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The grapevine microbiota: A review on the possible impacts of plant protection programs on phyllosphere microorganisms

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Abstract

Grapevine (*Vitis vinifera*) is one of the most important fruit crops, but it is susceptible to several pests and pathogens. Pesticide applications are required to prevent yield and quality losses, and fungicides represent the largest fraction of treatments in plant protection programs under conventional, integrated, or organic disease management. Thus, alternative fungicides are being developed for the sustainable control of grapevine pathogens, such as biocontrol agents and natural products. In addition to toxicological and ecotoxicological tests, there is considerable interest in verifying possible effects of plant protection products on non-target microorganisms. This review highlights recent advances in understanding the impact of plant protection programs and alternative fungicides on the taxonomic composition of bacterial and fungal communities of the grapevine phyllosphere. Amplicon sequencing studies revealed that the grapevine microbiota is primarily shaped by the plant compartment, vineyard location, and sampling time. Plant protection programs generally have only a minor impact on the taxonomic composition of microbial communities on grapevine bunches, leaves, and wood, suggesting the resilience of indigenous phyllosphere microorganisms. However, the effects of fungicide treatments can vary with fungicide dosage, application frequency, grapevine cultivar, and environmental conditions, indicating that further long-term studies with more frequent applications are required to clarify the effects of chemical and biological treatments on non-target microorganisms at different sampling times.

KEYWORDS
biocontrol, biofungicides, integrated disease management, plant microbiome

1 | INTRODUCTION

Grapevine (*Vitis vinifera*) is one of the most widely cultivated fruit crops worldwide, with significant relevance for agricultural, economic, and industrial sectors. Grapevine ranks as the third most valuable crop worldwide, with an estimated global market size of 73.7 billion USD in 2023 (FAOSTAT, 2025; https://www.fao.org/faostat/en/#data/QV). However, most grapevine cultivars, both for table grape and wine grape production, are frequently affected by numerous pathogens (Armijo et al., 2016) and insect pests (Jambagi & Kambrekar, 2023)

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that negatively impact plant growth, fruit yield, and fruit quality. For example, downy mildew and powdery mildew are two of the most important grapevine pathogens that colonize green plant tissues (Koledenkova et al., 2022; Pirrello et al., 2019). Moreover, considerable yield quality grape losses can also be caused by grey mould (Armijo et al., 2016), black rot, anthracnose (Pirrello et al., 2019), Eutypa dieback, esca disease, Botryosphaeria dieback (Kenfaoui et al., 2022), bacterial canker (Benčič et al., 2025), and Pierce's disease of grapevine (Kyrkou et al., 2018; Rapicavoli et al., 2018). Among insect pests, *Empoasca vitis* (Hemiptera: Cicadellidae) and *Lobesia botrana* (Lepidoptera: Tortricidae) are considered major pests in viticulture (Mani, 2022; Reineke & Thiéry, 2016). Moreover, *Scaphoideus titanus* (Hemiptera: Cicadellidae) and *Hyalesthes obsoletus* (Hemiptera: Cixiidae) are key vectors of the grapevine flavescence dorée and bois noir phytoplasma, respectively (Pagliari et al., 2019; Reineke & Thiéry, 2016). Thus, frequent pesticide applications are required to prevent losses caused by infections from bacterial, fungal, and oomycete pathogens or by attacks from insect pests, and fungicides represent the largest fraction of pesticide treatments in viticulture, with an average of 12–15 applications per year, and up to 25–30 applications under environmental conditions favourable to pathogen infection (Pertot et al., 2017). For example, grapevine downy mildew is commonly controlled through intensive fungicide use, with an average of 7.9 treatments per year in France (Chen et al., 2020). Conversely, the management of insect pests is usually moderate in viticulture, with 1–4 insecticide applications per year, except in table grape production, where up to 8–10 applications may be required (Pertot et al., 2017), suggesting that fungicides are the main contributors to plant protection programs and could potentially impact non-target indigenous bacterial and fungal communities of the grapevine phyllosphere.

Synthetic chemical fungicides are commonly used in conventional and integrated disease management (Shabeer et al., 2023; Russo et al., 2019; Xiao et al., 2020), whereas copper- and sulfur-based products are the main fungicides allowed under organic and biodynamic management (Pertot et al., 2017). The overuse of chemical fungicides and insecticides raises concerns about potential adverse effects on human health and the environment (Fantke et al., 2012), prompting a growing interest in sustainable alternatives for plant protection in viticulture. Alternative fungicides are being tested as spray treatments for the sustainable control of grapevine phyllosphere pathogens, including biocontrol agents (e.g., *Ampelomyces quisqualis*, *Bacillus* sp., *Lysobacter capsici*, *Saccharomyces cerevisiae*, *Streptomyces* sp., and *Trichoderma* sp.), plant extracts (e.g., *Artemisia* sp., *Larix* sp., *Origanum vulgare*, and *Salvia officinalis*), inorganic compounds (e.g., potassium bicarbonate, silicon nanoparticles, and 3-aminobutyric acid), and organic compounds (e.g., laminarin, chitin, chitosan, and COS-OGA) (Clippinger et al., 2024; Nadalini & Puopolo, 2024; Pertot et al., 2017).

Toxicological and ecotoxicological tests for chemical and biological fungicides are required during the registration process, but limited information is available regarding the potentially harmful effects of plant protection products on non-target indigenous microorganisms of the plant phyllosphere (Clippinger et al., 2024). The hypothesis is that fungicides sprayed to control grapevine phyllosphere pathogens could negatively affect the taxonomic composition of bacterial and fungal communities associated with grapevine leaf, bunch, and wood tissues. This review highlights recent advances in understanding the impact of plant protection programs and alternative fungicides on the taxonomic composition of bacterial and fungal communities associated with grapevine leaf, bunch, and wood tissues as revealed by amplicon sequencing analyses to evaluate possible side effects on non-target microorganisms and outline future research directions on studies of the grapevine microbiome.

