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Perspective

Action of *Pythium oligandrum* on Grapevine Trunk Diseases and its impact on microbial communities

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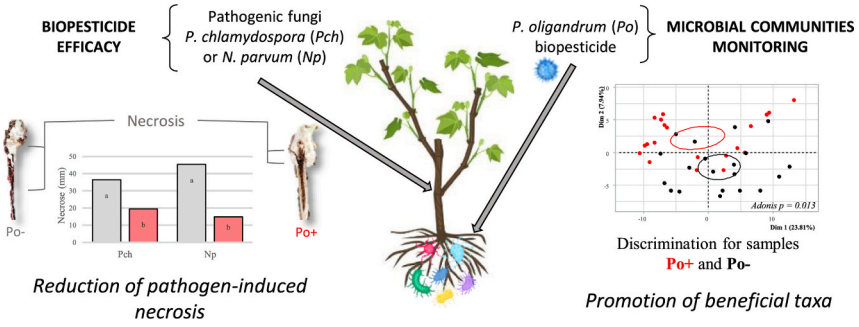
HIGHLIGHTS

- No *P. oligandrum* biosolution for Grapevine Trunk Diseases.
- *P. oligandrum* biopesticide efficacy and environmental impact assessment.
- The biopesticide reduced necrosis size by 47–67 % depending on the pathogen.
- *P. oligandrum* did not significantly alter rhizosphere composition.
- The biopesticide has promoted taxa with functions that are beneficial to plants.

GRAPHICAL ABSTRACT



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ABSTRACT

Grapevine Trunk Diseases (GTDs) have become a major challenge for viticulture. Since the ban of sodium arsenate (the only approved pesticide) in 2001, the need for alternative methods, such as biocontrol, has become increasingly urgent. One promising microorganism is the oomycete *Pythium oligandrum,* which is known to improve plant health by increasing natural defences and reducing disease incidence by up to 40 %. In order to use this microorganism in vineyards, it need first to be formulated and tested to ensure its safety. An experiment was therefore carried out in a greenhouse under semi-controlled conditions to assess the efficacy of the *P. oligandrum* biopesticide produced and its impact on microbial communities using a high-throughput sequencing approach. Vines were treated with the biopesticide and were inoculated with two fungi involved in wood diseases: *Neofusicoccum parvum* (involved in *Botryosphaeria* dieback) and *Phaeomoniella chlamydospora* (involved in Esca). During a three-month experiment, samples of the rhizosphere environment were collected to assess possible changes in microbial communities, either as part of GTDs or as a result of the action of *P. oligandrum*. The results indicated a minimal effect of the *P. oligandrum* biopesticide on the total microbial community of the vine rhizosphere. However, the treatment appeared to enhance several beneficial taxa that play a role as plant growth promoting rhizobacteria (PGPR) or biocontrol agents (BCA). This improvement, together with the direct effect of *P. oligandrum*, may explain the reduction in necroses caused by *N. parvum* and *P. chlamydospora* following the biopesticide application.

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1. Introduction

Grapevine Trunk Diseases (GTDs) have become a major concern in viticulture since the ban of the only approved pesticide, sodium arsenate, in 2001. These complex diseases are caused by a diverse group of fungal pathogens that infect the woody tissues of grapevines, leading to chronic infections that severely affect vine vigour, longevity, and yield. Esca and *Botryosphaeria* dieback are among the most important diseases (Fontaine et al., 2016). *Neofusicoccum parvum* is one of the 21 species involved in *Botryosphaeria* dieback, and *Phaeomoniella chlamydospora* is a key pathogen in the group of microorganisms responsible for Esca (Fischer, 2006; Mesguida et al., 2023). The final stage of these two diseases is the death of the vine, driven by an apoplectic symptom (Bertsch et al., 2013).

One of the alternatives for the management of GTDs is biological control. In the last decade, about 1600 microorganisms with potential biocontrol activities have been studied (Mesguida et al., 2023). Most of these studies have focused on bacterial and fungal strains, but some species of oomycetes have also been investigated for the biocontrol of GTDs pathogens (Mondello et al., 2018). Among the promising oomycetes, *Pythium oligandrum* is a rhizospheric microorganism that colonises the root system of many cultivated plant species, including grapevine (Benhamou et al., 2012; Yacoub et al., 2020). Its biocontrol activity is attributed to both direct effects on pathogens (*via* antibiosis or mycoparasitism) and indirect effects through resistance induction and plant growth promoting ability (Gerbore et al., 2014). In recent years, *P. oligandrum* has been studied as a solution to control GTDs in a controlled environment. These studies have demonstrated the ability of *P. oligandrum* to improve plant health by increasing natural defences and reducing diseases caused by pathogenic fungi such as *N. parvum* and *P. chlamydospora* by up to 40 % (Ouhaibi-Ben Abdeljalil et al., 2021; Yacoub et al., 2020, 2016).

Despite its efficacy, no commercial product based on P. oligandrum has been yet developed. The microorganisms most commonly used as biocontrol agents (BCAs) are *Trichoderma spp*. and *Bacillus subtilis*. In vineyards, these BCAs are mainly used by application to pruning wounds or by inoculation of vine cuttings (Bertsch et al., 2013; Gramaje et al., 2018; Mondello et al., 2018).

The aim of this study is to gain a better understanding of the role of plant microbiota in plant health and, more generally, in the effectiveness of biocontrol treatments. It is also important that this biopesticide is environmentally safe and has little impact on indigenous microbial communities. Although the effects of conventional pesticides have been well studied, very few studies have examined the effects of biological control on microbial communities. However, understanding the effects of microorganism-based treatments on existing microbial communities is essential to ensure sustainable and safe vineyard management practices.

