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RESEARCH ARTICLE

Eruca vesicaria leaf extracts and intercropping mitigate the Kiwifruit Vine Decline Syndrome and modulate the rhizosphere pathobiome

Giovanni Mian · Paolo Ermacora · Marta Martini · Luke Bell · Francesco Spinelli · Guido Cipriani

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Abstract

Background Kiwifruit Vine Decline Syndrome (KVDS) is the most significant soil-borne disorder affecting *Actinidia spp.*, impacting both yield and economics, often forcing farmers to switch crops once the diseases occur. The *Oomycota* phylum is a key component of the root rhizosphere pathobiome. Aside from proper irrigation—preventing KVDS-favourable conditions—there are no effective management strategies. Yet, the lack of soil treatments and concerns over fumigants drive the search for sustainable alternatives.

Methods In this study, leaf extracts from rocket (*Eruca vesicaria* subsp. *sativa*) were tested for their potential to control KVDS. The isothiocyanates, key components of rockets, are known to counteract soil-borne pathogens. The extracts were applied in different concentrations to kiwifruit plants (*Actinidia chinensis* var. *deliciosa* 'Hayward') grown in pots with KVDS-promoting soil. Additionally, kiwifruit plants were intercropped with rocket ('Astra', ~20 plants per pot) to evaluate whether intercropping could also help control KVDS.

Results The highest dose of rocket leaf extract and intercropping reduced KVDS symptoms by 70–80% compared to untreated plants ($p < 0.05$). To further investigate the mode of action of rocket extracts and intercropping, their effects on the rhizosphere *Oomycota* pathobiome were analysed using a metabarcoding approach. To achieve this aim, an in-house reads reference dataset was created and implemented due to the lack of public reference databases. Data were processed via QIIME2, identifying six out of twelve oomycetes at species level. Treatments altered the pathocommunity, reducing several pathogens—most

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notably *Phytopythium vexans* ($p < 0.05$). Concurrently, *Globisporangium intermedium* increased in treatments with the highest extract dose and intercropping, correlating with symptom reduction (r^2: -0.86).
Discussion Our results suggest *G. intermedium* being less pathogenic than *P. vexans* yet competes for the same root niche. In fact, *P. vexans* highly correlated with symptoms display (r^2: 0.97). Lastly, *P. asiatica* was also identified, another oomycete strongly associated with the dysbiosis. These findings suggest that rocket-based treatments could be a promising strategy for KVDS management, potentially applicable in field conditions.

Keywords *Actinidia* · Biocontrol · Oomycetes · Root system · Salad rocket

Introduction

Kiwifruit cultivation is very successful, and it is widely spread with an increase of total fresh fruit production from 577 to 887 Mt/year in the last 20 years (Richardson et al. 2023; Bassi et al. 2023). One of the major issues of this crop is the fact that it is facing several phytoiatric problems and, among them, Kiwifruit Vine Decline Syndrome (KVDS) (Donati et al. 2020; Mian et al. 2022a) poses the highest risks (Spinelli and Mian, 2024). The first records of KVDS date to 2012 and the acreage affected by this syndrome constantly increased leading to losses exceeding €300 million in 2020 (Savian et al. 2020a, b). In Italy, KVDS was firstly observed in Verona province (Veneto, North-eastern of Italy), where several kiwifruit orchards (±50 ha) suddenly began to wilt during summer 2012 (Tacconi et al. 2015). Furthermore, in just few seasons, the syndrome rapidly spread, exceeding 1,200 ha in 2016 (Tacconi et al. 2019). Meanwhile, during 2014–2015, two other outbreaks occurred in two regions of Northern Italy; namely Friuli-Venezia Giulia and Piedmont regions (Donati et al. 2020; Savian et al. 2020a, b). To date, the disease is present in the majority of kiwifruit producing areas within Italy (thus, southern most part as well). At any rate, reports of declines with KVDS-like symptoms are also found worldwide (e.g., Turkey) (Polat et al. 2017; Türkkan et al. 2022), and unofficially both in New Zealand and Chile.

In this context of kiwifruit decline, environmental stressors such as waterlogging, anoxia, soil-borne pathogens, and reduced soil fertility are key drivers of rhizosphere microbiome imbalances (Donati et al. 2020), characterized by reduced microbial diversity, the loss of beneficial taxa, and the proliferation of potentially pathogenic organisms—conditions collectively referred to as microbiome dysbiosis (Savian et al. 2020a, b). As a consequence, in KVDS affected vines, the root system is severely compromised, exhibiting extensive rot and a lack of absorbent roots. As a result, the aerial parts of the plant collapse during at the beginning of the hot season when the vines have a high evapotranspiration rate. As the etiological agents of KVDS, several soil-borne pathogens have been identified, with oomycetes appearing to be the most significant. *Phytophthora* spp., *Pythium* spp., and *Phytopythium* spp. have been frequently isolated from infected plants (Mian et al. 2023a; Prencipe et al. 2020; D'Ippolito et al. 2021), alongside some fungi, such as *Desarmillaria tabescens* (Donati et al. 2020).

For KVDS and alike-diseases, at the moment, the control measures for soil-borne pathogens are currently very limited, due to the difficulties to find out effective measures. In fact, this is a time-consuming and economically costly approach that needs to begin at the laboratory scale, proceed through greenhouse conditions, and finally be tested in the field. No effective chemical treatments or soil fumigants are available (under the Law) to manage KVDS, leaving growers with few options (Bellostas et al. 2007). This underscores the urgent need for alternative, eco-friendly strategies that can sustainably mitigate the impact of root pathogens. Approaches such as biological control, the use of organic soil amendments, and microbiome engineering are gaining attention as promising solutions to restore rhizosphere health and improve plant resilience. In this context, a promising strategy to counteract the syndrome is the intercropping with specific plants, as well as the use of plant extracts (e.g., grounded leaves, intercropping with the whole plant, etc.), practices already adopted to control several soilborne pathogens (Morales-Rodríguez et al. 2016; Cerritos-Garcia et al. 2021). These strategies are compatible with EU laws for organic farming and does not increase environmental pollution (Poveda et al. 2020). Under this biological control standpoint, several plants from the *Brassicaceae* family, especially rocket *(Eruca vesicaria* subsp. *sativa),* also known as argula, has been widely studied for their

secondary metabolites; e.g., glucosinolates (GSLs) (Bell and Wagstaff 2014), which can exert a biocontrol effect against soil-borne pathogens (Cerritos-Garcia et al. 2021). Upon hydrolysis by endogenous enzymes (myrosinases), GSLs produce a variety of biologically active substances that possess fungicidal, insecticidal, herbicidal, and nematocidal effects (Bednarek et al. 2009). In this sense, rockets are particularly distinctive from a phytochemical perspective (Martínez-Sánchez et al. 2006). Previous studies demonstrated that GSLs can inhibit, in vitro, the growth of the *Oomycetes* involved in KVDS disorder (e.g., *Phytopythium vexans*, *Phytopythium chamaehyphon* and *Phytophthora citrophthora* (Mian et al. 2023b). However, other phytohormones, including cytokinins, gibberellic acid, strigolactones, and brassinosteroids, have demonstrated significant roles in plant–microbe interactions, particularly in fostering rhizobia and arbuscular mycorrhizal fungi (AMF) associations (Efimova 2022; Li et al. 2021; Sharma et al. 2022; Duret et al. 2024; Soliman et al. 2022; González Rojas and Salazar Orellana 2024). Thus, *Eruca* and its GSLs may influence the growth of intercropped kiwifruit plants through multiple mechanisms (Unger et al. 2024), including enhanced stress resilience and promoted resistance to biotic and abiotic stresses, potentially mediated by secondary metabolite production or modulation of hormonal pathways (Gao et al. 2023). Furthermore, intercropping systems involving rockets could actively shape the assembly of a beneficial microbiome in the rhizosphere (Duret et al. 2024), thereby improving plant health and productivity.