2 | THE GRAPEVINE PHYLLOSPHERE MICROBIOTA

The plant and its associated microbiota (e.g., bacteria, fungi, viruses, archaea, and protists) form a holobiont, which acts as a collective ecological unit that functions in concert, and plant–microbe interactions can have positive, negative, or neutral effects on the host (Berg et al., 2020). For example, plant-associated microbial communities can improve plant growth, plant health, crop productivity, and tolerance to abiotic and biotic stresses (Trivedi et al., 2020). In particular, aboveground plant compartments provide unique environments for the phyllosphere microbiota (e.g., flowers, fruits, leaves, and wood) (Compant et al., 2019), and indigenous microbial communities of the grapevine phyllosphere can affect must fermentation, contributing to wine quality and regional distinctiveness (microbial terroir) (Belda et al., 2017; Gilbert et al., 2014).

The grapevine phyllosphere microbiota is commonly analysed by amplicon sequencing, which is a culture-independent approach for taxonomic profiling of microbial communities by amplifying and sequencing specific marker genes (Morgan et al., 2017). These markers contain conserved regions for universal primer design and hypervariable regions that allow the identification of different taxa within the community (Table 1), such as the 16S rRNA gene (nine hypervariable regions; V1–V9) for bacteria, and the ITS1-5.8S rRNA-ITS2 region or the 26S rRNA gene for fungi (Morgan et al., 2017). The most common high-throughput next-generation sequencing methods include sequencing by synthesis (Illumina), single-molecule real-time sequencing (SMRT; Pacific Biosciences, PacBio), and nanopore sequencing (Oxford Nanopore) (Hajieghrari & Nejati-Jahromi, 2025). However, amplicon sequencing analysis of environmental samples allows a safe taxonomic annotation only at the genus level (Gupta et al., 2019), providing incomplete information on microbial species and their functions. Thus, shotgun metagenomic studies are required to identify gene functions of grapevine-associated microorganisms by fragmenting and sequencing all DNA extracted from a given sample (Nwachukwu & Babalola, 2022). Shotgun metagenomic approaches have been used to characterize the grapevine phyllosphere microbiota (Castanera et al., 2024; Zhang et al., 2025), but these approaches, along with metatranscriptomics, have not yet been applied to investigate the effects of plant protection programs on microbial functional traits.

TABLE 1 Studies on the impact of fungicide treatments and plant protection programs on bacterial and fungal communities associated with grapevine bunches, leaves, and wood, sorted by year of publication.

Plant compartment	Microbial communities	Plant protection program	Plant protection products	Sequencing details[a]	Grapevine genotype	Season	Reference
Leaf	Bacteria and fungi	Conventional, alternative fungicide	*Lysobacter capsici* AZ78, penconazole, and untreated plants	454 pyrosequencing of bacterial 16S (V5-V9) and fungal ITS1-ITS2 regions	Pinot gris	2012	(Perazzolli et al., 2014)
Leaf	Bacteria and fungi	Alternative fungicide	Laminarin, nutrient broth, water-treated plants, and untreated plants	454 pyrosequencing of bacterial 16S (V6-V8) and fungal ITS2 regions	Pinot noir	Greenhouse	(Cappelletti et al., 2016)
Bunch	Bacteria and fungi	Biodynamic, conventional, organic	Copper, cyazofamid, cymoxanil, cyprodinil, dimetomorph, famoxadon, fludioxonil, folpet, metiram, metrafenone, myclobutanil, pyraclostrobin, pyrimethanil, quinoxyfen, spiroxamine, sulfur algae extracts, clay, plant extracts, potassium-phosphonate, potassium hydrogen carbonate, and yeast extracts	454 pyrosequencing of bacterial 16S (V1-V2) and fungal ITS1 regions	Riesling	2010, 2011	(Kecskeméti et al., 2016)
Wood and bunch (and must)	Fungi	Biodynamic, conventional	Not specified	454 pyrosequencing of fungal 26S (D1/D2) region	Sauvignon blanc	Not specified	(Morrison-Whittle et al., 2017)
Bunch and leaf	Fungi	Conventional, organic	Not specified	Illumina sequencing of ITS2 and D2/LSU fungal regions	Carménère	2014	(Castañeda et al., 2018)
Bunch	Fungi	Conventional, organic	Bordeaux mixture, copper sulphate, copper-oxychloride, fosetyl-al, copper sulfate, metalaxyl-m14, copper-oxychloride, quinoxyfen, myclobutanil, coformulants, spiroxamina, sulfur (tiovit jet and microthiol disperss), and untreated plants	Illumina sequencing of ITS2 and 26S rDNA D1/D2 fungal regions	Montepulciano	2016	(Agarbati, Canonico, Ciani, & Comitini, 2019)
Bunch (and must)	Fungi	Conventional, organic	Bordeaux mixture, copper sulphate, copper-oxychloride, coppery sulfur, cyclohexanol, 1,2-propanediol, abamectin, 2,6-diterbutyl-p-icresol, iprovalicarb, technical copper oxychloride, fosetyl-al, copper sulfate, metalaxyl-m14, copper-oxychloride, phosphorus pentoxide, potassium oxide,	Illumina sequencing of fungal 26S (D1/D2) region	Montepulciano and Verdicchio	2016	(Agarbati, Canonico, Mancabelli, et al., 2019)

(Continues)

TABLE 1 (Continued)