This study aims to understand the role of plant microbiota play in plant health, focusing on how the indigenous microbial communities of the grapevine contribute to overall plant health, particularly when a biocontrol treatment based on P. oligandrum is applied. In addition, the study seeks to assess the environmental safety of this biocontrol treatment. A key objective is to assess whether the use of P. oligandrum as a biopesticide has any unintended effects on the indigenous microbial communities in the vine rhizosphere. To answer this question, we assessed the impact of the biopesticide on vine microbial communities using a high-throughput sequencing for a microbial community characterisation.

2. Materials and methods

2.1. Microorganisms used

Two pathogens involved in GTDs, *Phaemoniella chlamydospora* (*Pch*, S037) and *Neofusicoccum parvum* (*Np*, COU02), were selected from the INRAE-UMR 1065 SAVE collection in Bordeaux. As previously described, the strains were cultivated on malt agar medium and incubated for one month at 22 °C in the dark before inoculating the vines (Yacoub et al., 2020, 2016).

The biocontrol agent used in this study was the oomycete *Pythium oligandrum* (*Po*, strain I-5180), which was selected for its ability to control GTDs pathogens (Gerbore et al., 2014). The BCA was prepared at Greencell (Saint Etienne Chomeil, France). As previously described (Benhamou et al., 2012), the strain was produced on malt extract agar, with a static fermentation step in a molasses-based medium. After production, the active substance derived from the P. oligandrum strain was standardised with maltodextrin (a co-formulant) to achieve a final concentration of 1.5 10^5 oospores per gram in the final biopesticide product.

2.2. Experimental design

Grafted vines (Merlot as scion and SO4 as rootstock) were supplied by Mercier (https://www.pepinieres-mercier.com). These vines were planted in 2-litre pots and grown for 4 months in a greenhouse. The potting soil used was Klassman RHP 15 commercial potting mix (70 % fair peat of sphaine, 15 % cold black peat, 15 % pearlite and Danish clay). The plants were watered by subirrigation and fertilised twice a week (nutrient solution N/P/K 20/20/20). The vines were incubated for one month (until the 3–4 leaf stage) before biopesticide treatment and pathogen inoculation.

To evaluate the efficacy of the biopesticide against *P. chlamydospora* and *N. parvum* attacks, 90 biopesticide-treated (Po) and 90 non-treated (NT) plants were considered, randomly distributed according to the biopesticide treatment. The different inoculation modalities were uninoculated plants and plants inoculated with one of the pathogens. For each modality, 30 replicates were considered.

To assess the effect of the biopesticide on the indigenous microbial communities, 40 additional plants were used, randomised according to the biopesticide treatment, with 20 plants treated with the biopesticide (Po) and 20 plants untreated (NT). For each treatment, the different inoculation modalities were undrilled and uninoculated plants (NPni), drilled and uninoculated plants (Pni) and plants inoculated with one of the pathogenic fungi (Np or Pch). Five replicates were considered for each modality.

The different modalities were summarized in Table 1.

2.3. Biopesticide treatment and pathogen inoculation

At the 3–4 leaf stage, the vines were either treated with the biopesticide *P. oligandrum* or left untreated. Treatment consisted of applying 50 mL of water containing the equivalent of 1.5 10^4 oospores per vine to the soil, administered twice at five-day intervals. One week after the second biopesticide treatment, the vines were inoculated, or not with the pathogen *P. chlamydospora* or *N. parvum*. Inoculated plants were drilled in the dorsal–ventral axis and the resulting hole (2 mm diameter, 5 mm deep) was filled with a plug of mycelium from one of the two pathogens tested for inoculated plants, and with a sterile malt agar plug for drilled and uninoculated plants, as a drilling control. The inoculation site was immediately sealed with parafilm to prevent contamination. Some vines were not drilled as a negative control.

2.4. Evaluation of plant protection induced by the biopesticide

At the end of the efficacy trial, 3 months after pathogen inoculation, 30 plants per modality were collected. The stem of each plant was then cut lengthwise and the length of necrosis caused by *P. chlamydospora* or *N. parvum* was measured. The rate of necrosis was obtained by calculating the ratio between the length of necrosis with biopesticide treatment and the length of necrosis for untreated vines. Statistical analyses (Tukey HSD test and Student's *t*-test) were performed using R software

(version 4.2.2).

2.5. Sampling for microbial community analysis

At the end of the experiment, the rhizosphere environment (roots and soil adhering to the root surface) was sampled from 5 replicates per modality. These samples were collected to fill a 50 mL tube and stored at −80 °C prior to DNA extraction.

2.6. Sample treatment and sequencing analyses

Rhizosphere samples were ground using a 6875D Freezer/Mill® cryogenic grinder and stored at −80 °C prior to DNA extraction. DNA was extracted from 60 mg aliquots using the Invisorb® Spin Plant Mini Kit (Invitek molecular) according to the manufacturer's instructions. DNA extracts were then quantified using a Nanodrop (ND-1000, Thermoscientific, Labtech) and sent to the ADNId laboratory / Qualtech group (Montpellier, France) for high-throughput rDNA amplicon sequencing.

Bacterial and fungal communities were characterised from the V5-V6 variable region of the 16S rRNA gene using primers 799f (AC MGG ATT AGA TAC CCK G) and 1115R (AGG GTT GCG CTC GTT G) and from the ITS1 region of the fungal rRNA operon using primers 7ITS1f (CTT GGT CAT TTA GAG GAA GTA A) and ITS2r (GCT GCG TTC TTC ATC GAT GC) (Redford et al., 2010). DNA amplification was performed by PCR in a total volume of 15 µl containing 1X master mix, Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany), 0.13 µM of each primer, and 2 µl genomic DNA. The PCR conditions were: 94 °C for 2 min, 35 cycles of 30 s at 94 °C, 52 °C for 30 s and 72 °C for 1 min, followed by 7 min at 72 °C. PCR amplifications were verified by electrophoretic migration on a 1.5 % agarose gel.

