

# The composition and structure of bacterial and fungal communities in kiwifruit are influenced by photoselective nets

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## ABSTRACT

Photoselective nets in agriculture are typically designed to modify the light spectrum, intensity, and microclimate around crops, influencing plant growth, productivity, and quality. However, knowledge regarding their impact on the microbiota of plants and fruits remains limited. This study assessed the impact of pearl, grey, and yellow photoselective nets on the microbial communities present on kiwifruit surfaces using amplicon high-throughput sequencing of ITS and 16S metagenomic DNA. Kiwifruit pathogens associated with postharvest rot, such as *Alternaria*, *Didymella*, and *Cladosporium*, were significantly more prevalent on kiwis grown without nets. Additionally, different net types influenced microbial diversity, richness, and network structure. Pearl nets promoted bacterial richness and fungal diversity, while yellow nets enhanced overall diversity and resilience in both microbial communities. Grey nets resulted in evenness in fungal communities but led to less robust bacterial networks. Kiwifruit yield increased under photoselective nets compared to outside. At harvest, fruit dry matter, firmness, pH, total soluble solids, and titratable acidity were similar across treatments. However, fruits under yellow and grey nets showed significantly lower firmness compared to other treatments. Understanding these effects may contribute to optimizing fruit production and shelf-life management.

## 1. Introduction

Photoselective nets filter specific wavelengths of sunlight being an agricultural practice designed to modify the light environment around crops, by altering the light spectrum that reaches the fruit or other specific crop. This modification can affect fruit development and quality, influencing attributes such as color by affecting pigment synthesis, texture and firmness, reducing sunburn and heat stress, avoiding pests and diseases and overall productivity (Basile et al., 2012; Legarrea et al., 2012; Manja and Aoun, 2019; Snelgar et al., 1991; Vuković et al., 2022). Experimental evidence shows that photoselective nets may influence fruit size, sweetness, and shelf stability. Experimental data suggests the nets may also influence the bacterial disease caused by *Pseudomonas syringae* pv. *actinidiae* (Psa), the most severe disease in kiwifruit

production (Moura et al., 2022). Fresh fruits harbor a diverse array of microorganisms crucial to their health (Kuruppu et al., 2024). However, our understanding of the dynamics, composition, and functions of fruit microbiota remains limited. Various factors, including environmental conditions, microbial contamination, and postharvest treatments, influence microbial community development and, hence, fruit shelf life (Kuruppu et al., 2024; Kusumaningrum Dewi et al., 2015). Fungal pathogens from several genera can cause fruit decay and produce harmful mycotoxins (Kuruppu et al., 2024). Many factors may influence fruit shelf-life and storage time, and one of them is certainly the microbiota associated with fruit tissues (Dai et al., 2022; Gao et al., 2023). These microbial communities, including bacteria, yeasts, and molds, interact with the fruit in ways that can enhance or degrade its quality. Beneficial microorganisms can improve fruit quality by

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promoting the synthesis of bioactive compounds, defending against pathogens (Di Francesco et al., 2018; Gao et al., 2021; Zhao et al., 2023). Conversely, pathogenic microorganisms can cause spoilage, leading to discoloration, off-flavors, and textural changes, resulting in significant postharvest losses (Gao et al., 2021). Kiwifruit is no exception, and this, together with the fact that photosensitive nets influence the incidence of severe diseases like Psa, as already mentioned, makes this fruit a suitable model for studying the influence of photosensitive nets on the fruit microbiome.

Postharvest treatments, including washing, chemical treatments, and biocontrol applications, are crucial for reducing the microbial load and extending storage duration. Employing techniques such as microbial antagonists or biocontrol agents to manage surface microbiota can prolong the shelf life of fruit by inhibiting spoilage organisms and pathogens (Dukare et al., 2019). These treatments have discernible impacts on the composition of the epiphytic community, as evidenced by the application of *Wickerhamomyces anomalus*, which significantly diminished decay incidence caused by pathogens such as *Penicillium expansum* and *Botrytis cinerea*, thereby decelerating the deterioration of kiwifruit quality (Zhao et al., 2023) or application of the yeast *Aureobasidium pullulans* that was able to significantly reduce the incidence of deterioration of kiwifruit previously inoculated with *B. cinerea* (Di Francesco et al., 2018). Understand the composition and structure of these microbial communities is therefore critical, as epiphytic and endophytic microorganisms, which colonize fruit tissues, may also offer biocontrol against postharvest pathogens. In the case of kiwifruit, known postharvest pathogens include *P. expansum*, *B. cinerea* (Michailides and Elmer, 2000), *Botryosphaeria dothidea* (Zhou et al., 2015), *Alternaria alternata* (Ruan et al., 2022; Tsahouridou and Thanassouloupoulos, 2000), *Diaporthe* spp., and *Didymella glomerata* (Pan et al., 2018), whose growth can induce kiwifruit rot.

Fruit deterioration is significantly influenced by factors such as host characteristics, environmental conditions, and interactions within the microbiota. Typically, an increase in the pathogen load leads to the development of diseases, disrupting the beneficial microbiota. This process is often triggered or accelerated by factors such as ripening, harvesting, mechanical injuries, transportation methods, and storage conditions (Kuruppu et al., 2024).

In this work, we evaluated the effect of photosensitive nets (pearl, yellow, and grey) on the taxonomic composition of the epiphytic bacteriome and mycobiome of kiwifruit, as well as its microbial diversity and structure of microbial networks. The effect of the nets on Psa disease severity, crop yield and fruit quality, was also evaluated.

## 2. Materials and methods

### 2.1. Field set-up and experimental design

The experiment was conducted in a kiwifruit orchard (*A. deliciosa*, 'Hayward') in Quinta das Picas, Portugal, using photosensitive nets (Iridium®, Agritech, Eboli, Italy) in pearl, yellow, and grey, installed in a gable roof configuration. The experiment followed a randomized block design, comprising four treatments (nets of three different colors and a control without nets) with three replicates each. Detailed information regarding the soil characteristics, orchard setup, irrigation system, and specific experimental design can be found in previously published works (Moura et al., 2022; Ribeiro et al., 2024).

### 2.2. Pollination and biometric measurements

The pollination was performed as previously described (Moura et al., 2022).

Psa disease severity was assessed on 18 leaves per kiwifruit plant from June to October 2022, using a 0–5 severity scale based on the percentage of the leaf surface showing necrosis (Vanneste et al., 2012). A score of 0 indicated 0 % necrosis, while scores from 1 to 5 represented

the following necrosis percentages: 1 = 1–10 %, 2 = 11–25 %, 3 = 26–50 %, 4 = 51–75 %, and 5 = 76–100 %.

