*-viniferin levels were similar across phenological stages (F = 1.91, p = 0.176) (Table 3).

Anatomical differences in berry skin at harvest were observed between HW and PCC (Table 4, supporting information Fig. S3). Overall, the number of cell layers varied from 7 to 11 in the skin, remained constant at 2 in the epidermis, and ranged between 5 and 9 in the hypodermis. The number of hypodermal and total skin cell layers was significantly higher under PCC (Chisq = 6.83, p = 0.009 and Chisq = 5.72, p = 0.02 respectively). Thickness values spanned from 140.2 to 317.6 *μ*m for the skin, 1.4 to 2.8 *μ*m for the cuticle, 124.2

to 294.7 μm for the hypodermis, and 10.5 to 23.7 μm for the epidermis. PCC resulted in significantly thicker cuticle (2.29 ± 0.05 μm, Chisq = 28.87, $p < 0.001$) and epidermis (18.8 ± 0.57 μm, Chisq = 11.2, $p = 0.008$) compared to HW (1.85 ± 0.05 μm and 16.2 ± 0.54 μm, respectively; $p < 0.01$). Hypodermis and total skin thickness tended to be greater under PCC (210 ± 8.67 μm and 231 ± 8.72 μm) than HW (192 ± 8.17 μm and 210 ± 8.43 μm), but these differences were not statistically significant for hypodermis thickness ($p > 0.05$).

Table 2 Enzymatic and non-enzymatic antioxidant activity in Tannat leaves and berry skin under herbicide weeding (HW) and permanent cover crop (PCC) at flowering, veraison, and harvest 2021. Values represent means ± SE. Different letters indicate significant differences between soil management within phenological stages and organs (Tukey's test, $p < 0.05$). DW: Dry Weight; GAE: Gallic Acid Equivalent

Antioxidant compound	Organ	Soil management	Flowering	Veraison	Harvest
Superoxide dismutase-SOD (U mg protein-1)	Leaves	HW	0.763 ±0.284 a	0.279 ± 0.274 a	0.584 ± 0.274 a
		PCC	0.782 ±0.284 a	0.502 ± 0.274 a	0.597 ± 0.274 a
	Berry skin	HW		3.020 ± 0.284 a	4.637 ± 0.284 a
		PCC		3.362 ± 0.284 a	4.863 ± 0.284 a
Peroxidase-POD (U mg protein-1)	Leaves	HW	0.122 ±0.02 a	0.052 ±0.02 a	0.072 ± 0.02 a
		PCC	0.128 ± 0.02 a	0.064 ± 0.02 a	0.038 ± 0.02 a
	Berry skin	HW		0.254 ± 0.02 a	0.223 ± 0.02 a
		PCC		0.271 ± 0.02 a	0.160 ± 0.02 b
Catalase-CAT (mmol H2O2 min-1 mg protein -1)	Leaves	HW	0.089 ± 0.11 a	0.052 ± 0.11 a	0.006 ± 0.11 a
		PCC	0.069 ± 0.11 a	0.107 ± 0.11 a	0.064 ± 0.11 a
	Berry skin	HW		0.244 ± 0.11 a	1.152 ± 0.11 a
		PCC		0.231 ± 0.11 a	1.810 ± 0.11 a
Ascorbate	Leaves	HW	0.003 ± 0.01 a	0.005 ± 0.01 a	0.003 ± 0.01 a

peroxidase-APX (mmol min-1 mg protein -1)		PCC	0.002 ± 0.01 a	0.005 ± 0.01 a	0.004 ± 0.01 a
	Berry skin	HW		0.026 ± 0.01 a	0.081 ± 0.01 a
		PCC		0.035 ± 0.01 a	0.279 ± 0.01 b
Total phenols-TPC (mg GAE g -1 DW)	Leaves	HW	128 ± 7 a	139 ± 7 a	177 ± 7 a
		PCC	126 ± 7 a	141 ± 7 a	194 ± 7 b
	Berry skin	HW		293 ± 7 a	320 ± 7 a
		PCC		141 ± 7 a	316 ± 7 a

Table 3 Phytoalexin content (ε-viniferin and trans-resveratrol, mg g^{-1} DW) in leaves and berry skin of Tannat grapevines under herbicide weeding (HW) and permanent cover crop (PCC) at flowering, veraison, and harvest 2021. Values represent means ± SE. Different letters indicate significant differences between soil management within phenological stages and organs (Tukey's test, $p < 0.05$)

Phytoalexin	Organ	Soil Management	Flowering	Veraison	Harvest
e-Viniferin	Leaves	HW	6 ± 1.5 a	6.5 ± 1.5 a	7.2 ± 1.5 a
		PCC	5.5 ± 1.5 a	9.5 ± 1.5 a	9.8 ± 1.5 a
	Berry skin	HW		3.3 ± 0.6 a	2.7 ± 0.6 a
		PCC		3.5 ± 0.6 a	2.5 ± 0.6 a
trans-Resveratrol	Leaves	HW	3.7 ± 0.7 a	4.9 ± 0.7 a	4.7 ± 0.7 a
		PCC	3.6 ± 0.7 a	6.2 ± 0.7 a	6.3 ± 0.7 a
	Berry skin	HW		2.9 ± 0.5 a	3.9 ± 0.5 a
		PCC		2.7 ± 0.5 a	3.1 ± 0.5 a

Table 4 Anatomical characterization of Tannat berry skin under HW (herbicide weeding) and PCC (permanent cover crop) at harvest 2021. Values represent means ± SE. Different letters indicate significant differences between soil management (Tukey's test, $p < 0.05$)

Variable	HW	PCC
Cuticle thickness (mm)	1.9 ± 0.1 a	2.3 ± 0.1 b
Epidermis thickness (mm)	16.2 ± 0.5 a	18.8 ± 0.6 b
Hypodermis thickness (mm)	192 ± 8 a	210 ± 9 a
Skin thickness (mm)	210 ± 8 a	231 ± 9 b
Epidermis cell layer number	2 ± 0	2 ± 0
Hypodermis cell layer number	6.2 ± 0.2 a	6.8 ± 0.2 b
Skin cell layer number	8.2 ± 0.2 a	8.8 ± 0.2 b

5.5.5. Defense Responses and Disease Metrics

PCA test indicated that PC1 and PC2 were statistically significant, explaining 34.0% (95% CI: 35.4 – 62.5%) and 30.7% (95% CI: 21.7 – 37.7%) of the total variance, respectively (Fig. 3). All 22 variables were included in the PCA, and those with significant loadings ≥ 0.30 on PC1 were cuticle thickness (Cut_thick), POD activity in leaves (PODL), Ascorbate peroxidase activity in berry skins (APXB), catalase activity in leaves (CATL), latent infection incidence (Inc_latinf), and AUDPC at harvest (AUDPCh) (Supporting Information, Table S2).

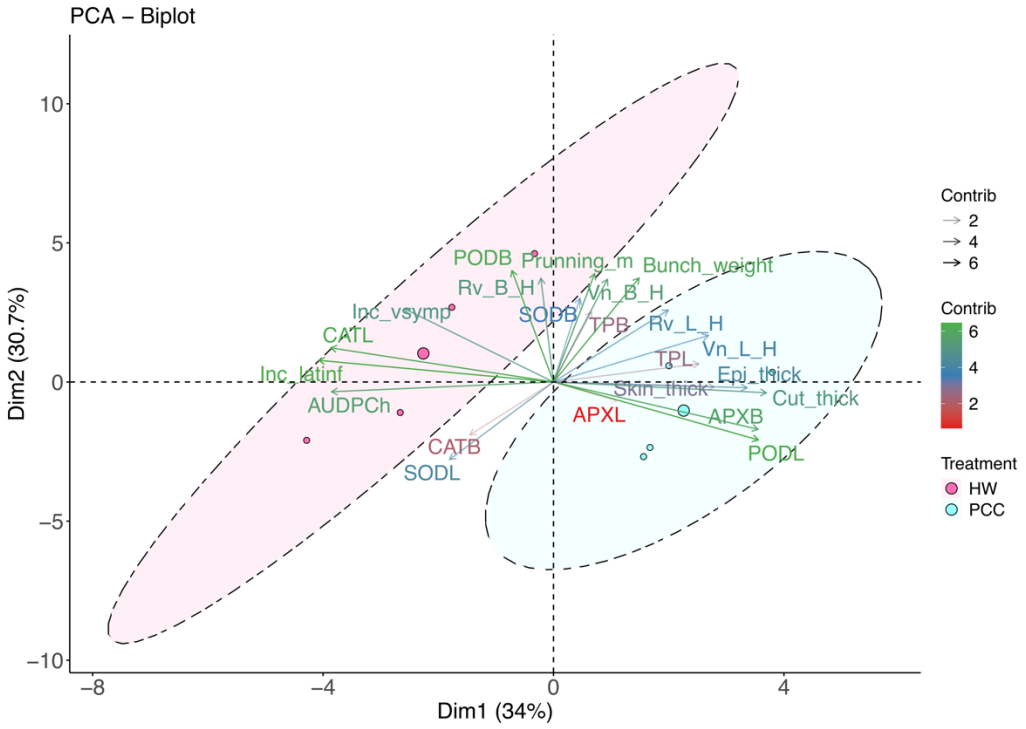

Fig. 3 Principal component analysis (PCA) biplot of grapevine traits under different under-vine soil management: herbicide weeding (HW) and permanent cover crop (PCC). Vectors represent the contribution of each variable, indicated

by different colors. Colored ellipses indicate sample groupings: HW (red) and PCC (turquoise). Variables included: incidence of visible symptoms (Inc_vsymp), latent B. cinerea infections (Inc_latinf), area under the disease progress curve at harvest (AUDPCh), cuticle thickness (Cut_thick), epidermis thickness (Epi_thick), skin thickness (Skin_thick), trans-resveratrol in leaves (Rv_L_H) and berry skin (Rv_B_H), ε-viniferin in berry skin (Vn_B_H) and leaves (Vn_L_H), catalase in leaves (CATL) and berry skin (CATB), peroxidase in leaves (PODL) and berry skin (PODB), ascorbate peroxidase in leaves (APXL) and berry skin (APXB), superoxide dismutase in leaves (SODL) and berry skin (SODB), total phenolics in leaves (TPL) and berry skin (TPB), bunch weight (Bunch_weight), and pruning weight (Prunning_m).

5.5.6. Microbial communities in Response to Under-Vine Management

Rhizosphere microbial community composition was affected by under-vine soil management at harvest but not at flowering (Table 5, supporting information Fig. S5). During flowering, the block effect explained a substantial portion of the diversity for prokaryotic ($R^2 = 0.65$, $p = 0.012$) and for fungal communities ($R^2 = 0.50$, $p = 0.067$). At harvest, soil management had a significant impact on both prokaryotic ($R^2 = 0.42$, $p = 0.025$) and fungal communities ($R^2 = 0.47$, $p = 0.006$), while the block effect was not significant.

Table 5 PERMANOVA results for prokaryotic and fungal communities during flowering (2020) and harvest (2021), based on Weighted UniFrac distance (prokaryotes) and Bray-Curtis distance (fungi).

Dataset	Factor	F	R2	Pr (> F)
Prokaryotic-flowering 2020	Soil Management	2.025	0.14	0.099
	Block	3.1735	0.65	0.012
Prokaryotic-harvest 2021	Soil Management	4.53	0.42	0.025
	Block	1.1	0.31	0.447
Fungal-flowering 2020	Soil Management	1.4957	0.16	0.106
	Block	1.5112	0.50	0.067
Fungal- harvest 2021	Soil Management	6.1308	0.47	0.006
	Block	1.2997	0.29	0.400

Based on ALDEx2 analysis, several prokaryotic and fungal taxa were identified as discriminant for PCC, while only a few fungal taxa were associated with HW (Fig. 4; Supporting Information, Tables S3 and S4). Prokaryotic taxa associated with PCC included seven ASVs of *Pseudomonas*, two of *Rahnella1*, and one ASV each of *Pseudarthrobacter*, *Chitinophaga*, *Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium*, and a member of the phylum Chloroflexi not identified at the genus level. Prokaryotic taxa associated with HW comprised one ASV of *Pseudomonas*, three of *Paenarthrobacter*, and three of *Rhodococcus*. Fungal taxa associated with PCC included two ASVs of Calophoma (including one *C. rosae*), and one ASV each of *Paraphoma ledniceana*, *Rhexocercosporidium panacis*, *Knufia tsunedae*, *Mortierella lapis*, *Fusarium tricintum*, *Mycoarthris corallina*, *Penicillium sp.*, *Cladosporium herbarum*, and two ASVs belonging to Ascomycota not identified at the genus level. Fungal taxa associated with HW included six ASVs of *Pseudogymnoascus*

appendiculatus, and one ASV each of *Cryptococcus nyarrowii*, *Arthropsis hispanica*, *Tremella sp.*, *Vishniacozyma sp.* and *Tricellula sp.*

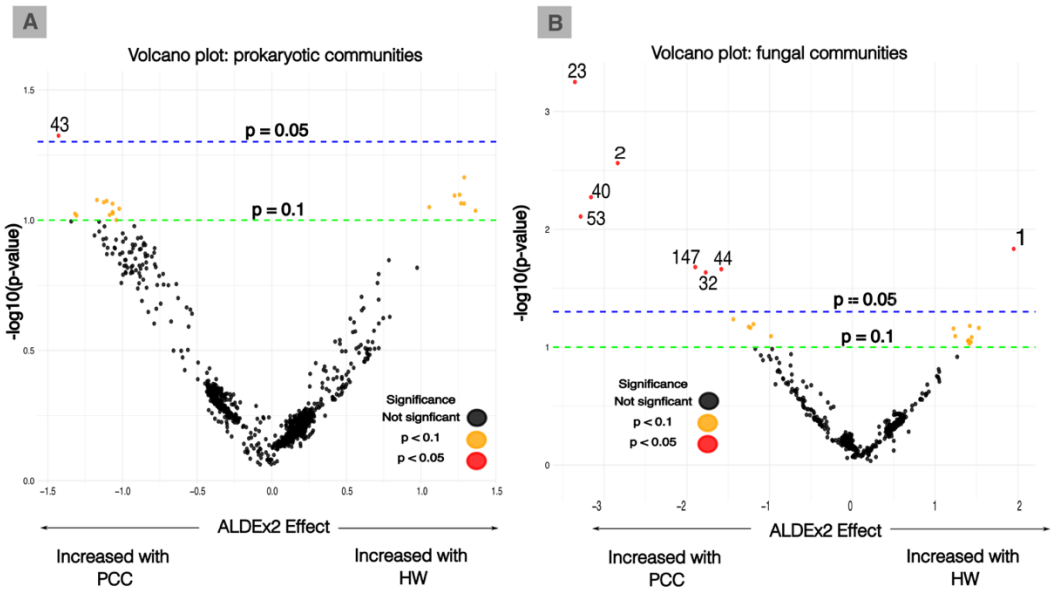

Fig. 4 Differential abundance of prokaryotic and fungal taxa identified using ALDEx2. The analysis shows the effect of under-vine soil management at harvest on (A) prokaryotic and (B) fungal taxa, comparing herbicide weeding (HW) and permanent cover crop (PCC). The x-axis represents effect size (log-ratio), and the y-axis shows the $-\log_{10}$ of FDR-adjusted p-values. Numbers next to points correspond to ASV identifiers: 43- *Pseudarthrobacter sp.*, 23- *Calophoma sp.*, 2- *Rhexocercosporidium panacis*, 40- *Paraphoma ledniceana*, 53- *Penicillium sp.*, 147- *Knufia tsunedae*, 44- *Mortierella lapis*, 32- *Calophoma rosae*, 1- *Pseudogymnoascus appendiculatus*.

5.5.7. Effect of soil management on microbial communities in relation to disease and defense responses

The dbRDA model for prokaryotic communities explained 71.3% of the total variation, with dbRDA1 axis accounting for 60.7% of the constrained variance (Fig. 5A). A permutation test for dbRDA axes showed that dbRDA1 was significant ($F = 4.53$, $p = 0.045$), while the remaining axes were not ($p > 0.1$). Among the variables, Cut_thick was the only significant predictor ($F = 3.23$, $p = 0.024$), whereas incidence of visible symptoms (Inc_vsymp), APXB, and total phenolics in leaves (TPL) did not show significant effects ($p > 0.1$).

For the fungal communities, the model explained 75.4% of the total variation, with dbRDA1 axis accounting for 68.7% of the constrained variation. dbRDA1 was significant ($F = 6.31$, $p = 0.036$), while subsequent axes were not ($p > 0.05$) (Fig. 5B). Cut_thick was a significant predictor of fungal community composition ($F = 3.73$, $p = 0.028$), while Inc_vsymp showed a marginally significant trend ($F = 2.29$, $p = 0.089$), and APXB and TPL were not significant ($p > 0.1$) (Fig. 5B).

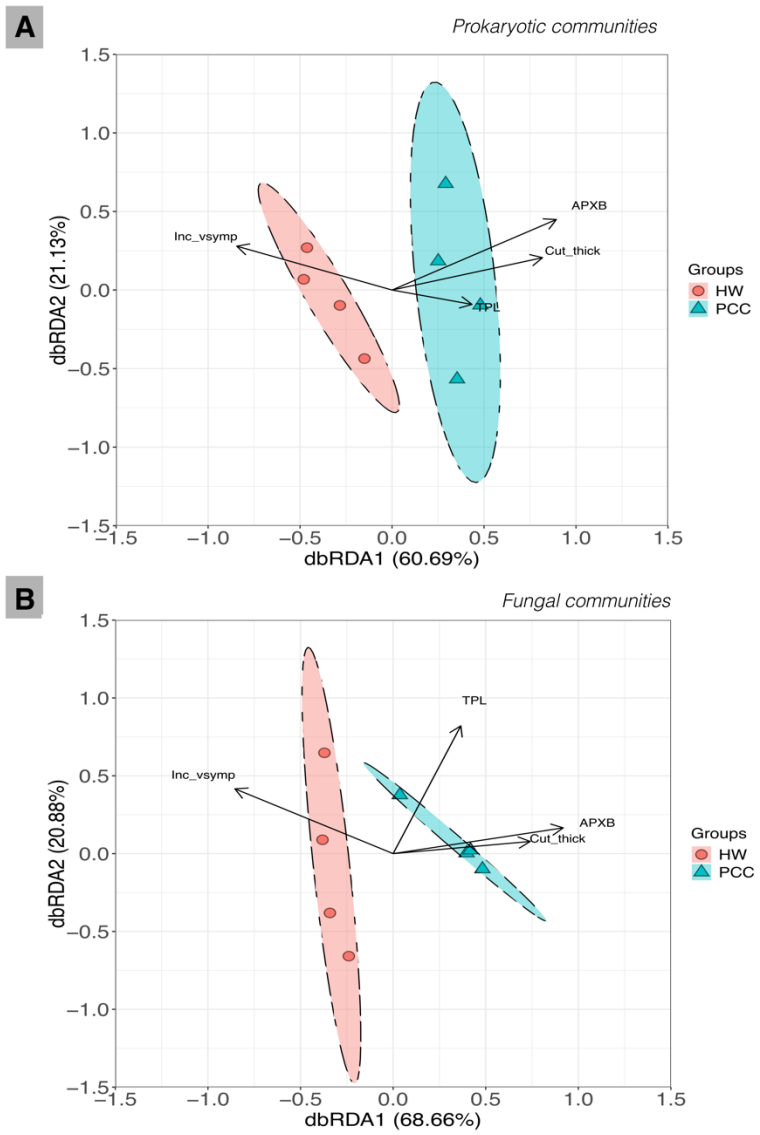

Fig. 5 Distance-based redundancy analysis (dbRDA) showing the effect of under-vine soil management on grapevine rhizosphere in relation to disease and defense responses. (A) prokaryotic communities based on weighted UniFrac distances and (B) fungal communities based on Bray-Curtis dissimilarity. Colored ellipses indicate treatment groups: herbicide weeding (HW) and permanent cover crop (PCC). Arrows indicate the direction and strength of explanatory variables. Variables: incidence of visible symptoms (Inc_vsymp),

cuticle thickness (Cut_thick), ascorbate peroxidase in berry skin (APXB), and total phenolic content in leaves (TPL).