Based on the above-mentioned instances that *Eruca vesicaria* subsp. *sativa* possesses bioactive properties capable of influencing plant–microbe interactions, we hypothesized that both the application of rocket leaf extract and intercropping with rockets would mitigate the incidence and severity of KVDS, via modulate the rhizosphere oomycete community (suppressing pathogenic taxa and fostering a more balanced microbial assemblage). Accordingly, the current study aimed to evaluate the effects of these treatments on (i) the root growth of 'Hayward' kiwifruit plants, (ii) the expression of KVDS symptoms in the root apparatus, and (iii) the composition and structure of the rhizosphere oomycete pathocommunity. To this end, high-throughput sequencing and bioinformatic analyses were employed to investigate how these biological interventions shape microbial dynamics and contribute to plant health under KVDS-conducive conditions.

Materials and methods

Experimental setup

The experiment was performed in a controlled glasshouse environment at the University of Udine (Italy). One year old micro propagated, self-rooted plants of *Actinidia chinensis* var. *deliciosa* 'Hayward' were cultivated in 4 L pots (20 cm diameter-at the top-, 13 cm height) containing soil collected from naturally affected KVDS vines, located in a historical infected orchard in Friuli-Venezia Giulia region (FVG, northeastern Italy; GPS coordinates: 45°59′55.4 N, 12°46′06.6 E). The site was chosen based on previous research that identified it as conducive to KVDS development (Mian et al. 2022a). Soil was collected at a depth of 5 to 20 cm near symptomatic vines and thoroughly mixed prior the experiment. In this study, seven biological replicates were used for each treatment. Prior transplantation in KVDS-promoting soil, roots were disinfected using a 1% sodium hypochlorite solution for 10 min, followed by thorough rinsing with sterile water. The following treatments were applied: i) **NTC** (Non-Treated Control), ii) **T1:** Application of *E. vesicaria* leaf extract at a concentration of 1.9 g plant^{-1}, designed to simulate conditions used in prior in vitro studies on oomycete control (Mian et al. 2023a); iii) **T2:** Application of *Eruca* leaf extract at 16 g plant^{-1}, reflecting concentrations found in commercial products like Biofence®; iv) **T3 (Intercropping):** Incorporation of *Eruca* plants into the soil as an intercropping strategy. In each pot, 25 g of *E. vesicaria* 'Astra' seeds were sown, resulting in complete soil coverage with rocket plants. Biofence® is a commercial and well-known soil biofumigant (repository: https://www.agristoresrl.com/prodottiagricoltura.php?p=biofence).Hence, T2 treatment mimic the application of this latter, as it is widely used under soil-borne diseases standpoint.

Each treatment consisted of 7 biological replicates (kiwifruit plants). Waterlogging conditions were simulated in three cycles to recreate environmental stress factors associated with KVDS, according to Mian and colleagues (Mian et al. 2023a, b). During

the first cycle, the leaf extracts were applied to T1 and T2. Rockets were grown for ten days in seedling trays and then transplanted to larger trays. Plants were grown for another twenty days and then leaves were harvested together. Sampling for each plant took approximately one minute from the cutting of the leaves at the petiole to being placed in zipper-top poly bag freezer bags on dry ice inside a polystyrene container (with lid). Thirty days was chosen as the optimum point of harvest as it reflects the typical number of days commercial growers grow their crop after sowing. Furthermore, leaves were freeze dried and grounded (Mian et al. 2023a, b). Hence, leaf extracts were manually incorporated in the soil of the pot, well spread around the radical system. For the intercropping treatment (T3), *E. vesicaria* plants ('Astra') were mulched and incorporated into the soil at flowering stage, maximizing GSL content (Bellostas et al. 2007).

Assessment of KVDS root symptoms and bio metric indices

For each test-plant, the percentage of roots with necrosis or rat-tail appearance was assessed when the peak of symptoms occurred on the NTC. Assessment was independently performed, in blind treatment conditions, by 8 trained plant pathologists. For the symptoms' evaluation, Quantitative Descriptive Analysis was adopted. Thus, symptoms were visually categorized in 8 disease classes based on visual incidence of symptomatic roots, as follow: 1: 0–12.5%, 2: 12.5–25%, 3: 25–37.5%, 4: 37.5–50%, 5: 50–62.5%, 6: 62.5–75%, 7: 75–87.5%, 8: 87.5–100%. The relative differences between vines were analysed and confirmed, submitting the judgments to statistical analysis using the ANOVA method (F-test p-value < 0.0001).

Biometric indices were also recorded. The root systems were photographed with a digital camera, and the photos were processed with ImageJ (National Institutes of Health, USA) to convert them to binary values (Tomasi et al. 2020). Furthermore, photos were analysed by using RhizoVision Explorer (Seethepalli and York 2020). Image features were extracted using the image analysis function, which worked in batch mode to process feature images, including the derived metrics overlaid on the segmented image. Segmentation was performed by simple thresholding of the greyscale values for each pixel, optimized based on the imaging method. Following segmentation, the edges were smoothed to remove small irregularities that could falsely contribute to root length. The smoothed image was then skeletonized using a distance transformation, followed by the identification of the medial axes. From this skeleton, the number of root tips, volume (L), network area (mm^2), and total root length (m) were calculated (Mattupalli et al. 2019).

Sample collection, and separation of root rhizosphere

Root samples were collected from potted vines in the following phases: 1. before the transplantation of the plants into KVDS-inducing soil, and 2. when over 50% of control plants showed canopy decay. These samples were stored and later analysed for metabarcoding to assess changes in the rhizosphere *Oomycota* pathocommunity, since it is the most important for KVDS development. At sampling time n.2, the roots of 7 plants were randomly taken and processed to separate the rhizosphere from root endosphere following literature (Savian et al. 2022). After uprooting, the samples were kept at 4 °C and quickly processed. Roots were put into 50 mL Falcon tubes containing 30 mL of epiphyte removal buffer pH 6.5 (6.75 g of KH_2PO_4, 8.75 g of K_2HPO_4, and 1 mL of Triton X-100, to 1 L of sterile water). For each sample approximately 6–10 g of roots plus soil were collected. The samples were then sonicated at 600 Hz with a cycle of 30 s sonication and 30 s without sonication for 10 min at 4 °C in an ultrasound bath (Merck, Germany). Root tissues were then removed, since it is known that these bio products primarily influence the soil/rhizosphere microbiota. The tubes containing buffer and rhizosphere soil particles, were centrifuged at $4{,}000 \times g$ for 10 min at 4 °C. The supernatant was removed, and the rhizosphere fraction (0.2–1.0 g) was transferred into a 2 mL tube, centrifuged at $6{,}000 \times g$ for 2 min to remove excess of epiphytic buffer, before the immersion in liquid nitrogen and storage at −20 °C.