Plant compartment	Microbial communities	Plant protection program	Plant protection products	Sequencing details[a]	Grapevine genotype	Season	Reference
			quinoxyfen, myclobutanil, coformulants, spiroxamina, sulfur, terpene alcohols, sodium salt of an aromatic polymer, and untreated plants				
Wood	Fungi	Conventional, organic	Blad-containing oligomer, copper oxychloride, fosetyl-aluminium, penconazole, potassium permanganate, and sulfur	Illumina sequencing of fungal ITS2 region	Cabernet Sauvignon	Greenhouse	(Del Frari et al., 2019)
Bunch and wood	Bacteria	Biodynamic, conventional	Copper sulphate, dimethomorph, dithiocarbamate, metalaxyl, penconazole, potassium permanganate, sulfur	Illumina sequencing of bacterial 16S (V3-V4) region	Dolcetto and Sangiovese	2015	(Vitulo et al., 2019)
Leaf	Bacteria and fungi	Organic, alternative fungicide	Copper, *Lactobacillus plantarum* MW-1	Illumina sequencing of bacterial 16S (V3-V4) and fungal ITS1 regions	Shiraz	2016, 2017	(Gobbi et al., 2020)
Bunch	Fungi	Conventional, organic	Acibenzolar-s-methyl, calcium oxide, chito-oligosaccharides and oligogalacturonides, copper hydroxide, electrolyzed water, fosetyl-al, laminarin, metiram, potassium phosphonate, sulfur, and untreated plants	Illumina sequencing of fungal ITS1-ITS2 region	Nebbiolo	2017	(Rantsiou et al., 2020)
Leaf	Bacteria and fungi	Alternative fungicide	Tagatose and untreated plants	454 pyrosequencing of 16S (V6-V8) and ITS2 regions	Pinot gris and Schiava gentile	2014	(Perazzolli et al., 2020)
Wood	Bacteria and fungi	Organic	Copper-based product (lc2017) and untreated plants	Illumina sequencing of bacterial 16S (V4) and fungal ITS1 regions	Chardonnay	2019	(Mondello et al., 2022)
Bunch	Fungi	Biodynamic, conventional, organic	Not specified	Illumina sequencing of fungal ITS2 region	Montepulciano	2020	(Perpetuini et al., 2022)
Bunch	Bacteria and fungi	Conventional, alternative fungicide	Cyflufenamid, difenoconazole, fluopyram, folpet, metiram, ozonized oleic acid, proquinazid, sulfur, tebuconazolev, and untreated plants	Illumina sequencing of bacterial 16S (V3-V4) and fungal ITS2 regions	Portugieser	2019	(Stahl et al., 2022)
Wood	Fungi	Conventional, alternative fungicide, organic	Boscalid, copper oxychloride, pyraclostrobin, sodium bentonite, *Trichoderma atroviride* I-1237, and water-treated plants	Illumina sequencing of fungal ITS1 region	Cabernet Sauvignon and Syrah	2022	(Del Frari et al., 2023)

TABLE 1 (Continued)

Plant compartment	Microbial communities	Plant protection program	Plant protection products	Sequencing details[a]	Grapevine genotype	Season	Reference
Bunch	Fungi	Conventional, organic	Copper, dimetomorf, mancozeb, mefenoxam, spiroxamine, and sulfur	Illumina sequencing of fungal ITS2 region	Trebbiano Abruzzese	2020	(Rossetti et al., 2023)
Leaf	Fungi	Alternative fungicides	*Rugulopteryx okamurae* extract, *Ulva ohnoi* extract, and untreated plants	Illumina sequencing of fungal ITS2 region	Tempranillo	Greenhouse	(Zarraonaindia et al., 2023)
Bunch	Fungi	Conventional, organic	Not specified	Illumina sequencing of fungal ITS2 region	Acadie blanc	2018, 2019, 2020, 2021	(Bunbury-Blanchette et al., 2024)
Bunch (and must)	Bacteria and fungi	Organic, alternative fungicide	*Bacillus pumilus* QST 2808, and sulfur	Illumina sequencing of bacterial 16S (V3-V4) and fungal ITS regions	Tempranillo	Not specified	(González-Arenzana et al., 2024)
Leaf	Bacteria and fungi	Conventional, alternative fungicides	*Bacillus subtilis* OCASB12 (JQ240640.1), myclobutanil, pyraclostrobin, and untreated plants	Illumina sequencing of bacterial 16S (V3-V4) and fungal ITS1 regions	Red Globe	2020	(He et al., 2024)
Bunch, leaf, and wood	Fungi	Biodynamic, integrated, organic	Ametoctradin, copper, difenoconazol, dimethomorph, dithianon, fluopyram, fluxapyroxad, folpet, kaliumphosphonat, mancozeb, mandipropamid, metrafenone, myclobutanil, oxathiapiprolin, potassium hydrogencarbonate, proquinazid, sulfur, tebuconazol, and zoxamide	Illumina sequencing of fungal ITS2 region	Riesling	2021	(Steng et al., 2024)
Leaf	Bacteria and fungi	Conventional	Cyflufenamid, fluopyram, metrafenone, myclobutanil, quinoxyfen, tebuconazole, and trifloxystrobin	Illumina sequencing of bacterial 16S (V4) and fungal ITS2 regions	Autumn King	2020	(Yang et al., 2024)
Wood	Bacteria and fungi	Alternative fungicides	*Trichoderma atroviride* I-1237 and untreated plants	Illumina sequencing of bacterial 16S (V4) and fungal ITS1 regions	Chardonnay, Cabernet Sauvignon, Melon de Bourgogne	Not specified	(Yacoub et al., 2024)

[a]Amplicon sequencing approaches and sequenced regions of the bacterial 16S rRNA and/or fungal internal transcribed spacer (ITS) are reported for each study.

Although the effects of grapevine genotypes, plant compartments, phenological stage, vineyard location, and environmental conditions on bacterial and fungal communities of the grapevine phyllosphere have been previously reviewed (Bettenfeld et al., 2022; Legesse et al., 2025; Minerdi & Sabbatini, 2025), a key question remains whether plant protection programs can influence the taxonomic composition of the grapevine phyllosphere microbiota. The taxonomic composition of grapevine-associated microbial communities has been analysed in response to treatments with biological, chemical, and alternative fungicides across different plant protection programs (e.g., biodynamic, conventional, integrated, and organic management) under greenhouse or field conditions with various