5.6. Discussion

Our study confirms that permanent under-vine cover crops (PCC) decrease *Botrytis* bunch rot (BBR) incidence at harvest, despite not highly conducive climatic conditions, like in previous findings (Coniberti et al., 2023; Coniberti, Ferrari, Disegna, García Petillo, et al., 2018). Disease incidence and intensity were negatively associated with biochemical and anatomical defenses, such as APX activity in berry skin, POD activity in leaves, TPC in leaves, and thickness of epidermis, cuticle and skin. Also, a shift in the grapevine rhizosphere microbiome within PCC was observed, as previously reported for the same experiment (Bernaschina et al., 2023).

Managements aiming at maintaining soil coverage under the vine, among them organic mulches or permanent cover crops, are suggested as a strategy for BBR management (Jacometti et al., 2007). This approach focuses first on reduction of primary inoculum by accelerating the decomposition of vine debris where *B. cinerea* overwinters and by promoting the competition of soil biota with *B. cinerea* in vine material (Jacometti, et al. 2007). Also, the presence of cover crops in vineyards alleys reduced the number of *B. cinerea* conidia which escapes from the ground by more than 85% in comparison to a bare soil (Hasanaliyeva et al., 2024). In any case, there is an absence of compelling evidence that the use of UVCC is effective for reducing BBR, particularly given that BBR is an airborne polycyclic disease where *B. cinerea* conidia are widespread in the air (Elmer & Michailides, 2007). Moreover, our experimental design with PCC and HW implemented in adjacent rows, the reduction in

primary inoculum in one alternating row is probably not sufficient to account for the observed decline in disease intensity associated with PCC.

Under-vine cover crops have also been associated with lower BBR, where competition leads to a reduction of vine vigor resulting in improved canopy aeration, lower plant nitrogen content and reduced water availability (Coniberti, Ferrari, Disegna, García Petillo, et al., 2018; Thomidis et al., 2016). However, in our experiment we did not observe significant differences in vine vigor (estimated by pruning weight per meter and shoot elongation rate) between treatments nor differences in free amino nitrogen (FAN) content in berries. Water stress has been proposed as an abiotic factor plausible to reduce aerial diseases in grapevine and olive trees (Coniberti et al. 2018b; Conde-Innamorato et al. 2024). Conde-Innamorato et al. (2024) showed that olives with moderate water stress presented significantly less disease incidence and severity of olive anthracnose caused by *Colletotrichum* spp. This moderate water stress was associated with increased activity of the enzymes related to hydrogen peroxide scavenging (CAT and POX) and cuticle thickness. Although in our study an irrigation strategy was implemented to avoid severe and differential water stress between PCC and HW, a difference ≤ 0.1 MPa in midday Ψstem was observed between the phenological stages of berries pea-sized and the beginning of bunch closure. Unfortunately, it is not possible to ensure or deny that this difference in PCC Ψstem at this time is responsible for the reduction of BBR and increased defense responses observed at harvest.

Biochemical defense mechanisms in grapevine against *B. cinerea* are well characterized, particularly the accumulation of stilbene phytoalexins like *trans*-resveratrol and its oligomer *ε*-viniferin and the production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) (Verhagen et al. 2010; Aziz et al. 2016). The synthesis of these phytoalexins in leaves and berry skins can be

triggered by various stimuli including UV radiation, chemical elicitors, microbe-associated molecular patterns (MAMPs), fungal pathogens, and beneficial microbes through induced systemic resistance (ISR) (Verhagen et al. 2010, 2011; Gruau et al. 2015; Aziz et al. 2016). However, in the present study, phytoalexin concentrations did not differ between treatments at any phenological stage or in either organ examined. Activation of antioxidant enzymes has been linked to increased plant tolerance to biotic stress (Lobato et al. 2011). Under biotic and abiotic stress, plants often accumulate ROS, which can damage cellular macromolecules if not tightly regulated (Ramezani et al. 2017). The plant's oxidative stress response system, comprising both enzymatic and non-enzymatic antioxidants, plays a crucial role in neutralizing oxidative damage (Mohammadi et al. 2021). Enzymes such as CAT, APX, and glutathione peroxidase convert H_2O_2 into water, thereby limiting oxidative stress. In line with this, our study showed that berries from PCC exhibited higher APX activity in the berry skin and elevated TPC in leaves compared to those under HW.

Several structural traits of grape berries have been associated with increased resistance to *B. cinerea* (Deytieux-Belleau et al. 2009). Characteristics such as the number and thickness of epidermal and hypodermal cell layers, as well as cuticle and wax content, have shown positive correlations with disease resistance (Comménil et al. 1997; Gabler et al. 2003). Additionally, ROS production may contribute to cell wall reinforcement during pathogen attack (Lamb and Dixon 1997). In our study, berry skins from PCC exhibited thicker epidermis and cuticle. These anatomical characteristics were consistent with values reported in Tannat and other grape varieties (Gabler et al. 2003; Battista et al. 2015; Navarro et al. 2021; Salvarrey et al. 2024). Taking together, our findings suggest that the lower BBR observed in PCC may be mediated by a combination of biochemical and anatomical defense responses.

A shift in the grapevine rhizosphere microbiome associated with soil management may also explain the lower BBR incidence and severity (Bettenfeld et al. 2022; Bender at al 2016). Bernaschina et al. (2023) reported that rhizospheric microbial communities of Tannat grapevines under PCC were different from HW, with several differential taxa responsive to PCC, like *Pseudomonas*, *Rahnella1* and *Penicillium*. Similarly, in the present study, PCC influenced the composition of prokaryotic and fungal communities at harvest, again associated with a higher abundance of ASVs belonging to these genera, known for their potential beneficial traits and to induce systemic resistance (ISR) in plants. Several species and strains of *Pseudomonas* have been recognized as effective biocontrol agents against diseases in grapevines and other crops (Ganeshan & Kumar, 2005; Gruau et al., 2015; Niem et al., 2020; Takishita et al., 2018; Tao et al., 2020; Walsh et al., 2001). Their biocontrol activity operates through both direct and indirect mechanisms. Direct mechanisms include the production of antimicrobial compounds (e.g., phenazines, pyrrolnitrin, and 2,4-diacetylphloroglucinol), siderophores that limit iron availability to pathogens, and the secretion of hydrolytic enzymes such as chitinases, proteases, and β-glucanases that degrade fungal cell walls (Guzmán-Guzmán & Santoyo, 2022; Mehmood et al., 2023; Singh et al., 2022). Indirect mechanisms involve the induction of plant defense responses and competition for nutrients and space, further enhancing plant protection (Gruau et al., 2015; Hiddink et al., 2005; Niem et al., 2020; Takishita et al., 2018; B. W. M. Verhagen et al., 2010). In addition, some species within the genera *Penicillium* and *Rahnella1* have been reported to exhibit plant-beneficial traits, including antagonistic activity against phytopathogens and contributions to disease suppression (F. Chen et al., 2007; El-Hendawy et al., 2005; Murali & Amruthesh, 2015; Tarroum et al., 2022).

While we observed enhanced defense mechanisms in grapevines with PCC, definitively attributing this to ISR triggered by a modified microbiome is challenging. Field testing ISR is complex, especially when the ISR-inducing microbes are a diverse community rather than a single species (Lee et al., 2021; Prigigallo et al., 2022). Additionally, disentangling the timing of defense activation in natural environments is difficult due to the constant interplay of various biotic and abiotic stresses that plants encounter simultaneously, including drought or excessive precipitations, extreme temperatures, pests, and beneficial microbes. A comprehensive approach is needed to address these issues in future studies.

In summary, the enhanced response of the grapevines to *B. cinerea* when managed with PCC is a complex response and the stacked effect of several factors explains this improvement. Beyond the reason why PCC management reduces the incidence of *Botrytis* in grapevines, in the context of agroecological crop protection (ACP, Deguine et al. 2023), where the promotion of soil health and aerial and subterranean biodiversity are the pillars for a healthy agroecosystem, soil management is a promising tool. The use of PCC not only allows to reduce the use of fungicides and herbicides in the agroecosystem but also provides numerous ecosystem services such as population regulation (pests, weeds and pathogens) and water regulation and nutrient cycling (reducing erosion and nutrient losses through wash-off and run-off) (Vanden Heuvel & Centinari, 2021). To ensure successful adoption by wine growers, further research is necessary to identify the most suitable cover crop species and management practices for specific vineyard conditions. This future research should explore how UVCC can be optimized to maximize its contribution to enhanced vineyard health, vine performance, and overall sustainability.

Data availability: Data generated in this study are included either in the article, supplementary information and in the NCBI repository under the BioProject accession number PRJNA889760 for prokaryotic data and PRJNA903835 for fungal data.

Statements and declarations

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Conflict of interest: The authors have no competing interests to declare that are relevant to the content of this article.

5.7. Supplementary information

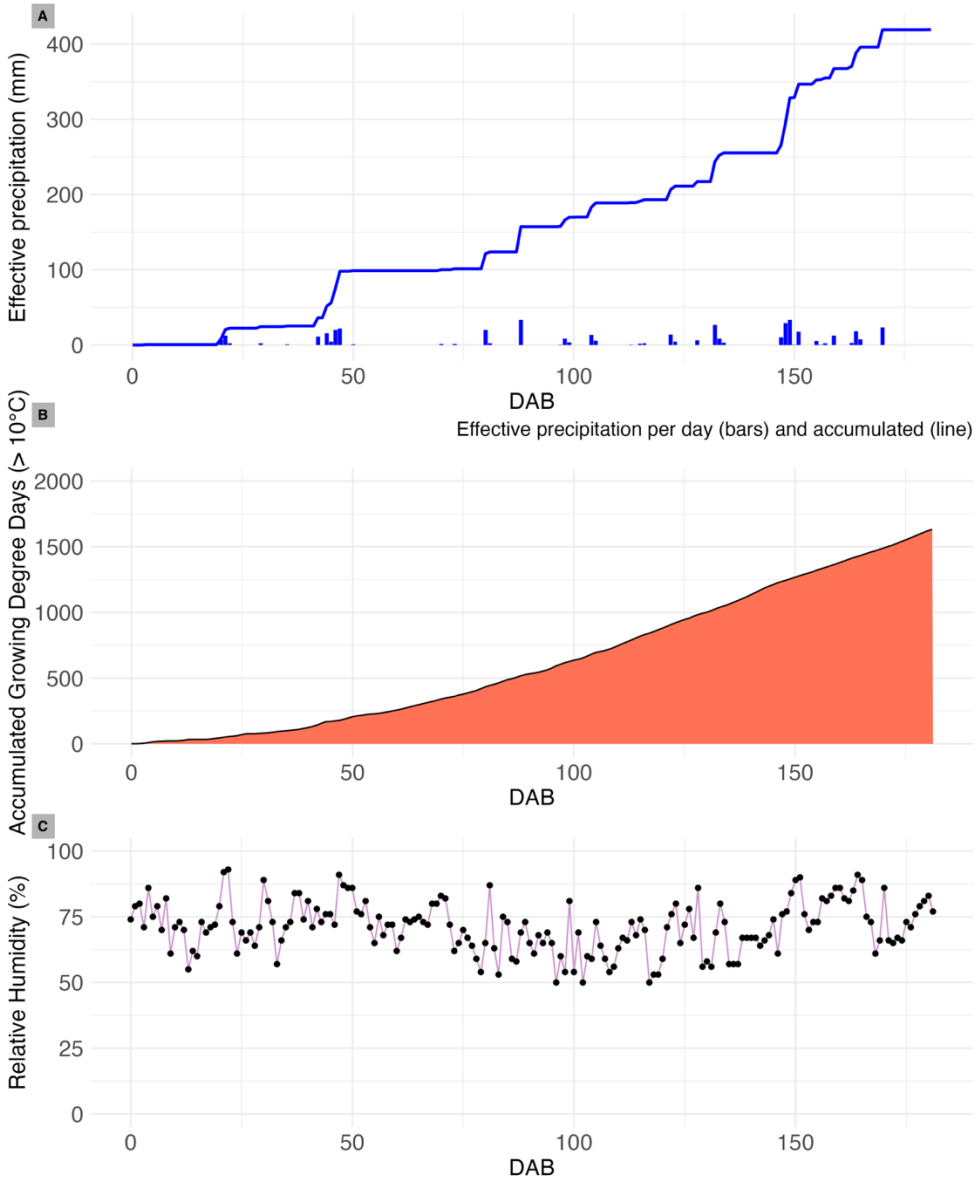

Fig. S1 Meteorological conditions during the grapevine growing season (September 2020–March 2021): (A) Effective and accumulated precipitation (mm), (B) Accumulated Growing Degree Days (GDD, base temperature >10°C), and (C) Relative humidity (%). DAB: Days after bud break

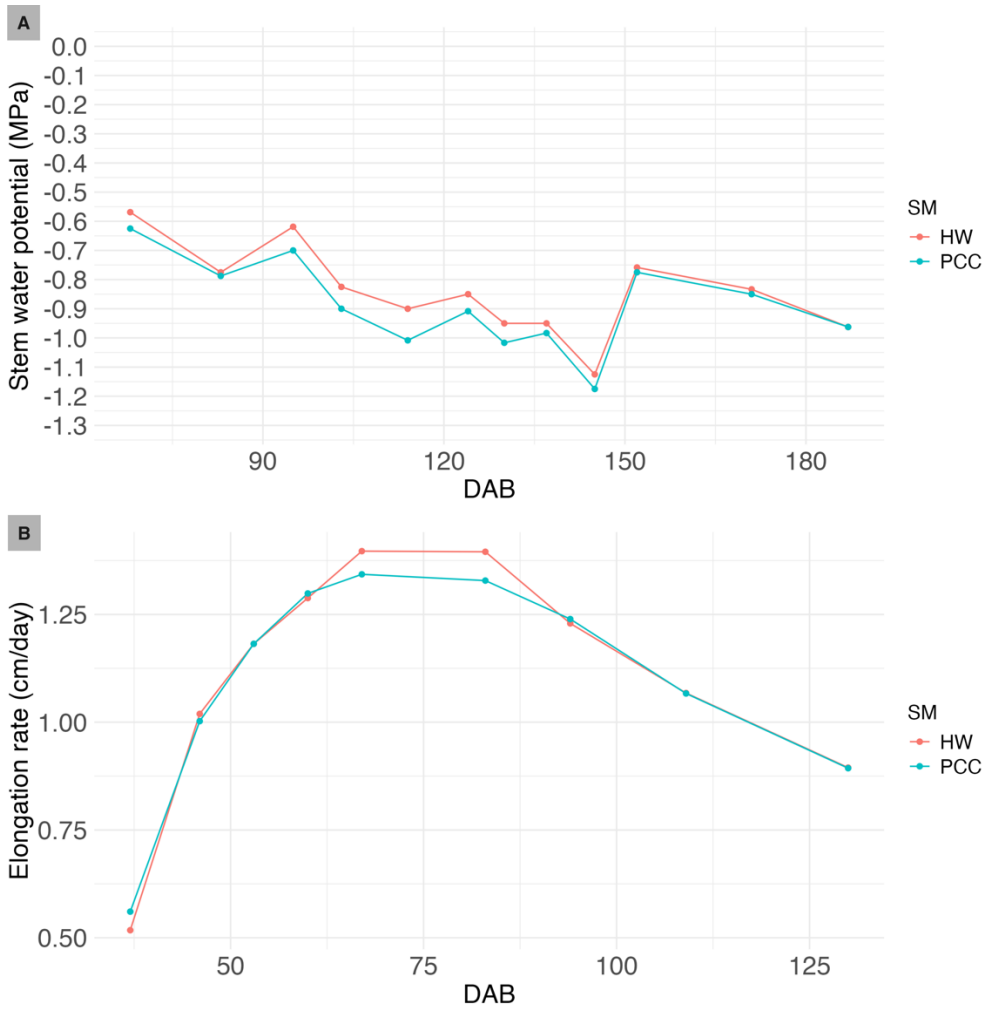

Fig. S2 Physiological responses of Tannat grapevines under two under-vine soil management practices during the 2020/21 season in an experimental vineyard in southern Uruguay: (A) Mid-day stem water potential (Ψstem, MPa). (B) Shoot elongation rate. Treatments: permanent cover crop (PCC) and herbicide weeding (HW). DAB: Days after bud break