Harvest occurred on 17th and 18th November 2022, once the total soluble solids in the orchard reached at least 6.5 °Brix. For each fruit grade, the fresh weight was recorded, with four standard classes defined by EU regulations (EU, 2011): extra  $\geq 90$  g·fruit<sup>-1</sup>; class I,  $70$  g·fruit<sup>-1</sup> < class I <  $89$  g·fruit<sup>-1</sup>; class II,  $65$  g·fruit<sup>-1</sup> < class II <  $69$  g·fruit<sup>-1</sup>; and waste  $\leq 64$  g·fruit<sup>-1</sup>. At harvest for each plant treatment replicate, 10 fruits were evaluated for dry matter (DM, %; according to NP EN 12, 145:1999(IPQ, 1999a)), firmness (Newton, N; measured with a penetrometer with an 8 mm diameter tip), pH (according to NP EN 1132:1996 (IPQ, 1996)), total soluble solids content (TSS, °Brix; according to NP EN 12,143:1999(IPQ, 1999b)), and titratable acidity (TA, % of citric acid in fresh fruit; according to NP EN 12,147:1999 (IPQ, 1999c)).

Statistical analyses were conducted using SPSS software (SPSS, Chicago, IL, USA). To evaluate the significance of differences between treatments for the various measured parameters (dependent variables), one-way ANOVA was performed, with the Duncan test used as a post hoc test for mean separation ( $p \leq 0.05$ ).

### 2.3. Preparation of kiwi samples for microbial DNA extraction

A total of 40 kiwis, 10 grown under each net color used—pearl, grey, and yellow—and a control grown in the open field (no net), were used to perform the extraction of microbial flora associated with their surface. A set of 5 replicates with two kiwis each was used for the extractions. Each extraction was performed in triplicate, and the DNA obtained was pooled before any use. A total of 250 mL of sterile Phosphate Buffer Saline (PBS) pH 7.0 with 0.01 % (v/v) Tween 80® (PanReac AppliChem) was added to each sterile bag containing two kiwis. A washing procedure was performed for 30 min on an orbital shaker (B. Braun Biotech International, Melsungen, Germany) at room temperature (100 rpm). The bags were then placed for 30 s in an ultrasound bath using the Jet Program option (Soltec, Milan, Italy). After removing the kiwi fruits, the bag contents were filtered through a 0.22 µm sterile membrane (PALL, Michigan, EUA). The filter was placed in a sterile 50 mL Costar tube and 25 mL of PBS Tween 80 was added. The tube was agitated horizontally for 30 min (150 rpm), followed by sonication for 30 s, and centrifugation for 5 min at 10,000 rpm at 4 °C. A disposable pipette was used to remove 20 mL of supernatant without disturbing the pellet and membrane. The pellet was resuspended with the remaining 5 mL, which was also used to wash the membrane. After splitting the total volume into three sterile 1.5 mL polypropylene tubes, these were centrifuged for 5 min at 10,000 rpm at 4 °C. The supernatant was discarded, and the pellet of each tube was resuspended in 800 µL of CD1 solution from the DNeasy® PowerSoil® Pro-Kit (QIAGEN, Hilden, Germany).

### 2.4. Microbial DNA extraction, purification, and sequencing

DNA was extracted from each sample obtained in 2.2 using the DNeasy® PowerSoil® Pro-Kit and according to the instructions of the manufacturer, using a bead beater (Benchmark Scientific, Sayreville, USA) at 4000 rpm for two cycles of 30 s, with at least 30 s of ice-cooling between the cycles. Three replicate tubes for each sample were processed and the purified DNA from the three independent sample extractions was combined and stored at –80 °C for future downstream procedures. DNA processing (extraction, purification, quality control, PCR, and library preparation) followed previously published procedures (Fernandes et al., 2024). The pooled DNA from each extraction was quantified by spectroscopy (Nanodrop, manufactured by Thermo Fisher Scientific, located in Massachusetts, USA) and by fluorimetry (QUBIT 3.0, manufactured by Thermo Fisher Scientific, located in Massachusetts, USA).

PCR amplification with the DNA from each sample was performed using the primers, 341F (5' -CCTAYGGGRBGCASCAG-3') and 806R (5'

-GGACTACNNGGTATCTAAT-3), targeting from V3 to V4 hypervariable regions of the 16S rRNA gene for bacterial identification and the primers ITS5 (5' -GGAAG- TAAAAGTCGTAACAAGG-3') and ITS2 (5' -GCTGCGTTCTTCATCGATGC -3') for fungal DNA amplification (White et al., 1990). The produced amplicons were then normalized, pooled, end-repaired, A-tailed, and ligated with Illumina adapters. Sequencing was conducted on a paired-end Illumina platform at Novo-gene Company Limited (located in Cambridge, United Kingdom) to generate paired-end reads.

## 2.5. Analysis of sequencing data

The merging, quality filtering, alignment to SILVA138, and OTU clustering of sequencing reads were performed as previously described (Fernandes et al., 2024). The paired-end reads were merged using FLASH (V1.2.7). The raw tags underwent quality filtering using the QIIME (Caporaso et al., 2010) and were compared with the reference SILVA138 Database (Quast et al., 2013) for rRNA sequence data, and Unite\_INSDC (Abarenkov et al., 2022) for ITS sequence data, using the UCHIME algorithm (R. C. Edgar et al., 2011) to identify and eliminate chimaera sequences, and taxonomically annotated against the databases using QIIME and the Mothur method at each taxonomic rank. For bacterial 16S sequences, host-associated sequences were filtered using the subset\_taxa function from the phyloseq R package v1.52.0 (McMurdie and Holmes, 2012). Lefse analyses were performed using run\_lefse function from the microbiomeMarker package 1.13.2 (Cao et al., 2022) with CPM pre-sample normalization, Kruskal-Wallis p value cutoff of 0.05, and lda score cutoff of 4. These analyses were carried out using R version R-4.5.0 (R Team, 2013). For sequence analysis, the UPARSE (Edgar, 2013) software (Uparse v7.0.1090) was used, considering all the effective tags. Sequences with a similarity of  $\geq 97\%$  were assigned to the same Operational Taxonomic Units (OTUs). The abundance data of OTUs were normalized based on the sample with the lowest number of sequences. Alpha-diversity metrics reflecting richness and evenness, including the Shannon index (Shannon, 1948), observed features and Simpson diversity (Simpson, 1949), were calculated using the normalized data. These analyses were conducted using QIIME (version 1.9.1), which was also used to assess beta-diversity. Non-parametric tests to analyse differences between data groups, ANOSIM, were performed with vegan package and ggplot2 package within R. Functional annotation was conducted using the FAPROTAX database (Louca et al., 2016).

Co-occurrence networks were constructed to explore the complexity of relationships between the communities from the different colors of nets. To ensure comparability between samples regardless of their sequencing depth, a rarefaction at 70,000 reads was performed in all samples. Samples were divided by color of the net to construct four networks for each gene sampled (ITS and 16S) with a minimum appearance in 4 samples with Pearson coefficient connecting only taxa with a minimum absolute correlation of 0.3 and the following parameters: Centered log-ratio transformation for normalization, a pseudo count of 0.5 added to all counts as zero treatment, and fast greedy modularity optimization using Netcomi R package (Peschel et al., 2021). Network properties compared include network size (number of nodes), clustering coefficient (degree to which nodes in a graph tend to cluster together with arithmetic mean of the local clustering coefficient defined by Barrat et al. (2004), modularity (clustering in modules as computed in igraph package (Csardi and Nepusz, 2006) positive edge percentage (percentage of edges with positive estimated association), edge density (proportion between potential and realized paths), natural connectivity (robustness measure of complex networks, corresponding to the average eigenvalue of the adjacency matrix) and vertex/edge connectivity (number of nodes (vertices) or edges (connections) that need to be removed to be removed to disconnect the network)), average dissimilarity (mean of values from dissimilarity matrix) and average path length (mean of shortest paths).