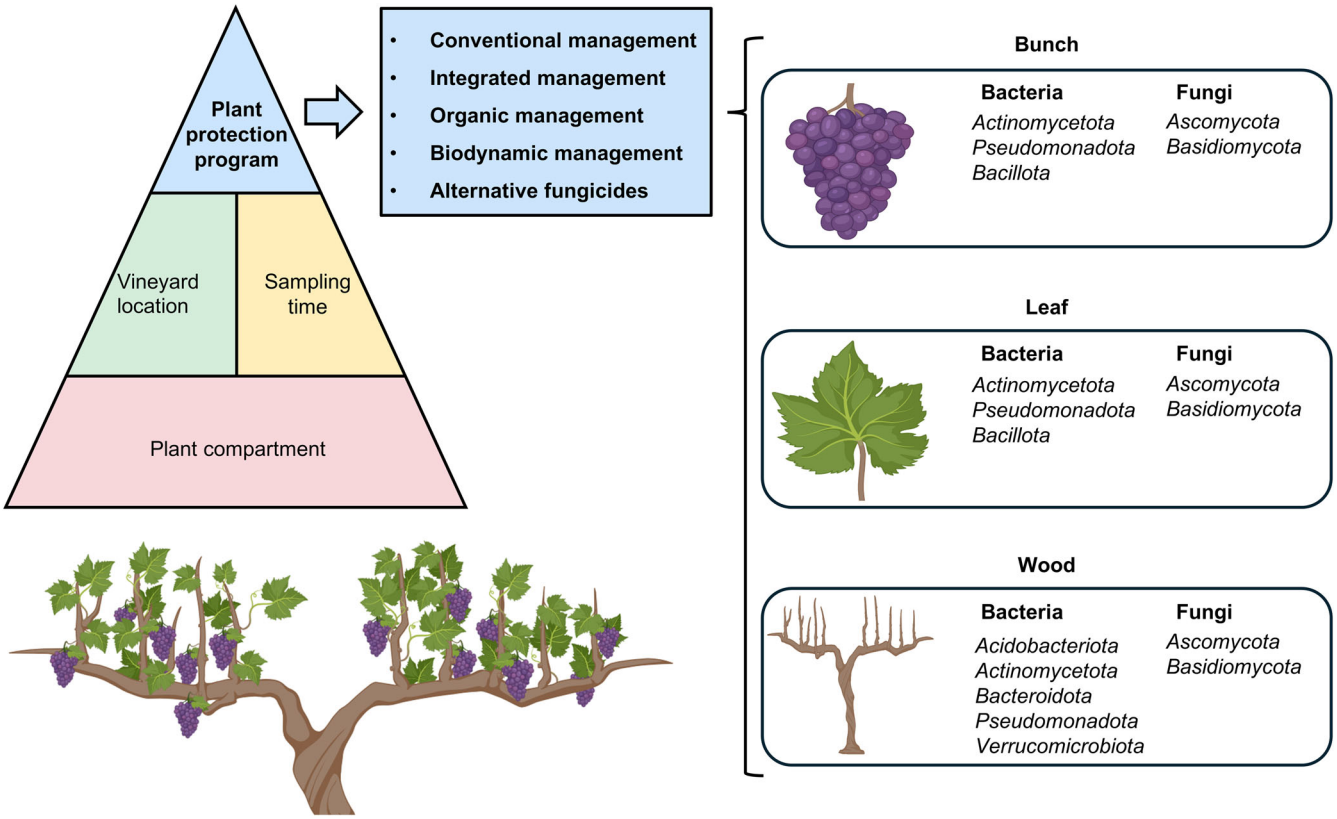

FIGURE 1 Overview of main factors affecting the composition of bacterial and fungal communities associated with the grapevine leaf, bunch, and wood. Amplicon sequencing studies revealed that phyllosphere microbiota composition is primarily shaped by plant compartment, vineyard location, and sampling time, while plant protection programs generally exert only minor effects. Dominant bacterial and fungal phyla associated with each plant compartment are reported according to amplicon sequencing studies investigating the impact of plant protection programs on bacterial and fungal communities of the grapevine phyllosphere (Table 1), while less abundant taxa are omitted.

environmental conditions (Table 1), and the grapevine phyllosphere microbiota was dominated by the phyla *Acidobacteriota* (formerly *Acidobacteria*), *Actinomycetota* (formerly *Actinobacteria*), *Bacillota* (formerly *Firmicutes*), *Bacteroidota*, *Pseudomonadota* (formerly *Proteobacteria*), *Verrucomicrobiota* (formerly *Verrucomicrobia*), *Ascomycota*, and *Basidiomycota* (Figure 1).

Major effects of the plant compartment were found on the taxonomic composition of bacterial and fungal communities in studies investigating the impact of fungicide treatments on the phyllosphere microbiota of grapevine (Castañeda et al., 2018; Morrison-Whittle et al., 2017; Steng et al., 2024; Vitulo et al., 2019), and amplicon sequencing data were analysed individually for bunch, leaf, and wood samples. On grapevine bunches, *Pseudomonadota* (e.g., *Massilia* sp., *Pseudomonas* sp., and *Sphingomonas* sp.) and *Actinomycetota* have been consistently reported as dominant bacterial phyla, whereas members of the phylum *Bacillota* (e.g., families *Bacillaceae* and *Paenibacillaceae*) have been less frequently detected (González-Arenzana et al., 2024; Kecskeméti et al., 2016; Stahl et al., 2022; Vitulo et al., 2019). Fungal communities associated with grapevine bunches were dominated by the phylum *Ascomycota*, such as *Dothideomycetes* (e.g., *Alternaria* sp., *Aureobasidium* sp., and *Cladosporium* sp.), *Leotiomycetes* (e.g., *Botrytis*), and *Saccharomycetes* (e.g., *Hanseniaspora* sp., *Lachancea* sp., *Metschnikowia* sp., *Pichia* sp., *Saccharomyces* sp., *Starmerella* sp., and *Zygosaccharomyces* sp.) (Agarbati, Canonico, Ciani, & Comitini, 2019; Agarbati, Canonico, Mancabelli, et al., 2019; González-Arenzana et al., 2024; Kecskeméti et al., 2016; Perpetuini et al., 2022; Rantsiou et al., 2020; Rossetti et al., 2023). Moreover, the phylum *Basidiomycota* (e.g., *Filobasidium* sp., *Rhodotorula* sp., *Sporobolomyces* sp., and *Vishniacozyma* sp.) was less abundant, although consistently detected, on grapevine bunches (Agarbati, Canonico, Ciani, & Comitini, 2019; Bunbury-Blanchette et al., 2024; González-Arenzana et al., 2024) (Figure 1).

Studies investigating the impact of plant protection programs on the leaf microbiota showed that bacterial communities were dominated by the phyla *Actinomycetota*, *Pseudomonadota*, and *Bacillota*, whereas members of the classes *Clostridia* and *Bacteroidia* were less frequently found (Figure 1) (Cappelletti et al., 2016; Gobbi et al., 2020; He et al., 2024; Perazzolli et al., 2014; Perazzolli et al., 2020; Yang et al., 2024). In particular, leaf-associated bacterial communities were dominated by *Enterobacteriaceae* (e.g., *Enterobacter*, *Erwinia*, and *Pantoea*) and *Pseudomonadaceae* (e.g., *Pseudomonas*) (Cappelletti et al., 2016; Gobbi et al., 2020; Perazzolli et al., 2014; Perazzolli et al., 2020). Moreover, *Ascomycota* (e.g., *Chaetomiaceae*, *Erysiphaceae*, and *Pleosporaceae*) and *Basidiomycota* (e.g., *Filobasidiales*) have been reported as dominant fungal phyla of the

leaf microbiota (Figure 1) (Cappelletti et al., 2016; Castañeda et al., 2018; Gobbi et al., 2020; He et al., 2024; Perazzolli et al., 2014; Perazzolli et al., 2020; Yang et al., 2024; Zarraonaindia et al., 2023). The dominant fungal genera were *Alternaria*, *Aureobasidium*, *Cryptococcus*, *Sporobolomyces*, and *Vishniacozyma*, while *Aspergillus*, *Botrytis*, *Cladosporium*, *Erysiphe*, and *Filobasidium* were less frequently detected (Cappelletti et al., 2016; Castañeda et al., 2018; Gobbi et al., 2020; He et al., 2024; Perazzolli et al., 2020; Steng et al., 2024; Yang et al., 2024).