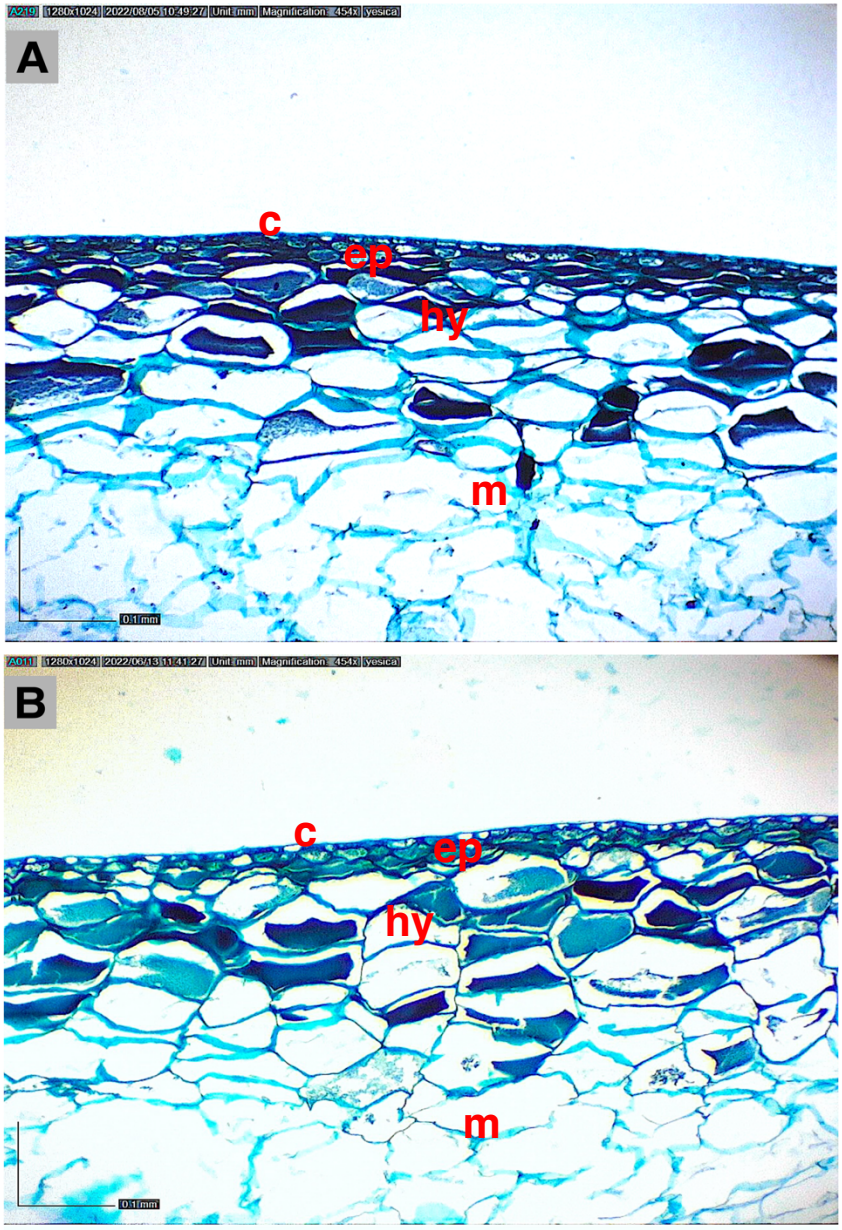

Fig. S3 Anatomical characterization of Tannat berry skin under herbicide weeding (HW) and permanent cover crop (PCC) at harvest 2021. (A) Cross-section of berry skin from HW (10×). (B) Cross-section from PCC (10×). Abbreviations: c = cuticle, ep = epidermis, hy = hypodermis, m = mesocarp (pulp)

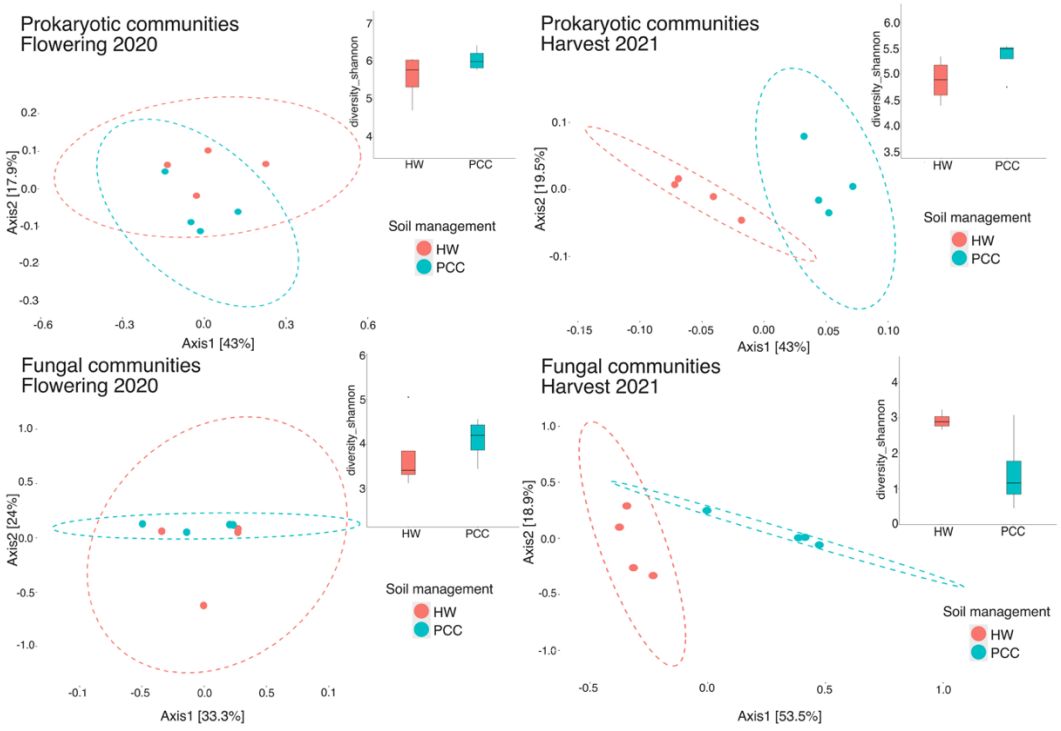

Fig. S4 Alpha (Shannon index, boxplots) and beta diversity (Principal Coordinates Analysis, PCoA) of prokaryotic and fungal rhizosphere communities in grapevine under different under-vine soil management: permanent cover crop (PCC) and herbicide weeding (HW)

Table S1 Variables included in the principal component analysis (PCA), with corresponding abbreviations used in Figure 3.

Variable	Reference
Incidence of visible symptoms at harvest	Inc_vsymp
Incidence of latent infections at harvest	Inc_latinf
Area under disease progress curve at harvest	AUDPCh
Cuticle thickness of berry skin at harvest	Cut_thick
Epidermis thickness of berry skin at harvest	Epi_thick
Berry Skin thickness at harvest	Skin_thick
Trans-Resveratrol in leaves at harvest	Rv_L_H
Trans-resveratrol in berry skin at harvest	Rv_B_H
ε-viniferin in berry skin at harvest	Vn_B_H
ε-viniferin in leaves at harvest	Vn_L_H
Catalase activity in leaves at harvest	CATL
Catalase activity in berry skin at harvest	CATB
Peroxidase activity in leaves at harvest	PODL
Peroxidase activity in berry skin at harvest	PODB
Ascorbate peroxidase activity in leaves at harvest	APXL
Ascorbate peroxidase activity in berry skin at harvest	APXB
Superoxide dismutase in leaves at harvest	SODL
Superoxide dismutase in berry skin at harvest	SODB
Total phenolic content in leaves at harvest	TPL
Total phenolic content in berry skin at harvest	TPB
Bunch weight at harvest	Bunch_weight
Pruning weight	Pruning_m

Table S2 Significant principal component (PC) loadings of disease severity and defense response variables contribute to differences between under-vine soil management. Bold values indicate loadings ≥ l0.30l.

Variables	PC 1 (34%)	PC2 (30.7%)
Cuticle thickness	**0.31**	-0.03
Peroxidase activity in leaves	**0.30**	-0.18
Ascorbate peroxidase activity in berry skin	**0.30**	-0.15
Catalase activity in leaves	**-0.32**	0.10
Latent infections incidence	**-0.34**	0.07
AUDPC at harvest	**-0.33**	-0.03
Trans-resveratrol in berry skin at harvest	-0.02	**0.33**
ε-viniferin in berry skin at harvest	0.08	**0.33**
Peroxidase activity in berry skin	-0.06	**0.36**
Superoxide dismutase in berry skin	0.04	0.27
Superoxide dismutase in leaves	-0.15	-0.25
Bunch weight	0.12	**0.33**
Pruning weight	0.06	**0.35**

Table S3 Taxonomic annotation of prokaryotic amplicon sequence variants (ASVs) identified as differentially abundant by ALDEx2 (adjusted p-value < 0.1). ASVs are shown with their effect size, taxonomic classification, and the treatment group in which they were enriched: permanent cover crop (PCC) or herbicide weeding (HW)

Taxa	ALDEx2_Effect	adjP_value	Phylum	Family	Genus	Species	Enriched group
ASV43	-1,428182	0,047379	Actinobacteriota	*Micrococcaceae*	*Pseudarthrobacter*	NA	PCC
ASV158	1,288731	0,068489	Actinobacteriota	*Micrococcaceae*	*Paenarthrobacter*	NA	HW
ASV138	1,257885	0,079872	Actinobacteriota	*Micrococcaceae*	*Paenarthrobacter*	NA	HW
ASV148	1,224182	0,080418	Actinobacteriota	*Micrococcaceae*	*Paenarthrobacter*	NA	HW
ASV82	-1,170734	0,083658	Proteobacteria	*Pseudomonadaceae*	*Pseudomonas*	NA	PCC
ASV431	-1,106109	0,084395	Proteobacteria	*Yersiniaceae*	*Rahnella1*	NA	PCC
ASV357	-1,124753	0,085318	Proteobacteria	*Yersiniaceae*	*Rahnella1*	NA	PCC
ASV144	1,267764	0,086146	Actinobacteriota	*Nocardiaceae*	*Rhodococcus*	NA	HW
ASV13	1,288299	0,086307	Actinobacteriot	*Nocardiaceae*	*Rhodococcus*	NA	HW

1			a				
ASV524	-1,066197	0,086388	Proteobacteria	*Pseudomonadaceae*	*Pseudomonas*	NA	PCC
ASV212	1,054479	0,089062	Proteobacteria	*Pseudomonadaceae*	*Pseudomonas*	NA	HW
ASV99	-1,021089	0,090364	Proteobacteria	*Pseudomonadaceae*	*Pseudomonas*	NA	PCC
ASV163	1,364621	0,091961	Actinobacteriota	*Nocardiaceae*	*Rhodococcus*	NA	HW
ASV105	-1,066017	0,093078	Proteobacteria	*Pseudomonadaceae*	*Pseudomonas*	NA	PCC
ASV414	-1,06422	0,094219	Bacteroidota	*Chitinophagaceae*	*Chitinophaga*	NA	PCC
ASV391	-1,317946	0,094415	Proteobacteria	*Rhizobiaceae*	*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*	NA	PCC
ASV95	-1,086382	0,095468	Proteobacteria	*Pseudomonadaceae*	*Pseudomonas*	NA	PCC
ASV523	-1,307629	0,096026	Chloroflexi	*NA*	*NA*	NA	PCC
ASV108	-1,040929	0,099872	Proteobacteria	*Pseudomonadaceae*	*Pseudomonas*	NA	PCC

Table S4 Taxonomic annotation of fungal amplicon sequence variants (ASVs) identified as differentially abundant by ALDEx2 (adjusted p-value < 0.1). ASVs are shown with their effect size, taxonomic classification, and the treatment group in which they were enriched: permanent cover crop (PCC) or herbicide weeding (HW)

Taxa	ALDEx2_Effect	adjP_value	Phylum	Family	Genus	Species	Enriched group
ASV23	-3,365524639	0,000561327	p__Ascomycota	*f__Didymellaceae*	*g__Calophoma*	*NA*	PCC
ASV2	-2,861924517	0,002738 7	p__Ascomycota	*f__Helotiales_fam_Incertae_sedis*	*g__Rhexocercosporidium*	*s__panacis*	PCC
ASV40	-3,176011172	0,005333116	p__Ascomycota	*f__Phaeosphaeriaceae*	*g__Paraphoma*	*s__ledniceana*	PCC
ASV53	-3,298781941	0,007776771	p__Ascomycota	*f__Aspergillaceae*	*g__Penicillium*	*NA*	PCC
ASV1	1,808408866	0,014644927	p__Ascomycota	*f__Pseudeurotiaceae*	*g__Pseudogymnoascus*	*s__appendiculatus*	HW
ASV147	-1,94734860	0,020908729	p__Ascomycota	*f__Trichomeriaceae*	*g__Knufia*	*s__tsunedae*	PCC

	5						
ASV44	-1,639439277	0,021756651	p__Mortierellomycota	*f__Mortierellaceae*	*g__Mortierella*	*s__lapis*	PCC
ASV32	-1,823904182	0,023189867	p__Ascomycota	*f__Didymellaceae*	*g__Calophoma*	*s__rosae*	PCC
ASV5	-1,4974536	0,057949816	p__Ascomycota	*f__Nectriaceae*	*g__Fusarium*	*s__tricinctum*	PCC
ASV49	-1,261066002	0,06369056	p__Ascomycota	*f__Helotiales_fam_Incertae_sedis*	*g__Mycoarthris*	*s__corallina*	PCC
ASV127	1,291191967	0,065987387	p__Ascomycota	*f__Pezizomycotina_fam_Incertae_sedis*	*g__Tricellula*	*NA*	HW
ASV38	-1,316279129	0,06717736	p__Ascomycota	*NA*	*NA*	*NA*	PCC
ASV47	-1,295774849	0,068309071	p__Ascomycota	*f__Cladosporiaceae*	*g__Cladosporium*	*s__herbarum*	PCC

ASV20	1,397908205	0,068646022	p__Basidiomycota	*f__Bulleribasidiaceae*	*g__Vishniacozyma*	*NA*	HW
ASV24	1,099405428	0,069441796	p__Basidiomycota	*f__Cryptococcaceae*	*g__Cryptococcus*	*s__nyarrowii*	HW
ASV76	1,118630194	0,080502582	p__Ascomycota	*f__Onygenales_fam_Incertae_sedis*	*g__Arthropsis*	*s__hispanica*	HW
ASV29	-1,054055414	0,080567983	p__Ascomycota	*NA*	*NA*	*NA*	PCC
ASV52	1,3131319	0,082356199	p__Ascomycota	*f__Pseudeurotiaceae*	*g__Pseudogymnoascus*	*s__appendiculatus*	HW
ASV61	1,275522993	0,08735666	p__Ascomycota	*f__Pseudeurotiaceae*	*g__Pseudogymnoascus*	*s__appendiculatus*	HW
ASV54	1,280756642	0,08856288	p__Ascomycota	*f__Pseudeurotiaceae*	*g__Pseudogymnoascus*	*s__appendiculatus*	HW
ASV67	1,268351586	0,08860233	p__Ascomycota	*f__Pseudeurotiaceae*	*g__Pseudogymnoascus*	*s__appendiculatus*	HW
ASV58	1,303781616	0,090348846	p__Ascomycota	*f__Pseudeurotiaceae*	*g__Pseudogymnoascus*	*s__appendiculatus*	HW
ASV319	1,285652725	0,093321967	p__Basidiomycota	*f__Tremellaceae*	*g__Tremella*	*NA*	HW

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6. Soil management and water status modulate rhizosphere microbiota and reduce *Botrytis* bunch rot in grapevine[3]

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6.1. Resumen

Los efectos del manejo del suelo bajo la fila de la vid y del estado hídrico sobre las comunidades microbianas de la rizósfera de vid y la susceptibilidad a la podredumbre gris del racimo (BBR) fueron evaluados en un experimento en macetas con *Vitis vinifera* cv. Tannat. Las vides fueron sometidas a dos manejos del suelo bajo la línea —cultivo de cobertura permanente (PCC) y desmalezado manual (MW)— y dos regímenes hídricos —restricción de agua durante 20 días antes del envero y sin restricción de agua. Las muestras de rizósfera se analizaron para evaluar la diversidad y composición de comunidades procarióticas y fúngicas mediante secuenciación de alto rendimiento de las regiones 16S rRNA e ITS2. La diversidad de la composición de comunidades fue afectada significativamente por el manejo del suelo y el estado hídrico, en particular para las comunidades procarióticas. En contraste, las comunidades fúngicas mostraron una respuesta limitada a estos factores. El manejo con PCC, especialmente bajo restricción hídrica previa al envero, redujo la incidencia e intensidad de BBR, potencialmente a través de la mejora en las defensas estructurales (por ejemplo, cutícula de la baya más gruesa) y cambios en las

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comunidades microbianas que favorecieron rizobacterias promotoras del crecimiento vegetal (PGPR). Las predicciones funcionales revelaron rutas asociadas a mecanismos de defensa vegetal, como la biosíntesis de alcaloides isoquinolínicos y de ácido jasmónico, enriquecidas bajo condiciones de PCC. Estos hallazgos subrayan el potencial de integrar PCC y restricción hídrica para promover una viticultura sostenible, reducir la severidad de la enfermedad y fomentar interacciones microbianas beneficiosas.

Palabras clave: diversidad microbiana, vid, *Botrytis cinerea*, cultivo de cobertura, restricción hídrica.

6.2. Summary

The effects of soil management and water status on grapevine rhizosphere microbial communities and susceptibility to Botrytis bunch rot (BBR) were evaluated in a pot experiment using *Vitis vinifera* cv. *Tannat*. Grapevines were subjected to two under-vine soil management—permanent cover crops (PCC) and manual weeding (MW)— and two water regimes —20 days pre-veraison water restriction and no water restriction. Rhizosphere samples were analyzed for prokaryotic and fungal community diversity and composition through high-throughput sequencing of 16S rRNA and ITS2 regions. Community composition diversity was significantly influenced by soil management and water status, particularly for prokaryotic communities. In contrast, fungal communities showed limited response to these factors. PCC management, especially under pre-veraison water restriction, reduced BBR incidence and intensity, potentially through enhanced structural defenses (e.g., thicker berry cuticle) and shifts in microbial communities favoring plant growth-promoting rhizobacteria (PGPR). Functional predictions revealed pathways associated with plant defense mechanisms, such as isoquinoline alkaloid and jasmonic acid biosynthesis, enriched under PCC conditions.

These findings underscore the potential of integrating PCC and water restriction to promote sustainable viticulture by reducing disease severity and fostering beneficial microbial interactions.

Keywords: microbial diversity, grapevine, *Botrytis cinerea*, cover crop, water restriction.

6.3. Introduction

Viticulture is an economically and culturally important activity in several regions of the world (Costa et al., 2016), including Uruguay. Currently, the sector faces considerable challenges, such as extreme climatic conditions and widespread fungal diseases that severely affect grape production, leading to historically low wine production in 2023 (International Organization of Vine and Wine, 2023). Among the major phytosanitary challenges in viticulture, Botrytis bunch rot (BBR) or grey mold, caused by *Botrytis cinerea*, represent a significant threat to grape quality and yield (Elmer & Michailides, 2007).