PCR products from the bacterial and fungal communities were pooled and purified using the Agencourt® AMPure® XP kit (Beckman Coulter, Italy, Milano). Each PCR product was tagged in a second PCR reaction using the Nextera XT DNA Library Prep Kit (Illumina Inc., San Diego, CA). Tagged PCR products were purified using the Agencourt® AMPure® XP kit (Beckman Coulter, Italy, Milan) and quantified using the Tecan Nanoquant spectrometer. Equal amounts of DNA for each sample were pooled and then purified using the Agencourt® AMPure® XP kit (Beckman Coulter, Italy, Milan). The DNA library was controlled on an Advanced Analytical fragment analyser and quantified using SYBR Green-quantitative polymerase chain reaction on real-time PCR. Finally, the DNA library was sequenced on an Illumina Miseq instrument using 2 × 250-bp technology and V2 chemistry (Illumina Inc., San Diego, CA).

2.7. Bioinformatic and statistical analyses

The resulting sequence reads were processed using the Frogs pipeline (Escudié et al., 2018). Briefly, forward and reverse reads were filtered and merged using vsearch with the following parameters: minimum amplicon size: 220; maximum amplicon size: 480; mismatch rate: 0.1. Merged sequences were clustered using swarms and chimera sequences were removed. Sequences were assigned to clusters at 97 % similarity and only clusters with a minimum abundance of 5.10^{-5} % of the total abundance were retained. Taxonomic assignment was performed using the SILVA (Silva.nr_v123, https://www.arb-silva.de/) and ITS UNITE Eukaryote (https://unite.ut.ee/) databases. Sequencing data were deposited in GenBank under the accession numbers PP703244 – PP704333 for bacterial sequences and PP766267 – PP766425 for fungal sequences. To compare the data from each sample, the high-throughput sequencing results were normalised to the sample with the lowest total counts.

Statistical analyses were performed using R software (version 4.2.2). Differential statistical analyses at the genus level were performed using the Namco interface (Dietrich et al., 2022). Analyses were based on a Kruskal-Wallis test ($p < 0.05$) with a Benjamini-Hochberg correction and selected genera were retained for PCA and correlogram analyses performed in R software. For the correlogram, only genera with a significant correlation (based on the Spearman method) with necrosis size were retained ($p < 0.05$).

3. Results

3.1. Effect of Pythium oligandrum on pathogen-induced necrosis

Analyses of necrotic lesions induced by *Pch* and *Np* (Fig. 1) statistically confirmed that untreated plants (negative controls) exhibited the longest necrosis, with *Pch* at 36 ± 13 mm and *Np* at 45 ± 17 mm. When the grafted vines were treated with the full dose of the *P. oligandrum* biopesticide, the size of the necrotic lesions was significantly reduced, up to 40 % of the growth inhibition for *Pch* and up to 65 % of the growth inhibition for *Np* (19 ± 13 mm for *Pch* and 15 ± 6 mm for *Np*). Soil treatment with half the biopesticide dose was significantly effective, with a slight reduction in efficacy for *Np* (20 ± 11 mm).

3.2. Microbial community diversity

3.2.1. Composition of the microbial diversity

Microbial diversity metrics were calculated for the different modalities (Table 2). Based on the Chao1 and Observed species indices, the coverage reached at least 91 %, meaning that the sequencing was sufficient to capture the diversity of the microbial community. There were no statistical differences in diversity among different measures, whereas the average Shannon index was 3.81 ± 0.31.

An Illumina sequencing approach was used to characterise the microbial diversity. For the bacterial community, a total of 579,025 bacterial sequences were obtained from the 40 samples collected, belonging to 921 clusters from 17 bacterial phyla. The relative abundance of major bacteria (> 1 %) at the phyla level is shown in Fig. 2a. The main phyla of total sequences for the different treatments considered were Proteobacteria (54 %), Bacteroidota (22 %) and Actinobacteria (11 %). The only significant difference was found for the phylum Actinobacteria, where Po-Pni was statistically different from the three treatments Po-NPni, NT-Np and NT-NPni.

The fungal community was 2.5 times higher than the bacterial one, with a total of 1,474,484 fungal sequences were obtained, belonging to 208 clusters from 7 identified phyla (Fig. 2b). The most represented phyla were Ascomycota (53 %), Basidiomycota (12 %) and Glomeromycota (8.5 %). More specifically, a higher representation of Basidiomycota can be highlighted for untreated but pathogen-inoculated plants

Table 1
Experimental design. NT: non-treated, *Po*: *P. oligandrum* biopesticide, *Np: N. parvum*, *Pch*: *P. chlamydospora*, NPni: undrilled and uninoculated, Pni: drilled and uninoculated.