DNA preparation

Total genomic DNA from 200 mg of rhizosphere of each individual sample was obtained using the Quick-DNA Fecal/Soil Microbe Miniprep kit (Zymo

Research Corp., US) (Savian et al. 2022), following the manufacturer recommendations. Concentration and quality of the DNA samples were verified using NanoDrop ND-1000 spectrophotometer (ThermoFisher Scientific, Italy,); successively DNA samples were diluted to 25 ng/µL (Mian et al. 2023a, b).

Oomycetes-specific ITS amplicon library preparation and sequencing

PCR (Polymerase Chain Reaction) was used to selectively amplify the internal transcribed spacer (ITS) region of the oomycetes with the primer pair ITS3Oo/ITS4ngs (Riit et al. 2016), designed to avoid co-amplification of DNA of vascular plants and modified only to add the Nextera Miseq adapters. The PCR mixture contained the following ingredients: 12.5 µL of KAPA HiFi Hot Start Ready Mix (2×) (Roche sequencing store, Italy), 0.375 µL of each primer 20 µM and approximately 50 ng of genomic DNA, water to a final volume of 25 µL. The following thermal cycling scheme was adopted: initial denaturation at 95 °C for 3 min, 37 cycles of: 20 s at 98 °C, 15 s at 59 °C, and 15 s at 72 °C, final extension at 72 °C for 3 min. Amplicons were detected by gel electrophoresis (2% agarose gel in 1×TBE buffer at 120 V for 120 min). The resulting PCR products were sent to the BMR Genomics s.r.l. (Padova, Italy) for purification and sequencing on Illumina MiSeq platform using a 2×300 bp paired end protocol. A total of 32 samples were sequenced, including samples taken before transplanting plants into KVDS promoting soils.

Bioinformatics and statistical analysis

The Illumina output was received as demultiplexed FASTQ files. Quantitative Insights Into Microbial Ecology 2 (QIIME2) (Bolyen et al. 2019) software was used for subsequent bioinformatic analysis. Forward and reverse reads were not concatenated. The data were imported as an artefact, trimmed to remove the primer sequences and nucleotides with low quality scores, filtered for quality and chimeras, denoised, and merged using the DADA2 plugin (Callahan et al. 2016), yielding ASVs (amplicon sequence variants). Taxonomical classification of oomycete sequences was carried out using a Naive Bayes classifier trained on a curated reference database of plant-associated oomycetes (Savian et al. 2022). Taxa were assigned based on a minimum sequence similarity threshold of 99% for species-level resolution, by training via a Naive Bayes classifier. To ensure consistency and accuracy in taxonomic assignment, the nomenclature was aligned with the most recent updates in oomycete taxonomy proposed in literature (Yang et al. 2017). In the case of sequences assigned to chloroplasts and mitochondria, with a frequency < 2 (singletons) or non-identified, these were filtered out from the dataset. The rarefaction curves on species richness were calculated using QIIME2. All biological replicates (7) per each treatment were used, and all rarified data were further considered for analysis. Thus, after rarefaction, R package 'vegan' (Dixon 2003) and 'ape' (Paradis and Schliep 2019) were used to calculate alfa-diversity indices, through richness (number of observed features per sample) and diversity (Shannon entropy and Specie richness) (Colautti et al. 2024). Differences in alfa-diversity (specie richness and Shannon entropy between treatments was assessed using Kruskal–Wallis test, with results considered significant when the p-value was < 0.05. To graphically represent the microbiological differences between the treatments, a Principal Coordinates Analysis (PcoA) using the Bray–Curtis dissimilarity matrix was constructed using the vegan package evaluating the presence of a significative effect of the treatment with a PERmutational Multivariate Analysis Of Variance (PERMANOVA) based on 4,999 permutations. The meta-MDS package (Ssekagiri et al. 2017) was used to analyse the microbial community data. To determine the optimal number of clusters (k) representing distinct groups within the data, we applied the elbow method (Rahmatbakhsh et al. 2021). The elbow method involves calculating the within-cluster sum of squares (WCSS) for different numbers of clusters. WCSS measures how tightly the data points within each cluster are grouped together. By plotting WCSS values against increasing numbers of clusters (k), we observe a curve that typically decreases as (k) increases because adding more clusters reduces variation within clusters. The key point in this plot is the "elbow"—the point at which adding more clusters no longer significantly decreases the WCSS. This "elbow" indicates an optimal balance between having enough clusters to

capture meaningful differences in microbial communities and avoiding overfitting by creating too many small or redundant clusters. In this study, using the elbow method allowed us to objectively select the number of clusters that best represent differences in microbial community composition among treatments. This ensures that our ecological interpretations are based on a statistically supported number of groups rather than arbitrary choices (Laporte et al. 2022).

To analyse root indices in the different samples, one-way analysis of variance (ANOVA) was performed by using "R" software (version 4.0.3 2022-10-10) (www.R-project.org). Statistical analysis to determine significant differences was carried out using the Tukey HSD test ($p < 0.05$). Figures were generated using 'ggplot2' library, ensuring consistent theming and annotation. Data were transformed and normalized where appropriate prior to visualization to improve interpretability. Finally, the correlation matrix between symptoms and oomycetes present in the samples was implemented in "R" by using the cor_matrix function (Kolde and Kolde 2015), plotted with "corrplot".

Results

Radical system symptoms and biometric indexes

Extensive root necrosis and reduction of feeder roots were observed primarily in NTC and T1 plants (Fig. 1S). Concerning KVDS symptom incidence, the highest values were observed in NTC and T1 with no statistically significant difference between these two treatments. T2 and T3 plants exhibited similar symptom levels, statistically lower than NTC and T1 groups (Fig. 1A). Concerning root volume, it showed significantly higher values in the T2 and T3 groups (with no significant differences between these two treatments) when compared to the NTC and T1 groups (Fig. 1B). Similar results were observed for the number of root tips and network area (Figs. 1C) and 1D). Notably, the total root length displayed statistically significant variations across all treatments, with the highest total root length observed in the T2 group, followed by T3, T1, and NTC (depicted in Fig. 1E). An example of RhizoVision work is reported in Fig. 2S that also shows the pipeline used to phenotype the radical system.

Oomycetes taxonomy in the rhizosphere compartment

A total of 3,081,589 reads was obtained through Illumina MiSeq sequencing. The distribution of these sequences was uniform across all the samples examined. Notably, all rarefaction curves exhibited a plateau, indicating that the sample sizes were adequate and well-suited for subsequent analysis, thereby ensuring optimal coverage. It is noteworthy that Oomycetes were detected across all examined samples.

