ROOT ROT PATHOGENS SHAPE THE AVOCADO RHIZOSPHERE MICROBIOME – IMPACTING TREE AND SOIL HEALTH

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INTRODUCTION

Dematophora necatrix (previously referred to as *Rosellinia necatrix*) is the causal agent of white root rot (WRR), an economically important disease of avocado in South Africa, Spain and Israel (1). It has recently been demonstrated that *D. necatrix* produces antimicrobial compounds (2), which can kill off beneficial microbes in the host rhizosphere - the soil layer just adjacent to plant roots. The rhizosphere microbiome contains various bacteria, fungi, and oomycetes which may offer protection against pathogens, assist plants in coping with abiotic and biotic stresses and play a crucial role in overall plant health (3). We hypothesised that *D. necatrix* infection will change the microbial composition of the avocado rhizosphere soil, with beneficial bacteria and fungi being more enriched in the soil of healthy trees. This study aimed to:

- assess and compare the microbial communities found in the rhizosphere of healthy and WRR-infected avocado trees in South African orchards;
- assess the impact of *D. necatrix* infection on the soil nutrient composition which may affect tree health; and
- isolate microbes from the avocado rhizosphere soil with antagonistic potential against *D. necatrix* which could potentially be used as novel biocontrol agents.

MATERIALS AND METHODS

A survey of putative WRR-diseased and -healthy avocado trees was carried out in two separate orchards in Tzaneen, Limpopo, South Africa. For Orchard A, trees were 30-year old 'Hass' grafted onto Duke7, which were planted 5-7 m apart. For Orchard B, trees were 10-year old 'Carmen-Hass' grafted onto Dusa®, which were planted 3-4 m apart. The trees were assessed using a visual scoring system for above-ground stress symptoms, including chlorotic leaves, defoliation, poor fruit set, dieback of branches, and the presence or absence of white mycelial mass around the tree trunk. Putative WRR-infected trees (symptomatic) were found in spots across the orchards, whereas asymptomatic trees were positioned near symptomatic trees either within or across rows. Avocado trees were categorised as WRR symptomatic (WRR-S), WRR asymptomatic (WRR-AS), and healthy/non-infected (WRR-N) using the *D. necatrix* species-specific qPCR assay (4). All trees tested positive for the presence of *Phytophthora cinnamomi*.

Rhizosphere soil samples were collected from 60 trees in total (30 trees per orchard consisting of 10 WRR-S, 10 WRR-AS, and 10 WRR-N) and DNA extracted for metabarcoding. The microbial communities within the avocado rhizosphere samples were characterised by PacBio sequencing of the 16S rRNA (bacterial) and ITS (fungal) gene regions. The physicochemical properties of the rhizosphere soil were also analysed. Orchard A has red loamy/clayey soil and Orchard B a reddish-brown clayey/loamy soil. Both orchards are in the mid-slope to foot-slope range, which influences the distribution of water and soil nutrients. Rhizosphere soil samples obtained from WRR-N trees in Orchard B were used to isolate culturable fungi and bacteria. Using a dual-culture assay, 20 fungal and 22 bacterial isolates were screened for their ability to inhibit the growth of *D. necatrix* isolate ARP-2017-Rn2, a South African isolate previously shown to be highly virulent on avocado[5].

RESULTS AND DISCUSSION

Enrichment analysis demonstrated that the rhizosphere of uninfected trees had significantly more beneficial microbes as compared to either asymptomatic or symptomatic *D. necatrix* infected trees.

Contrary to our hypothesis, *D. necatrix* did not significantly alter the microbiome in the two avocado orchards studied. This is consistent with previous findings for *P. cinnamomi*, which similarly also did not affect species richness or diversity in the avocado rhizosphere[6]. Bacterial (Fig. 1) and fungal (Fig. 2)