Designation	NT-NPni	NT-Pni	NT-Np	NT-Pch	Po-NPni	Po-Pni	Po-Np	Po-Pch
Po	no	no	no	no	yes	yes	yes	yes
Drilling	no	yes	yes	yes	no	yes	yes	yes
Pathogen inoculation	no	no	Np	Pch	no	no	Np	Pch
Necrosis measurement	yes	no	yes	yes	yes	no	yes	yes
Sequencing analyses	yes	yes	yes	yes	yes	yes	yes	yes

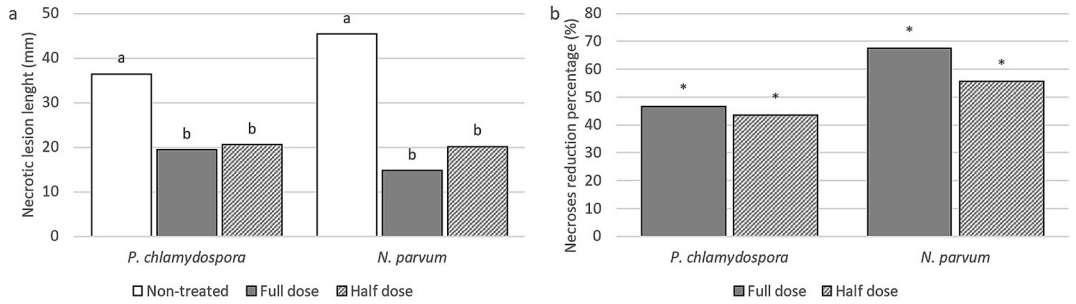

Fig. 1. Efficacy of *P. oligandrum* biopesticide against *P. chlamydospora* and *N. parvum* on necrosis size. Necrotic lesion measurements (a) with different letters were significantly different (Tukey HSD test, $p < 0.05$). The percentage of necrotic reduction (b) marked with an asterisk shows a significant reduction in necrosis (Student's *t*-test, $p < 0.05$).

Table 2
Microbial community diversity analysis. 5 samples per modality, means and standard deviations are provided. %Coverage = (number of observed species/ Chao1) * 100.

Modality	Chao1	Observed species	%Coverage	Shannon
Po-Np	806 ± 30	752 ± 35	93.2 ± 1.9	3.86 ± 0.26
Po-NPni	787 ± 40	731 ± 32	93.0 ± 1.3	3.82 ± 0.29
Po-Pch	769 ± 46	707 ± 40	92.1 ± 2.4	3.77 ± 0.24
Po-Pni	750 ± 64	691 ± 75	92.1 ± 3.3	4.03 ± 0.40
NT-Np	768 ± 39	710 ± 45	92.5 ± 2.2	3.80 ± 0.39
NT-NPni	771 ± 46	707 ± 50	91.6 ± 1.9	3.86 ± 0.42
NT-Pch	761 ± 79	702 ± 73	92.3 ± 2.9	3.61 ± 0.31
NT-Pni	772 ± 42	715 ± 54	92.5 ± 2.4	3.74 ± 0.06

(average of 20 % compared to 9.2 % for other modalities). In addition, the phylum Glomeromycota seems to be associated with *Pch,* with a higher relative abundance in plants inoculated with this pathogen, with or without biopesticide treatment (relative abundance of 16 % in these samples and 6.0 % for others).

At the genus level, the top 10 relatively abundant bacteria (Fig. 3a) accounted for 30 % (Po-NPni) to 46 % (Po-Pni). The dominant genera were *Steroidobacter* (11.5 % ± 2.60), *Streptomyces* (5.47 % ± 2.71) and *Novosphingobium* (5.05 % ± 2.10). The genus *Streptomyces* is significantly higher for Po-Pni (11.28 %) than for NT-NPni, NT-Pni and Nt-Np modalities (mean of 4.65 %). The other significant difference was found for the genus *Ideonella*, where Po-Np (1.97 %) was statistically different from NT-Pch (0.96 %).

For the top 10 fungi (Fig. 3b), the total relative abundances were ranged from 44 % (NT-Pch) to 67 % (Po-Pch). The dominant genera were *Ilyonectria* (25.7 % ± 5.93), *Rhizophagus* (8.23 % ± 4.72) and *Hymenoscyphus* (7.56 % ± 2.69). No significant differences were found for the fungal genera but Auricularia was only detected for the NT-NPni, NT-Pch, NT-Np and Po-Np modalities.

3.2.2. *Effect of treatment and inoculation on microbial communities*

A Principal Component Analysis (Fig. 4) was performed to assess the differences in the microbial communities of the rhizosphere samples based on two modalities: treatment with the biopesticide (Fig. 4a) and inoculation or not with the pathogens (Fig. 4b). The 198 clusters retained for this PCA are presented in Supplementary Table 1 with their associated coordinates for the two axes 1 and 2. The main plot represents 32 % of the total variability. In the first case (Fig. 4a), there was a discrimination of the two treatment modalities (NT and Po) along the second axis (Dim 2). In the second case (Fig. 4b), an overlap is observed for the ellipses of the samples inoculated with *Pch* and *Np*, while the undrilled or drilled and uninoculated samples were dissociated from the others. These results showed the effect of *Po* on microbial communities, but also that even when a pathogen is inoculated into plants, it can have an effect on rhizosphere microbial communities.