Management of *B. cinerea* mainly rely on chemical control, however, in seasons with highly conducive conditions for disease development, fungicides, even when rationally applied, may be insufficient (Elmer & Michailides, 2007). Additionally, environmental and health impacts of fungicides have led to increasing restrictions on their use, reinforcing the need for alternative disease suppression strategies (Hobbelen et al., 2014; Jacometti et al., 2010) .

The importance of the rhizosphere in plant health and productivity has been widely studied and recognized (Berendsen et al., 2012; Raaijmakers et al., 2009). This dynamic soil compartment, directly interacting with plant roots, harbors high microbial activity, with microorganisms playing key roles in nutrient cycling and plant protection against biotic and abiotic stresses

(Mendes et al., 2013). Plants influence the composition of their rhizosphere microbiome through the secretion of rhizodeposits, selectively recruiting microorganisms that provide benefits under specific environmental conditions (Berendsen et al., 2012; Pieterse et al., 2014). Given this intricate plant-microbe relationship, the concept of manipulating the rhizosphere to enhance plant growth and resilience has emerged. This approach, known as rhizosphere engineering, offers a promising ecological strategy for achieving more sustainable agricultural production (Dries, Hendgen, et al., 2021; Hakim et al., 2021).

Rhizosphere engineering through soil management can be a promising approach to modulate grapevine-associated microbial communities and improve plant health. It is well established that the composition and structure of the grapevine holobiont microbiota is influenced by vineyard management (Bettenfeld et al., 2022a). Among them, the use of cover crops has been shown to influence the abundance, activity, and composition of bulk and rhizosphere soil microbiome, fostering beneficial microorganisms (Bernaschina et al., 2023; Kim et al., 2020; Lumini et al., 2010; Sharma et al., 2018; Vukicevich et al., 2016).

Additionally, water availability plays a crucial role in modulating grapevine physiology and microbiota composition, further influencing plant resilience and disease susceptibility (Bettenfeld et al., 2022a; Carbone et al., 2021; Coniberti, Ferrari, Disegna, García Petillo, et al., 2018; Guilpart et al., 2017; Preece et al., 2019). Moderate water stress has been associated with enhanced plant defense responses, including increased phenolic compound accumulation and reinforcement of fruit skin structures (Conde-Innamorato et al., 2024; Sun et al., 2023), which may contribute to reduce the incidence of fungal diseases. The use of under-vine cover crops compared to maintaining bare soil has been associated with reduced *B. cinerea* incidence and severity at harvest in previous studies (Coniberti et al., 2023; Coniberti, Ferrari, Disegna, García Petillo, et al., 2018). It has been hypothesized that this effect is linked to water stress induced by cover crops, as their influence on vine

vigor and bunch compactness are known to affect disease severity. However, in scenarios where vigor, bunch compactness, and berry nitrogen levels are comparable, vines with under-vine cover crops have exhibited lower *B. cinerea* incidence (Coniberti et al., 2023). This suggests that additional mechanisms, beyond water stress and canopy structure, may contribute to disease suppression.

We hypothesize that under-vine soil management and water status influence BBR incidence and intensity through the modulation of grapevine rhizosphere microbiota. To test this hypothesis, we conducted a pot experiment using *Vitis vinifera* cv. Tannat, subjected to two soil management (permanent cover crop and manual weeding) and two irrigation regimes (no restriction and 20-day pre-veraison restriction). The microbial community's diversity and composition were analyzed using high-throughput sequencing of the 16S rRNA and ITS2 regions, along with the incidence and intensity of BBR. Additionally, potential defense mechanisms induced by the treatments were explored, including the accumulation of antioxidant compounds and changes in berry skin anatomy.

6.4. Materials and methods

6.4.1. Experimental site and vineyard management

The experiment was carried out during the 2020–2021 growing season on *Vitis vinifera* cv. Tannat grafted onto SO4 rootstock at INIA Las Brujas experimental station, located in Canelones, southern Uruguay (34°44′ S, 56°13′ W; 29 m a.s.l.). The site experiences a humid subtropical climate (Cfa, Köppen-Geiger classification; Castaño et al. 2011), with a mean annual temperature of 16.8 °C and an average annual precipitation of 1276 mm. Weather conditions throughout the season were monitored using a local automated weather station (http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico).

A total of 64 grapevines were cultivated in 100 L pots filled with a soil and compost mixture (70:30, v/v). Plants were arranged within an

experimental vineyard following a 2.5 m (inter-row) × 0.7 m (intra-row) spacing in north–south-oriented rows and trained using a vertical shoot positioning system (see Supplementary Information, Fig. S1). The study employed a split-plot randomized block design with four replicates. The main-plot factor was under-vine soil management (SM): manual weeding (MW) or a permanent cover crop (PCC). MW consisted of bare soil-maintained weed-free by hand weeding, while PCC was implemented using *Festuca arundinacea* (Schreb.) 'Tall fescue', sown in March 2019 at a rate of 6 g/m² to establish full ground cover.

Subplots received one of two irrigation regimes: a water restriction treatment (WR) applied for 20 days starting at the pepper-size stage, and a no-restriction control (NR). Water was supplied via drip irrigation using 4 L/h emitters spaced every 0.3 m beneath the canopy. All other viticultural practices, including canopy management, fertilization, and disease control (excluding *Botrytis cinerea*) were conducted in accordance with Uruguayan integrated grape production guidelines (DGSA, 2022).

6.4.2. Soil and Rhizosphere Sampling and DNA extraction

Soil and root samples were obtained at two phenological stages—flowering (November 2020) and harvest (March 2021)—corresponding to stages 23 and 38, respectively, based on the modified Eichhorn and Lorenz scale (Coombe, 1995) . For each treatment and phenological stage, four composite soil samples were collected (one per plot), summing up to 32 composite samples in total. Each composite sample was made by pooling eight individual soil cores (0–15 cm depth, 2 cm diameter), collected with a soil auger. These were thoroughly mixed and sieved through a 2 mm mesh after manually removing roots. Vine roots were gently brushed to detach loosely adhering soil and pooled. Rhizosphere soil was extracted from 5 g of roots using a Stomacher followed by centrifugation, according to Schreiter et al. (2014) protocol. The resulting rhizosphere pellets were stored at −20°C until further processing for TC-DNA extraction.

Total community DNA (TC-DNA) was isolated from 500 mg (wet weight) of frozen rhizosphere pellets using the FastDNA™ Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) in combination with the FastPrep-24 bead-beating system, according to the manufacturer's protocol. DNA quality was verified via agarose gel electrophoresis, and concentrations were quantified with a Nanodrop 2000 spectrophotometer (Invitrogen, USA).

6.4.3. Amplicon sequencing, sequence processing and taxonomic classification

The prokaryotic communities associated with the rhizosphere at both flowering and harvest stages were analyzed by sequencing the V3–V4 region of the 16S rRNA gene on an Illumina MiSeq platform (2 × 300 bp, paired-end) at Macrogen Inc. (Korea), using primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'), as described by Takahashi et al. (2014). The fungal community structure was assessed in parallel by targeting the ITS2 rRNA region using primers 3F (5'-GCATCGATGAAGAACGCAGC-3') and 4R (5'-TCCTCCGCTTATTGATATGC-3'), following Schmidt et al. (2013) with sequencing also performed on the MiSeq platform at Macrogen Inc.

Initial quality assessment of raw sequence reads was performed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and the results were compiled into a single summary report using MultiQC (Ewels et al., 2016). Amplicon sequence variants (ASVs) were inferred using the DADA2 v1.24.0 pipeline (Callahan et al., 2016), which included quality filtering, trimming, error correction, merging of paired end reads, and chimera removal. For 16S rRNA gene sequences, forward and reverse reads were truncated at 280 bp and 250 bp, respectively. For fungal ITS2 reads, only reverse reads were trimmed at 245 bp. Sequences with expected error rates exceeding 3 in either direction were excluded. The remaining DADA2 settings were kept at default. Paired reads were merged with a minimum

overlap of 20 bp and no mismatches allowed. Chimeric sequences were detected and removed during processing.

Taxonomic assignment of ASVs was conducted using the SILVA SSU rel. 138.1 database (Quast et al., 2013) for prokaryotic sequences and the UNITE dynamic release (sh_general_release_dynamic_04.04.2024) for fungal ITS2 sequences (Nilsson et al., 2019). Non-target sequences identified as chloroplasts, mitochondria, or non-fungal eukaryotes were filtered out. Subsequent data handling and visualization were performed with the phyloseq v1.34.0 package (McMurdie & Holmes, 2013).

Sequence data was analyzed under the framework of R software v4.3.0 (https://www.r-project.org/). Before performing beta diversity analyses, the phyloseq object was rarefied to an equal sequencing depth across all samples to minimize biases associated with differences in library size. Shannon Index was estimated for rhizosphere samples using the R package microbiome v1.12.0 (Lahti et al., 2017). To test the effect of the soil under vine management on prokaryotic and fungal communities, a non-parametric multivariate analysis of variance (PERMANOVA) based on weighted Unifrac dissimilarity index was run with 10000 permutations using the R package vegan v2.5.7 (Oksanen et al., 2020). A pairwise PERMANOVA was performed to test for differences between treatments. The analysis of multivariate homogeneity of group dispersions was done using the function betadisper from vegan package.

Differential abundance analysis at the genus level was performed using the ANCOM-BC package (Lin & Peddada, 2020). The analysis was based on count data with taxonomic classification aggregated to the genus level. The fixed-effects formula included the variable ‘Water status for flowering data and ‘Soil management’ for harvest data, to account for its influence on prokaryotic abundance. Structural zeros were identified, and negative log-ratio bounds were employed to improve robustness. To correct for multiple testing, p-values were adjusted using the Holm method and the significance threshold was set at 0.05 (alpha = 0.05). Samples with low prevalence

(prv_cut = 0.10) or insufficient library size (lib_cut = 1000) were excluded from the analysis. A sensitivity analysis using pseudocounts was conducted and multiple group comparisons (pairwise, Dunnett's test, and trend analysis) were performed.

Functional predictions of the prokaryotic community were obtained with PICRUSt2 (Douglas et al., 2019) and results analyzed using the R package ggpicrust2 package (Yang et al., 2023) . The predicted metagenomes were obtained and the filtered KO (KEGG Orthology) table was used as input. Differential abundance analysis was performed using the Linear Models for Differential Abundance (LinDA) method, with 'Water status" (WR/NR) and 'Soil management' (PCC/MW) as the grouping variable. KEGG pathways were annotated using KO-to-KEGG mapping, and results were organized by pathway name. To control for multiple testing, p-values were adjusted using the Benjamini-Hochberg (BH) method.

6.4.4. Disease assessment

At harvest, the incidence and severity of natural infections of Botrytis bunch rot (BBR) were visually assessed. Incidence was defined as the percentage of grape bunches exhibiting visible symptoms, while severity corresponded to the bunch area affected. A 5-point quantitative scale was used to classify the level of infection. Disease intensity, an index which includes both healthy and diseased bunches, was calculated with the formula: $SI = \Sigma (ni \times si)/N$, where 'ni' corresponds to the number of bunches in each severity category, si' is the numerical value of the respective class, and 'N' is the total number of bunches assessed.

To evaluate natural latent infections of *Botrytis cinerea*, 60 berries were sampled from each treatment and plot at both veraison and harvest. Berries were subjected to a brief freezing period (2 h), followed by surface disinfection. Subsequently, they were incubated under controlled conditions (22 °C for 15 days) to allow the development of latent infections, following the protocol described by Sanzani et al. (2012). The incidence of latent infection

was expressed as the proportion of infected berries relative to the total number analyzed.

For artificial leaf inoculation, the method of Aziz et al. (2016) was followed with minor adjustments. Twenty fully expanded young leaves per plot (80 leaves per treatment) were collected at veraison and harvest, immediately transported in moist, refrigerated bags, and processed in the laboratory. After washing with sterile distilled water, leaves were placed on moist filter paper inside Petri dishes. A single wound was made on the abaxial surface of each leaf using a sterile needle, and a 10 µL drop of a *B. cinerea* conidial suspension (1×10^6 conidia/mL) was applied to the wound site. Dishes were incubated at 22 °C under a 16-hour photoperiod. Disease development was monitored at 4, 7, and 12 days post-inoculation, recording both incidence (proportion of symptomatic leaves) and severity (percentage of leaf area affected). The area under the disease progress curve (AUDPC) was calculated to integrate disease severity over time for the artificial inoculation assays.

6.4.5. Defense responses

The enzymatic activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) were quantified in leaves and berry skin following the same protocols described in Chapter No. 5 of the thesis. Total phenolic content (TPC) was determined using the Folin–Ciocalteu method, with absorbance measured at 725 nm, according to Sánchez-Rangel et al. (2013).

The anatomical characterization of berry skin was performed at harvest, following the same procedure detailed in Chapter 5 of the thesis. Briefly, five berries per plot were sampled and processed using paraffin embedding, sectioning, and Safranin–Fast Green staining. Microscopic analysis focused on cuticle and epidermis thickness. ImageJ software (Schneider et al., 2012) was used for image processing and analysis.

6.4.6. Statistical analysis

Statistical analyses were performed under the framework of R software v4.3.0 using generalized linear mixed models (GLMM) with the glmmTMB package (Brooks et al., 2017). Response variables were modeled as functions of soil management (SM), water status (WS; defined by the irrigation regime), and their interaction (SM × WS), with Block included as a random intercept. Model diagnostics were conducted with DHARMa, and fixed effects were evaluated using Type II Wald chi-square tests. Marginal means were estimated for pairwise comparisons. Disease incidence was analyzed using beta-binomial GLMMs with a logit link. Disease intensity was analyzed using a zero-inflated beta regression, where severity categories were converted to their midpoints to obtain continuous proportions for parametric modeling (Chiang & Bock, 2022). Disease progression was quantified as the Area Under the Disease Progress Curve (AUDPC), calculated with the audpc function of the agricolae R package. The model included SM, WS, SM × WS, and phenological stage as fixed effects, with a random intercept for Block, using a Gamma distribution with a log link.

Enzymatic, non-enzymatic, and anatomical variables were analyzed with GLMMs using a Gamma distribution and log link. For enzymatic and non-enzymatic variables, phenological stage was also included as a fixed effect.

6.5. Results

6.5.1. Diversity of grapevine rhizosphere microbial communities

Shannon diversity index of rhizosphere microbial communities was primarily influenced by phenological stage (Table 1, Supporting Information Table S1). For prokaryotic communities, Shannon diversity significantly increased from flowering to harvest across all treatments ($p < 0.001$), with no significant effects of soil management nor water status. In fungal communities, Shannon diversity was also significantly higher at harvest

compared to flowering ($p = 0.021$) and showed a trend toward interaction between water status and phenological stage ($p = 0.078$). Neither soil management nor water status alone had a significant effect on microbial diversity for either community type.

Table 1 Shannon diversity index of prokaryotic and fungal communities in the rhizosphere of *Vitis vinifera* L. cv. Tannat at flowering and harvest during the 2020/21 season, under two under-vine soil management practices—manual weeding (MW) and permanent cover crop (PCC)—and two irrigation regimes: no restriction (NR) and 20-day pre-veraison restriction (WR). Values are means ± SE.

Community	Phenological stage	Soil management. Water status			
		MW. NR	MW.WR	PCC.WR	PCC.NR
Prokaryotic	Flowering 2020	5.81 ± 0.09	5.87 ± 0.09	5.64 ± 0.09	5.66 ± 0.09
	Harvest 2021	6.38 ± 0.09	6.29 ± 0.09	6.18 ± 0.09	6.23 ± 0.09
Fungal	Flowering 2020	3.73 ± 0.39	2.71 ± 0.39	3.66 ± 0.39	2.77 ± 0.39
	Harvest 2021	3.70 ± 0.39	4.00 ± 0.39	4.07 ± 0.39	3.96 ± 0.39

Diversity composition of microbial communities in the grapevine rhizosphere showed different trends for prokaryotic and fungal communities at flowering and harvest (Table 2). For prokaryotic communities, soil management (SM) had a moderate but not statistically significant impact at flowering ($F = 1.8229$, $R^2 = 0.10$, $p = 0.068$). However, water status showed a significant effect ($F = 2.7852$, $R^2 = 0.15$, $p = 0.008$), while neither the interaction between soil management and water status nor the block effects were significant. By the time of harvest, SM significantly affected prokaryotic communities ($F = 5.4047$, $R^2 = 0.27$, $p = 0.001$), whereas water status and the interactions between factors did not show significant impacts.

Regarding fungal communities, water status had an impact on community composition at flowering ($F = 2.7065$, $R^2 = 0.15$, $p = 0.078$), although this effect was not statistically significant. SM, block, and the interactions did not exhibit significant effects. At harvest, neither SM, water status, nor their interaction showed significant impacts on fungal communities.

Table 2. PERMANOVA results based on Weighted UniFrac distance (prokaryotic communities) and Bray–Curtis distance (fungal communities) at flowering and harvest during the 2020/21 season.

Dataset	Factor	F. Model	R^2	Pr (> F)
Prokaryotic- flowering 2020	Soil Management	18.229	0.10	0.068
	Water status	27.852	0.15	0.008
	Block	13.488	0.07	0.190
	Soil management*Water status	14.832	0.08	0.146
Prokaryotic- harvest 2021	Soil Management	54.047	0.27	0.001
	Water status	17.801	0.09	0.091
	Block	0.8706	0.13	0.580
	Soil management*Water status	10.488	0.05	0.307
Fungal- flowering 2020	Soil Management	14.929	0.08	0.147
	Water status	27.065	0.15	0.078
	Block	13.666	0.22	0.172
	Soil management*Water status	0.8380	0.04	0.509
Fungal- harvest 2021	Soil Management	12.113	0.08	0.243
	Water status	11.980	0.08	0.277
	Block	0.9749	0.19	0.505
	Soil management*Water status	0.7005	0.04	0.770

6.5.2. Response of prokaryotes in the grapevine rhizosphere

A differential abundance analysis identified taxa significantly influenced (responders) by treatment effects that impacted community composition, as determined by PERMANOVA. The analysis focused on WS at flowering and SM at harvest for prokaryotic communities. Eighteen genera were identified as responders in both conditions. Taxa such as *SH-PL14*, *Sphingobium* and *Paenibacillus* among others were enriched under WR conditions. Conversely, taxa including *UTBCD1*, *Fimbriimonas*, and *Rahnella1* were more abundant in NR conditions (Figure 1A). At harvest, soil management also influenced microbial composition, with 13 and 16 genera identified as responders of

PCC and MW respectively (Figure 1B). Under PCC, taxa such as *Aeromonas* and *Pantoea* among others were significantly more abundant.