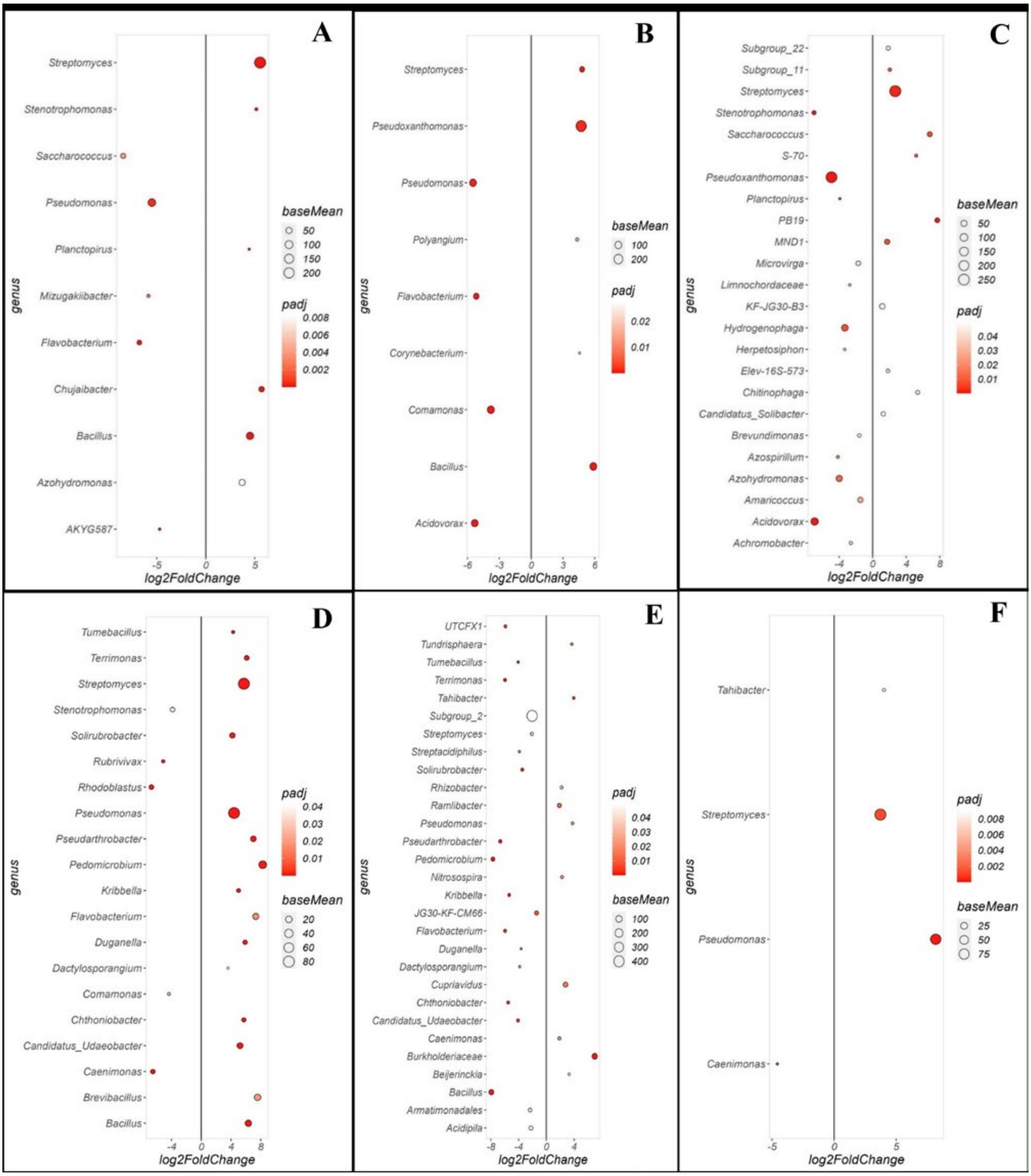

Figure 1: Comparison of specific bacterial genera that were either enriched or depleted between different groups of soil samples collected in the rhizosphere in Orchards A and B. Comparison of rhizosphere soil samples between (A) WRR-S vs WRR-N, (B) WRR-S vs WRR-AS, (C) WRR-AS vs WRR-N from Orchard A and (D) WRR-S vs WRR-N, (E) WRR-S vs WRR-AS, (F) WRR-AS vs WRR-N from Orchard B. The group mentioned first was used as a reference when determining statistical differences; in the figure it will always have a positive log2fold change and thus be enriched in the reference group. The fold-change is shown on the X-axis and the genera are listed on the Y-axis. Each coloured dot represents a genus population that is significantly more or less abundant ($p \leq 0.05$). The size of the circle shows the average abundance, and the colour intensity of the circle shows *padj* value (significance level). A larger circle indicates higher relative abundance in each population. This figure was taken from Magagula *et al*., 2025 (17)

genera in both orchards exhibited distinct enrichment patterns when comparing communities between the WRR-S, WRR-AS, and WRR-N trees. *Fusarium* spp. were prevalent in both orchards, particularly in WRR-S samples. *Ilyonectria* was also present in both avocado orchards; which is concerning given that this genus is linked to root rot, stem lesions, damping off, branch and crown cankers, fruit disease, reduced yield, and increased susceptibility to other pathogens[7]. The co-occurrence of *D. necatrix* and *P. cinnamomi*, *Ilyonectria*, and opportunistic pathogens like *Fusarium*, suggests a disrupted microbial community structure that impacts avocado tree health. Known beneficial microbial genera *Trichoderma*, *Pseudomo-*