Pseudomonadota (e.g., *Massilia* sp., *Pantoea* sp. *Pseudomonas* sp., and *Sphingomonas* sp.) and *Actinomycetota* have been consistently reported as dominant bacterial phyla of wood-associated bacterial communities in studies investigating the impact of plant protection programs, and members of the phyla *Acidobacteriota*, *Bacteroidota*, and *Verrucomicrobiota* have also been detected (Figure 1) (Mondello et al., 2022; Vitulo et al., 2019; Yacoub et al., 2024). Fungal communities associated with grapevine wood were dominated by the phylum *Ascomycota* (e.g., *Alternaria* sp., *Aureobasidium* sp., *Cadophora* sp., *Candida* sp., *Cladosporium* sp., *Debaryomyces* sp., *Diaporthe* sp., *Epicoccum* sp., *Fusarium* sp., *Neofusicoccum* sp., and *Phaeomoniella* sp.) followed by *Basidiomycota* (e.g., *Cryptococcus* sp., *Malassezia* sp., *Naganishia* sp., *Peniophora* sp., and *Sporidiobolus* sp.) (Del Frari et al., 2019; Del Frari et al., 2023; Mondello et al., 2022; Morrison-Whittle et al., 2017; Steng et al., 2024; Yacoub et al., 2024).

3 | IMPACTS OF PLANT PROTECTION PROGRAMS ON BACTERIAL AND FUNGAL COMMUNITIES ASSOCIATED WITH GRAPEVINE BUNCHES

Impacts of plant protection programs have been analysed on the taxonomic composition of the bunch-associated microbiota in various grapevine cultivars (Table 1), including Acadie blanc (Bunbury-Blanchette et al., 2024), Carménère (Castañeda et al., 2018), Dolcetto and Sangiovese (Vitulo et al., 2019), Montepulciano and Verdicchio (Agarbati, Canonico, Ciani, & Comitini, 2019; Agarbati, Canonico, Mancabelli, et al., 2019; Perpetuini et al., 2022), Nebbiolo (Rantsiou et al., 2020), Portugieser (Stahl et al., 2022), Sauvignon blanc (Morrison-Whittle et al., 2017), Riesling (Kecskeméti et al., 2016; Steng et al., 2024), Tempranillo (González-Arenzana et al., 2024), and Trebbiano Abruzzese (Rossetti et al., 2023).

In amplicon sequencing studies investigating the impact of plant protection programs on the bunch microbiota, the taxonomic composition of bacterial and fungal communities was primarily influenced by vineyard location and sampling time, indicating that environmental conditions and phenological stages exert a stronger influence on bunch-associated microbial communities compared to disease management practices (biodynamic, conventional, integrated, and organic management) (Bunbury-Blanchette et al., 2024; Kecskeméti et al., 2016; Stahl et al., 2022). Additionally, an effect of the season was observed on the composition of bacterial and fungal communities on grapevine bunches in Germany (Kecskeméti et al., 2016) and Canada (Bunbury-Blanchette et al., 2024), further supporting the strong impact of environmental conditions on the phyllosphere microbiota.

Plant protection programs can partially influence the composition (e.g., alpha-diversity and beta-diversity) of bacterial and fungal communities associated with grapevine bunches, although to a lesser extent than vineyard location, sampling time, and season (Figure 1 and Table 1) (Agarbati, Canonico, Ciani, & Comitini, 2019; Agarbati, Canonico, Mancabelli, et al., 2019; Bunbury-Blanchette et al., 2024; Castañeda et al., 2018; González-Arenzana et al., 2024; Morrison-Whittle et al., 2017; Perpetuini et al., 2022; Rossetti et al., 2023; Stahl et al., 2022; Steng et al., 2024; Vitulo et al., 2019). For example, Steng et al. (2024) found higher relative abundances of mycoparasitic genera (e.g., *Sporobolomyces* and *Rhodotorula*) in bunch-associated fungal communities under integrated management (treatments with ametoctradin, difenoconazol, dimethomorph, dithianon, fluopyram, fluxapyroxad, folpet, kaliumphosphonat, mancozeb, mandipropamid, metrafenone, myclobutanil, oxathiapiprolin, potassium hydrogencarbonate, proquinazid, sulfur, tebuconazol, zoxamide) compared to organic and biodynamic management (treatments with copper and sulfur) in Germany (cultivar Riesling), while they found no differences between organic and biodynamic management. Similarly, beta-diversity of bunch-associated bacterial communities in northern Italy (cultivars Dolcetto and Sangiovese) differed between conventional management (treatments with copper, dimethomorph, dithiocarbamate, metalaxyl, penconazole, potassium permanganate, and sulfur) and biodynamic management (treatments with copper and sulfur), with members of the family of *Chitinophagaceae* detected only under biodynamic management (Vitulo et al., 2019). Moreover, alpha-diversity and beta-diversity of fungal communities associated with grapevine bunches were affected by conventional management (e.g., copper, dimetomorf, mancozeb, mefenoxam, spiroxamine, and sulfur) and organic management (e.g., copper and sulfur) in central Italy (cultivar Trebbiano Abruzzese) (Rossetti et al., 2023) and Canada (cultivar Acadie blanc) (Bunbury-Blanchette et al., 2024), with some genera occurring predominantly under conventional management (e.g., *Saccharomyces*, *Sporidiobolales*, and *Mucor*) or organic management (e.g., *Aspergillus*, *Penicillium*, and *Symmetrospora*). Lower fungal richness was found on grapevine bunches under conventional management compared to biodynamic management in New Zealand (cultivar Sauvignon blanc), with differential abundances of the genera *Columnosphaeria*, *Davidiella*, *Hanseniaspora*, *Chalara*, and *Trichothecium* (Morrison-Whittle et al., 2017). Similarly, lower yeast biodiversity was observed under conventional management compared to organic management in central Italy (cultivars Montepulciano and Verdicchio) (Agarbati, Canonico, Ciani, & Comitini, 2019; Agarbati, Canonico, Mancabelli, et al., 2019) and in southern Italy (cultivar Montepulciano) (Perpetuini et al., 2022), with some taxa detected predominantly under conventional management (e.g., *Lachancea thermotolerans*), organic management (e.g., *Starmerella bacillaris* and *Cladosporium ramotenellum*), and biodynamic management (e.g., *Cladosporium cladosporioides*, *Saccharomyces* sp., and *Pseudopithomyces* sp.), indicating that lower microbial diversity of bunch-associated microbial communities was observed under conventional management compared to organic