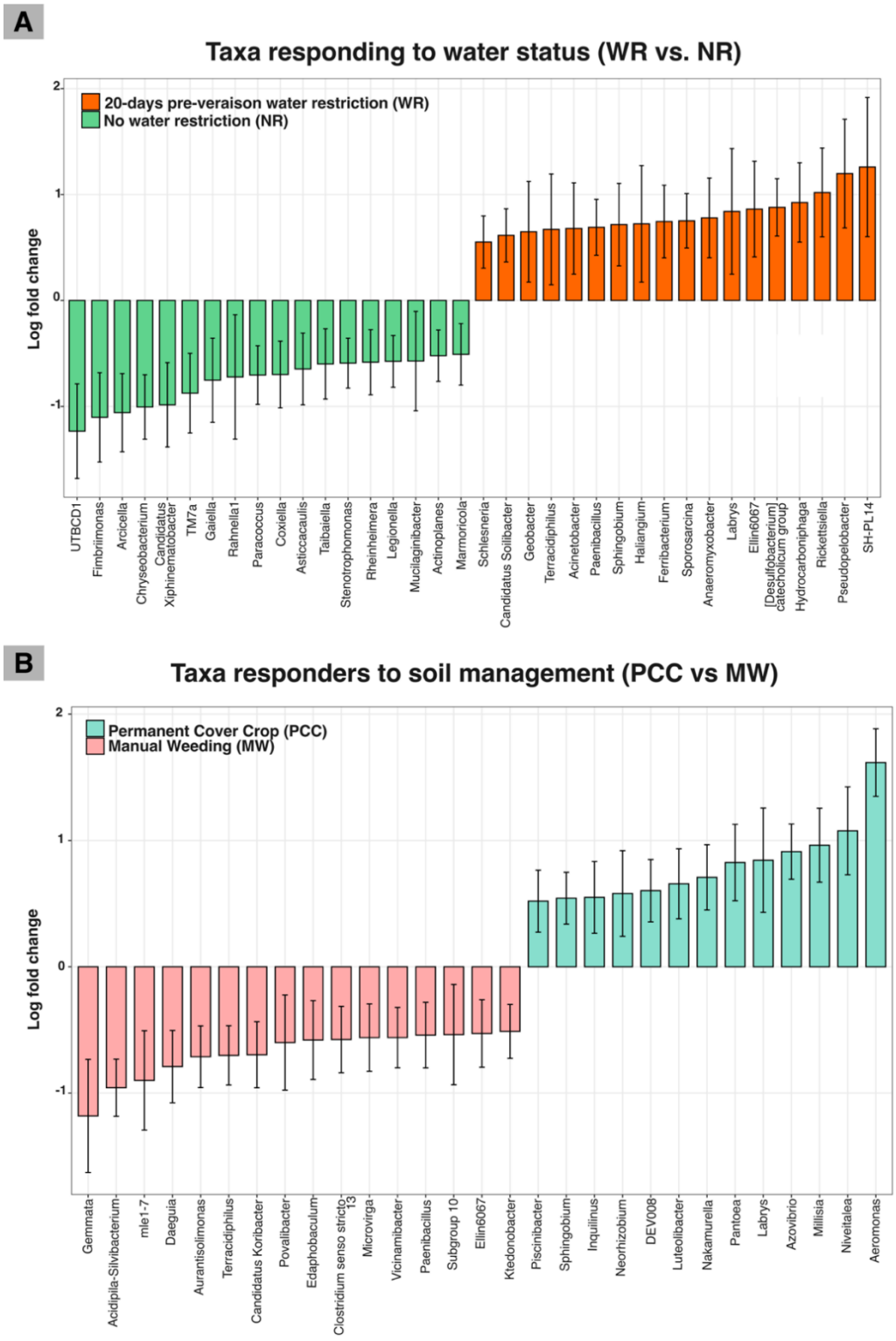

Figure 1. Differential abundance of prokaryotic taxa identified using ANCOM-BC. (A) Log fold change (LFC) of taxa responding to water status at

flowering (20-day pre-veraison restriction, WR, vs. no restriction, NR). (B) Log fold change (LFC) of taxa responding to under-vine soil management at harvest (permanent cover crop, PCC, vs. manual weeding, MW).

The functional prediction of the prokaryotic communities at flowering and harvest revealed distinct patterns influenced by water status and soil management (Figure 2). The PCA for water status indicated a slight separation between samples under NR and WR conditions along the second principal component (PC2, explaining 12.9% of the variance). In contrast, the PCA for SM demonstrated a clear separation between MW and PCC treatments along PC2 (14.9%), highlighting differences in microbial pathway composition associated with soil management. At flowering, the “D-Arginine and D-ornithine metabolism” pathway showed a significant increase in activity under WR (p-adjusted = 0.028). At harvest, pathways such as "Isoquinoline alkaloid biosynthesis" (p-adjusted = 0.003), "Bacterial chemotaxis" (p-adjusted = 0.002), and "alpha-Linoleic acid metabolism" were significantly enriched in the PCC treatment. In contrast, "Sesquiterpenoid and triterpenoid biosynthesis" exhibited reduced activity under PCC compared to MW (p-adjusted = 0.003).

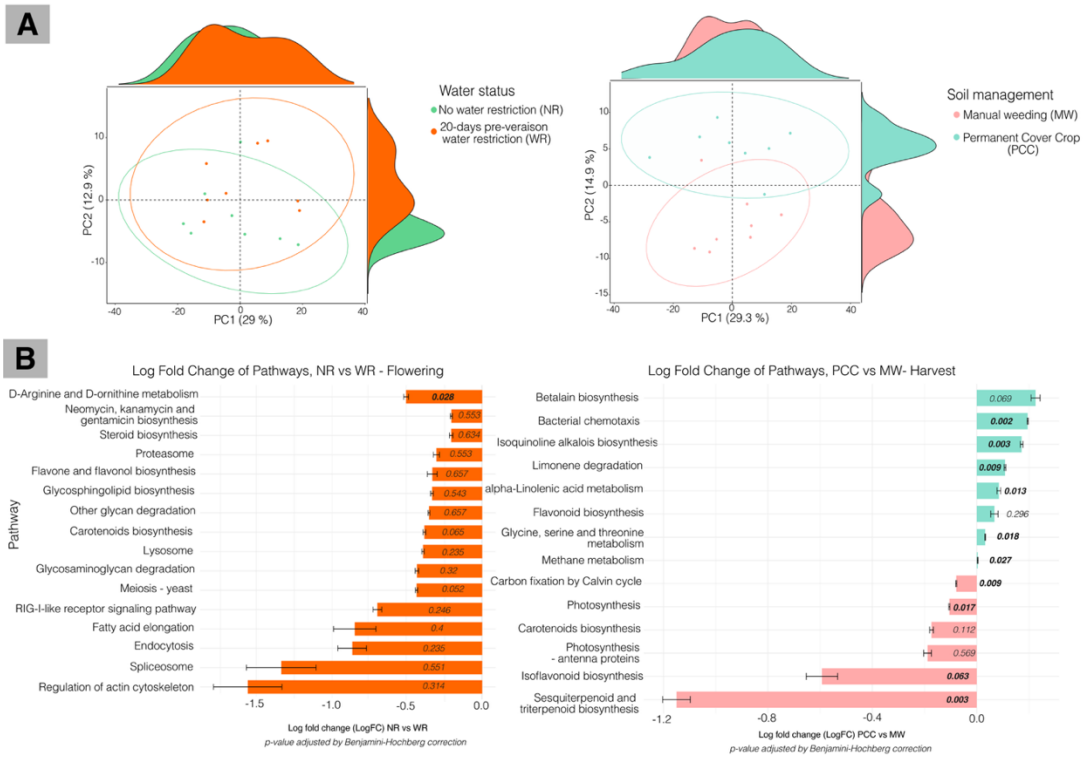

Figure 2. Functional prediction (PICRUSt2) of prokaryotic communities from the rhizosphere of *Vitis vinifera* L. cv. Tannat under two irrigation regimes—no restriction (NR) and 20-day pre-veraison restriction (WR)—and two under-vine soil management—manual weeding (MW) and permanent cover crop (PCC)—at flowering and harvest during the 2020/21 season. (A) Principal component analysis (PCA) of functional pathway abundance. (B) Log fold change (LogFC) of pathways with p-values adjusted using the Benjamini–Hochberg correction.

6.5.3. Soil management and water status influence on grape must composition and vine vigor

Soil management and water status influenced free amino nitrogen (FAN) levels (SM: $\chi^2 = 4.5$, $p = 0.03$; WS: $\chi^2 = 15.2$, $p < 0.001$). Additionally, water status significantly affected pH ($\chi^2 = 7.6$, $p = 0.006$) and pruning weight ($\chi^2 = 9.46$, $p = 0.002$) (Supplementary Material, Table S2).

6.5.4. *Botrytis* bunch rot incidence, intensity and AUDPC

The incidence of BBR in bunches was significantly influenced by SM ($\chi^2 = 41.8$, $p < 0.001$) and WS ($\chi^2 = 6.5$, $p = 0.01$), but not by factors interaction ($\chi^2 = 0.02$, $p = 0.879$) (Figure 3A and B). Specifically, PCC exhibited the lowest BBR incidence (0.20 ± 0.05), which was significantly different from MW plots (0.71 ± 0.06). Regarding WS, water-restricted (WR) plants showed lower BBR incidence (0.35 ± 0.07) than not restricted plants (NR) (0.53 ± 0.07).

Disease intensity (%) was significantly influenced by SM ($\chi^2 = 17.5$, $p < 0.001$), WS ($\chi^2 = 7.9$, $p = 0.005$) and interaction ($\chi^2 = 4.34$, $p = 0.04$) (Figure 3C). Specifically, PCC under WR exhibited the lower disease intensity (1.05 ± 0.39) in comparison with PCC managed with NR (PCC.NR: 5.57 ± 1.73), and manual weeding (MW.WR: 8.38 ± 2.45, MW.NR: 11.57 ± 3.5).

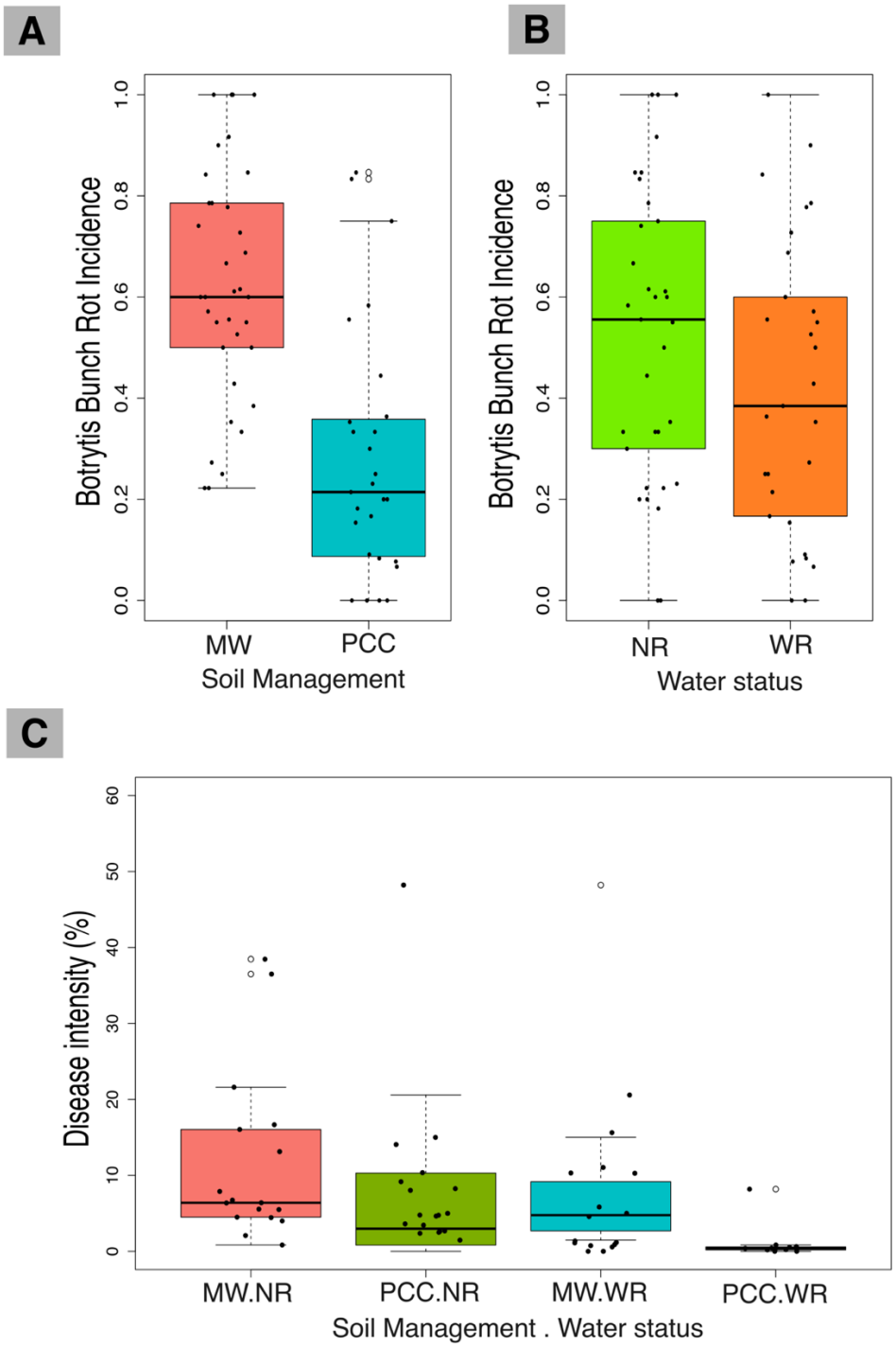

Figure 3. Botrytis bunch rot (BBR) incidence and intensity (incidence × severity, %) at harvest 2021 in *Vitis vinifera* L. cv. Tannat. (A) BBR incidence under two under-vine soil management: permanent cover crop (PCC) vs.

manual weeding (MW). (B) BBR incidence under two water status conditions: no restriction (NR) vs. 20-day pre-veraison restriction (WR). (C) BBR intensity for the interaction between soil management (PCC, MW) and water status (NR, WR).

The analysis of AUDPC for grapevine leaves inoculated with *B. cinerea* revealed significant effects of SM ($\chi^2 = 19.1$, $p < 0.001$), WS ($\chi^2 = 22.9$, $P < 0.001$), and phenological stage ($\chi^2 = 11.5$, $p < 0.001$), with no significant interaction between soil management and water status. At both veraison and harvest, the lowest AUDPC values were observed under PCC (16.3 ± 1.9 and 25.3 ± 2.8) compared to MW (28.7 ± 3.1 and 44.6 ± 5.0). Similarly, water restricted plants exhibited lower AUDPC values (15.9 ± 1.7 and 24.7 ± 2.8) than non-restricted plants (29.4 ± 3.3 and 45.6 ± 5.1). These findings highlight that both soil management and water status independently and significantly reduce disease progression, with PCC and WR plants consistently associated with the lowest AUDPC values.

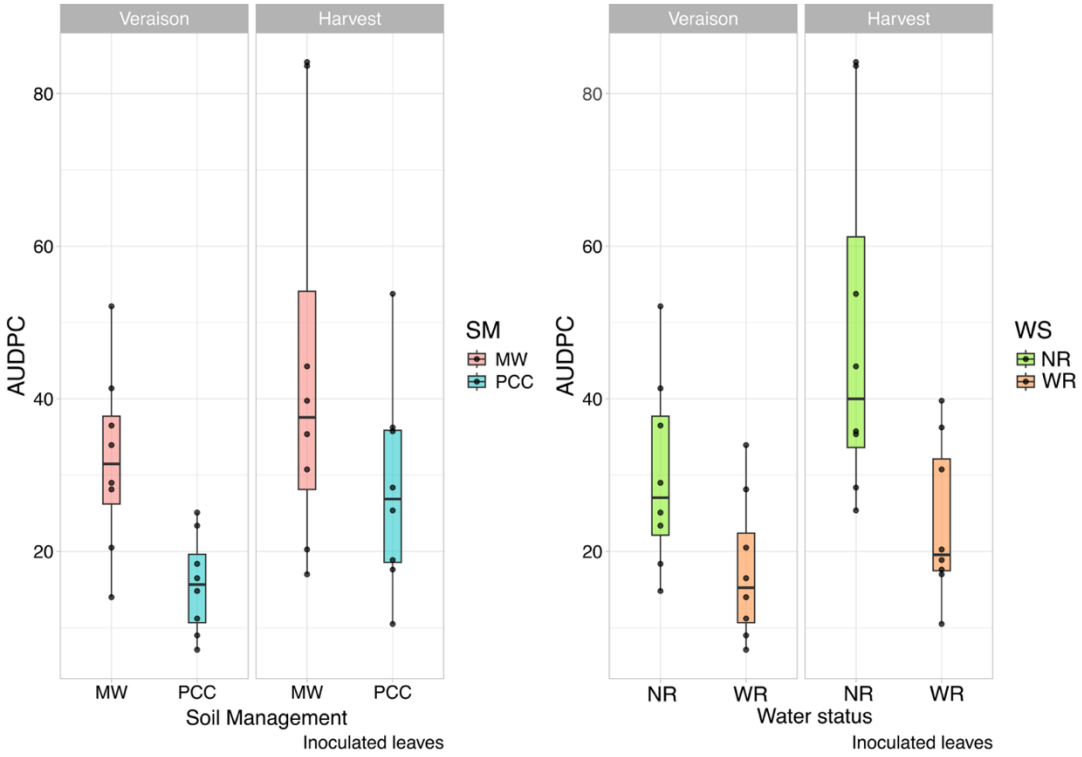

Figure 4. Area under the disease progress curve (AUDPC) for *Vitis vinifer*a L. cv. Tannat leaves inoculated with *Botrytis cinerea* at veraison and harvest during the 2020/21 season. (A) Under-vine soil management: manual weeding (MW) vs. permanent cover crop (PCC). (B) Water status: no restriction (NR) vs. 20-day pre-veraison restriction (WR).

6.5.5. Defense responses

The enzymatic and non-enzymatic antioxidant responses in grapevine leaves and berry skin varied significantly across phenological stages, soil management (SM) and water status (WS) (Supplementary information, Table S3).

Peroxidase (POD) activity in leaves was significantly affected by phenological stage ($\chi^2 = 524$, $p < 0.001$), WS ($\chi^2 = 35$, $p < 0.001$), and their interaction ($\chi^2 = 28$, $p < 0.001$). In berry skin, POD was influenced by phenological stage ($\chi^2 = 33$, $p < 0.001$), WS ($\chi^2 = 7$, $p = 0.007$), and the interaction SM x phenological stage ($\chi^2 = 6$, $p = 0.009$).