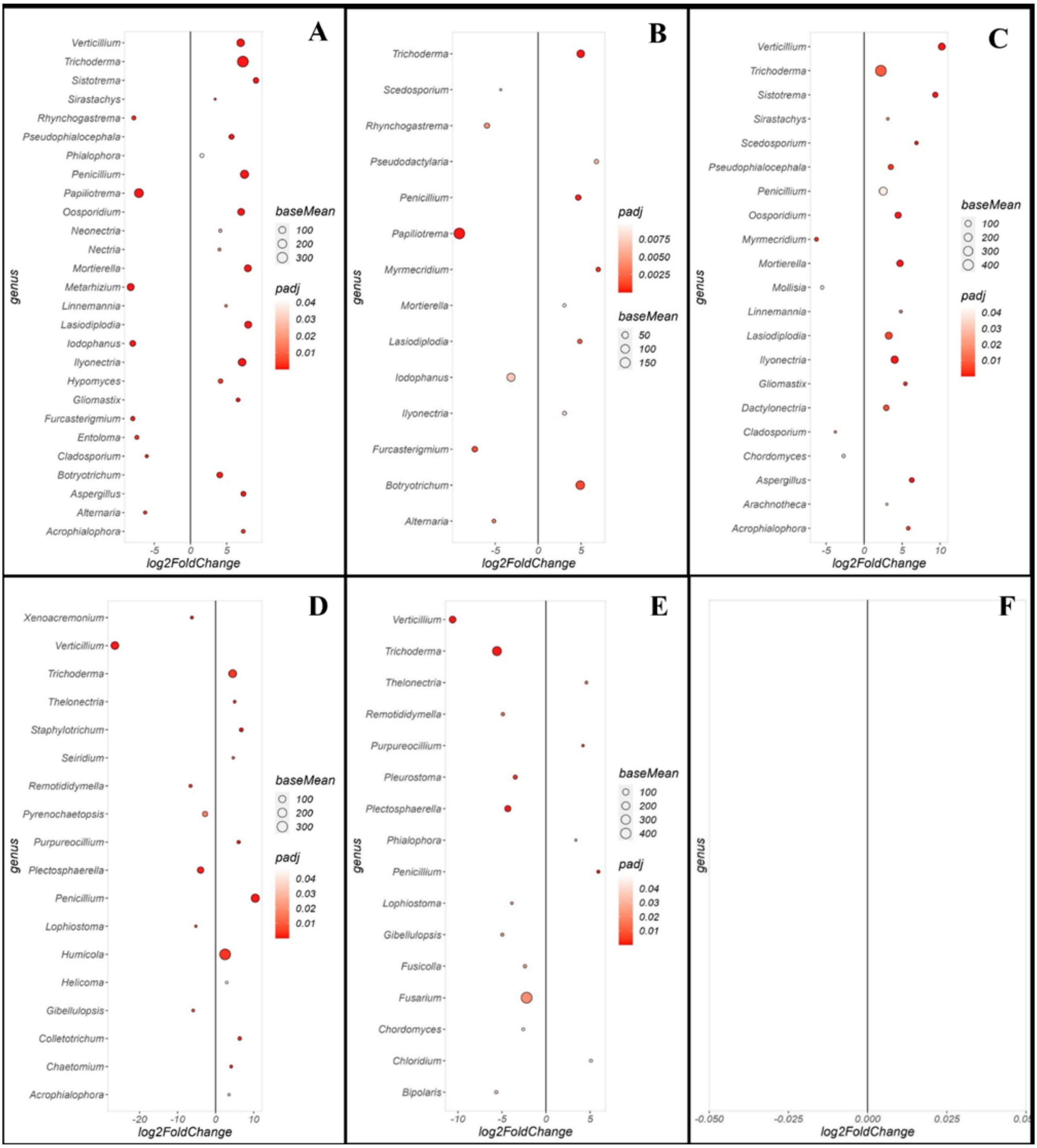

Figure 2: Comparison of specific fungal genera that were either enriched or depleted between different groups of soil samples collected in the rhizosphere at Orchards A and B. Comparison of rhizosphere soil samples between (A) WRR-S vs WRR-N, (B) WRR-S vs WRR-AS, (C) WRR-AS vs WRR-N from Orchard A and (D) WRR-S vs WRR-N, (E) WRR-S vs WRR-AS, (F) WRR-AS vs WRR-N from Orchard B. The group mentioned first was used as a reference when determining statistical significant differences; in the figure it will always have a positive log2fold change and thus be enriched in the reference group. The fold-change is shown on the X-axis and the genera are listed on the Y-axis. Each coloured dot represents a genus population that is significantly more or less abundant ($p \leq 0.05$). The size of the circle shows the average abundance, and the colour intensity of the circle shows *padj* value (significance level). A larger circle indicates higher relative abundance in each population. This figure was taken from Magagula *et al*., 2025 (17)

nas, *Bacillus*, and *Streptomyces*, were enriched in non-infected trees. The presence of *Streptomyces* in WRR-N may offer disease-suppressive potential, as a reduction in *Streptomyces* sp. has been linked to the lack of disease suppression in strawberry plants[8]. *Bacillus* sp. are known to suppress Phytophthora root rot in avocado[9]. As expected, *Trichoderma* was abundant and enriched in WRR-N soil in the two orchards. These findings highlights the importance of beneficial fungi in maintaining soil health and suppressing disease.

Healthy soils promotes lower incidence of white root rot disease

The study demonstrated a strong association between fungal and bacterial microbial community structure and rhizosphere soil properties in WRR-N samples compared to WRR-S and WRR-AS samples (Figs. 3 and 4). Soil pH and Fe were strongly related with several bacteria and fungi, emphasising the key role pH plays in shaping microbial diversity and driving processes like nitrification and denitrification[10]. This distinction may be attributed to enhanced microbial activity, which facilitates nutrient cycling and organic matter decomposition, ultimately improving soil fertility and plant growth[11]. The observed association highlight the intricate relationship between soil physicochemical properties and microbial ecology, reinforcing the importance of maintaining healthy soils for sustainable productivity. WRR-N soils clustered on the opposite side to WRR-AS and WRR-S soils of the Principal Component Analysis plots, together with several soil properties; this may indicate patterns likely to support a decreased incidence of WRR.