The analysis focused on the 50 most frequently occurring features, defined as those representing at least 0.01% of the total read count across all samples. ASVs were classified into 11 Operational Taxonomic Units (OTUs), aligned with phylogenetically defined clusters established in the extensive study conducted by Robideau and colleagues (Robideau et al. 2011). The use of OTUs was chosen due to the limited availability of comprehensive reference databases for oomycetes. This approach allows for robust biodiversity analysis and ecological pattern detection without relying on precise taxonomic annotations, which are challenging for less-studied groups like oomycetes (Foster 2020). In total, two dominant OTUs captured considerable information, contributing to 59–93% of the total hits across each sample. However, their relative proportions exhibited variations in relation to the treatments. These prominent OTUs were identified as *Phytopythium vexans* and *Globisporangium intermedium*. Notably, *Pp. vexans* predominated across all treatments, with a marked reduction in abundance observed from the Non-Treated Control (NTC) group to treatments T1, T2, and T3. Conversely, the presence of *G. intermedium* exhibited significantly heightened levels in treatments T1, T2, and T3 compared to the NTC group.

Further identification efforts unveiled the presence of additional microorganisms classified as oomycetes belonging to the *Peronosporaceae* family, *Pp. chamaehyphon*, *Phytophtora asiatica*, *Saprolegniaceae* spp., *Lagena radicicola*, various species of *Pythium, Phytopythium*, *Saprolegnia megasperma*, and *Phytophthora*. Intriguingly, it's noteworthy that *Pp. chamaehyphon* was absent in T3 (Fig. 2).

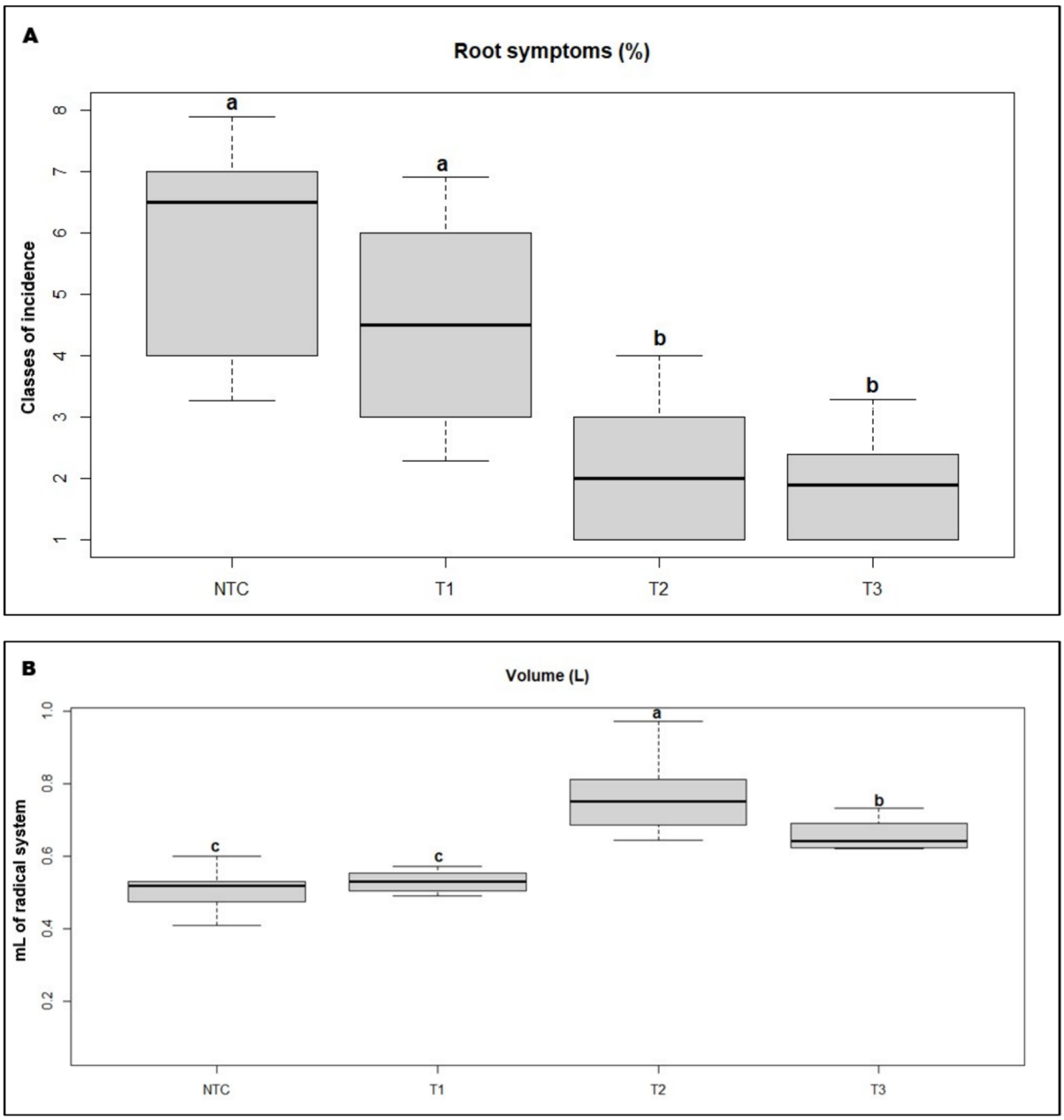

Fig. 1 **A** Root system symptoms, **B**) Root system volume; **C**) Number of root tips found in the radical system; **D**) Root system network area; and **E**) Total root length, of NTC, T1, T2 and T3. Statistical analysis was carried out applying the Tukey HSD test ($p < 0.05$). Different letters mean a statistical difference for the p value considered. Data are plotted using 'R', library 'ggplot2'. NTC (Non-Treated Control), T1: leaf extract at a concentration of 1.9 g plant^{-1}, T2: leaf extract at 16 g plant^{-1}, T3 (Intercropping)