management or biodynamic management. In contrast, no differences in alpha-diversity and beta-diversity of bacterial and fungal communities were observed under conventional, organic, and biodynamic management in other studies conducted in Germany (cultivar Riesling) (Kecskeméti et al., 2016), Italy (cultivar Nebbiolo) (Rantsiou et al., 2020), and Chile (cultivar Carménère) (Castañeda et al., 2018), indicating the resilience of some bunch-associated microbial communities and the variable effects of plant protection programs, depending on the fungicide dosage, application frequency, grapevine cultivar, vineyard location, sampling time, and season (Table 1). However, fungicide treatments can cause slight changes in the relative abundances of some bunch-associated taxa, indicating that some microbial taxa are more responsive to disease management practices also in resilient communities. For example, some specific taxa (e.g., *Alternaria* sp., *Didymellaceae*, *Heterophoma* sp., and *Vishniacozyma* sp.) were less abundant under conventional management (treatments with copper, metiram, and sulfur) compared to untreated grapevine bunches (cultivar Nebbiolo) (Rantsiou et al., 2020), while other taxa (*Alternaria alternata*, *Sporidiobolus pararoseus*, and *Pseudomonas* sp.) were more abundant under conventional management (treatments with cyazofamid, cymoxanil, cyprodinil, dimetomorph, famoxadon, fludioxonil, folpet, metiram, metrafenone, myclobutanil, pyraclostrobin, pyrimethanil, quinoxyfen, spiroxamine, and sulfur) compared to biodynamic management (treatments with algae extracts, copper, clay, plant extracts, potassium-phosphonate, potassium hydrogen carbonate, sulfur, and yeast extracts; cultivar Riesling) (Kecskeméti et al., 2016). Moreover, a lower occurrence of *Aureobasidium pullulans* was observed under conventional management compared to organic and biodynamic management in central Italy (Perpetuini et al., 2022) and Chile (Castañeda et al., 2018), supporting that fungicide treatments can only slightly affect the relative abundances of some specific taxa depending on the plant protection programs applied (e.g., dosage, frequency, and mode of action of chemical and biological fungicides) and the taxonomic composition of indigenous microbial communities. As a consequence, negligible impacts of fungicide treatments were found on the yeast biodiversity of grape musts and during spontaneous fermentations (Agarbati, Canonico, Mancabelli, et al., 2019; Bagheri et al., 2015; Castrillo Cachón et al., 2019; Englezos et al., 2022; Morrison-Whittle et al., 2017; Xu et al., 2020).

Other studies have investigated the effects of alternative fungicides (e.g., biocontrol agents and natural products), and Stahl et al. (2022) observed changes in bacterial beta-diversity on grapevine bunches (Germany, cultivar Portugieser) treated with ozonized oleic acid compared to a plant protection program based on fungicides used in organic and conventional management (e.g., cyflufenamid, difenoconazole, fluopyram, folpet, metiram, proquinazid, sulfur, and tebuconazole). Moreover, the alpha-diversity of bacterial and fungal communities was higher on grapevine bunches treated with *Bacillus pumilus* QST 2808 compared to sulfur-based fungicides in Spain (cultivar Tempranillo) (González-Arenzana et al., 2024), suggesting no negative impacts of treatments with biocontrol agents on the bunch-associated microbiota. Thus, disease management practices exert a minor effect on the taxonomic composition of bacterial and fungal communities on bunches. Moreover, lower diversity of the bunch microbiota has often been reported under conventional management compared to organic and biodynamic management, as well as under treatments with alternative fungicides.

4 | IMPACTS OF PLANT PROTECTION PROGRAMS ON BACTERIAL AND FUNGAL COMMUNITIES ASSOCIATED WITH GRAPEVINE LEAVES

The taxonomic composition of the leaf-associated microbiota was analysed under different plant protection programs in various grapevine cultivars (Table 1), such as Autumn King (California, USA) (Yang et al., 2024), Carménère (Chile) (Castañeda et al., 2018), Pinot gris (Italy) (Cappelletti et al., 2016; Perazzolli et al., 2014; Perazzolli et al., 2020), Pinot noir (Italy) (Cappelletti et al., 2016), Schiava gentile (Italy) (Perazzolli et al., 2020), Shiraz (South Africa) (Gobbi et al., 2020), Red Globe (Chile) (He et al., 2024), Riesling (Germany) (Steng et al., 2024), and Tempranillo (Spain) (Zarraonaindia et al., 2023).

In amplicon sequencing studies investigating the impact of plant protection programs, vineyard location and sampling time were the main factors influencing the taxonomic composition of the leaf-associated microbiota, whereas disease management practices (biodynamic, conventional, integrated, and organic management) had only minor effects (Cappelletti et al., 2016; Gobbi et al., 2020; Perazzolli et al., 2014; Perazzolli et al., 2020; Yang et al., 2024). For example, vineyard location and sampling time affected the beta-diversity of leaf-associated bacterial and fungal communities in California (Yang et al., 2024). Similarly, leaf-associated bacterial and fungal communities varied according to the vineyard location in Italy (Perazzolli et al., 2014; Perazzolli et al., 2020), and fungal communities also differed according to sampling time in South Africa (Gobbi et al., 2020). Temporal changes in bacterial and fungal community composition of grapevine leaves are likely driven by grapevine phenological stages and environmental conditions (Yang et al., 2024), such as the rainfall amount, maximum temperatures, and mean temperatures (Perazzolli et al., 2014). Moreover, the influence of vineyard location could result from differences in the agricultural practices, microbial terroir, and microclimatic conditions (Perazzolli et al., 2020; Steng et al., 2024; Yang et al., 2024), indicating that these factors could exert selective pressure, leading to the consequent adaptation of the grapevine microbiota.