Ascorbate peroxidase (APX) activity in leaves was significantly influenced by phenological stage ($\chi^2 = 217$, $p < 0.001$), WS ($\chi^2 = 93$, $p < 0.001$), and by several interactions: SM × WS ($\chi^2 = 37$, $p < 0.001$), WS × phenological stage ($\chi^2 = 10$, $p = 0.005$), and SM × WS × phenological stage ($\chi^2 = 27$, $p < 0.001$). In berry skin, APX was influenced by phenological stage ($\chi^2 = 22$, $p < 0.001$), SM ($\chi^2 = 4$, $p = 0.048$), and the interaction SM x WS ($\chi^2 = 5$, $p = 0.02$).

Catalase (CAT) activity in leaves was significantly affected by phenological stage ($\chi^2 = 22$, $p < 0.001$), SM ($\chi^2 = 42$, $p < 0.001$), WS ($\chi^2 = 112$, $p < 0.001$), and by several interactions: SM x WS ($\chi^2 = 48$, $p < 0.001$), SM x phenological stage ($\chi^2 = 22$, $p < 0.001$). In berry skin, CAT was influenced by phenological stage ($\chi^2 = 64$, $p < 0.001$) and SM ($\chi^2 = 4$, $p = 0.04$).

Superoxide dismutase (SOD) in leaves was only affected by phenological stage ($\chi^2 = 354$, $p < 0.001$). In berry skin, SOD was significantly influenced by SM ($\chi^2 = 12$, $p < 0.001$) and its interaction with phenological stage ($\chi^2 = 10$, $p = 0.001$).

Total phenol content (TPC) in leaves was strongly affected by phenological stage ($\chi^2 = 30$, $p < 0.001$) and by the following interactions: SM x phenological stage ($\chi^2 = 5$, $p = 0.02$) and WS x Time ($\chi^2 = 11$, $p < 0.001$). In berry skin, TPC was influenced by phenological stage ($\chi^2 = 30$, $p < 0.001$) and by the following interactions: SM x phenological stage ($\chi^2 = 5$, $p = 0.02$) and WS x Time ($\chi^2 = 11$, $p < 0.001$).

The anatomical characterization of berry skin at harvest revealed significant effects of SM on cuticle thickness ($\chi^2 = 19.7$, $p < 0.001$) and its interaction with water status ($\chi^2 = 8.04$, $p = 0.004$). Water status by itself had no effect ($\chi^2 = 2.31$, $p = 0.128$). Berry skin from PCC under water restriction showed a thicker cuticle than berries from the rest of treatments. The epidermis thickness was not affected by SM ($\chi^2 = 3.5$, $p = 0.06$), WS ($\chi^2 = 3.4$, $p = 0.06$) nor the interaction ($\chi^2 = 0.35$, $p = 0.554$) (Table 3).

Table 3. Anatomical characterization of Tannat berry skin at harvest 2021 under two under-vine soil management practices—manual weeding (MW) and permanent cover crop (PCC)—and two irrigation regimes: 20 days pre-veraison water restriction (WR) and no water restriction (NR). Values represent means ± SE. Different letters indicate significant differences between soil management (Tukey's test, $p < 0.05$)

Anatomical variable	MW.WR	MW.NR	PCC.WR	PCC.NR
Cuticule thickness (mm)	1.52± 0.06 a	1.59± 0.07 a	1.98±0.08 b	1.66±0.08 a
Epidermis thickness (mm)	19.4 ± 0.51 a	19.9 ± 0.56 a	20 ± 0.52 a	21 ± 0.63 a

6.5.6. Multivariate Analysis of Defense Responses and Disease Metrics

Variation patterns among 33 variables related to defense mechanisms and disease metrics, showed that the first two PCs were significant, explaining in combination 44.3% of the total variance in the dataset. PC1 alone explained 22.9% of the total variation (95% CI: 24.2-37.1), while PC2 explained 21.4% (95% CI: 19.6-28.1) (Figure 5). Variables with significant loadings on PC1 included total phenolic content at different stages in leaves and berries (TPC_F_I, TPC_H_I, TPC_V_I, TPC_H_b), disease metrics (DI_H, INC_H, AUDPC_V), and catalase activity (CAT_F_I, CAT_V_b). PC2 had significant loadings for antioxidant enzyme activities (catalase, peroxidase, ascorbate peroxidase, and superoxide dismutase) at various stages in leaves and berries, along with cuticle thickness (CUT_THICK). These loadings highlight the main variables influencing each axis, suggesting the drivers of observed patterns.

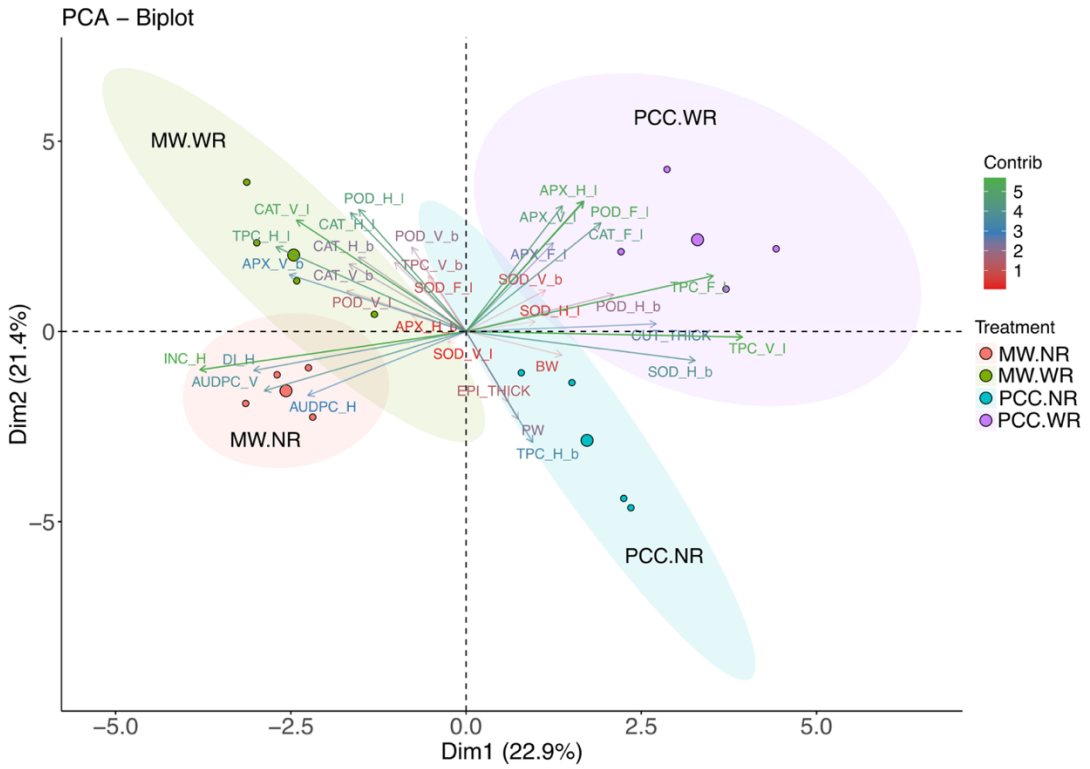

Figure 5. PCA biplot of antioxidant activity, canopy characteristics, and disease metrics measured in leaves and berries of *Vitis vinifera* L. cv. Tannat under two under-vine soil management—manual weeding (MW) and permanent cover crop (PCC)—and two water status treatments—no restriction (NR) and 20-day pre-veraison restriction (WR). Colored ellipses denote treatment combinations (MW.NR: red; MW.WR: green; PCC.NR: blue; PCC.WR: purple).

6.5.7. Influence of Disease Metrics and Cuticle Thickness on Prokaryotic Communities

The influence of disease metrics (disease incidence, disease intensity, and AUDPC at veraison and harvest) together with an anatomical trait (cuticle thickness) on microbial community composition, based on the weighted UniFrac distance matrix, was significant overall ($F = 2.62$, $p = 0.002$). These explanatory variables accounted for 56.7% of the total variation in community composition, while residuals explained the remaining

43.3%. The dbRDA plot shows the distribution of this explained variation within the constrained space. Specifically, dbRDA1 and dbRDA2 captured 67.5% and 13.2% of the constrained variance, respectively (Figure 6). An axis-wise permutation test further revealed that only the first constrained axis (dbRDA1) was statistically significant ($F = 8.83$, $p = 0.001$), whereas the subsequent axes (dbRDA2–dbRDA5) were not ($p > 0.05$). Testing each environmental variable sequentially showed that cuticule thickness - CUT_THICK ($F = 2.5923$, $p = 0.019$) and AUDPC at veraison - AUDPC_V ($F = 6.2793$, $p = 0.003$) were statistically significant. Other predictors, including disease intensity at harvest - DI_H ($F = 1.44$, $p = 0.150$), AUDPC at harvest- AUDPC_H ($F = 0.92$, $p = 0.393$), and incidence at harvest- INC_H ($F = 1.87$, $p = 0.082$), were not significant, although INC_H showed a marginal effect.

The enzymatic responses were not included in the dbRDA model due to the complexity and high dimensionality of these variables. With multiple enzymes measured across three sampling moments, the dataset would contain many variables, potentially leading to issues such as multicollinearity, overfitting, and difficulty in interpreting the dbRDA results. Additionally, enzyme activity responses varied significantly across different enzymes and sampling times, which could obscure the relationships with microbial community composition. By focusing on key variables, the analysis was able to achieve a more robust and interpretable model, allowing clearer insight into the primary factors driving microbial community changes.

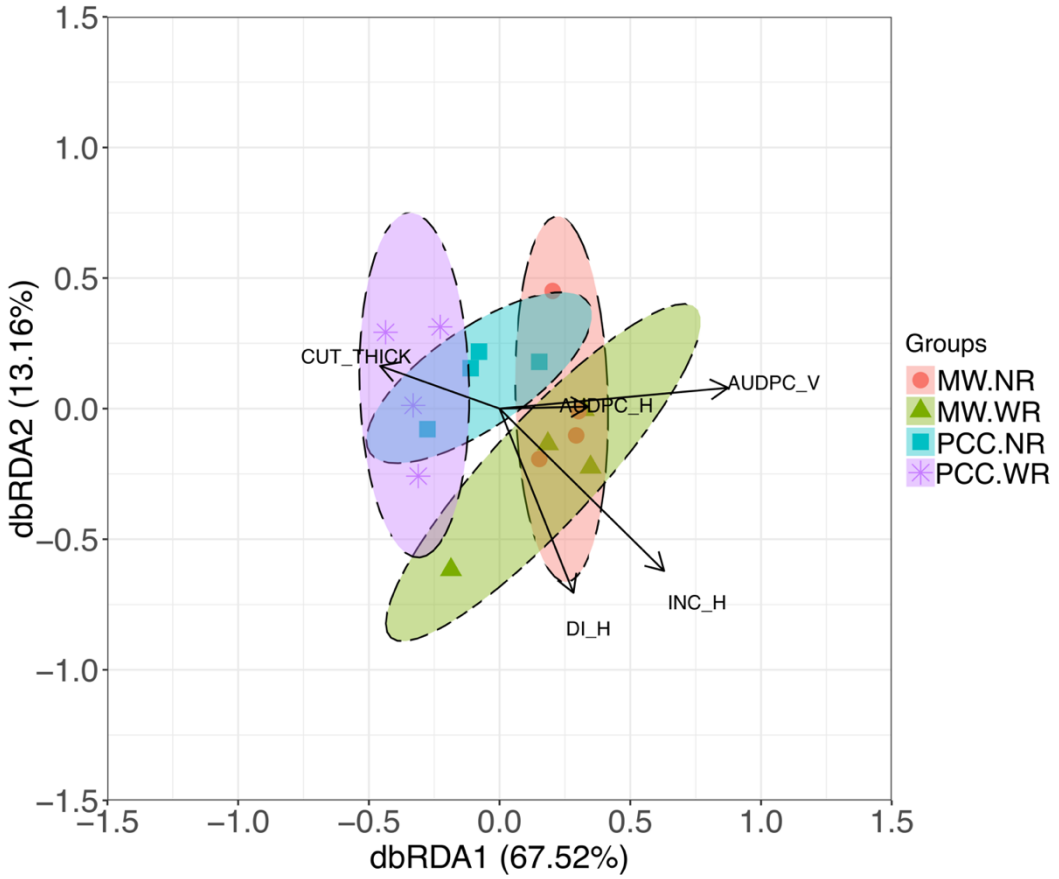

Figure 6. Distance-based redundancy analysis (dbRDA) of grapevine rhizosphere prokaryotic communities constrained by under-vine soil management and water status, including disease metrics and cuticle thickness, during the 2020/21 season. Treatments: manual weeding (MW) vs. permanent cover crop (PCC); water status defined by irrigation regime—no restriction (NR) and 20-day pre-veraison restriction (WR). Colored ellipses denote treatment combinations (MW.NR, MW.WR, PCC.NR, PCC.WR; red, green, blue, and purple, respectively). Arrows indicate the direction of each explanatory variable; arrow color (green → red) denotes increasing contribution strength.

6.6. Discussion

6.6.1. Permanent cover crop and water status reduce Botrytis bunch rot and modulate grapevine rhizosphere

Our study highlights a significant impact of SM on grapevine rhizosphere microbial communities, particularly prokaryotic communities, and on grapevine health in relation to susceptibility to Botrytis bunch rot (BBR). Water restriction exerted a more pronounced and statistically significant effect on prokaryotic community composition at flowering while soil management significantly influenced prokaryotic communities at harvest. The lack of significant interaction effects suggests that the influences of soil management and water status on microbial diversity are likely to be independent.

The lack of significant changes in the composition of fungal communities in the grapevine rhizosphere under different SM and WS levels in this experiment is surprising. This result contrasts with findings from field experiments and other pot-experiments, where fungal microbiota often exhibit sensitivity to these factors (Bernaschina et al., 2023; Carbone et al., 2021). Possibly, the controlled nature of this pot experiment, which may not fully reproduce the environmental complexity, soil heterogeneity and fungal interactions present under field conditions, could play a role in shaping fungal community dynamics. In addition, the use of a homogenized substrate—composed of equal parts soil and compost and sieved for mixing—may have further reduced environmental variability and masked potential treatment effects for this community.

The incidence of BBR was significantly affected by both SM and WS, although no significant interaction between these factors was detected. The reduction in BBR incidence was more pronounced under different SM (72%) than between WS levels (34%). In contrast, disease intensity (which considers severity) was impacted by both factors and their interaction, indicating that PCC, particularly when combined with a pre-veraison water

restriction, reduced the incidence of symptoms but also its severity at harvest.

This effect could be attributed to PCC's potential role in promoting beneficial microbial communities or enhancing plant resilience given its association at harvest with a higher abundance of plant growth-promoting rhizobacteria (PGPR), such as *Pantoea*. This genus is known for its role in enhancing systemic resistance, potentially reinforcing grapevine defenses against initial infections (Walterson & Stavrinides, 2015). Notably, *Pantoea* was also identified as a responder to PCC in the rhizosphere of Tannat grapevines in a previous study under field-conditions (Bernaschina et al., 2023), underlining its consistent association with this management practice. Certain strains of *Pantoea agglomerans* have demonstrated high efficacy in inducing systemic responses-ISR- and reducing symptoms of gray mold disease caused by *B. cinerea* (Aziz et al., 2016; Magnin-Robert et al., 2013). In the other hand, MW management promoted an increased abundance of Acidobacteria, which, although valuable for nutrient cycling (Kalam et al., 2020; Ward et al., 2009), may not offer the same protective effects against *Botrytis* infection. These results underscore the potential of PCC management, especially under moderate WS, to create a rhizosphere environment that enhances resistance to BBR by fostering beneficial microbial taxa.

Pre-veraison water status also impacted the prokaryotic community's composition, with water restricted plants showing a higher abundance of taxa such as *Sphingobium* (a PCC-responsive taxon at harvest) and *Paenibacillus* (an MW-responsive taxon at harvest) in their rhizosphere. These taxa may contribute to plant resilience under water restriction conditions by enhancing nutrient availability and stress tolerance (Asaf et al., 2020; Dixit et al., 2022; Timmusk & Wagner, 1999), which could be crucial for maintaining plant health in water-limited conditions. Water restriction could be introducing an additional selective pressure, favoring stress-resilient taxa that support plant

health under challenging conditions, as evidenced by the reduced incidence of BBR in PCC-WR grapevines.

Although the composition of the prokaryotic community under different water status differed, the metabolic pathways predicted as significantly different between WS conditions were scarce. Only the “D-arginine and D-ornithine metabolism” pathway showed differences (ko00472; $p = 0.00022$, adjusted $p = 0.0278$) between NR and WR conditions. The increased activity of this pathway under WR suggests a potential adaptive role in the rhizosphere of grapevines under these conditions. The low differentiation among pathways between WS conditions could be attributed to functional redundancy, a phenomenon where multiple microbial taxa perform similar metabolic roles, ensuring the resilience of ecosystem functions even when community composition changes (Louca et al., 2018). It has been shown that despite taxonomic shifts in microbial communities under varying conditions, functions like nitrogen cycling or carbon fixation remain stable due to contributions from a diverse array of taxa (Louca et al., 2018). Such redundancy highlights the robustness of microbial communities, particularly in complex ecosystems like the rhizosphere, and could explain the limited number of significantly different pathways observed in this study. The limited functional differences may also reflect the inherent constraints of functional prediction tools like PICRUSt2, which infer functions based on the 16S rRNA gene profiles and annotated reference databases. While these tools are powerful, they are not immune to biases or gaps in database coverage. Some metabolic pathways may not be fully represented in the available reference genomes, potentially leading to an underestimation of functional differences (Djemiel et al., 2022; Douglas et al., 2019).

In the other hand, the functional prediction of metabolic pathways at harvest under different SM conditions revealed several pathways in the PCC communities that could be associated with plant defense mechanisms. A highly abundant pathway predicted in the PCC communities was the “isoquinoline alkaloid biosynthesis” (ko00950; $p = 0.0005$, adjusted $p =$

0.003) pathway, which is linked to the production of plant secondary metabolites ([PATH:map01060]). These metabolites often play defensive roles against pathogens and herbivores (R. N. Bennett & Wallsgrove, 1994; Goswami et al., 2016). In addition, the “α-linolenic acid metabolism” (ko00592; p = 0.0055, adjusted p = 0.013) pathway (M00113; [PATH:map00592]) plays a critical role in the biosynthesis of jasmonic acid (JA), a key signaling molecule in plant defense responses. Notably, in cucumber, activation of the α-linolenic acid pathway by specific treatments enhances resistance to *B. cinerea*, promoting increased JA synthesis (Jia et al., 2024). Beneficial microbes further contribute to plant immunity by triggering induced systemic resistance (ISR), which relies on both salicylic acid (SA) and jasmonic acid/ethylene (JA/ET) signaling pathways (Yu et al., 2022). Plants recruit microbes in their rhizosphere through exudates, and these exudates are known to vary its composition depending on the cultivar, the exposure to stress (biotic and abiotic), the plant growth stage and even along different root’s zones (Compant et al., 2010; Pieterse et al., 2014). In this context, bacterial chemotaxis (ko02030; p = 0.0002, adjusted p = 0.002) emerges as a crucial process, enabling microbial cells to detect and move along chemical gradients in their environment and to successfully colonize the rhizosphere (Compant et al., 2010).