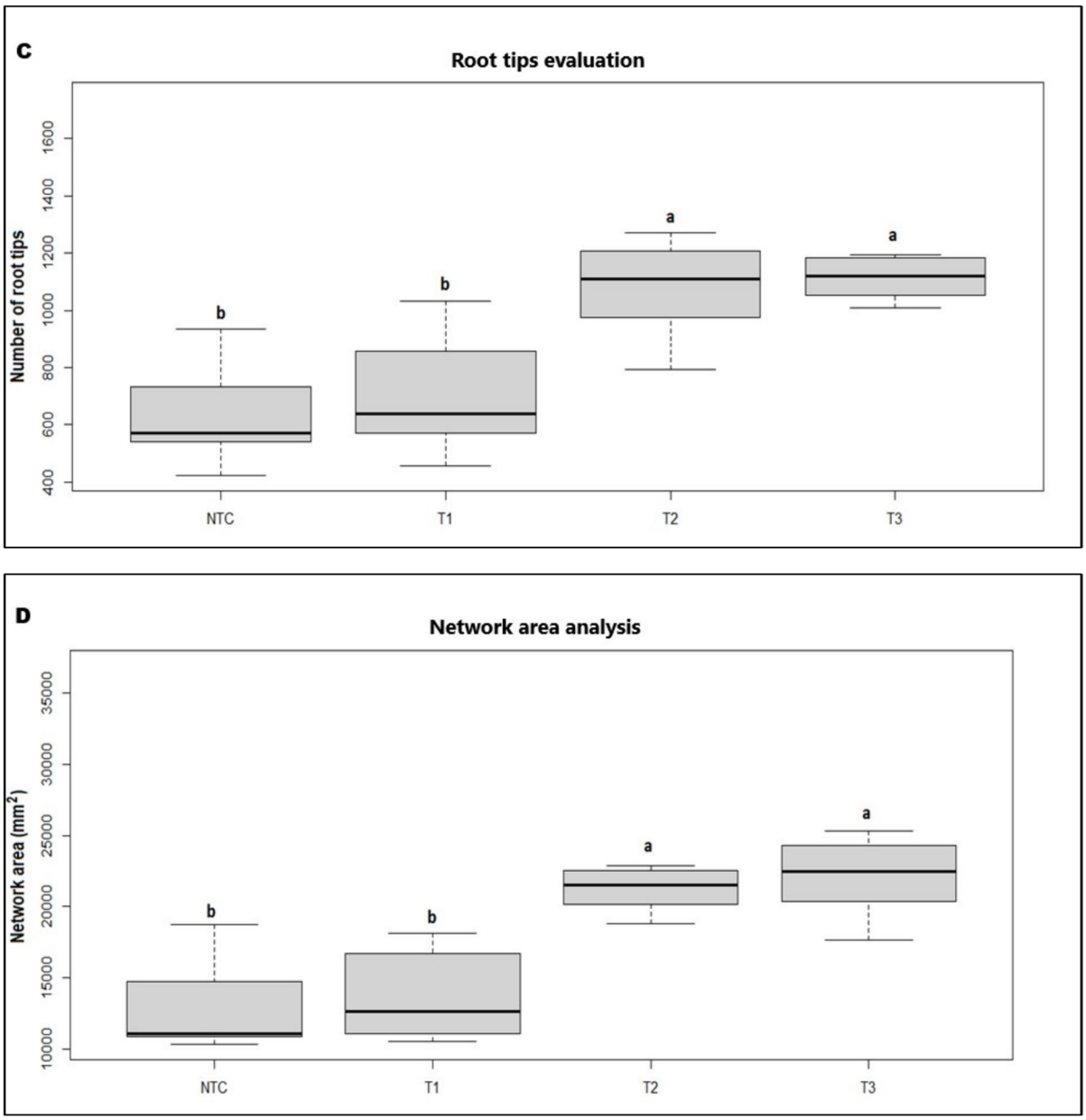

Fig. 1 (continued)

Alfa and beta diversity of the oomycetes in the rhizosphere of kiwifruit plants

Alfa diversity, which includes both species richness and the Shannon index, was assessed across treatments. No significant differences in species richness were observed among treatments (Fig. 7 – Species Richness). However, Shannon diversity was significantly higher in all treatments compared to the control, while no significant differences were found among the treatments themselves (Fig. 7 – Alfa Diversity – Shannon Index).

Using the elbow method, four clusters (groups with associated samples) were identified, as shown in

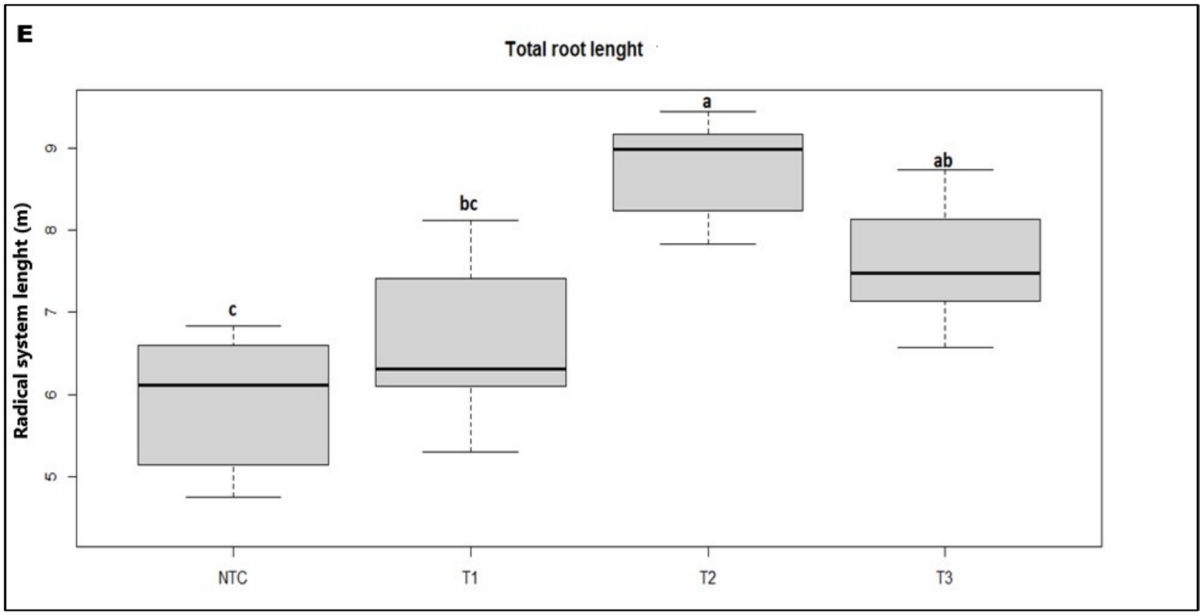

Fig. 1 (continued)

Fig. 3S. In the NMDS plot (Fig. 3), the Beta diversity analysis did not display a strong differentiation of clustering patterns among the treatments. While a slight tendency towards separation is observed, as indicated by the distribution of points corresponding to different clusters, the overall variability explained is substantial (approximately 70%). However, the PERMANOVA analysis confirmed the absence of statistically significant differences between treatments. This suggests that the application of rocket leaf extracts had no marked impact on the biodiversity patterns of oomycetes within the plant rhizosphere. Lastly, on the right hand side is showed a network that only visually group data points into clusters of similarity. This helps in understanding the structure and relationships within complex high throughput sequencing.

Correlation matrix between the oomycetes found in plant rhizosphere and KVDS symptoms

Considering the correlation between the oomycetes identified in the plant rhizosphere and the KVDS symptoms, *P. vexans* is notably linked to symptom manifestation with a substantial positive correlation coefficient of 0.97. Conversely, *G. intermedium* exhibits a strong negative correlation coefficient (−0.87) with symptoms. This implies that the presence of *P. vexans* corresponds to a higher incidence of symptoms, while the prevalence in population of *G. intermedium* is associated with a reduction in root damage. Moreover, other oomycete species categorized under the *Pythium* genus exhibited a robust positive correlation with symptoms, indicated by a correlation coefficient of 0.99. Surprisingly. *Phytopythium* spp. displayed a negative correlation coefficient of −0.73 with symptoms. This suggests that the presence of some species of *Phytopythium* genus is associated with a reduced symptom incidence previously measured and reported (Fig. 4).

Discussion

KVDS is a dysbiotic multifactorial syndrome caused by a combination of factors, both abiotic and biotic ones, which occur together causing the symptom development (Donati et al. 2020; Savian et al. 2020a, b; 2022; Mian et al. 2022b, 2023a). Recent evidences suggest a key role of plant microbiota (especially the pathobiome) in the crop health (Purahong et al. 2018; Trivedi et al. 2020). Plants are subjected to multiple stresses which may lead to a shift from eubiosis to dysbiosis.