Rhizosphere soil from healthy avocado trees contains microbes which directly antagonises *D. necatrix*

Dual-culture assays showed that bacterial genera such as *Pseudomonas*, *Bacillus*, and *Streptomyces* effectively inhibited mycelial growth of *D. necatrix*, consistent with their role in disease suppression and plant growth promotion (Fig. 5). *Pseudomonas* species have previously been shown to exhibit antifungal activity against *D. necatrix* by competing for space and root exudate nutrients[12]. Additionally in support of our findings, *B. subtilis* strains isolated from healthy avocado rhizospheres have demonstrated antifungal activity against *D. necatrix* in both *in vitro* and *in vivo* studies, directly attacking soil patho-

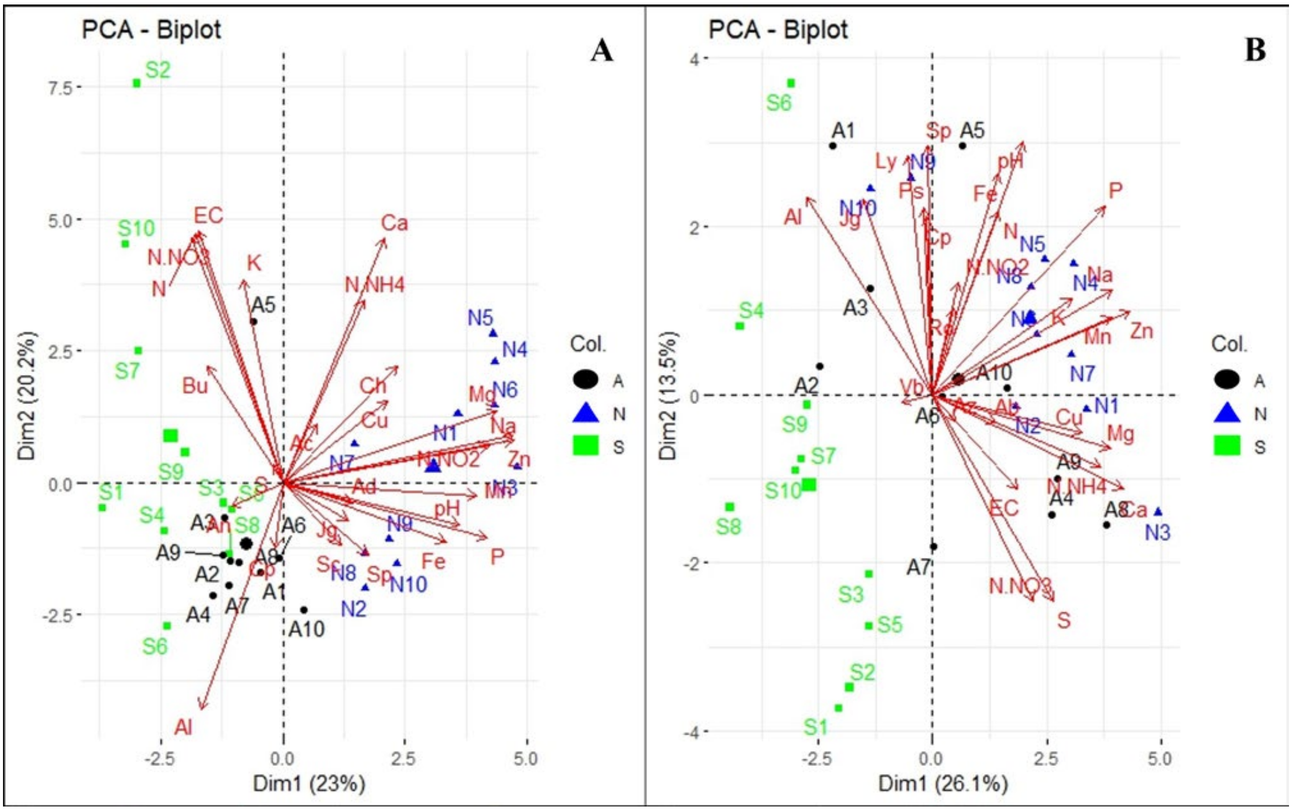

Figure 3: Principal Component Analysis (PCA) of abundant bacterial genera and soil physicochemical properties. (A) Orchard A and (B) Orchard B. The tree categories: WRR-S (S in green), WRR-AS (A in black) and WRR-N (N in blue). The top 0.6% bacterial genera: *Acidibacter* (Ac), *Acidothermus* (Ad), *Acinetobacter* (An), *Burkholderia-Caballeronia-Paraburkholderia* (Bu), *Chujaibacter* (Ch), *Cupriavidus* (Cp), *JG30-KF-AS9* (Jg), *SC-I-84* (Sc), *Sphingomonas* (Sp), *11-24* (Ab), *Azohydromonas* (Az), *Lysobacter* (Ly), *Pseudomonas* (Ps), *Rokubacteriales* (Ro), and *Vicinamibacteraceae* (Vb). Soil physicochemical properties: exchangeable cations (EC), potential of hydrogen (pH), Nitrogen dioxide ($N\text{-}NO_2$), Nitrate Nitrogen ($N\text{-}NO_3$), Ammonium nitrate ($N\text{-}NH_4$), Nitrogen (N), Potassium (K), Phosphorus (P), Magnesium (Mg), Calcium (Ca), Sodium (Na), Sulphur (S), Aluminium (Al), Copper (Cu), Manganese (Mn), Iron (Fe), Zinc (Zn). The arrow lengths in the plot represent the association strength between the soil physicochemical properties and the bacterial genera (the longer the arrows, the stronger the association). The perpendicular distance between microbes and soil physicochemical properties axes reflects their correlation (the smaller the distance, the stronger the association).