To explore these results further, we selected samples inoculated with

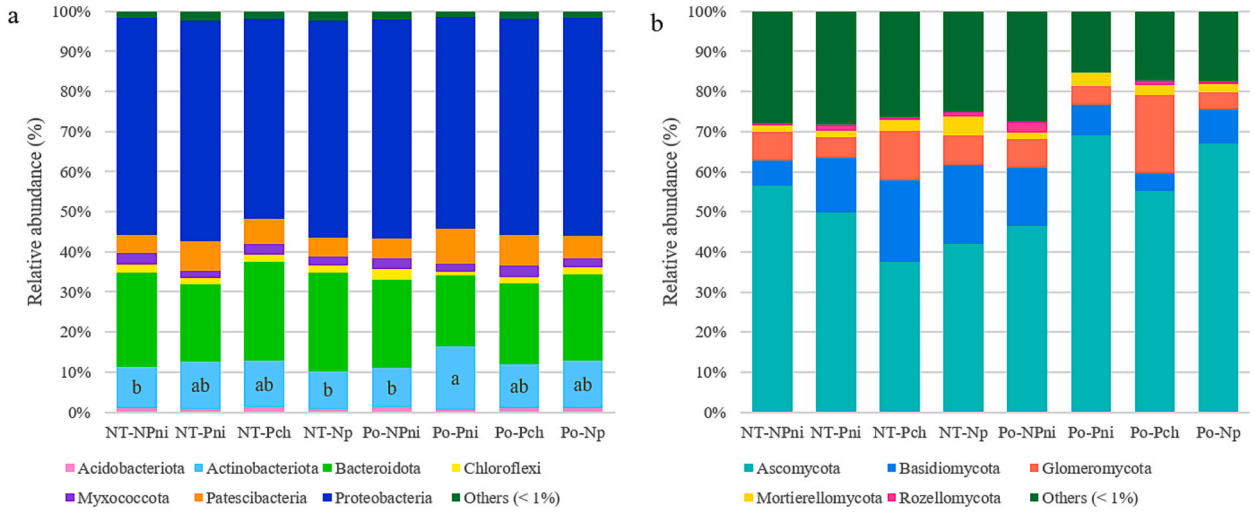

Fig. 2. Composition of bacterial (a) and fungal (b) communities characterised to the phylum level in the rhizosphere environment. NT: untreated vines, Po: treated with *P. oligandrum* biopesticide, NPni: undrilled and uninoculated, Pni: drilled and uninoculated, Pch: inoculated with *P. chlamydospora* and Np: inoculated with *N. parvum*. Others (phyla with relative abundances < 1 %) refers to (a) Abditibacteriota, Armatimonadota, Bdellovibrionota, Fibrobacterota, Firmicutes, Gemmatimonadota, Nitrospirota, Planctomycetota, Spirochaetota and Verrucomicrobiota; (b) Basidiobolomycota, Mucoromycota and unidentified sequences. Different letters indicate significantly different values at $p < 0.05$ (TukeyHSD test).

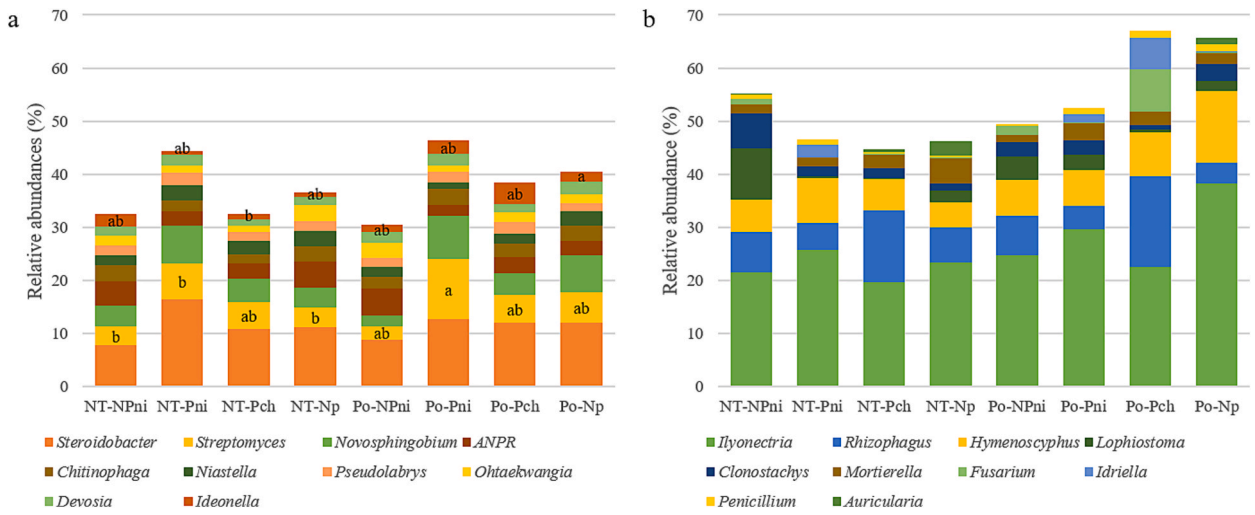

Fig. 3. Top 10 genera identified in the bacterial (a) and fungal (b) communities. NT: untreated vines, Po: treated with *P. oligandrum* biopesticide, NPni: undrilled and uninoculated, Pni: drilled and uninoculated, Pch: inoculated with *P. chlamydospora* and Np: inoculated with *N. parvum*. ANPR stands for the genus Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium. Different letters indicate values significantly different at $p < 0.05$ (TukeyHSD test).

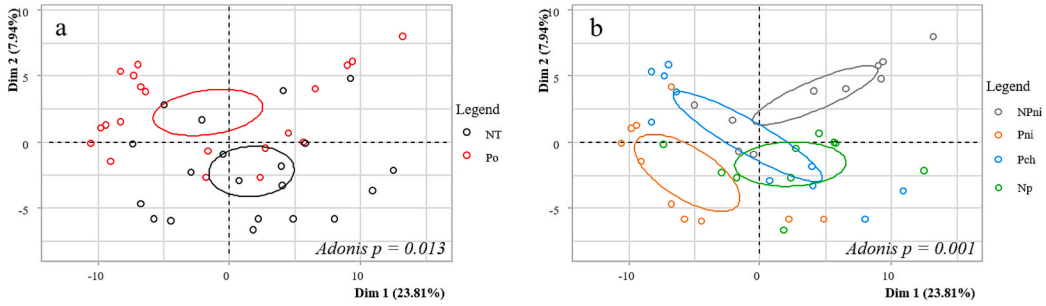

Fig. 4. Effect of biopesticide (a) and pathogen inoculation (b) on rhizosphere microbial communities. The Principal Component Analysis is based on the 141 bacterial and 57 fungal clusters retained after differential microbial community analyses of 40 samples. Confidence ellipses indicate the centre of the factors with 95 % of confidence, depending on the biopesticide treatment (a) and inoculation modalities (b). Adonis (Bray-Curtis) p-values indicate significant results. NT: untreated vines, Po: treated with biopesticide *P. oligandrum*, NPni: undrilled and uninoculated, Pni: drilled and uninoculated, Pch: inoculated with *P. chlamydospora* and Np: inoculated with *N. parvum*.