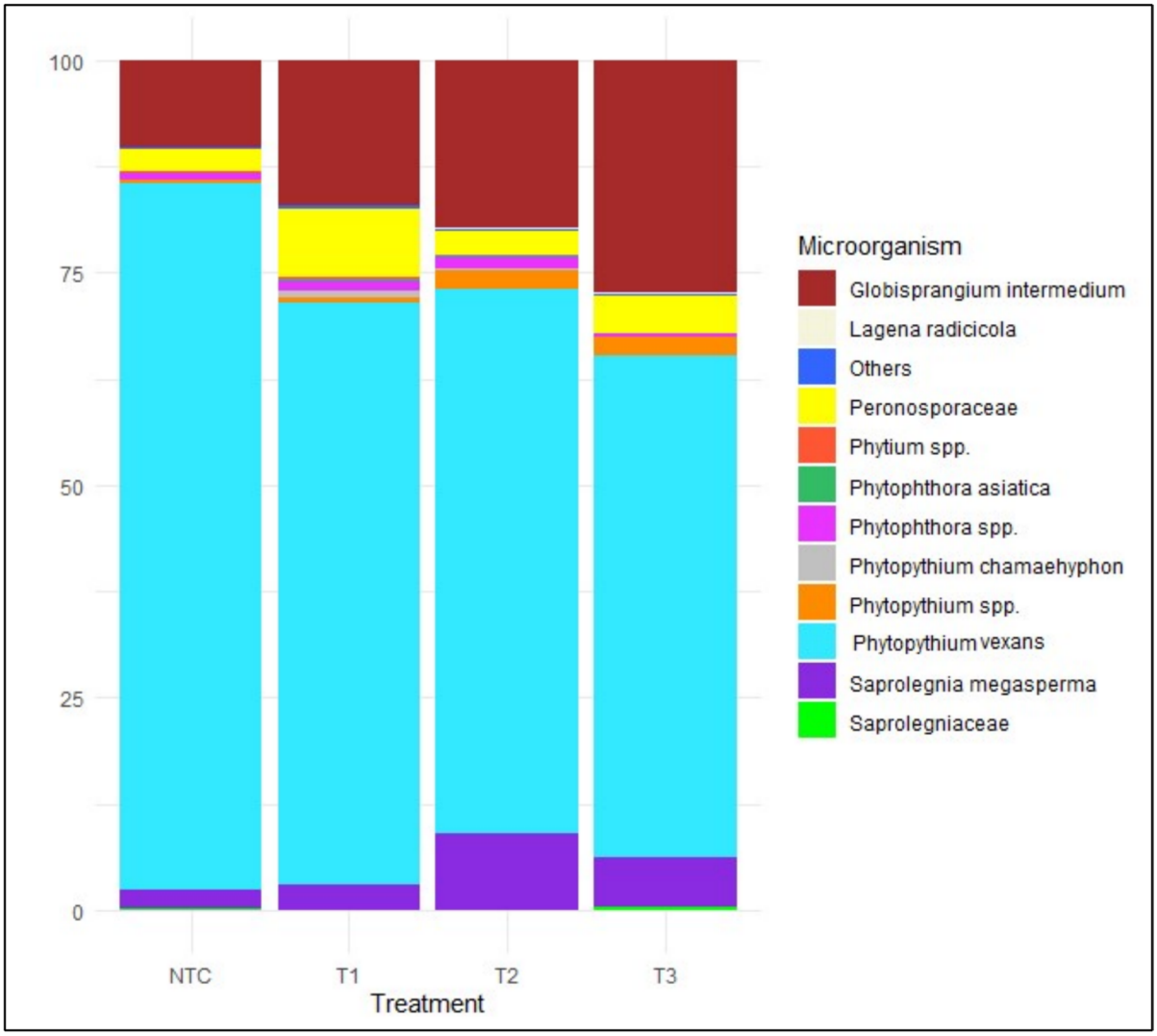

Fig. 2 Taxa bar plots of oomycetes found in plant rhizosphere of NTC, T1, T2 and T3. Data are plotted using 'R', library 'ggplot2'. NTC (Non-Treated Control), T1: leaf extract at a concentration of 1.9 g plant^{-1}, T2: leaf extract at 16 g plant^{-1}, T3 (Intercropping). Data are the relative abundances (%) of the most abundant annotated taxa

In KVDS, irrigation management, climatic conditions such as concentrated rainfall, and cultural strategies may promote dysbiosis. These factors not only directly influence plant responses but also interact with each other indirectly. For instance, the climates affect plant physiology, while simultaneously impacting cultural practices, soil properties, microbiota, pathogens, and ecological dynamics, all of which, in turn, influence plant responses. Consequently, even a minor shift in one of these factors can trigger cascading effects on the others, amplifying their impact on plant health and performance. Nonetheless, the rapid spread of KVDS within orchards and across kiwifruit production areas strongly suggests the involvement of an aggressive pathogen or a pathogenic consortium driving this phenomenon.

Oomycetes spp. spread and multiplication is strictly linked with gravitational water in the soil, which also facilitate KVDS which has been reported primarily in orchards characterized by periods of waterlogging (Savian et al. 2022). These aspects, together with the lack of effective control measures,