This figure was taken from Magagula *et al*., 2025 (17)

gens and stimulating plant defences[13]. Fungal genera such as *Trichoderma*, *Penicillium*, and *Mortierella* also inhibited *D. necatrix* in dual-culture assays (Fig. 6). *Trichoderma* is well known for promoting plant growth and suppressing soil-borne diseases, with previous studies demonstrating its efficacy against *D. necatrix in vitro* and in greenhouse trials (14, 15, 16). Our findings suggests that there is intense competition within the avocado rhizosphere, where *D. necatrix* must contend with other microbes (both beneficial and pathogenic) to establish infection.

CONCLUSIONS

Metabarcoding and enrichment analysis data revealed that *D. necatrix* altered the relative abundance of microbes in the rhizosphere, but did not affect overall microbial diversity. *Fusarium* was more predominant in the soil of WRR symptomatic trees, while beneficial microbes such as *Trichoderma* were enriched in the soil of WRR non-infected trees. Soil physicochemical analysis showed that asymptomatic trees were associated with healthier soil conditions despite infection. Culturable microbial isolates from genera such as *Pseudomonas*, *Bacillus*, *Trichoderma*, and *Penicillium* demonstrated significant inhibitory effects against *D. necatrix in vitro*, highlighting their potential for biocontrol. Future studies should prioritise greenhouse and field trials with promising fungal and bacterial isolates to develop effective biocontrol strategies for avocado orchards.

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REFERENCES

1. PLIEGO, C., LÓPEZ-HERRERA, C., RAMOS, C. & CAZORLA, F.M. 2012. Developing tools to unravel the biological secrets of *Rosellinia necatrix*, an emergent threat to woody crops. *Molecular Plant Pathology*, 13(3): 226-39.
2. CHAVARRO-CARRERO, E.A., SNELDERS, N.C., TORRES, D.E., KRAEGE, A., LÓPEZ-MORAL, A. *et al*. 2024. The soil-borne white root rot pathogen *Rosellinia necatrix* expresses antimicrobial proteins during host colonization. PLOS Pathogens