pathogens and treated or not with the biopesticide solution (Fig. 5). This was done to highlight clusters involved in the plant response to the pathogen inoculation and defence enhancement.

For the bacterial community (Fig. 5a-b), PCA explains 46 % of the total variability based on the 67 bacterial genera retained. The first axis (Dim. 1) strongly discriminates samples belonging to the modality treated and inoculated with Pch (Po-Pch). Rhizosphere samples of this modality had a higher representation of the genera *Mesorhizobium*, *Cellulomonas* or *Chthoniobacter*, whereas samples treated and inoculated with *Np* were associated with a higher proportion of the genera *Neochlamydia*, *Streptomyces*, *Nitrospira* or *Sphingomonas*.

The PCA based on the 25 fungal genera (Fig. 5c-d) explains 47 % of the total variability. No differences were found for untreated samples inoculated with *Pch* or *Np*. Conversely, there was discrimination for samples treated with the biopesticide, and inoculated with *Pch* or *Np*. The community from samples treated and inoculated with *Pch* was associated with a higher proportion of *Hyaloschypha*, *Arthrobotrys* or *Conlarium* genera, whereas samples treated and inoculated with *Np* were associated with a higher proportion of *Dactylonectria*, *Basidiomycota* and *Sebacinales* genera.

3.2.3. Link between disease and microbial clusters

Of the 84 bacterial and fungal genera retained for the PCA in Fig. 4, 22 genera were correlated with necrosis size ($p < 0.05$), as shown in the correlogram analysis (Fig. 6). A negative correlation between a genus and necrosis size indicates that this genus is less represented in the rhizosphere of plants with high necrosis, and therefore this genus is also better represented in plants with the lowest necrosis size, and therefore where the biopesticide could have the best effect. In this analysis, 18 bacterial genera were retained, half of which had a negative correlation with necrosis length and half of which had the opposite correlation. Among the bacterial genera of interest for biopesticide application (negatively correlated with necrosis size), genera from *Spirochaeta*, *Saccharimonadales*, *Sphingobacteriales*, *Lysobacter* and *Caulobacter* were the most negatively correlated with necrosis size. Of the four fungal genera retained for this analysis, three genera were negatively correlated with necrosis size: 1 *Dactylonectria,* 1 genus belonging to the order *Sebacinales* and 1 genus belonging to the phylum *Basidiomycota*.

4. Discussion

4.1. Efficacy of the biopesticide

The aim of the present study was to develop a new treatment based on the biocontrol agent *P. oligandrum*, to be applied directly to the vineyard by watering the vines. To date, this is the first study carried out with *P. oligandrum* formulated in a biopesticide treatment. This study showed that the treatment, applied in a greenhouse under controlled conditions, reduced necrosis induced by the pathogens *P. chlamydospora* and *N. parvum* by 47 % and 67 % respectively. We were able to confirm