## 3. Results and discussion

### 3.1. $\alpha$ -diversity analysis

Both the presence of nets and their color influence the complexity and richness of bacterial communities on the surface of kiwifruits, as observed in Table 1. Some differences are statistically significant, such as the number of observed species (Yellow vs Grey,  $p = 0.0211$  and Grey vs Pearl,  $p = 0.017$ ), phylogenetic diversity (Yellow vs Grey,  $p = 0.0196$  and Grey vs Pearl,  $p = 0.423$ ), and the Abundance-based Coverage Estimator (ACE), particularly when comparing Yellow vs Grey ( $p = 0.0249$ ) and Grey vs Pearl ( $p = 0.0176$ ) nets. Kiwis grown under grey nets present the lowest species richness, in contrast to kiwis grown under pearl nets, which present the highest species richness. The yellow nets seem to favor species diversity, while the absence of nets favors evenness among the species present.

In relation to fungal  $\alpha$ -diversity, and based on Table 2, kiwis grown under pearl nets show the highest species richness and phylogenetic diversity, in contrast to kiwis grown in open field, which present fungal communities with the lowest species richness and diversity. Grey nets seem to favor evenness, in opposition to yellow nets.

When comparing  $\alpha$ -diversity determined from the results of 16S and ITS sequencing, higher bacterial species richness and diversity are observed compared to fungi, with the kiwis grown under pearl nets showing the highest metrics.

### 3.2. $\beta$ -diversity analysis

The analysis using a simple binary index such as Jaccard reveal the grouping according to the color of the net, when observed in Principal Coordinates Analysis (PCoA) plots (Fig. 1). This grouping is particularly clear for the fungal community, especially when comparing the pearl net to the open field condition (No\_net), and even more evident for the bacterial community, clearly demonstrating the influence of the net color on the composition of bacterial communities.

### 3.3. Bacterial and fungal communities on the surface of kiwifruit

The microbial community from the surface of fruits is composed of a variable range of bacteria, yeasts, and molds (Dimkić et al., 2023) and its presence is highly influenced by environment and agricultural practices (Kusumaningrum et al., 2015). The microbiological characterization of samples of kiwifruits grown under different net colors and in open field revealed the presence of almost 688 genera of bacteria and 730 of fungi. Actinobacteria is the most abundant class observed, comprising 22.4 % to 41.0 % of all reads, followed by Alphaproteobacteria which accounts for 19.0 % (yellow nets) to 33.1 % (no nets) of reads (Fig. 2).

There are no highly abundant bacterial genera present in any sample, being *Pedobacter* (7.8 %–11.4 %) and *Methylobacterium-Methylorubrum* (7.0 %–10.2 %) the most abundant genera.

A significant number of fungal genera could not be identified and appear on the abundance histogram of Fig. 3, as *Fungi\_phy\_incertae\_sedis*. Among the identified genera, *Ascomycota* accounts for most of the fungal genera in all samples, ranging from 54.3 % (open field) to 70.9 % (yellow nets). Within *Ascomycota*, *Acremonium* is the most abundant genus on the surface of kiwis grown under nets, ranging from 33.5 %

**Table 1**

Summary of  $\alpha$ -diversity – based on taxonomic composition obtained by sequence analysis of the V3–V4 regions of the 16S rRNA gene.

Net color	Obs. species	Shannon	Simpson	Chao1	ACE
No net	1431	5.121	0.983	1610.891	1615.650
Pearl	1629	4.962	0.965	1856.999	1873.994
Grey	889	4.738	0.977	993.234	997.658
Yellow	1590	5.244	0.983	1755.594	1771.130

**Table 2**

Summary of  $\alpha$ -diversity – based on taxonomic composition obtained by sequence analysis of the ITS region.

Net color	Obs. species	Shannon	Simpson	Chao1	ACE
No net	657	4.340	0.872	772.614	798.150
Pearl	773	4.395	0.873	947.001	990.213
Grey	700	4.463	0.897	812.859	856.206
Yellow	759	4.074	0.831	917.012	960.077

(pearl nets) to 41.5 % (yellow nets) of all the reads, whereas *Alternaria* is the most abundant genus on the surface of kiwis grown in the open field (20.2 %), with its abundance in kiwis grown under nets always below 9.7 %. *Didymella* is also more abundant in kiwis grown in open-field (4.1 %) compared to those grown under nets (0.2–0.4 %).

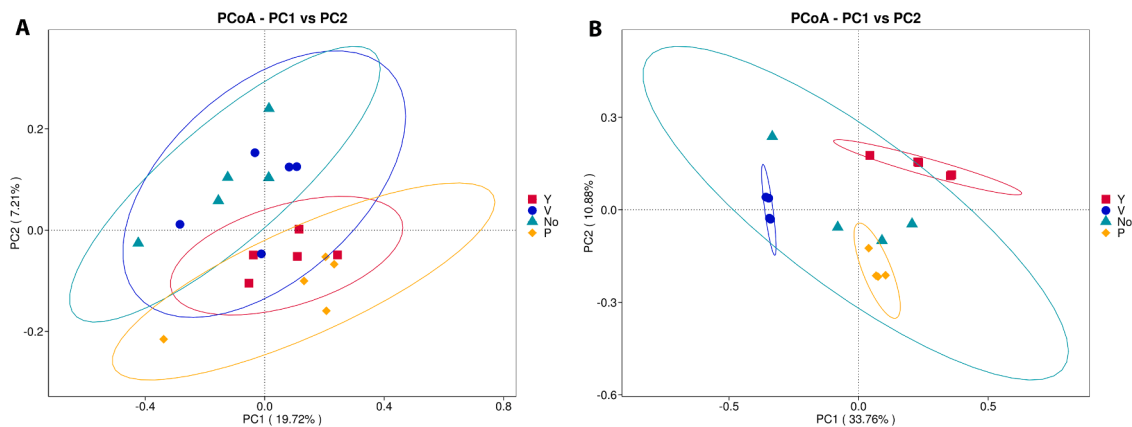
A Linear Discriminant Analysis (LDA) of Effect Size (LEfSe) with the results filtered for high significance and a minimum LDA effect score of four was used to identify potential biomarkers based on differentially abundant taxa between the microbiomes present on the surface of kiwis grown under different net colors. Higher abundance (positive LDA values) and lower abundance in Figs. 4 and 5 reflects the varying presence of specific microbial taxa on kiwis grown under different conditions. Only the statistically significant differences are presented

and in comparison with the kiwis grown under nets, the kiwis grown in open field are significantly more rich in *Sphingomonas*, which is the opposite found by Sui et al. (2021) when the kiwifruit microbiome is compared between open-field and rain-shelter cultivation. Additionally *Flavobacterium* abundance seems to be favored by the presence of grey nets in relation to all the other conditions tested.