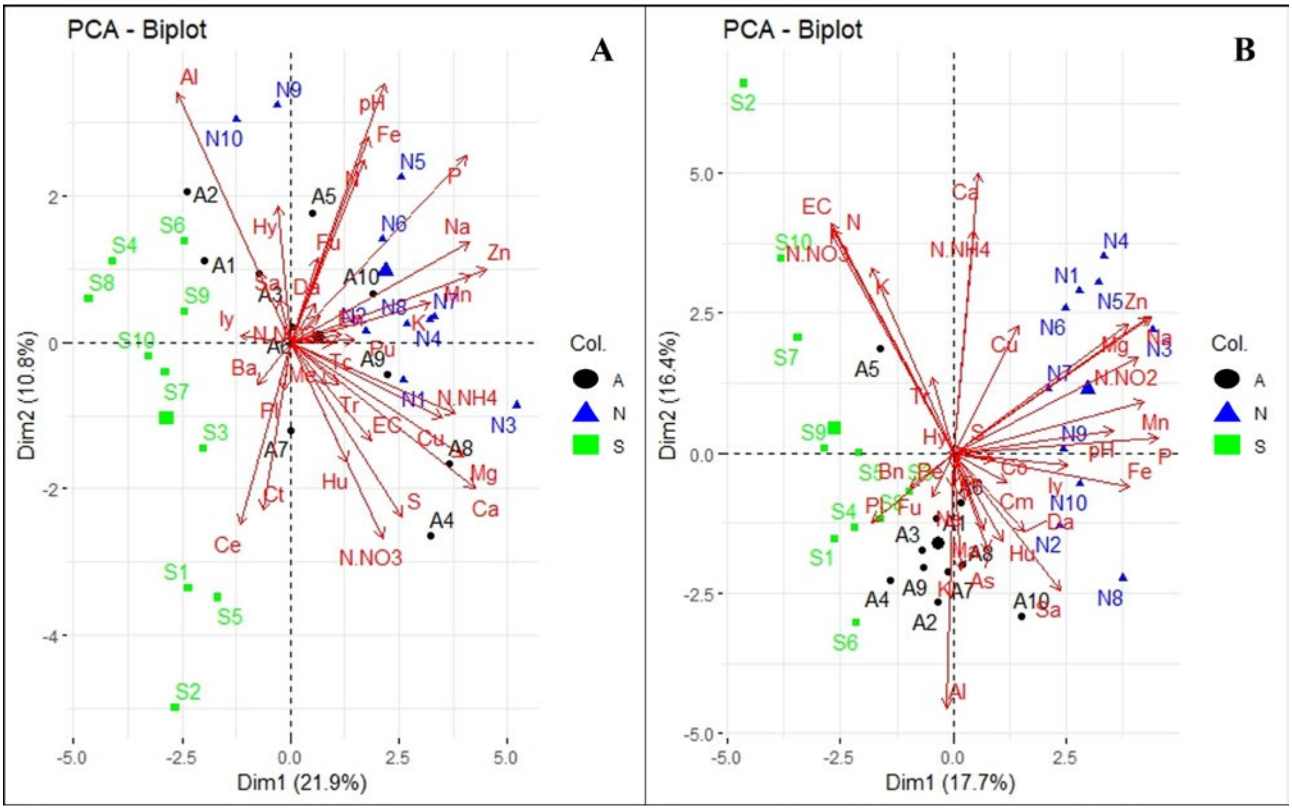

Figure 4: Principal Component Analysis (PCA) of abundant fungal genera and soil physicochemical properties. (A) Orchard A and (B) Orchard B. The tree categories: WRR-S (S in green), WRR-AS (A in black) and WRR-N (N in blue). The top 0.6% fungal genera: *Bartalinia* (Ba), *Cephalotrichum* (Ct), *Cercophora* (Ce), *Dactylonectria* (Da), *Enterocarpus* (En), *Fusarium* (Fu), *Humicola* (Hu), *Hyaloscypha* (Hy), *Ilyonectria* (Iy), *Metarhizium* (Me), *Plectosphaerella* (PI), *Pseudallescheria* (Pu), *Saitozyma* (Sa), *Trichocladium* (Tc), *Trichoderma* (Tr), *Aspergillus* (As), *Barnettozyma* (Bn), *Chloridium* (Cm), *Cosmospora* (Co), *Kendrickiella* (KI), *Mariannaea* (Ma), *Neopyrenochaeta* (Ne), *Paracremonium* (Pr), and *Penicillium* (Pe). Soil physicochemical properties: exchangeable cations (EC), potential of hydrogen (pH), Nitrogen dioxide (N-NO_2), Nitrate Nitrogen (N-NO_3), Ammonium nitrate (N-NH_4), Nitrogen (N), Potassium (K), Phosphorus (P), Magnesium (Mg), Calcium (Ca), Sodium (Na), Sulphur (S), Aluminium (Al), Copper (Cu), Manganese (Mn), Iron (Fe), Zinc (Zn). The arrow lengths in the plot represent the association strength between the soil physicochemical properties and the fungal genera (the longer the arrows, the stronger the association). The perpendicular distance between microbes and soil physicochemical properties axes reflects their association (the smaller the distance, the stronger the association).
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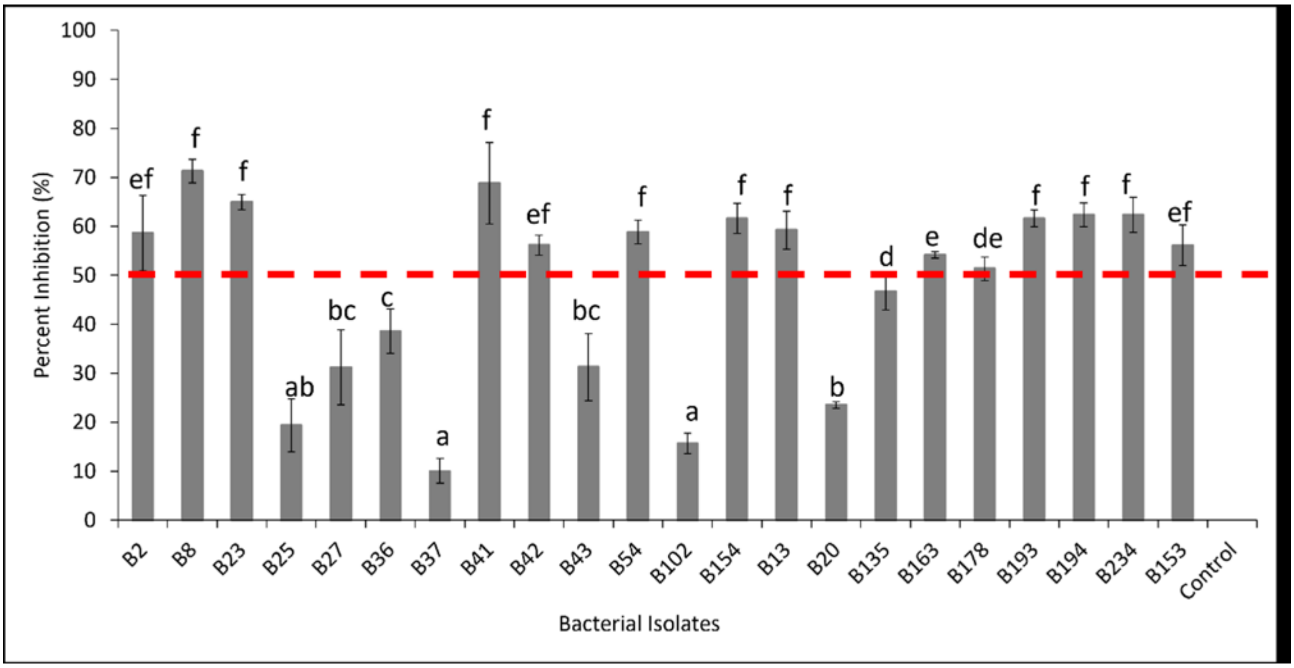