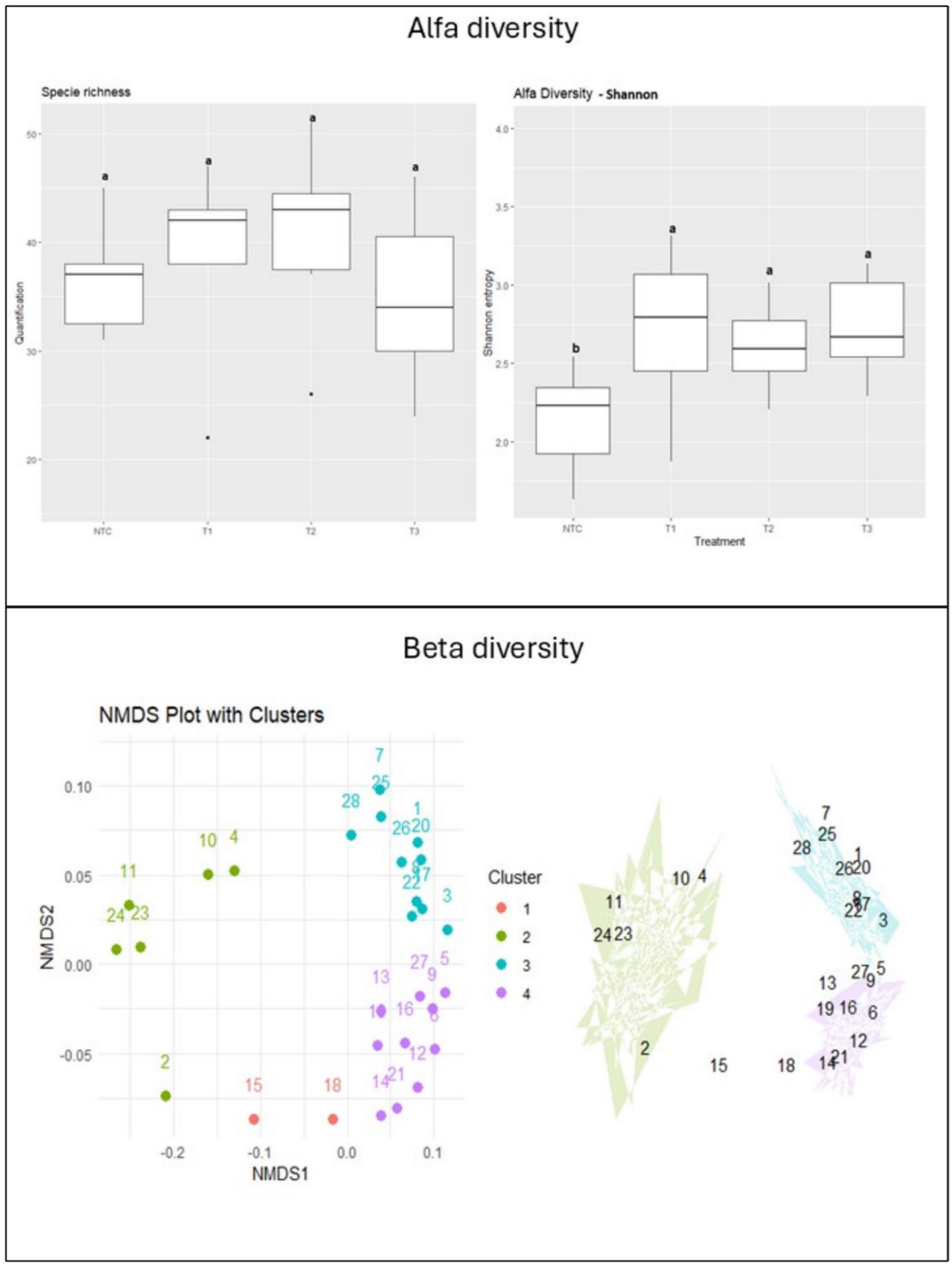

Fig. 3 Alfa and Beta analysis of NTC, T1, T2 and T3. Alfa diversity: Specie richness and Shannon of NTC, T1, T2 and T3. Statistical analysis was carried out applying the Kruskal–Wallis test ($p < 0.05$). Different letters mean a statistical difference for the p value considered. Beta diversity: statistical analysis was carried out applying the Bray–Curtis test ($p < 0.05$), and plotted with the "meta-MDS" function of the R vegan package. Numbers 1–7 are the replicates of NTC, 8 −14 are the replicates of T1, 15 −21 replicates of T2, and 22–28 replicates of T3. NTC (Non-Treated Control), T1: leaf extract at a concentration of 1.9 g plant^{-1}, T2: leaf extract at 16 g plant.$^{-1}$, T3 (Intercropping)

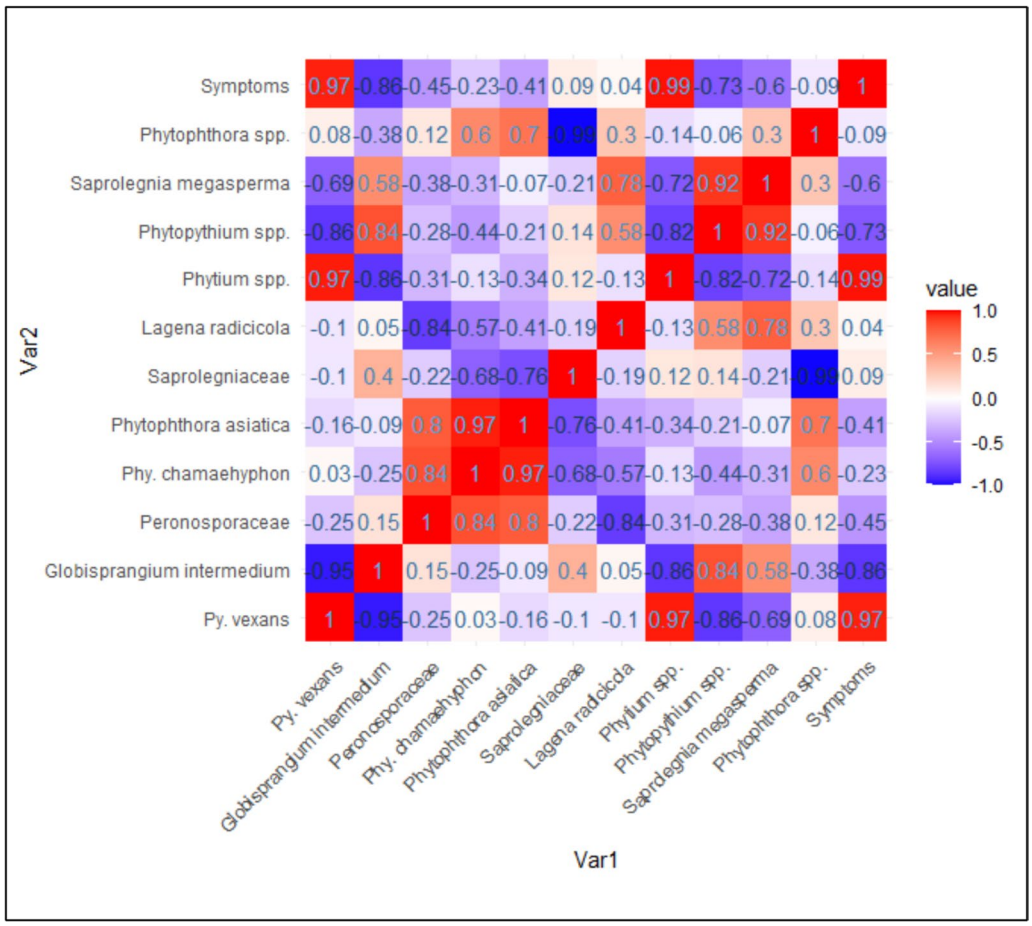

Fig. 4 Correlation heatmap between oomycetes and symptoms. The matrix was plotted after using the "pheatmap package". Colour scale is based on the strength of correlation

led us to investigate the effect of rocket leaf extract (T1 and T2) and rocket-kiwifruit consociation (T3) on KVDS development and Oomycetes population structure within the rhizosphere of *Actinidia chinensis* var. *deliciosa* sensitive genotype. In fact, biofumigation with rockets has already been shown to be effective in controlling soil borne pathogens in many crops (Bellostas et al. 2007; Morales-Rodríguez et al. 2016; Bell and Wagstaff 2019).

In our experimental conditions, the application of rocket leaf extracts notably resulted in the reduction of symptoms' incidence and the promotion of enhanced root system architecture (Fig. 1A, B, C, D, E) (Poulaki et al. 2024), especially when applied at the highest quantity (designed to simulate the application similar to that of a commercially recognized product -T2 (Biofence®). Interestingly, intercropping strategy (T3) also held comparable results with the most effective biofumigation treatment (T2) opening new opportunities for a sustainable, simple and low cost and labour management of KVDS and possibly other soil borne disease (Gullino et al. 2022). This was already demonstrated in other intercropping systems where disease incidence crop can be reduced by 55% (Chadfield et al. 2022).