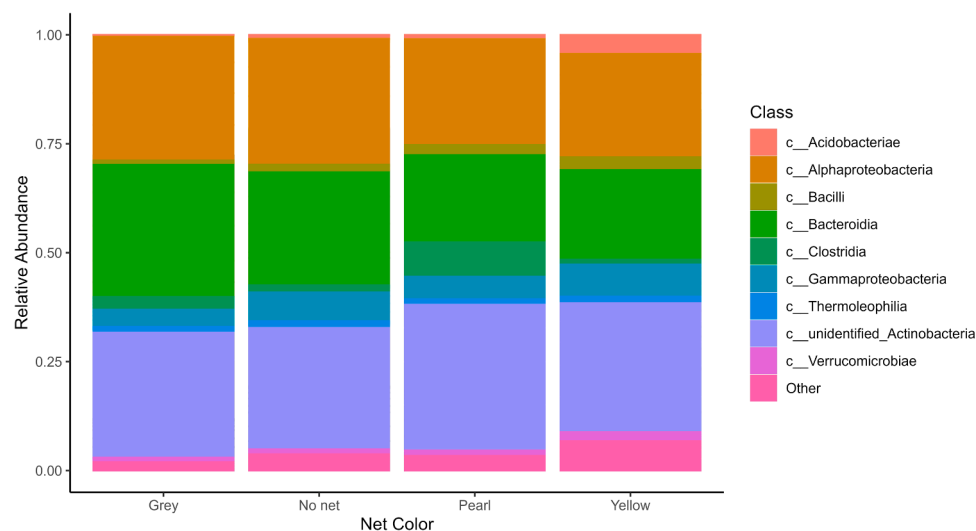
In relation to the fungal community, some significant results arise from the comparison between the population of kiwis grown in open field and under yellow nets. Some relevant taxon as *Didymella* and *Alternaria* are much more abundant at the surface of kiwis grown in open-field. These fungi are known plant pathogens and its association with kiwi diseases has been already described (Corazza et al., 1999; Pan et al., 2018; Ruan et al., 2022; Tshouridou and Thanassouloupoulos, 2000).

Analyzing the presence of bacterial taxa related to plant pathogenesis and based on the FAPROTAX database annotation results, we can verify that there are no significant variations in these bacterial taxa among the different samples. This feature (plant pathogenesis) is essentially associated with the presence of a low number of reads of *Rhodococcus fascians*, *Erwinia billingiae*, *Curtobacterium flaccumfaciens*, and *Rathayibacter tritici* in the various samples, but without significant differences between the kiwifruit samples grown in different conditions.

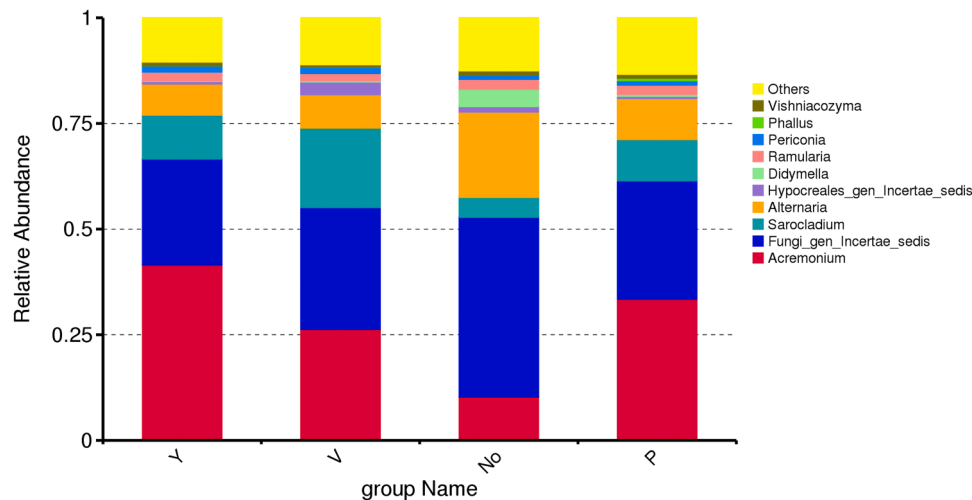
The ecological functions of fungal taxa were obtained using



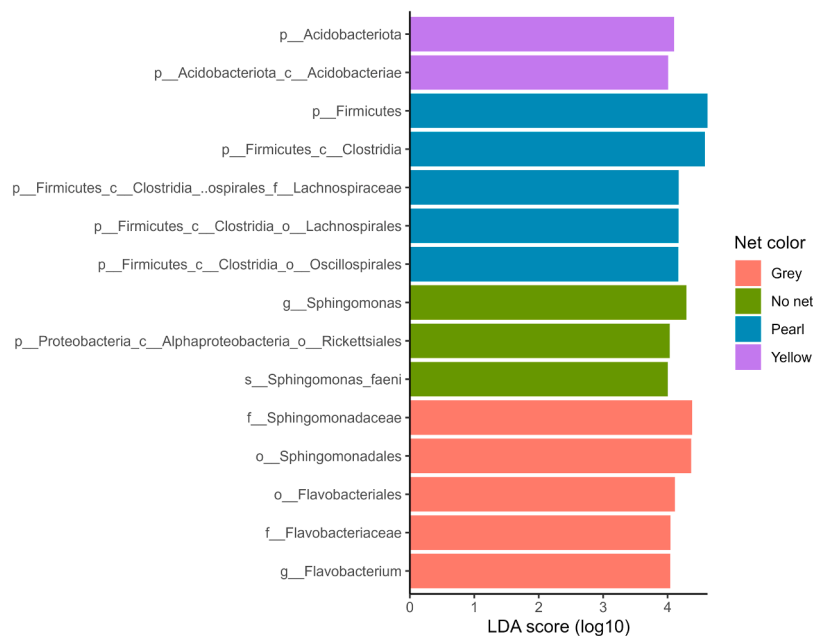
**Fig. 1.** Principal coordinates analysis (PCoA) showing the percent of variation explained in the microbial community. (A) Jaccard distance matrix based on ITS data; (B) Jaccard distance matrix based on 16S data. The ellipses represent distinct clustering of the samples obtained from kiwis grown under different net colors (Y-yellow, V-grey, P-pearl, and No-no net (open field)).



**Fig. 2.** Relative abundances (%) of the ten most dominant sequences assigned to a bacterial class level identified in kiwifruit samples grown under different net colors (Y-yellow, V-grey, P-pearl and No-no net (open field)).



**Fig. 3.** Relative abundances (%) of the ten most dominant sequences assigned to a bacterial class level (A) and fungal genus level (B) identified in kiwifruit samples grown under different net colors (Y-yellow, V-grey, P-pearl and No-no net (open field)).



**Fig. 4.** Linear Discriminant Analysis (LDA) scores computed for bacterial features differentially abundant on the surface of Kiwifruit grown under different net colors (Y-yellow, V-grey, P-pearl and No-no net (open field)).

FunGuild (Nguyen et al., 2016) and based on their species classification. The top-ten function abundance results can be observed in Fig. 6.

A t-test of the functional prediction results was used to test the main functional differences between different groups (yellow, grey, pearl nets and open-field (no net)). The results can be observed in Fig. 7.