On grapevine leaves, conventional, integrated, organic, and biodynamic plant protection programs can partially influence the beta-diversity of fungal communities (Figure 1 and Table 1) (Castañeda et al., 2018; Steng et al., 2024), indicating a minor impact of fungicide treatments on the taxonomic composition of microbial communities on grapevine leaves. In particular, Castañeda et al. (2018) found lower relative abundances of the family *Dothioraceae* on grapevine leaves under conventional compared to organic management (Chile, cultivar Carménère), while Steng et al. (2024) demonstrated higher relative abundances of mycoparasitic taxa (e.g., *Sporobolomyces roseus*,

S. ellipsoideus, and *Rhodotorula glutinis*) under integrated management (treatments with ametoctradin, difenoconazol, dimethomorph, dithianon, fluopyram, fluxapyroxad, folpet, kaliumphosphonat, mancozeb, mandipropamid, metrafenone, myclobutanil, oxathiapiprolin, potassium hydrogencarbonate, proquinazid, sulfur, tebuconazol, and zoxamide) compared to organic and biodynamic management (treatments with copper and sulfur) (Germany, cultivar Riesling), highlighting that changes in leaf-associated microbial composition may depend on the type of fungicide dosage, application frequency, grapevine cultivar, and environmental conditions (vineyard location, sampling time, and season; Table 1). However, impacts of two plant protection programs (treatment strategy with cyflufenamid, fluopyram, myclobutanil, and trifloxystrobin or with cyflufenamid, fluopyram, quinoxyfen, metrafenone, and tebuconazole) on alpha-diversity and beta-diversity of bacterial and fungal communities were not significant in California (cultivar Autumn King) (Yang et al., 2024), indicating that further long-term studies with more frequent applications are required to verify the possible resilience of some leaf-associated microbial communities.

Amplicon sequencing analyses were used to dissect the taxonomic composition of the bacterial and fungal communities associated with grapevine leaves (cultivars Pinot gris, Pinot noir, Red Globe, and Tempranillo) in response to treatments with alternative fungicides, such as algal extracts, biocontrol agents, and natural products (Cappelletti et al., 2016; Gobbi et al., 2020; He et al., 2024; Perazzolli et al., 2014; Perazzolli et al., 2020; Zarraonaindia et al., 2023). For example, treatments with alternative fungicides influenced the alpha-diversity and beta-diversity of the leaf microbiota in Italy (cultivar Pinot gris) (Perazzolli et al., 2020) and in Spain (cultivar Tempranillo) (Zarraonaindia et al., 2023). In particular, a rare sugar with antifungal properties (tagatose) increased (e.g., *Chroococcidiopsis*, *Exiguobacterium*, *Erwinia*, *Leifsonia*, *Methylobacterium*, *Pelomonas*, *Pseudomonas*, and *Rhodobium*) and decreased (e.g., *Erysiphe*, *Frischella*, *Fructobacillus*, and *Snodgrassella*) the relative abundances of some leaf-associated taxa (Perazzolli et al., 2020). Similarly, an alternative fungicide based on algal extracts (*Rugulopteryx okamurae*) increased the relative abundance of possible beneficial yeasts (e.g., *Sporobolomyces* and *Debaryomyces hansenii*) (Zarraonaindia et al., 2023), suggesting positive effects of alternative fungicides on the leaf-associated microbiota. Likewise, treatments with a protein derivative (nutrient broth) altered the beta-diversity of leaf-associated microbiota under controlled conditions in Italy (cultivar Pinot noir), modifying relative abundances of some bacterial taxa (e.g., *Enterobacteriaceae*, *Pseudomonadaceae*, *Exiguobacterium* sp., and *Lysobacter* sp.) and fungal taxa (e.g., *Alternaria* sp., *Aureobasidium* sp., and *Trichoderma* sp.) (Cappelletti et al., 2016). However, He et al. (2024) observed no significant changes in microbial alpha-diversity of grapevine leaves treated with the biological control agent *Bacillus subtilis* OCASB12 compared to untreated plants, increasing the relative abundances of some potential beneficial taxa (e.g., *Bacillus* sp. and *Chaetomium* sp.) in Chile (cultivar Red Globe). Similarly, chemical fungicides (e.g., copper and penconazole) and alternative fungicides (e.g., laminarin, *Lysobacter capsici* AZ78, and *Lactobacillus plantarum* MW-1) did not affect alpha-diversity and beta-diversity of bacterial and fungal communities on grapevine leaves in Italy (cultivar Pinot gris and Pinot noir) (Cappelletti et al., 2016; Perazzolli et al., 2014), and in South Africa (cultivar Shiraz) (Gobbi et al., 2020), indicating that the effects of biological fungicides on leaf-associated microorganisms may depend on the product applied and taxonomic composition and responsiveness of indigenous microbial communities. These results support that fungicide treatments can slightly affect the relative abundances of specific taxa according to the plant protection program applied. Moreover, some alternative fungicides (e.g., nutrient broth and tagatose) can act as selective nutritional factors and engineer indigenous microbial populations to suppress phytopathogens and increase the relative abundances of potential beneficial microorganisms (Cappelletti et al., 2016; Perazzolli et al., 2020).

5 | IMPACTS OF PLANT PROTECTION PROGRAMS ON BACTERIAL AND FUNGAL COMMUNITIES ASSOCIATED WITH THE GRAPEVINE WOOD

Impacts of plant protection programs have been analyzed on the taxonomic composition of the wood-associated microbiota in various grapevine cultivars (Table 1), including Cabernet sauvignon under greenhouse conditions (Del Frari et al., 2019) and under field conditions in Italy (Del Frari et al., 2023) and in France (Yacoub et al., 2024), Chardonnay in France (Mondello et al., 2022; Yacoub et al., 2024), Dolcetto and Sangiovese in Italy (Vitulo et al., 2019), Melon de Bourgogne in France (Yacoub et al., 2024), Riesling in Germany (Steng et al., 2024), Sauvignon blanc in New Zealand (Morrison-Whittle et al., 2017), and Syrah in Portugal (Del Frari et al., 2023).