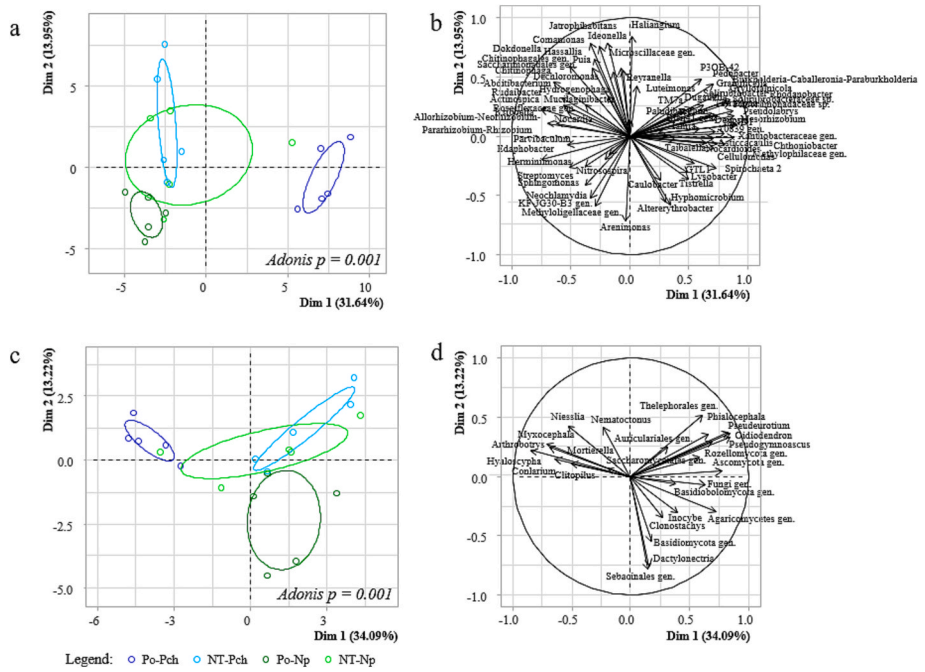

Fig. 5. Impact of the biopesticide on rhizosphere microbial communities of vines infected with pathogenic fungi. Principal Component Analyses are based on the 67 bacterial (a-b) and 25 fungal (c-d) genera retained after differential microbial community analyses of 20 samples. Distributions into correlation circles of bacterial (b) and fungal (d) genera involved in samples distribution are detailed in Supplementary Tables 2 and 3, respectively. Confidence ellipses indicate the centre of the factors with 95 % of confidence, depending on the biopesticide treatment and the inoculation modalities. Adonis (Bray-Curtis) p-values indicate significant results. Nt: untreated vines, Po: treated with *P. oligandrum* biopesticide, Pch: inoculated with *P. chlamydospora* and Np: inoculated with *N. parvum*.

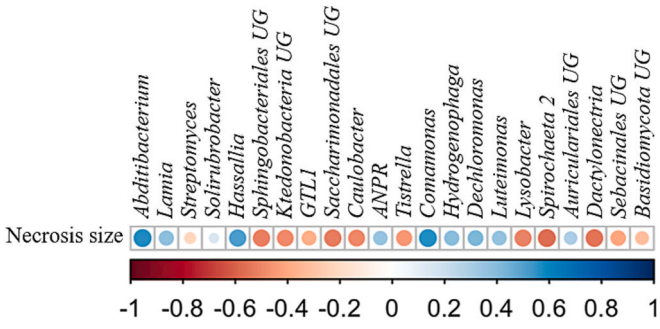

Fig. 6. Correlation matrix between microbial genera and necrosis size. Positive correlations are shown in blue and negative correlations in red. Colour intensity and circle size are proportional to the correlation coefficients. ANPR refers to *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* genus, and UG to unidentified genus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the results obtained by several authors, who observed that wood necrosis caused by these two pathogens was reduced after the application of *P. oligandrum* on young grafted vines (Daraignes et al., 2018; Yacoub et al., 2016). Therefore, the formulation used does not alter the efficacy of *P. oligandrum* as a biocontrol agent.

4.2. Effect of the biopesticide on microbial communities

After studying the efficacy of the biopesticide in protecting the vine against the two pathogens *N. parvum* and *P. chlamydospora*, the effect of its use on the indigenous microbial communities of the rhizosphere was assessed using an Illumina sequencing approach.

Comparison of the microbial communities in the different rhizosphere samples showed that the major phyla were present in all modalities, but at different abundances. Among all modalities, the relative abundances of Proteobacteria, Bacteroidota, and Actinobacteria were the three highest in the bacterial community. Proteobacteria and Actinobacteria have been described as the dominant phyla in the vineyard microbiome (Darriaut et al., 2022b; Samad et al., 2017), as well as in the rhizosphere of many plant species (Green and Bohannan, 2006; Kaiser et al., 2016; Singh et al., 2007). The rhizosphere is known to be a carbon-rich environment (Buée et al., 2009; Semenov et al., 1999; Yang et al., 2017), the preferred habitat of copiotrophic bacteria belonging to these two phyla (Fierer et al., 2007; Lienhard et al., 2014).

At the genus level, *Steroidobacter*, *Streptomyces* and *Novosphingobium* (formerly classified as *Sphingomonas*, (Takeuchi et al., 2001)) were identified as the predominant bacterial genera in this study. These first two genera have been described as predominant in Italian vineyard soils (Nerva et al., 2019), especially in bulk soils of asymptomatic plants (without Esca symptoms).

Regarding the fungal community, the Ascomycota, Basidiomycota and Glomeromycota phyla were the most abundant. Liu and Howell (2021) studied the fungal diversity during an annual growth cycle of grapevines and Ascomycota and Basidiomycota were the two dominant phyla, as observed in many other studies on grapevines (Berlanas et al., 2019; Carbone et al., 2021; Darriaut et al., 2022a; Samad et al., 2017; Swift et al., 2021; Zahid et al., 2021). With regard to the Glomeromycota phylum, its relative abundance was higher in vines infected with *P. chlamydospora* than in the other modalities. Arbuscular Mycorrhizal Fungi (AMFs) belong to the division Glomeromycota and form mutualistic symbiotic associations with most terrestrial plants, including vines (Trouvelot et al., 2015; Willis et al., 2013). AMFs have been shown to aid plant defence by mitigating abiotic and biotic stresses and improving plant growth and nutrition. In our study, the relative abundance of this division was not affected by the *P. oligandrum* biopesticide treatment. A study on legume plants showed comparable results, with a similar rate of mycorrhization for control and *P. oligandrum* treated plants, suggesting that the strong stimulation of various defence responses (PR proteins and secondary metabolism) by *P. oligandrum* did not affect the interaction between plant roots and AMFs (Hashemi et al., 2023). However, a recent study highlighted the higher level of AMFs in symptomatic Esca

vines (disease caused by *P. chlamydospora*) than in asymptomatic ones (Landi et al., 2021).

One of the most important fungal genera found in this study was *Ilyonectria*, a pathogenic fungus involved in black foot disease (Probst et al., 2022). The relative abundance of this genus was equivalent regardless of the treatment with *P. oligandrum*. It seems that the oomycete used in the biopesticide did not alter the development of this pathogen, as presented in a previous study for the field evaluation of biocontrol agents against black foot disease of grapevine (Del Pilar Martínez-Diz et al., 2021). Linked with the presence of *Ilyonectria*, the second most abundant fungal genus was *Rhizophagus*, an AMF known to fight against black foot disease and present in the rhizosphere microbiome associated with vine (Berlanas et al., 2019; Moukarzel et al., 2022).