Functional differences between the fungal communities associated with the surface of kiwifruits grown under colored nets and those grown in open fields arise from differences in their taxonomic composition. Most of the statistically significant differences, considering the taxa whose functions are properly annotated in the FunGuild database, fall upon a small number of taxa. *Acremonium* (guild: Animal Pathogen-Endophyte-Fungal Parasite-Plant Pathogen-Wood Saprotroph) is consistently much higher in samples from kiwifruits grown under colored nets (No net vs. yellow,  $p = 0.007$ ; No net vs. grey,  $p = 0.004$ ; No net vs. pearl,  $p = 0.012$ ). Conversely, *Alternaria* (guild: Animal Pathogen-Endophyte-Plant Pathogen-Wood Saprotroph) is always more abundant in the microbiome associated with kiwifruits grown in open fields (No

net vs. yellow,  $p = 0.002$ ; No net vs. grey,  $p = 0.001$ ; No net vs. pearl,  $p = 0.048$ ). *Didymella* (guild: Plant pathogen undefined saprotroph) is also more abundant in the microbiome associated with kiwifruits grown in open fields (No net vs. yellow,  $p = 0.031$ ; No net vs. grey,  $p = 0.035$ ; No net vs. pearl,  $p = 0.038$ ). Reads associated with *Cladosporium* (guild: Endophyte-Plant Pathogen) are likewise higher in open-field samples (No net vs. yellow,  $p = 0.018$ ; No net vs. grey,  $p = 0.027$ ).

*Acremonium*, a fungus belonging to the *Hypocreaceae* family is known to harbour some species with antifungal effects against several fungi, including *Botrytis cinerea* and *Botryosphaeria dothidea*, two known pathogenic fungi involved in postharvest kiwifruit rot (Di Francesco et al., 2018; Michailides and Elmer, 2000; Zhou et al., 2015). Its presence is particularly high in kiwis grown under yellow nets (41.5 % of all reads) and pearl nets (33.5 % of all reads), as well as in grey nets (26.3 % of all reads), whereas in kiwis grown in open-fields, only 10.3 % of all the reads are found to be associated with this genus.

*Alternaria*, *Didymella* and *Cladosporium*, all fungi genera associated

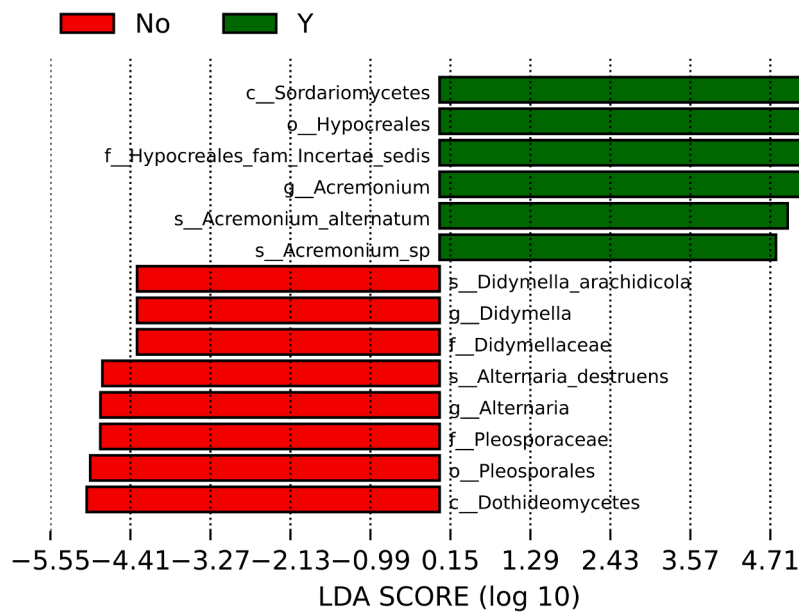


Fig. 5. Linear Discriminant Analysis (LDA) scores computed for fungal features differentially abundant on the surface of Kiwifruit grown under different net colors (Y-yellow, V-grey, P-pearl and No-no net (open field)).

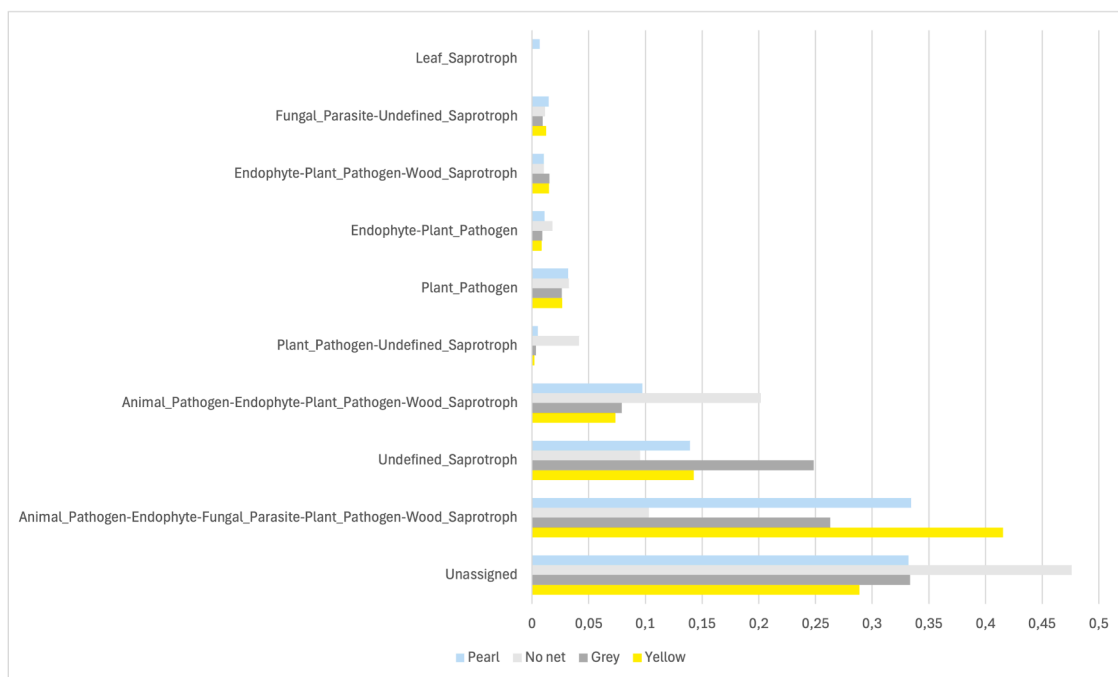


Fig. 6. Top-ten FunGuild functions prediction.

with postharvest deterioration of kiwifruit (Corazza et al., 1999; Pan et al., 2018; Rosado et al., 2019; Ruan et al., 2022; Tshouridou and Thanassouloupoulos, 2000) present consistently much higher read counts on kiwis grown in open-field than under any colored net.