Figure 5: Percent inhibition (%) of selected bacterial isolates showing antagonistic activity against *Dematophora necatrix*. B102 (*Serratia odorifera*), B13 (*Stremptomyces* sp.), B135 (*Pseudomonas baetica*), B153 (*Pseudomonas koreensis*), B154 (*Pseudomonas koreensis*), B163 (*Pseudomonas koreensis*), B178 (*Pseudomonas fluorescens*), B193 (*Psedomonas koreensis*), B194 (*Pseudomonas fluorescens*), B2 (*Bacillus subtilis*), B20 (*Serratia odorifera*), B23 (*Bacillus subtilis*), B234 (*Pseudomonas* sp.), B25 (*Microbactrium testaceum*), B27 (*Microbacterium* sp.), B36 (*Microbactrium testaceum*), B37 (*Microbactrium testaceum*), B41 (*Bacillus tequilensis*), B42 (*Bacillus subtilis*), B43 (*Microbacterium trichothecenolyticum*), B54 (*Pseudomonas* sp.), B8 (*Bacillus subtilis*), and Control (*Dematophora necatrix* only). The mean inhibition rates indicated by the same letter are not significantly different according to Tukey's test ($p \leq 0.05$).
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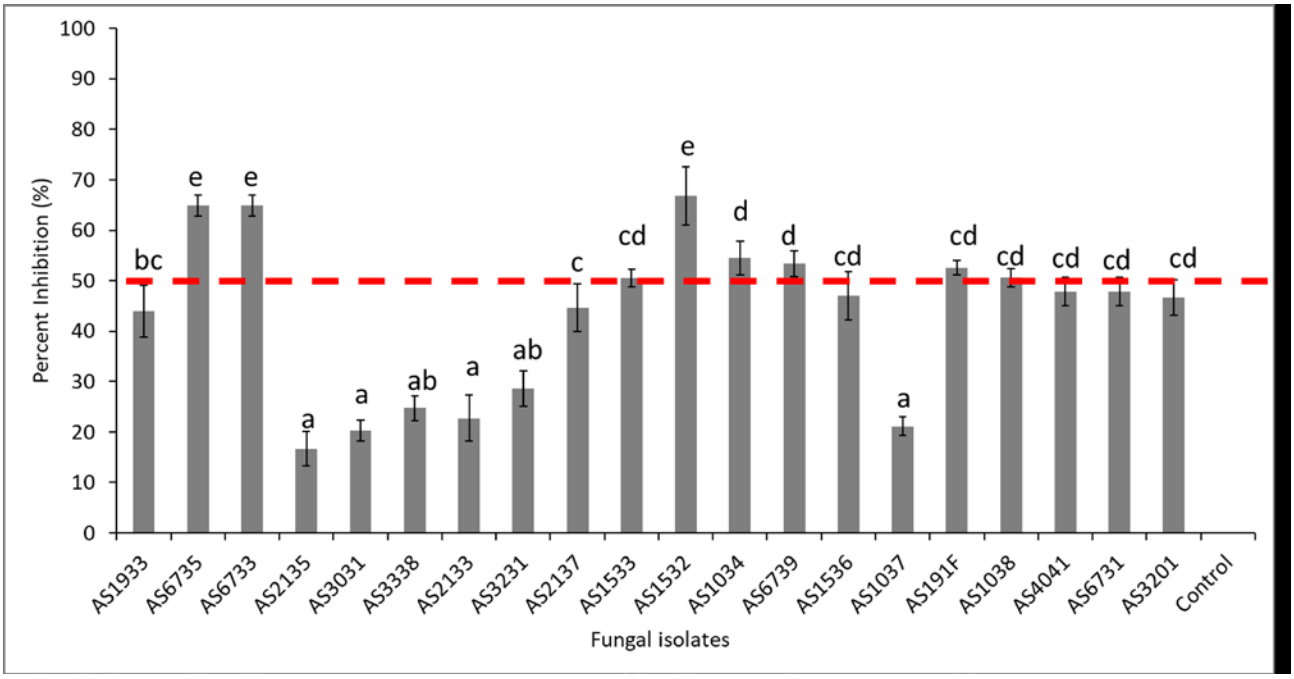

Figure 6: Percent inhibition (%) of selected fungal isolates showing antagonistic activity against *Dematophora necatrix*. AS1034 (*Mortierella alpina*), AS1037 (*Penicillium chrysogenum*), AS1038 (*Penicillium citreonigrum*), AS1532 (*Fusarium solani*), AS1533 (*Fusarium solani*), AS1536 (*Aspergillus flavus*), AS191F (*Linnemannia elongate*), AS1933 (*Cladosporium halotolerans*), AS2133 (*Dactylonectria novozelandica*), AS2135 (*Neopyrenochaeta acicola*), AS2137 (*Penicillium expansum*), AS3031 (*Subramaniula cuniculorum*), AS3201 (*Geomyces asperulatus*), AS3231 (*Purpureocillium lilacinum*), AS3338 (*Gloeotinia temulenta*), AS4041 (*Penicillium buchwaldii*), AS6731 (*Talaromyces variabilis*), AS6733 (*Fusarium solani*), AS6735 (*Trichoderma longibrachiatum*), AS6739 (*Podila minutissima*), and Control (*Dematophora necatrix* only). The mean inhibition rates indicated by the same letter are not significantly different according to Tukey's test ($p \leq 0.05$).
This figure was taken from Magagula *et al*., 2025 (17)