In amplicon sequencing studies investigating the impact of plant protection programs on the wood microbiota, the taxonomic composition of bacterial and fungal communities was primarily influenced by vineyard location and sampling time, indicating that environmental conditions exert a stronger influence on the wood microbiota compared to disease management practices (biodynamic, conventional, integrated, and organic management) (Del Frari et al., 2023; Vitulo et al., 2019; Yacoub et al., 2024). Additionally, the effect of wood health status was observed on the composition of fungal communities in France (Mondello et al., 2022), indicating that both environmental conditions and pathogen infections can shape the wood microbiota of grapevines.

Plant protection programs can slightly influence the taxonomic composition (e.g., alpha-diversity and beta-diversity) of bacterial and fungal communities associated with grapevine wood (Figure 1 and Table 1) (Del Frari et al., 2019; Del Frari et al., 2023; Mondello et al., 2022; Morrison-Whittle et al., 2017; Vitulo et al., 2019; Yacoub et al., 2024). In particular, the alpha-diversity of fungal communities was lower under conventional management compared to biodynamic management in New Zealand (cultivar Sauvignon blanc) (Morrison-Whittle et al., 2017), and the beta-diversity of bacterial communities differed between conventional management (treatments with copper,

dimethomorph, dithiocarbamate, metalaxyl, penconazole, potassium permanganate, and sulfur) and biodynamic management (treatments with copper and sulfur) in Italy (cultivars Dolcetto and Sangiovese) (Vitulo et al., 2019), indicating lower microbial diversity of wood-associated microbial communities under conventional management compared to biodynamic management. Likewise, treatments with a copper-based fungicide (LC2017) affected the beta-diversity of fungal communities, but not that of bacterial communities, compared to water-treated wood (France, cultivar Chardonnay) (Mondello et al., 2022), indicating only minor impacts of plant protection programs on wood-associated microbial communities. Moreover, no differences in alpha-diversity and beta-diversity were observed in fungal communities under conventional management (treatments with ametoctradin, difenoconazol, dimethomorph, dithianon, fluopyram, fluxapyroxad, folpet, kaliumphosphonat, mancozeb, mandipropamid, metrafenone, myclobutanil, oxathiapiprolin, potassium hydrogencarbonate, proquinazid, sulfur, tebuconazol, and zoxamide) compared to organic and biodynamic (treatments with copper and sulfur) management in Germany (cultivar Riesling), highlighting the resilience of wood-associated microbial communities, which may be attributed to the limited exposure of woody tissues to fungicide treatments (Steng et al., 2024).

Other studies have investigated the effects of alternative fungicides on the wood microbiota, and treatments with a natural product (blad-containing oligomer) did not affect the alpha-diversity and beta-diversity of fungal communities compared to control plants (treatments with potassium permanganate) and plants treated with fungicides used in conventional management (fosetyl-aluminium and penconazole) and organic management (e.g., copper and sulfur), although the relative abundances of some fungal taxa (e.g., *Candida friedrichii*, *C. sake*, *Diaporthe* spp, *Malassezia globosa*, and *M. restricta*) were affected by fungicide treatments (Italy, cultivar Cabernet sauvignon) (Del Frari et al., 2019). Moreover, Del Frari et al. (2023) observed changes in fungal alpha-diversity and beta-diversity on grapevine wood treated with a biocontrol agent (*Trichoderma atroviride* I-1237) compared to treatments with copper, boscalid, pyraclostrobin, sodium bentonite, or water, and the genera *Alternaria*, *Epicoccum*, *Phoma*, *Stemphylium*, and *Vishniacozyma* resulted as the most responsive to plant protection programs. Similarly, treatments with *T. atroviride* I-1237 slightly affected the taxonomic composition (alpha-diversity and beta-diversity) of bacterial and fungal communities on grapevine wood, depending on the sampling time and vineyard location (cultivars Chardonnay, Cabernet Sauvignon, and Melon de Bourgogne) (Yacoub et al., 2024), supporting that variable responses to plant protection programs can be associated with the fungicide applied (dosage and mode of action), application frequency, plant genotype (grapevine cultivar), and environmental conditions (vineyard location, sampling time, and season; Table 1). In particular, the relative abundances of some genera decreased (e.g., *Amycolatopsis*, *Dyadobacter*, *Filobasidium*, *Malassezia*, *Pantoea*, and *Sclerostagonospora*) or increased (e.g., *Bradyrhizobium*) in *T. atroviride* I-1237-treated compared to untreated plants (Yacoub et al., 2024), supporting that treatments with biocontrol agents can slightly affect the relative abundances of some specific grapevine-associated taxa.

6 | CONCLUSIONS AND FUTURE PERSPECTIVES

Amplicon sequencing analyses of bunches, leaves, and wood indicate that the grapevine microbiota is primarily shaped by the vineyard location and sampling time. Plant protection programs exert a minor impact on the taxonomic composition of microbial communities on grapevine bunches, leaves, and wood, indicating the resilience of some indigenous phyllosphere microorganisms. Lower microbial diversity of bacterial and fungal communities has often been reported under conventional management compared to organic management, biodynamic management, and treatments with alternative fungicides, suggesting that sustainable plant protection programs can preserve the microbial biodiversity of the crop phyllosphere. However, relative abundances of some taxa showed variable responses to plant protection programs, indicating that further long-term studies with more frequent applications are required to clarify the effects of chemical and biological fungicides on non-target microorganisms at different sampling times, with better consideration of fungicide dosage, application frequency, and the mode of action of active compounds.

Amplicon sequencing analysis is a tool for assessing the potential impacts of plant protection programs on the taxonomic composition of the crop phyllosphere microbiota, providing complementary information to the ecotoxicological characterization of chemical and biological fungicides. However, amplicon sequencing typically allows taxonomic annotation only at the genus level, indicating that network analyses and long-read sequencing will help to elucidate microbial interactions and improve the taxonomic identification of plant-associated microorganisms under different plant protection programs. Moreover, shotgun metagenomic approaches and metatranscriptomic analyses of the grapevine phyllosphere microbiota are required to characterize microbial gene functions that may be affected by fungicide treatments.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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