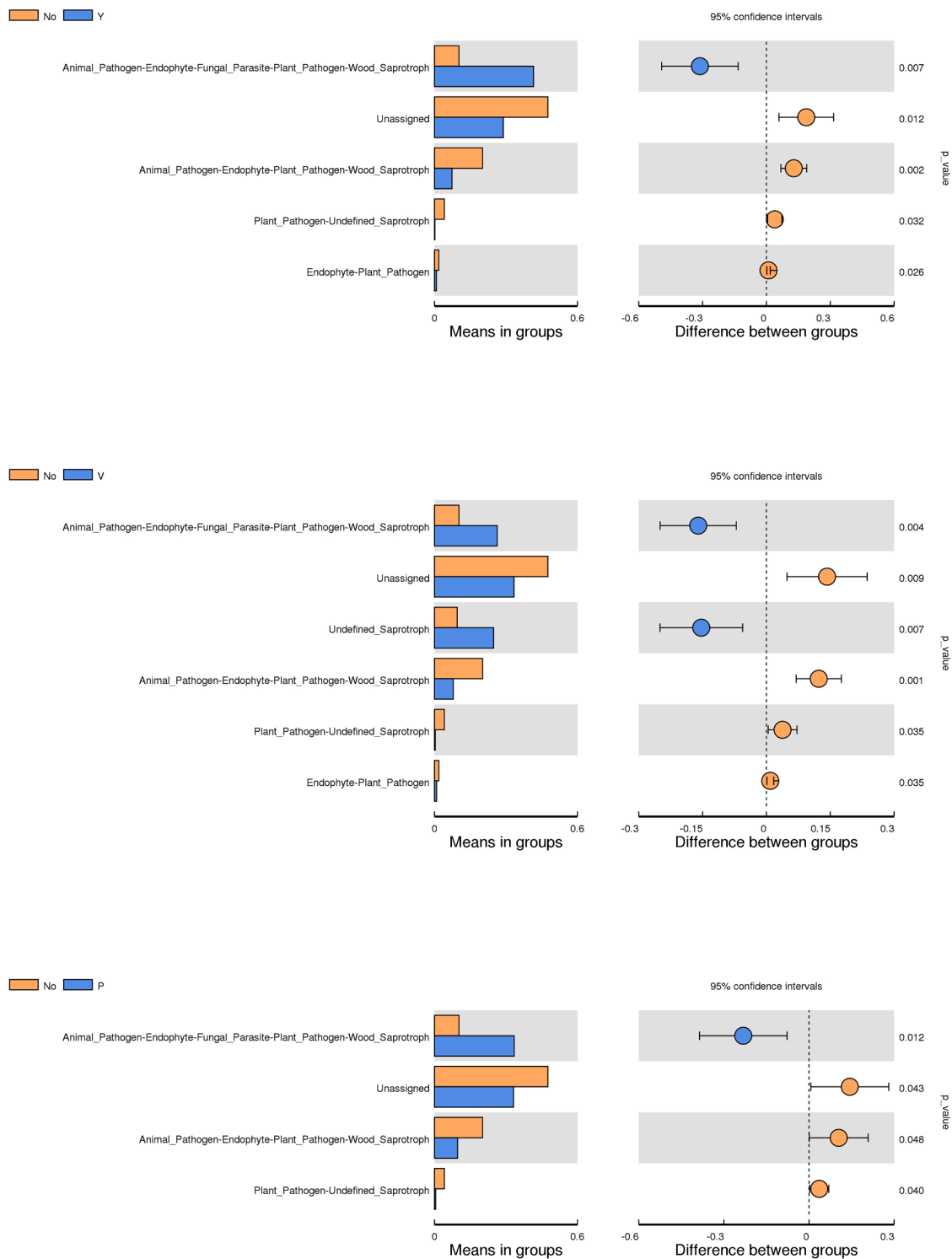
*Botrytis cinerea* which has been described as one of the main causes of postharvest kiwifruit rot (grey mold), was not found, but a very small amount of reads of *Botrytis caroliniana*, also a pathogenic fungus (Brauna-Morzevska et al., 2023), were found in kiwis from all the conditions used and without any statistically significant differences between the kiwis grown under different net colors.

Regarding other kiwi pathogenic taxa, while no statistically significant differences were observed between kiwifruit harvested under

varying conditions, the presence of *Pestalotiopsis* and *Verticillium albo-atrum* species is significant. These two fungi genera harbour species that are described to be associated with kiwifruit rot (Auger et al., 2009; Karakaya, 2001), and *V. albo-atrum* accounts for 3.8 % of all read counts in kiwis grown under grey nets. Additionally, trace amounts (<0.1 % of all read counts) of *P. expansum* and *D. passiflorae* were detected.

### 3.4. Co-occurrence networks

It is essential to consider that the microbial flora associated with a fruit consists of hundreds of different species with biological relationships among them, as they share the same environment and often



**Fig. 7.** T-test analysis of functional differences between groups (ITS data, FunGuild). The left panel shows the mean abundance of species with significant differences between groups. The right panel displays the 95 % confidence intervals of between-group variations, with each circle representing the lower limit (left), upper limit (right), and mean difference (center). Circle colors indicate the group with the higher mean, and the rightmost value represents the p-value of the significance test.

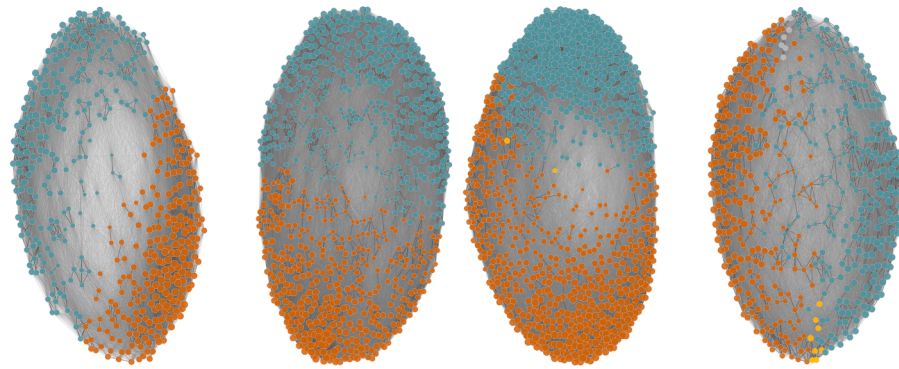
compete for the same nutrients. The stability of the microbial network and the relationships established among its members are fundamental for maintaining a beneficial community that prevents the development of undesirable pathogens that may affect the health of the fruit. The characteristics of this network will define its resistance to stresses and destabilizing factors (robustness) and its capacity to recover when destabilized (resilience).

The stability, connectivity, and diversity of the bacterial and fungal

co-occurrence networks were therefore analyzed. Notwithstanding the limitations of the analysis arising from the comparatively small sample size, distinct structural and interaction patterns are discernible within bacterial and fungal networks, as depicted in Fig. 8(A) and (B), and corresponding tables. These findings underscore the inherent robustness, resilience, and interaction dynamics of these networks.