20(1): e1011866. https://doi.org/10.1371/journal.ppat.1011866
3. BERENDSEN, R.L., PIETERSE, C.M. & BAKKER, P.A. 2012. The rhizosphere microbiome and plant health. *Trends in Plant Science*, 17(8): 478-86.
4. SCHENA, L., NIGRO, F. & IPPOLITO, A. 2002. Identification and detection of *Rosellinia necatrix* by conventional and real-time Scorpion-PCR. *European Journal of Plant Pathology*, 108: 355-366.
5. VAN DEN BERG, N., HARTLEY, J., ENGELBRECHT, J., MUFAMADI, Z., VAN ROOYEN, Z., MAVUSO, Z. *et al*. 2018. First report of white root rot caused by *Rosellinia necatrix* on *Persea americana* in South Africa. *Plant Disease*, 102: 1850. doi: 10.1094/PDIS-10-17-1637-PDN.
6. SOLÍS-GARCÍA, I.A., CEBALLOS-LUNA, O., CORTAZAR-MURILLO, E.M., DESGARENNES, D., GARAY-SERRANO, E., PATIÑO-CONDE, V. *et al*. 2021. Phytophthora root rot modifies the composition of the avocado rhizosphere microbiome and increases the abundance of opportunistic fungal pathogens. *Frontiers in Microbiology*, 11: 3484. doi: 10.3389/fmicb.2020.574110.
7. CHAVERRI, P., SALGADO, C., HIROOKA, Y., ROSSMAN, A. & SAMUELS, G. 2011. Delimitation of *Neonectria* and *Cylindrocarpon* (Nectriaceae, Hypocreales, Ascomycota) and related genera with *Cylindrocarpon*-like anamorphs. *Studies in Mycology*, 68: 57-78. doi: 10.3114/sim.2011.68.03.
8. KIM, D.R., CHO, G., JEON, C.W., WELLER, D.M., THOMASHOW, L.S., PAULITZ, T.C. *et al*. 2019. A mutualistic interaction between *Streptomyces* bacteria, strawberry plants and pollinating bees. *Nature Communication*, 10: 4802. doi: 10.1038/s41467-019-12785-3.
9. GUEVARA-AVENDAÑO, E., CARRILLO, J.D., NDINGA-MUNIANIA, C., MORENO, K., MÉNDEZ-BRAVO, A., GUERRERO-ANALCO, J.A. *et al*. 2018. Antifungal activity of avocado rhizobacteria against *Fusarium euwallaceae* and *Graphium* spp., associated with *Euwallacea* spp. nr. *fornicatus*, and *Phytophthora cinnamomi*. *Antonie van Leeuwenhoek*, 111: 563-572. doi: 10.1007/s10482-017-0977-5.
10. HAYATSU, M., KATSUYAMA, C. & TAGO, K. 2021. Overview of recent researches on nitrifying microorganisms in soil. *Soil Science and Plant Nutrition*, 67: 619-632. doi: 10.1080/00380768.2021.1981119.
11. CONDRON, L., STARK, C., O'CALLAGHAN, M., CLINTON, P. & HUANG, Z. 2010. The role of microbial communities in the formation and decomposition of soil organic matter. *Soil Microbiology and Sustainable Crop Production*, 81-118. doi: 10.1007/978-90-481-9479-7_4.
12. CALDERÓN, C.E., DE VICENTE, A. & CAZORLA, F.M. 2014. Role of 2-hexyl, 5-propyl resorcinol production by *Pseudomonas chlororaphis* PCL1606 in the multitrophic interactions in the avocado rhizosphere during the biocontrol process. *FEMS Microbiology Ecology*, 89: 20-31. doi: 10.1111/1574-6941.12319.
13. CAZORLA, F., ROMERO, D., PÉREZ-GARCÍA, A., LUGTENBERG, B., VICENTE, A.D. & BLOEMBERG, G. 2007. Isolation and characterization of antagonistic *Bacillus subtilis* strains from the avocado rhizoplane displaying biocontrol activity. *Journal of Applied Microbiology*, 103: 1950-1959.doi: 10.1111/j.1365-2672.2007.03433.
14. FREEMAN, S., SZTEJNBERG, A. & CHET, I. 1986. Evaluation of *Trichoderma* as a biocontrol agent for *Rosellinia necatrix. Plant and Soil*, 94: 163-170.
15. MAGAGULA, P., TAYLOR, N., SWART, V. & VAN DEN BERG, N. 2021. Efficacy of potential control agents against *Rosellinia necatrix* and their physiological impact on avocado. *Plant Disease*, 105: 3385-3396.
16. RUANO-ROSA, D., DEL MORAL-NAVARRETE, L. & LÓPEZ-HERRERA, C. 2010. Selection of *Trichoderma* spp. isolates antagonistic to *Rosellinia necatrix*. *Spanish Journal of Agricultural Research*, 8: 1084-1097. doi: 10.5424/sjar/2010084-1403.
17. MAGAGULA, P., SWART, V., FOURIE, A., VERMEULEN, A., NELSON, J.H., VAN ROOYEN, Z. & VAN DEN BERG, N. 2025. Avocado rhizosphere community profiling: white root rot and its impact on microbial composition. *Frontiers in Microbiology*, 16:1583797. doi: 10.3389/fmicb.2025.1583797