On a global scale, when considering the total microbial community composition, no differences were highlighted between treated and untreated vines (Supplementary Fig. 1). This lack of difference in total diversity is also confirmed by the alpha diversity metrics. This result shows the low impact of *P. oligandrum* biopesticide application after 3 months on the microbial community from the vine rhizosphere. Previously, Vallance et al. (2012) concluded from Single Strand Conformation Polymorphism (SSCP) analyses of bacterial communities from tomato plants that *P. oligandrum* did not permanently affect the indigenous bacterial communities, even when the oomycete colonised the rhizosphere. Other studies confirm the lack of changes after a bacterial inoculation (*Pseudomonas* sp.) on barley (*Hordeum vulgare*; Buddrus-Schiemann et al., 2010), maize (*Zea mays*; Ke et al., 2019) or potato (*Solanum tuberosum*; Roquigny et al., 2018).

However, after carrying out a differential analysis of the microbial community, some changes were observed between the microbial composition of vines treated or not with the biopesticide. Even if *P. oligandrum* cannot have a lasting effect on the indigenous bacterial communities, its application can change the abundance of some taxa (Fig. 4). Alternatively, it could be hypothesised that *P. oligandrum* induced changes in plant physiology and in the emission or composition of root exudates, which in turn influenced the microbial communities of the rhizosphere (Walker et al., 2003), since the quantity and quality of root exudates depend on biotic and abiotic factors (Jones et al., 2004).

4.3. Clusters linked with protection against GTDs

One of the results obtained is the change in microbial communities between the rhizosphere of treated vines and those inoculated with a pathogenic fungus (Fig. 5). In the case of *N. parvum* inoculation, the effect of the biopesticide slightly modulated the relative abundances of some genera. Among the changes, differences in the abundance of *Dactylonectria* and genera from the Basidiomycota phylum and the Sebacinales order, three fungal clusters involved in necrosis reduction were highlighted (Fig. 6). The genus *Dactylonectria* is known to be involved in black foot disease (Cobos et al., 2022). A recent study has analysed the effect of *P. oligandrum* on some vine pathogens such as *Dactylonectria torrensis*, and showed that even if the abundance of this pathogen in the rhizosphere was not modified, the severity of the disease decreased with the application of *P. oligandrum* (Del Pilar Martínez-Diz et al., 2021). Members of the Sebacinales are involved in nutrient uptake, help plants to survive under water, temperature or salinity stress, and confer resistance to toxins and pathogens (Shoresh et al., 2010; Varma et al., 2013). The effect of *P. oligandrum* against *N. parvum* was also associated with changes in the bacterial community, with an increase in the relative abundance of some clusters such as *Nitrospira*, *Sphingomonas* or *Streptomyces*. These last two genera are considered as PGPRs (Plant Growth Promoting Rhizobacteria), involved in different activities such as siderophore production, nitrogen fixation or phosphate solubilisation, and can be producers of antibiotics or antifungal compounds (Asaf et al., 2020; Lee et al., 2018). Bacteria of the genus *Nitrospira* have the ability to oxidise nitrite and are involved in the nitrogen cycle (Daims et al., 2015). Although they do not directly promote plant growth, they play an important role in maintaining soil health and nutrient availability. The increase in these fungal and bacterial genera, combined with the effect of *P. oligandrum*, can explain the reduction in necrosis of up to 60 %.

Regarding the effect of the biopesticide on the rhizosphere of vines inoculated with *P. chlamydospora*, a differentiation between the rhizosphere of vines treated with the biopesticide and those not treated were observed. When treated with the biopesticide, potential biocontrol agents from the fungal genera *Arthrobotrys* (Purba et al., 2022) and *Hyaloscypha* (Marian et al., 2022) increased. With regard to changes in bacterial genera, *Caulobacter*, *Lysobacter* and *Chthoniobacter* increased. *Caulobacter* and *Lysobacter* were also associated with the decrease in necroses in the correlogram analysis. The first genus, *Caulobacter*, is described as PGPR (Berrios, 2022), and *Lysobacter* is used as a biocontrol agent (Kilic-Ekici and Yuen, 2004; Liu et al., 2019). The genus *Chthoniobacter* is described in the literature as a genus capable of degrading many complex organic compounds, and it is therefore important for the proper functioning of the carbon cycle (Sangwan et al., 2004).

5. Conclusion

This study provided evidence that the *P. oligandrum* biopesticide, applied in a greenhouse under controlled conditions reduced necrosis induced by the pathogens *P. chlamydospora* and *N. parvum*. It would now be appropriate to test this biopesticide directly in the vineyard to see if it can also reduce disease symptoms when exposed to variable environmental conditions and applied to older vines.

In addition, the biopesticide seemed to promote several taxa that play a beneficial role for plants, as PGPR or biocontrol agents. This may explain the reduction in *Botryosphaeria* dieback and Esca disease, which may be additive to the biocontrol effect of *P. oligandrum*.

However, the biopesticide has a weak effect on the whole rhizosphere community. It would be interesting to observe the effects of *P. oligandrum* at the first time of its application, as it could induce an early change in the community, which, due to its resilience, would return to a stable state after 3 months of cultivation.

CRediT authorship contribution statement

Séverine Lopez: Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Alexandre Chataigner:** Validation, Investigation, Conceptualization. **Jessica Vallance:** Writing – review & editing, Data curation, Conceptualization. **Ahmed Taïbi:** Writing – review & editing, Resources. **Assia Dreux-Zigha:** Resources, Funding acquisition. **Marie-Cécile Dufour:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2025.105779.

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