In contrast to the predicted pathways enriched in PCC-associated prokaryotic communities, which are predominantly linked to plant defense and signaling, the pathways predicted as more abundant in MW communities reflect microbial processes centered on fundamental metabolic and ecological functions. The enrichment of Sesquiterpenoid and Triterpenoid Biosynthesis (ko00909; p = 0.0006, adjusted p = 0.003) suggests microbial strategies for competition and adaptation, as these metabolites play roles in antimicrobial activity and root-microbe signaling (Ma et al., 2016; Wang & Niu, 2019). These pathways emphasize the ecological adaptations of MW-associated microbes to maintain essential soil functions, contrasting with the

PCC communities, where pathways are more directly associated with enhancing plant defense and resilience.

6.6.2. Permanent cover crop and water restriction promote grapevine defense responses

Grapevines managed under PCC and subjected to WR exhibited significantly higher cuticle thickness of berry skin at harvest, suggesting enhanced structural defenses compared to those managed with MW or in NR conditions. This thicker cuticle likely acts as a physical barrier against pathogen invasion, potentially contributing to the lower disease incidence observed in PCC under WR (Gabler et al., 2003). However, the results for antioxidant activity showed considerable variability, with no clear trend across treatments, highlighting the complex interplay of abiotic and biotic factors that can trigger these responses (Mohammadi et al., 2021). The PCA indicated that enzymatic (e.g., APX) and non-enzymatic antioxidant activities (e.g., TPC) in leaves were elevated in some PCC-managed grapevines under WR. This response is likely part of the reactive oxygen species (ROS) scavenging mechanisms that mitigate oxidative stress and protect cellular integrity, potentially enhancing plant resilience under certain stress conditions (Hasanuzzaman, 2020). Conversely, MW-treated grapevines under WR displayed different antioxidant activities, including elevated catalase (CAT) activity in both leaves and berry skin, as well as peroxidase (POD) activity in leaves. These varied responses, observed across different enzymes and plant organs, indicate that antioxidant activity is a multifaceted defense mechanism influenced by numerous factors, including SM and environmental stressors. Given the variability in antioxidant responses, these results should be interpreted with caution, recognizing that both management practices and specific environmental conditions imposed by the pot-experiment can drive fluctuations in these defense pathways.

In Uruguayan vineyards, where most are non-irrigated, the predominant under-vine soil management involves maintaining bare soil to minimize

competition between spontaneous vegetation and grapevines for water and nutrients (Coniberti, Ferrari, Disegna, García Petillo, et al., 2018). This is primarily achieved through the application of herbicides, such as glyphosate and glufosinate ammonium, though their use is increasingly under discussion due to environmental and sustainability concerns. Some winegrowers have started exploring alternative approaches, including the use of mechanical in-row weeders to manage under-vine vegetation. In the context of sustainable viticulture, adopting permanent cover crops in combination with irrigation appears to be a more suitable strategy. This approach can help reduce herbicide use, mitigate the negative impacts of bare soil on soil health, and as demonstrated in this study, lower the incidence of Botrytis bunch rot.

In conclusion, our findings suggest that PCC supports beneficial microbial communities and enhances structural defenses- specially under water restriction- which collectively could provide a reduced incidence of Botrytis bunch rot. The findings of this study will contribute to a better understanding of the role of soil management and water availability in modulating rhizosphere microbiota and enhancing grapevine resistance to diseases, providing valuable insights for the development of sustainable vineyard management strategies.

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6.7. Supporting information

Figure S1. Experimental design. A- general view. B- grapevines in pots with manual weeding (MW), C- grapevines in pots with permanent cover crop (PCC).

Table S1. Type III Analysis of Variance Using Satterthwaite's Method for Shannon Index of Prokaryotic Communities and Fungal Communities in the Grapevine Rhizosphere (Tannat, *Vitis vinifera* L.) at Flowering in 2020 and Harvest in 2021.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)	
Prokaryotic communities- Shannon Index							
Soil management (SM)	0.05369	0.05369	1	6	2.4464	0.1688	
Water status (WS)	0.00056	0.00056	1	18	0.0257	0.8744	
Phenological stage (PS)	**2.21707**	**2.21707**	**1**	**18**	**101.0274**	**8.255e-09**	*******
SM:WS	0.00454	0.00454	1	18	0.2068	0.6547	
SM: PS	0.00922	0.00922	1	18	0.4201	0.5251	
WS: PS	0.00813	0.0813	1	18	0.3704	0.5504	
SM:WS: PS	0.01628	0.01628	1	18	0.7418	0.4004	
Fungal communities- Shannon Index							
Soil management (SM)	0.0503	0.0503	1	6	0.0798	0.78700	
Water status (WS)	1.4641	1.4641	1	18	2.3237	0.14479	
Phenological stage (PS)	**4.0443**	**4.0443**	**1**	**18**	**6.4190**	**0.02080**	*****
SM:WS	0.0390	0.0390	1	18	0.0618	0.80644	
SM: PS	0.0615	0.015	1	18	0.0976	0.75832	
WS: PS	**2.1981**	**2.1981**	**1**	**18**	**3.4887**	**0.07816**	**.**
SM:WS: PS	0.1486	0.1486	1	18	0.2539	0.63305	

Table S2. Effects of Soil Management (MW: manual weeding, PCC: permanent cover crop) and Water Status (NR: no water restriction; WR: 20 days pre-veraison water restriction) on Grape Composition and Vegetative Parameters in Tannat.

Soil Management-Water Status	MW-NR	MW-WR	PCC-NR	PCC-WR	SM effect	WS effect	SM:WS effect
Free amino Nitrogen (g/L)	123.83 ± 9.58	115.08 ± 7.53	119.08 ± 6.07	110.25 ± 10.11	*	**	ns
Total soluble solids (°Brix)	23.5 ± 0.43	23.56 ± 0.49	23.57 ± 0.38	23.62 ± 0.41	ns	ns	ns
Acidity (g/L)	6.57 ± 0.41	6.48 ± 0.31	6.68 ± 0.53	6.5 ± 0.5	ns	ns	ns
pH	3.54 ± 0.05	3.62 ± 0.06	3.58 ± 0.07	3.6 ± 0.07	ns	**	ns
Bunch weight (Kg)	0.23 ± 0.01	0.23 ± 0.01	0.25 ± 0.03	0.24 ± 0.01	ns	ns	ns
Prunning weight (kg/m)	0.62 ± 0.06	0.46 ± 0.05	0.61± 0.06	0.48 ± 0.05	ns	**	ns
Yield (kg/plant)	2.84 ± 0.24	2.84 ± 0.23	2.87 ± 0.40	2.52 ± 0.25	ns	ns	ns

Table S3. Antioxidant enzymatic (peroxidase-POD, ascorbate peroxidase-APX, catalase-CAT and superoxide dismutase-SOD) and non-enzymatic activity (total phenols content-TPC) in grapevine leaves and berry skin at flowering, veraison and harvest under different under-vine soil management (MW: weed management; PCC: permanent cover crops) and water status (NR: no water restriction; WR: 20 days pre-veraison water restriction).

Antioxidant compound	Organ	Soil management	Water status	Flowering	Veraison	Harvest
Superoxide dismutase-SOD (U mg protein-1)	Leaves	MW	WR	5.79 ± 1.22 a	39.78 ± 8.36 a	4.32 ± 0.9 a
			NR	4.8 ± 1.01 a	36.35 ± 7.65 a	5.01 ± 1.05 a
		PCC	WR	5.09 ± 1.07 a	40.4 ± 8.58 a	6.35 ± 1.34 a
			NR	5.03 ± 1.07 a	34.27 ± 7.19 a	5.11 ± 1.08 a
	Berry skin	MW	WR		28.3 ± 9.13 a	10 ± 3.27 a
			NR		26.3 ± 8.5 a	19.4 ± 6.27 ab
		PCC	WR		34.3 ± 11.2 a	41.4 ± 13.6 b
			NR		23.3 ± 7.79 a	43 ± 13.8 b
Peroxidase-POD (U mg protein-1)	Leaves	MW	WR	0.02 ± 0.01 b	0.06 ± 0.01 a	0.43 ± 0.09 b
			NR	0.01 ± 0.00 a	0.06 ± 0.01 a	0.33 ± 0.07 ab

		PCC	WR	0.04 ± 0.01 b	0.05 ± 0.01 a	0.36 ± 0.07 ab
			NR	0.01 ± 0.00 a	0.05 ± 0.01 a	0.20 ± 0.04 a
	Berry skin	MW	WR		0.28 ± 0.05 a	0.34 ± 0.07 ab
			NR		0.17 ± 0.04 a	0.29 ± 0.06 a
		PCC	WR		0.18 ± 0.04 a	0.69 ± 0.14 b
			NR		0.13 ± 0.04 a	0.35 ± 0.07 ab
Catalase-CAT (mmol H2O2 min-1 mg protein -1)	Leaves	MW	WR	3.56 ± 0.6 b	10.56 ± 1.6 b	11.81 ± 1.8 c
			NR	3.48 ± 0.5 b	7.61 ± 1.2 b	6.19 v 0.9 b
		PCC	WR	8.79 ± 1.4 c	7.34 ± 1.2 b	7.87 ± 1.2 bc
			NR	1.45 ± 0.2 a	1.85 ± 0.3 a	1.49 ± 0.2 a
	Berry skin	MW	WR		18.9 ± 6 a	4.36 ± 1.4 a
			NR		17.6 ± 5 a	3.88 ± 1.3 a
		PCC	WR		15.2 ± 5 a	3.04 ± 1 a
			NR		11.93 ± 4 a	1.82 ± 0.6 a

Ascorbate peroxidase-APX (mmol min-1 mg protein -1)	Leaves	MW	WR	0.09 ± 0.01 b	0.20 ± 0.03 b	0.48 ± 0.07 b
			NR	0.14 ± 0.02 bc	0.05 ± 0.00 a	0.39 ± 0.06 b
		PCC	WR	0.2 ± 0.03 c	0.25 ± 0.04 b	0.97 ± 0.15 c
			NR	0.05 ± 0.01 a	0.08 ± 0.04 a	0.20 ± 0.03 a
	Berry skin	MW	WR		0.88 ± 0.16 a	0.53 ± 0.09 b
			NR		0.63 ± 0.11 a	0.26 ± 0.05 a
		PCC	WR		0.52 ± 0.09 a	0.31 ± 0.06 ab
			NR		0.53 ± 0.1 a	0.37 ± 0.07 ab
Total phenols-TPC (mg GAE g -1 DW)	Leaves	MW	WR	127 ± 1.09 b	132 ± 1.59 a	199 ± 2.49 c
			NR	87 ± 1.6 a	127 ± 1.65 a	181 ± 2.26 b
		PCC	WR	157 ± 1.97 d	149 ± 1.87 b	176 ± 2.21 b
			NR	144 ± 1.8 c	151 ± 1.89 b	167 ± 2.09 a
	Berry skin	MW	WR		312 ± 7.2 a	318 ± 7.35 a
			NR		317 ± 7.33 a	341 ± 7.86 ab

		PCC	WR		315 ± 7.27 a	327 ± 7.56 a
			NR		297 ± 6.86 a	355 ± 8.2 b

6.8. References

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7. Consideraciones finales

7.1. Efecto del manejo del suelo en la salud del viñedo a partir de las relaciones suelo-planta-microbioma

Como primer aporte, este trabajo proporciona información sobre un área inexplorada en los viñedos uruguayos: la microbiota asociada al suelo y la rizósfera de la vid. La descripción de comunidades microbianas vinculadas a distintos hábitats en diversas regiones del mundo y su documentación permite valorar la importancia de esta biodiversidad, tanto en el contexto ecológico como en el agronómico (Bender et al., 2016; Guerra et al., 2020). A pesar del creciente acceso a herramientas de secuenciación avanzada, como la secuenciación de amplicones o el metabarcoding, aún existe un significativo desconocimiento sobre la diversidad microbiana en ciertas regiones, incluido Uruguay (Guerra et al., 2020; Jurburg et al., 2024).

En este sentido, distintos estudios han identificado los principales moduladores de la diversidad microbiana del suelo a escala global y muestran que bacterias y hongos responden de manera diferencial a los gradientes ambientales. Para las bacterias, el pH del suelo ha sido señalado como el predictor más consistente de su diversidad y composición, superando incluso a variables climáticas o espaciales (Delgado-Baquerizo et al., 2018; Fierer y Jackson, 2006; Rousk et al., 2010). En cambio, en el caso de los hongos, factores climáticos como la temperatura y la precipitación explican en mayor medida las variaciones observadas en riqueza y estructura de las comunidades (Tedersoo et al., 2014). Estas diferencias resaltan la necesidad de considerar múltiples factores —incluyendo los edáficos, climáticos y de manejo— al momento de evaluar la diversidad y funcionalidad de la microbiota en sistemas agrícolas.

En el tercer capítulo de esta tesis se analizó la microbiota del suelo en tres viñedos uruguayos ubicados en la zona sur del país, dentro de una misma categoría agroclimática (IHAS IFA2 ISA1) y bajo un manejo convencional del suelo bajo la vid caracterizado por el uso de herbicidas para mantener el suelo desnudo. Los resultados obtenidos evidenciaron que las diferencias en la composición de bacterias y hongos entre viñedos fueron leves, sin alcanzar significación estadística, a pesar de los contrastes en historia de cultivos, acceso al riego, altitud y características físico-químicas del suelo. Consideramos que el limitado rango geográfico del estudio, sumado a un manejo homogéneo del suelo durante varios años (viñedos de Tannat bajo un manejo convencional de suelos), pudo haber contribuido a la uniformidad observada en las comunidades microbianas del suelo.

Estudios previos han sugerido que la caracterización del microbioma del viñedo puede ser un indicador clave para diferenciar regiones vitícolas e incluso viñedos individuales (Gobbi et al., 2022). Este enfoque ha planteado la posibilidad de integrar la microbiota del suelo como un componente esencial en la definición de *terroirs* o zonas vitícolas (Belda et al., 2017; Zarraonaindia et al., 2015). Dada la relevancia del microbioma del suelo para la productividad y salud del viñedo, consideramos fundamental ampliar los estudios en esta línea, incluyendo un mayor número de viñedos distribuidos en diferentes regiones de Uruguay. Esto permitirá aportar un conocimiento más profundo sobre el microbioma del suelo y su relación con las particularidades agroecológicas y productivas del país.

Al incorporar un manejo alternativo del suelo bajo la vid, específicamente el uso de coberturas vegetales vivas permanentes, observamos que los efectos más significativos sobre la composición microbiana del suelo y de la rizósfera, así como sobre sus propiedades biológicas, físicas y químicas, se manifestaban claramente cuando esta práctica había sido implementada de manera sostenida durante varios años. Por el contrario, un solo año de cobertura vegetal permanente (CVP) bajo la

fila (estudios en viñedos comerciales) no resultó suficiente para generar cambios significativos en la microbiota ni en las demás propiedades evaluadas. Estos hallazgos resaltan la importancia de la temporalidad en la adopción de prácticas de manejo sostenible, especialmente en cultivos de larga duración como la vid, donde los cambios en el suelo y su microbiota requieren tiempo para consolidarse (Babin et al., 2019; Cuartero et al., 2021; Hartmann et al., 2015; Steng et al., 2024).

La implementación de CVP tuvo un impacto positivo en la salud del suelo, evidenciado por mayores niveles de actividad microbiana (estimada mediante la tasa de respiración del suelo), mayores concentraciones de proteína extraíble (ACE), carbono potencialmente oxidable (por permanganato de potasio) y carbono orgánico total. Asimismo, se observaron mejoras significativas en la estructura del suelo, reflejadas en una menor densidad aparente y una distribución más favorable del tamaño de los agregados. Estos indicadores no solo apuntan a una mejor funcionalidad del suelo (Moebius-Clune et al., 2016), sino también a su potencial para promover la sostenibilidad del sistema vitícola.

En relación con la microbiota seleccionada por la planta de vid en su rizósfera bajo diferentes manejos de suelo bajo la fila (capítulos 4, 5 y 6), nuestros resultados muestran que la implementación de una CVP tuvo un efecto significativo en la composición microbiana de bacterias y hongos, y promovió ciertos taxones específicos. Estos cambios fueron detectados mediante secuenciación de amplicones, tanto en experimentos a campo como en condiciones controladas en macetas, y corroborados para el caso de *Trichoderma* mediante técnicas clásicas de cultivo en medios semiselectivos. Sin embargo, las diferencias en diversidad alfa entre los manejos fueron escasas o inconsistentes, lo que sugiere que el efecto del manejo no se refleja tanto en la riqueza o equitatividad de las comunidades, sino más bien en cambios en la composición (diversidad beta) y en la presencia de grupos funcionales específicos.

Además, los patrones observados indican que ciertos momentos fenológicos, particularmente envero y cosecha, serían etapas clave para detectar cambios en la estructura y composición de las comunidades microbianas, posiblemente debido a variaciones en las condiciones del entorno rizosférico y en la fisiología de la planta. Las plantas modulan activamente el ensamblaje de su microbioma rizosférico a través de la secreción de exudados radiculares que contienen mezclas de compuestos como azúcares, alcoholes, fenoles y aminoácidos (Berendsen et al., 2012; Guyonnet et al., 2018). Estos compuestos, cuya producción varía a lo largo del desarrollo de la planta, actúan como señales que influyen en la colonización microbiana (Chaparro et al., 2014). En el caso de la vid, se sugiere que podría seleccionar subconjuntos específicos de microorganismos en distintas etapas fenológicas, ya sea por funciones particulares expresadas por el microbioma núcleo o por taxones raros, con el fin de optimizar su aptitud en el ecosistema del viñedo (Liu y Howell, 2021).