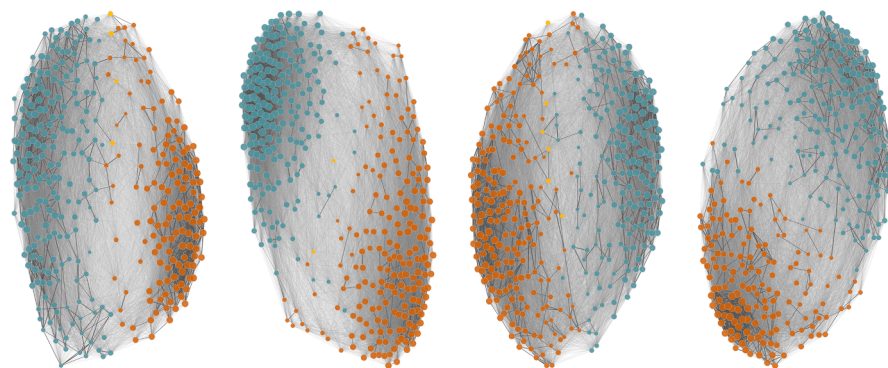
The bacterial community networks show distinct characteristics and resilience. Kiwis grown in the open field develop smaller but denser and

**A**



<b>16S</b>	<b>No net</b>	<b>Pearl</b>	<b>Yellow</b>	<b>Grey</b>
Network size	471	775	991	506
Clustering coefficient	0.780	0.740	0.751	0.734
Modularity	0.009	0.000	0.019	0.001
Positive edge %	50.916	49.972	50.326	50.046
Edge density	0.745	0.710	0.703	0.705
Natural connectivity	0.282	0.246	0.260	0.241
Vertex/Edge connectivity	233	466	351	299
Average dissimilarity	0.636	0.649	0.650	0.650
Average path length	0.664	0.677	0.682	0.679

**B**



<b>ITS</b>	<b>No net</b>	<b>Pearl</b>	<b>Yellow</b>	<b>Grey</b>
Network size	333	388	373	343
Clustering coefficient	0.748	0.792	0.721	0.720
Modularity	-0.001	0.003	0.001	0.001
Positive edge %	50.048	49.671	49.491	49.754
Edge density	0.698	0.732	0.674	0.675
Natural connectivity	0.240	0.274	0.227	0.226
Vertex/Edge connectivity	145	146	177	158
Average dissimilarity	0.654	0.649	0.659	0.660
Average path length	0.688	0.680	0.694	0.695

**Fig. 8.** Graphical representation and summaries of network global properties for Bacteria 16S (A) and Fungi ITS (B). Nodes are colored by module and edge intensity is related to the strength of the Pearson correlation.

more robust networks with high natural connectivity. Pearl nets promote larger, less clustered networks with higher connectivity, enhancing resilience and ecological competition. Yellow nets support the largest and most complex networks, balancing clustering and connectivity. Grey nets produce smaller, less robust networks similar to pearl nets but with lower connectivity. Overall, open-field kiwis exhibit the most robust networks, while pearl nets foster the most resilient ones, better recovering from environmental disturbances.

For fungal communities, kiwis grown in the open field develop the smallest but most robust networks, though with lower resilience. Pearl nets support the largest and most structured networks, indicating high

robustness. Yellow nets promote high diversity and the most resilient networks, while grey nets result in smaller, moderately structured networks. Overall, pearl nets enhance robustness, while yellow nets favor resilience.

Comparing the network structures between bacterial and fungal communities, the network size is larger in the bacterial community, indicating more complex bacterial interactions. Both communities have high clustering coefficients, but modularity is generally low, with slightly positive values in the networks from kiwis grown under pearl nets for the fungal community. As inferred by natural connectivity, the bacterial community forms a more robust network in kiwis grown in

open field, whereas the fungal network is more robust in kiwis grown under pearl nets.

### 3.5. Disease severity

The study on the effect of photosensitive nets on Psa disease investigates the pathogen's response to environmental conditions in spring, summer (June, July and August), and early autumn (September and November). The 2022 results support previous findings from 2020 to 2021 (Moura et al., 2022), confirming that photosensitive nets (pearl, yellow, and grey) significantly reduce Psa epiphytic infections. Disease severity increased from June to October (Fig. 9, with the lowest severity index in June and the highest in October). This trend aligns with other studies emphasizing the role of the environmental factors in disease progression (Moura et al., 2022; Vaz et al., 2018). In July, August, and September, disease severity was not significantly different among the three types of nets, but significantly higher disease severity was observed in July and August for the control plants grown without nets. The microclimate under the photosensitive nets, with increased mean maximum air temperature, decreased relative humidity, and reduced rainfall which influence bacterial proliferation (Scortichini et al., 2012) may contribute to explain a reduction in Psa disease severity. The use of photosensitive nets altering light conditions (Bastías et al., 2012), may influence microbial populations, that can potentially affect microbial species in the phyllosphere of kiwifruit plants (Correia et al., 2022; Vaz et al., 2018). The presence of species as *Pseudomonas putida* and *P. poae*, may compete or inhibit Psa colonization, thereby helping to suppress the disease (Froud et al., 2015). Furthermore, the spectral properties of the nets, particularly the higher blue:red radiation ratio in the pearl net, may influence the disease dynamics and can impact plant physiological responses, contributing to the observed decrease in disease severity, as highlighted by Correia et al. (2022). This specific light spectrum is thought to affect plant photomorphogenesis, potentially enhancing plant resistance, by activating defense responses, or by altering the bacterial pathogen's ability to infect the plant (Correia et al., 2022; Kook et al., 2013).

In conclusion, while the use of photosensitive nets appears to be effective in reducing Psa severity, further research is needed to fully understand how light affects not only the plant's physiology but also its microbial communities that will be essential for developing more effective strategies for controlling Psa and improving kiwifruit production.

### 3.6. Crop yield and fruit quality

Photosensitive nets and the presence of *P. syringae* pv. *actinidiae*

(Psa), can have significant impacts on both the yield and quality of kiwifruit (Gullo et al., 2021). In 2022, kiwi production was similar in control crops without net (33 t·ha<sup>-1</sup>) and those ones under the influence of photosensitive nets (37 t·ha<sup>-1</sup>), contrasting with results described for 2020 and 2021 by Moura et al. (2022), where kiwi production was higher outside (control without net). This change registered in 2022 in favor of plants under photosensitive nets, is most likely due to the better adaptation of the kiwifruit plants to the agroclimatic conditions under the influence of nets, and the improved water management in the orchard, over the three years of the experiment. Enhanced pollination management in 2022, including bumblebees, three artificial pollinations, and twice-daily ventilation, also may contributed to increased yields for all net treatments.

Recent studies showed that the same photosensitive nets could alter pollen characteristics, affecting size, fertility, and sugar/protein composition (Ribeiro et al., 2024). According to these authors the pearl net, was the one inducing the greater pollen traits modifications. Kiwifruit yield increase for each grade (Waste, Class II, Class I, Extra) in 2022 under the three photosensitive nets (pearl, yellow and grey) compared to outside and was primarily due to the higher weight of Class I kiwifruits on the crops under the effect of the nets (Fig. 10).

In the present study, at harvest, fruit dry matter content (DM), fruit firmness (N), pH, total soluble solids content (TSS, °Brix) and the titratable acidity (TA, %) were similar for all experimental crop treatments (Table 3). Occasional differences in firmness were noted particularly with fruits under the yellow and grey nets, which exhibited significantly lower firmness (41.2N and 40.7N, respectively) compared with fruits from the other treatments. The effect of net type on fruit quality attributes such as TSS, firmness, and DM content, is not always consistent and may depend on environmental factors like temperature, light, and humidity (Shahak et al., 2008). In some cases, there was little variation in TSS, pH, or TA, with most results being similar for netted and uncovered crops. In contrast, kiwifruits grown under the pearl net showed higher DM content than those grown under the yellow net, indicating that certain net types may promote higher nutrient accumulation in the fruit (Moura et al., 2022). Basile et al. (2014) showed that white nets led to higher TSS content in kiwifruits at harvest compared to the control group, along with increased dry matter and lower titratable acidity. Firmness is a key factor for kiwifruit storage and marketing, as softening is linked to senescence and fruit damage (Bastías and Corelli-Grappadelli, 2012).