Furthermore, the results suggest that biofumigation and intercropping had a the effect on the *Oomycota* population structure and diversity (Chen et al. 2023). It is important to note, however, that other potential effects contributing to these results cannot be excluded. For instance, there is the possibility that specific compounds within the extracts induced systemic resistance in the plants or promoted root

growth. Additionally, the treatments might have attracted beneficial microorganisms (e.g., promoting bacteria, mycorrhiza). This is plausible because the incorporation of plant residues and extracts into the soil can act as a carbon source or signal molecules for specific microbial taxa. Certain secondary metabolites from plants, including glucosinolates and phenolic compounds often present in biofumigant species, can selectively stimulate the proliferation of beneficial microbes such as plant growth-promoting rhizobacteria and/or arbuscular mycorrhizal fungi. In this sense, further research is needed to fully understand the mechanisms underlying the observed results and to explore the broader microbiological and physiological impacts of the treatments.

At any rate, concerning the alpha-diversity, as measured by the Shannon Index (Fig. 3), underscored a strong relationship with the diverse treatments applied. The presence of elevated alpha diversity within a microbiome sample holds significant importance in the context of ecological and functional dynamics (Reese and Dunn 2018). In our study, the higher alfa diversity observed in specific treatments (particularly in T2 and T3) correlates with measurable improvements in symptoms incidence reduction (Fig. 4). In our study, the observed differences in alpha diversity specifically reflect the diversity of oomycetes within the rhizosphere. The higher Shannon diversity index in treatments T2 and T3 suggests a shift in community composition, potentially characterized by increased evenness or a redistribution of species dominance (Fig. 3). One possible explanation is that these treatments favoured the presence of hypo-virulent species, thereby reducing the dominance of highly virulent pathogens. This restructuring of the oomycete community aligns with the observed improvements in root health and disease suppression. Notably, biofumigation and intercropping significantly increased the Shannon diversity index, which, despite being the only metric showing statistical differences, corresponded with reduced symptom incidence and enhanced root architecture. These findings emphasize the potential role of microbial diversity, particularly within the oomycete community, in shaping a more resilient rhizosphere. This suggests that a greater variety of coexisting species may not only enhance microbial interactions but also foster functional redundancies that buffer against environmental stressors, including pathogen invasion. In fact, treatments involving biofumigation and intercropping demonstrated their efficacy by significantly increasing Shannon alpha diversity, simultaneously reducing symptom severity and improving root architecture. These outcomes underline the functional link between higher microbial diversity and a healthier rhizosphere capable of suppressing soil-borne pathogens. Although the Shannon index was the only metric to show statistical differences, its alignment with symptomatology provides robust evidence that T2 and T3 treatments are superior in promoting both plant health and microbiome diversity.

Taking into account the taxonomical composition (Fig. 2), two particularly prominent oomycetes garnered attention, contributing to a substantial portion of the total hits across each sample (59–93% of taxon). The two prominent OTUs were identified as *Pp. vexans* and *G. intermedium*. While *Pp. vexans* prevailed across all treatments, its abundance decreased from the NTC to treatments T1, T2, and T3. Conversely, the prevalence of *G. intermedium* increases in T1, T2, and T3 compared to the NTC group, with the highest prevalence observed in T3.This is in accordance with previous study regarding KVDS. In fact, Türkkan and co-workers (Türkkan et al. 2022) found out a higher presence of *Pythium*-like species in *Actinidia* diseased plants, but they determined that the pathogenicity of *G. intermedium* was lower than that of *Pp. vexans*, which corroborates the negative correlation we found between this species and the symptomatology (Fig. 4). Furthermore, it was reported the strong capability of *Pp. vexans* in generating KVDS symptoms has been reported (Prencipe et al. 2020). It is also evident that there is a significant correlation between specific oomycetes in the plant rhizosphere and the symptoms observed. These findings highlight the importance of understanding the role of these specific oomycetes in plant health and symptom development, which could have implications for disease management and crop protection strategies. Nevertheless, since nearly all oomycetes classified within the genera *Pythium*, *Phytopythium*, and *Globisporangium* spp. are plant pathogens, it is crucial to understand their competitive interactions during plant colonization. For instance, *G. intermedium* has demonstrated an ability to establish dominance with increasing applications of rocket extracts (and intercropping). Due to its relatively lower

pathogenicity, fewer symptoms were observed compared to scenarios where *Pp. vexans* dominates—a species that is significantly more pathogenic than *G. intermedium* (Prencipe et al. 2020; Türkkan et al. 2022). Furthermore, in this dynamic, if *Pp. vexans* increases—possibly due to its greater competitiveness or better adaptation to specific conditions—*Globisporangium* spp. might decrease as it experiences competition (Gómez-Pérez and Kemen 2021).

Further taxonomic classification confirmed the presence of various oomycetes, predominantly *Phytophthora asiatica/sojae* and, generally, *Phytophthora* spp. (Tacconi et al. 2015; Savian et al. 2022; Mian et al. 2023a). *Phytophthora* species are well-known plant pathogens, with *P. asiatica/sojae* previously associated with root and collar rot in multiple hosts (Ghaderi and Karami 2024), though its specific impact on kiwifruit remains underexplored. Interestingly, *Phytopythium chamaehyphon* and certain *Pythium* species were notably absent in treatments T2 and T3. While *Pythium* spp. includes both pathogenic and saprotrophic species, some members are known to cause damping-off and root rot in kiwifruit. Lastly, in a recent study conducted in a comparable environment, Cardacino and collaborators (2025) corroborated our findings, identifying *Phytophthora*, *Pythium*, and *Globisporangium* as the most abundant oomycetes in their research. *Globisporangium* species, formerly classified within *Pythium*, include both plant-pathogenic and non-pathogenic taxa, some of which are known to infect kiwifruit roots. These findings reinforce the relevance of oomycete diversity in shaping rhizosphere health and disease outcomes in kiwifruit cultivation (Cardacino et al. 2025). At any rate, for our research, future step has to elucidate each community (fungi and bacteria) having a role, positive or negative, in KVDS and alike syndromes, as well as trying strategies to preserve crop yield and quality (Mian et al. 2025).

Conclusions and remarks

Our research highlights the potential of rocket (*Eruca vesicaria* subsp. *sativa*) leaf extracts and intercropping to mitigate KVDS symptoms' incidence. The adoption of these green alternatives offers a promising solution in light of the challenges associated with chemical control of soil-borne diseases and the EU legislation that aims to significantly reduce the use of chemical pesticides and their risks. This is particularly critical during the establishment of new orchards or in orchards facing early-stage infestations. Moreover, our findings underscore the complexity of interactions within the rhizosphere, revealing how alterations in microbial communities, driven by biocontrol strategies, can influence plant health. Given the current lack of effective management strategies for KVDS, rocket-based biocontrol systems emerge as a highly promising approach. Further research is warranted to elucidate the specific microbial and physiological mechanisms driving the observed protective effects, such as the recruitment of beneficial microorganisms (fungi and/or bacteria) or the induction of systemic resistance. Moreover, field-scale trials are needed to assess the consistency, scalability, and long-term impact of these strategies under real agricultural conditions, including different soil types, climatic areas, and cropping systems.

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Declarations

Conflicts of interest The authors declare no conflict of interest.

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