Un ejemplo destacado de los taxones asociados a la rizósfera de vid bajo CVP son los géneros *Pantoea, Pseudomonas* y *Rahnella1*, que se identificaron consistentemente en distintos experimentos (campo y macetas), durante dos temporadas consecutivas (2019-2020 y 2020-2021), utilizando diferentes análisis estadísitcos de abundancia diferencial (LDA-LefSe y ANCOM-BC, ALDEx2). Este hallazgo refuerza la robustez de los resultados y resalta el impacto del manejo del suelo en la modulación microbiana.

Sin embargo, dada la alta variabilidad de los factores que afectan la composición de las comunidades microbianas del suelo y la rizósfera (Berlanas et al., 2019; Bettenfeld et al., 2022; Burns et al., 2015; Liang et al., 2019; Rivas et al., 2021; Rousk et al., 2010; Tedersoo et al., 2014), resulta esencial realizar estudios de largo plazo que evalúen si el aumento en la abundancia relativa de los taxones microbianos promovidos por la CVP se mantiene a través del tiempo (años y etapas fenológicas) y se replica en diferentes viñedos. Comprender si estos grupos microbianos se establecen

de manera persistente bajo este manejo será clave para determinar su relevancia funcional. El próximo desafío será profundizar en estudios que permitan descifrar el papel ecológico y agronómico de los géneros o grupos microbianos promovidos de manera consistente por la CVP. Esto incluirá evaluar sus funciones específicas en el ecosistema del suelo, su contribución a la productividad y salud del viñedo y su potencial para mejorar la sostenibilidad de la viticultura en el contexto del cambio climático y las crecientes demandas de prácticas agrícolas sostenibles.

El impacto positivo de la CVP en la salud del cultivo, específicamente en la reducción de la incidencia y severidad de la podredumbre gris del racimo (PGR), fue corroborado a través de diversos experimentos realizados en diferentes temporadas. Incluso en condiciones menos favorables para el desarrollo de la enfermedad, como en la campaña 2019-2020, se observó una disminución en las infecciones latentes de *Botrytis cinerea* en las bayas tanto en envero como en cosecha. Entre los posibles mecanismos de defensa que podrían explicar estas diferencias, destaca la influencia de la anatomía de la baya, particularmente el incremento en el espesor de la cutícula y la epidermis, lo que podría actuar como una barrera física más efectiva frente a la infección (Deytieux-Belleau et al., 2009; Gabler et al., 2003). Por otro lado, aunque la respuesta antioxidante tanto enzimática como no-enzimática en bayas y hojas parece jugar un papel relevante, la alta variabilidad de los resultados obtenidos dificulta establecer tendencias claras asociadas a los diferentes manejos. Las plantas utilizan antioxidantes enzimáticos y no enzimáticos para eliminar el exceso de especies reactivas del oxígeno (ROS) y mantener la homeostasis redox celular durante el estrés oxidativo (Zandi y Schnug, 2022). La activación de estos mecanismos antioxidantes es una respuesta general frente a diversos factores bióticos y abióticos (Ramezani et al., 2017), los cuales en experimentos de campo y macetas pueden estar actuando sinergísticamente, lo que dificulta la identificación de respuestas claras.

Aún no está claro cómo el manejo del suelo bajo la fila, y en particular la CVP, promueve los mecanismos que regulan las respuestas de la vid frente a *Botrytis cinerea* en este estudio. Como se observó en el experimento a campo (capítulos 4 y 5), separar el efecto de la presencia de la CVP del estrés hídrico resulta complejo debido a la competencia que establece la especie usada como CVP con la vid, especialmente durante temporadas con precipitaciones por debajo del promedio histórico, a pesar de la implementación de un manejo estratégico del riego. Sin embargo, el experimento en macetas (capítulo 6) permitió separar estos efectos y observar que una restricción hídrica moderada en la etapa preenvero, en combinación con la CVP, jugó un papel importante en la reducción de la intensidad de la enfermedad al momento de la cosecha. Por otro lado, la reducción de la incidencia y severidad de la PGR también se produjo bajo condiciones de CVP sin estrés hídrico, lo que sugiere que otros factores adicionales pueden estar involucrados.

En el contexto de una viticultura convencional y orientada a la producción de uvas de alta calidad para la elaboración de vinos finos y caracterizada por el uso de herbicidas bajo la fila, la demanda de fertilización no suele ser elevada (Abad et al., 2021). Sin embargo, al introducir un cultivo de cobertura basado en gramíneas, la competencia por agua y nutrientes —particularmente nitrógeno— puede ocasionar una reducción significativa en el contenido de nitrógeno total y mineralizable del suelo (Abad et al., 2021), lo que a su vez puede impactar en el crecimiento vegetativo y el rendimiento de la vid (Chou y Heuvel, 2019; Vanden Heuvel y Centinari, 2021). Estudios previos han reportado una correlación positiva entre los niveles de nitrógeno asimilable por levaduras (YAN por su sigla en inglés: *yeast amino nitrogen*) y azúcares en las bayas y el porcentaje de bayas infectadas por *B. cinerea* (Mundy y Beresford, 2007). No obstante, la relación entre un mayor contenido de nitrógeno y una mayor susceptibilidad a enfermedades sigue siendo objeto de debate (Mundy y Beresford, 2007; Sun et al., 2020). Por un

lado, se ha observado que la adición de nitrógeno al suelo en viñedos puede incrementar la susceptibilidad a *B. cinerea*, debido a efectos indirectos sobre el vigor y el microclima, así como efectos directos en la anatomía y bioquímica del hollejo, como una reducción en su peso o densidad y un menor contenido de fenoles (Mundy, 2008). Por otro lado, un bajo contenido de nitrógeno en las bayas no necesariamente las hace menos susceptibles a la infección, ya que *B. cinerea* posee genes que le permiten crecer incluso con bajos niveles de nutrientes celulares (Mundy, 2008). En referencia al efecto de la CVP en la reducción de PGR a través de la reducción del vigor y del nitrógeno en bayas, al analizar los resultados de los diferentes experimentos realizados en este trabajo, junto con otros estudios relacionados (Coniberti et al., 2023; Coniberti et al., 2018), se puede observar que, bajo condiciones similares de vigor, estado hídrico, peso y compactación de racimos, madurez y contenido de nitrógeno en hojas y bayas, la presencia de una CVP reduce consistentemente la incidencia de PGR. Estos hallazgos refuerzan el potencial de la CVP como una herramienta de manejo sostenible para mitigar una de las principales enfermedades en los viñedos uruguayos, más allá de los efectos directos del manejo del agua y nutrientes en el vigor y el microclima.

Al controlar factores clave como el estrés hídrico, el contenido de nitrógeno, el microclima y el vigor de la vid, surge la pregunta de qué otros elementos podrían estar explicando la variación en la susceptibilidad a *Botrytis cinerea* observada bajo diferentes manejos de suelo. Las comunidades microbianas asociadas al suelo y a la rizósfera emergen como posibles actores en esta dinámica. Sin embargo, aunque este trabajo permite inferir ciertas relaciones entre los manejos de suelo, las comunidades microbianas, los mecanismos de defensa y la reducción de la incidencia de *Botrytis*, no es posible establecer causalidades con los datos y metodología empleados en este trabajo. Es necesario profundizar en el estudio de estos mecanismos mediante otros enfoques, como la

microbiología clásica cultivable para aislar y caracterizar funcionalmente los microorganismos que pueden estar desdencadenando estas respuestas y la transcriptómica para analizar la expresión génica de la planta bajo los diferentes manejos cuando es infectada por el patógeno. Una estrategia de investigación robusta debería incluir experimentos controlados que combinen estas herramientas -ómicas (genómica, transcriptómica, metabolómica, etc), idealmente integrados con análisis de redes funcionales para vincular las interacciones microbianas con los cambios fisiológicos y bioquímicos en la planta. Solo mediante este tipo de enfoques será posible determinar cómo las comunidades microbianas juegan un papel directo en la modulación de la susceptibilidad a *Botrytis cinerea* y cómo éstas pueden ser manejadas para mejorar la salud del viñedo de manera sostenible.

En el contexto de una viticultura sustentable, esta tesis sugiere que el uso de coberturas vegetales bajo la fila representa una estrategia prometedora para mejorar la salud del viñedo. Si la menor incidencia de podredumbre de racimos causada por *Botrytis cinerea* bajo manejo con cobertura vegetal se explicara exclusivamente por una reducción del vigor y de los niveles de nitrógeno o por el estrés hídrico, podrían considerarse otras técnicas desvigorizantes, como el descalzado y calzado de la vid, el deshojado, el uso de portainjertos enanizantes, podas severas de invierno o podas en verde, así como la aplicación de herbicidas, combinadas con un manejo adecuado del estrés hídrico en momentos clave (Cataldo et al., 2021; Coniberti et al., 2014; Dry y Loveys, 1998; Junquera et al., 2012; Romero et al., 2022). Sin embargo, en nuestras condiciones, donde las precipitaciones pueden ser excesivas en ciertos años, estas alternativas presentan limitaciones importantes. Por otro lado, aunque las técnicas desvigorizantes pueden ser más fáciles de implementar y menos costosas que el uso de coberturas vegetales, también pueden generar efectos negativos sobre la salud del suelo y de las plantas, tales como la rotura de raíces y la erosión en suelos desnudos entre otros impactos adversos. En

cambio, el manejo con coberturas vegetales no solo contribuye a la reducción de la incidencia de PGR, sino que también ofrece múltiples beneficios adicionales como se ha mencionado anteriormente.

En conjunto, los resultados de esta tesis respaldan la hipótesis general de que la CVP bajo la fila influye sobre las comunidades microbianas del suelo y la rizósfera mediante la promoción de grupos potencialmente supresores de enfermedades aéreas como la PGR. Aunque no fue posible establecer una relación causal directa, la consistencia de los datos obtenidos en distintos escenarios experimentales y temporadas sugiere una asociación entre la presencia de CVP, una comunidad microbiana rizosférica de bacterias y hongos diferencial, cambios en la anatomía y bioquímica del hollejo y una menor incidencia de la enfermedad.

Los resultados de esta tesis están alineados con la visión de la Protección Vegetal Agroecológica, que reconoce cómo la simplificación del agroecosistema —característica de esquemas convencionales con suelos desnudos y alta dependencia de agroquímicos— puede llevar a la disfuncionalidad del sistema, lo que favorece la aparición de enfermedades y compromete su resiliencia (Deguine et al., 2023). En contraste, la implementación sostenida de CVP se perfila como una estrategia capaz de restaurar procesos ecológicos clave, reforzar ciclos biológicos fundamentales para la salud del suelo y la planta y modular la diversidad de la composición de las comunidades microbianas, lo que favorecería mecanismos de defensa natural en la vid.

Resumiendo, la CVP se posiciona como una herramienta prometedora para una viticultura más sostenible, aunque su adopción requiere estudios más profundos y específicos. Es necesario evaluar aspectos como la selección adecuada de especies —explorando alternativas a la festuca o combinaciones óptimas de gramíneas y leguminosas—, el manejo de la cobertura para minimizar la competencia con la vid (momento y técnica de segado o terminación) y su efecto sobre otras enfermedades relevantes

como el mildiu de la vid causado por *Plasmopara viticola*. Asimismo, es fundamental analizar cómo estas estrategias impactan en el rendimiento y la calidad de la uva y el vino. Solo con evidencia científica sólida que demuestre beneficios agronómicos, sanitarios y ecológicos será posible incorporar la CVP como una práctica estándar en sistemas vitícolas sustentables.

7.2. Reflexiones sobre la estrategia, metodología y herramientas de investigación

Una de las principales limitaciones metodológicas del presente estudio fue el número de muestras y repeticiones realizadas a lo largo de varios años. Aunque se reconoce la importancia de una mayor cantidad de datos para obtener resultados más robustos y representativos, los costos asociados al proceso de muestreo y análisis de secuencias de amplicones, junto con las limitaciones logísticas, son factores determinantes. La extracción y procesamiento de la rizósfera, que incluye actividades como el muestreo de suelos, su desagregación y tamizado y la identificación y extracción de raíces de vid en cantidades adecuadas (5 g) en tiempos limitados para evitar alteraciones en las propiedades microbianas, requirió una considerable inversión de tiempo y mano de obra idónea. Estas restricciones pueden haber influido en la capacidad de capturar la variabilidad temporal y espacial completa de las comunidades microbianas del suelo. Por lo tanto, investigaciones futuras podrían considerar estrategias que optimizen los recursos y capacidades analíticas, mediante la colaboración y articulación con instituciones nacionales y extranjeras, que amplien el alcance sin comprometer la calidad del análisis.

Los estudios metataxónomicos a partir de muestras de rizósfera realizados en este estudio presentaron desafíos significativos relacionados con la extracción, cantidad y calidad del ADN. En particular, las muestras de rizósfera contenían una alta concentración de inhibidores, como ácidos

húmicos y fúlvicos, polifenoles, entre otros, que interfiereron en el proceso de extracción de ADN y ocasionaron fallos en las reacciones posteriores, como la amplificación por PCR. Este problema fue particularmente crítico en la rizósfera de la vid de los viñedos comerciales debido a las características químicas y físicas del suelo y la materia orgánica asociada. En este estudio, utilizamos un kit de extracción específico para suelos que permitió obtener ADN de buena calidad en la mayoría de los casos. Sin embargo, no se emplearon kits de purificación adicionales, lo que pudo incrementar el riesgo de fallos en las PCR por la presencia de inhibidores residuales. Esta experiencia subraya la necesidad de equilibrar las restricciones económicas con la calidad requerida para los análisis metataxónomicos. En investigaciones futuras, sería ideal considerar la implementación de estrategias de purificación posextracción más accesibles, que permitan mejorar la reproducibilidad y robustez de los análisis en estudios similares.

Otra de las complejidades metodológicas en el estudio del microbioma de suelos y rizósfera en diferentes manejos dentro de un mismo viñedo, así como en la comparación entre viñedos, es la autocorrelación espacio-temporal. Este fenómeno surge debido a que las comunidades microbianas suelen estar influenciadas no solo por los tratamientos y condiciones específicas, sino también por factores ambientales y características intrínsecas del suelo que varían con la ubicación y el tiempo. En un mismo viñedo, la proximidad geográfica entre parcelas puede generar patrones microbianos similares y dificultar la diferenciación clara entre los efectos del manejo y los condicionantes ambientales. Por otro lado, al comparar viñedos, diferencias inherentes en el clima, el tipo de suelo y la historia agrícola pueden introducir sesgos que complican la interpretación de los resultados. Para mitigar este problema, se emplearon diseños experimentales con bloques aleatorizados y análisis estadísticos específicos (modelos mixtos) que consideran la dependencia espacial en los datos. Una posible solución para investigaciones futuras sería incorporar herramientas

geoespaciales más precisas que permitan descomponer y modelar mejor las contribuciones relativas de los factores ambientales y de manejo. Este enfoque podría ayudar a diferenciar con mayor claridad los efectos de las prácticas de manejo sobre las comunidades microbianas del suelo y la rizósfera.

Una de las decisiones metodológicas importantes en este estudio fue el uso de ASV (*amplicon sequence variants*) en lugar de OTU (*operational taxonomic units*) para el análisis de las comunidades microbianas. Las ASV ofrecen una resolución más alta al identificar variantes exactas de secuencias, lo que permite una comparación más precisa entre muestras (Callahan et al., 2017). Sin embargo, se ha señalado que las OTU superan a las ASV en la recuperación de la diversidad fúngica, un hallazgo que es particularmente evidente para marcadores largos (Tedersoo et al., 2022). En este sentido, diversos autores coinciden en que el uso de marcadores más largos mejora la resolución taxonómica tanto para el gen 16S rRNA como para la región ITS, al reducir errores de asignación. Por otro lado, sugieren que el análisis de la región ITS completa permite una asignación taxonómica más precisa de hongos y otros eucariotas en comparación con la subregión ITS2, que fue la usada en nuestro caso. Las ASV pueden sobreestimar la riqueza de algunas especies comunes en hongos (especies cercanamente emparentadas del filo Ascomycota) cuando se usa ITS como marcador (por su alta variabilidad intraespecifica con genomas haploides), mientras que subestiman la riqueza de especies raras, lo que podría sesgar los resultados en análisis de diversidad alfa. A pesar de esta limitación, este sesgo no afecta los análisis de diversidad beta o de composición microbiana, que son el énfasis principal de nuestro trabajo. Sumado a esto, Kauseraud (2023) destaca que la ausencia de una brecha clara en los barcoding en la región ITS podría ser menos crítica en estudios localizados espacialmente, como el nuestro. Esto se debe a que se espera que la variación intraespecífica dentro de la región ITS sea menor en áreas geográficas más pequeñas.

Como lo demostraron Botnen et al. (2018), como se cita en Estensmo et al. (2021), los patrones de diversidad beta son altamente estables y robustos tanto para las regiones ITS como para 16S. Estos estudios sugieren que los taxones más abundantes, ya sean generados como ASV o OTU, son los que determinan los patrones de comunidad y muestran distribuciones similares independientemente de los métodos de procesamiento de datos utilizados. Por lo tanto, consideramos que el uso de ASV fue adecuado para los objetivos planteados, ya que permitió diferenciar la estructura y composición de las comunidades microbianas entre tratamientos y viñedos.

La elección de métricas de distancia es un aspecto crítico en los análisis de diversidad beta, ya que influye en la interpretación de las relaciones entre las comunidades microbianas. En este estudio, se emplearon tanto la distancia Bray-Curtis como la Unifrac (*weighted*), dependiendo del contexto de cada capítulo. La distancia Bray-Curtis, utilizada en los capítulos 4 y 5, es una métrica basada únicamente en la abundancia y composición de las comunidades, lo que resulta útil para identificar diferencias directas en las comunidades microbianas sin considerar relaciones evolutivas. Por otro lado, la distancia Unifrac, utilizada en el capítulo 6 para las comunidades procariotas, incorpora información filogenética, lo que permite una interpretación más detallada de las diferencias en función de la historia evolutiva de los organismos presentes.

Aunque consideramos que Unifrac es, en general, más adecuada por su capacidad para integrar relaciones filogenéticas, su uso con marcadores ITS en hongos ha sido cuestionado (Tedersoo et al., 2022). Esto se debe a que la región ITS es muy variable, lo que dificulta alinear secuencias de diferentes taxones más allá del nivel de género y no refleja adecuadamente las relaciones evolutivas. Por esta razón, se optó por emplear Bray-Curtis cuando el enfoque estaba en la composición y abundancia relativa de ambas comunidades procarioticas y fúngicas, mientras que Unifrac fue utilizado en

los análisis donde la perspectiva filogenética añadía valor, como en el caso de las comunidades procariotas en el experimento en macetas (capítulo 6).

La utilización de ambas métricas permitió abordar las preguntas de investigación desde diferentes perspectivas, resaltando las fortalezas y limitaciones de cada enfoque.

8. Bibliografía general

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