## 4. Conclusions

Photosensitive nets are used in agriculture to modify the light environment around crops by filtering specific wavelengths of sunlight and

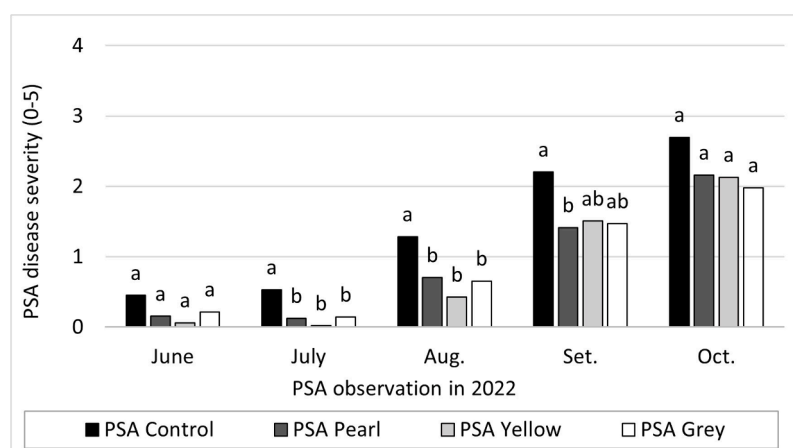
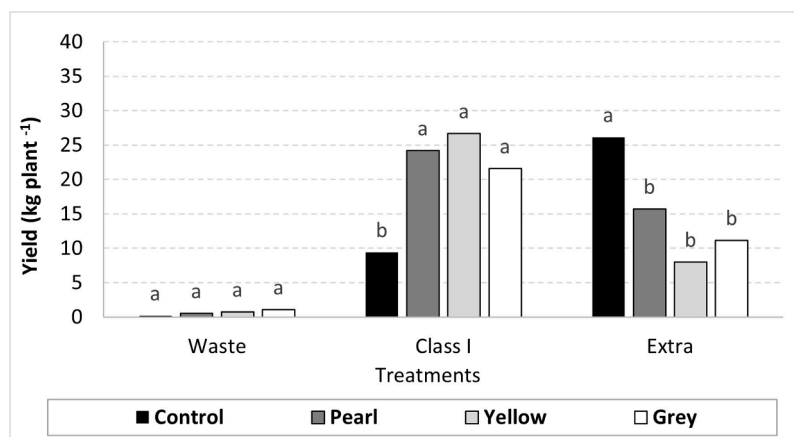


Fig. 9. Psa disease severity outside (control) and under the three photo-selective nets (pearl, yellow and grey) observed in 2022 according to a visual disease severity scale (0–5). For each month, bars with different letters are significantly different ( $p < 0.05$ ).



**Fig. 10.** Kiwifruit yield ( $\text{kg}\cdot\text{plant}^{-1}$ ) for each grade (Waste, Class II, Class I, Extra) for uncovered plants (control) and plants under the three photo-selective nets (pearl, yellow and grey), in 2022. For each grade, bars with different letters are significantly different ( $p < 0.05$ ).

**Table 3**

Effect of pearl, yellow, and grey nets on kiwifruit dry matter, firmness, pH, total soluble solids content and acidity (2022 harvest).

	Control	Photoselective Nets		
		Pearl	Yellow	Grey
Dry Matter (%)	14.6 a	15.0 a	14.2 a	14.4 a
Firmness (N)	49.1 a	46.6 a	41.2 b	40.7 b
pH	3.4 a	3.3 a	3.3 a	3.3 a
Total Soluble Solids (Brix)	8.7 a	7.4 a	7.4 a	7.8 a
Titrateable Acidity (%)	1.6 a	1.5 a	1.3 a	1.5 a

Note: Means in the same row followed by different letters are significantly different ( $p < 0.05$ ) between net types.

protecting crops from certain environmental conditions. How these nets might influence the development of the microbial flora is fundamental as a comprehensive understanding of microbial ecology and interactions within the fruit environment is essential for effective disease management, which can result in increased shelf life and a more sustainable production. In our study, the influence of photoselective nets on the microbial development associated with the surface of kiwi fruits was analyzed. The results showed that the presence of nets has a strong impact on the microbial community that establishes on the surface of kiwi, with a much greater accumulation of pathogenic microorganisms typically associated with postharvest kiwi rot, such as *Alternaria*, *Dimydelia*, and *Cladosporium*, found on kiwis grown without nets. Conversely, the levels of *Acremonium*, a genus of fungi with species that have biocontrol functions against some kiwi pathogens, were significantly higher on kiwis grown under photoselective nets. The influence of the nets is also evident in the structure and organization of the microbial communities, particularly in terms of diversity, species richness, and evenness. Pearl nets favor bacterial species richness and promote higher species richness and diversity in the fungal community, unlike the open field. Pearl nets also favor the creation of more resilient bacterial networks and create more robust fungal networks. Yellow nets favor bacterial species diversity and promote more diverse and resilient fungal networks. Additionally, yellow nets favor the creation of more resilient bacterial networks. The absence of nets favors bacterial species evenness, while kiwis grown in the open field have robust but less resilient fungal networks. Although the present study focuses exclusively on kiwi fruit, the impact of photoselective nets on the microbiome of other types of plantations and fruits may also be relevant and characterizing microbial communities of fruits obtained from diverse agricultural practices is pivotal for developing sustainable fruit production systems. Such knowledge will ultimately contribute to enhanced fruit quality and extended shelf life, factors that should be taken into consideration in

future similar studies on microbiome characterization and the influence of photoselective nets. Furthermore, this study was limited to analyzing the microbiome present on the fruit's surface, but the endophytic flora may also be highly relevant, making its future study particularly important.

The pearl photoselective net reduced the disease severity over the spring-autumn seasons 2022 in July, August and September without negative impacts in the final crop yield. In fact, the yield of Class I grade fruits of crops under the nets (pearl, yellow and grey) was significantly higher compared to the uncovered crops. At harvest, photoselective nets did not affect fruit quality.

The presented work provides data confirming the influence of net color on the development of certain microorganisms and the establishment of their relational structure on the surface of kiwifruit. Further research with a larger sample size, encompassing multiple seasons and distinct climatic conditions, would strengthen these findings and provide a better understanding of how these microbiological changes influence the shelf life of kiwifruit.

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### CRediT authorship contribution statement

**Paulo Fernandes:** Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Concha Cano-Díaz:** Writing – review & editing, Formal analysis, Investigation. **Rui Pinto:** Writing – review & editing, Investigation. **Isabel Mourão:** Writing – review & editing, Methodology. **Luís Miguel Brito:** Writing – original draft, Methodology. **Luísa Moura:** Writing – original draft, Supervision, Methodology, Conceptualization, Project administration.

### 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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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.crmicr.2025.100424](https://doi.org/10.1016/j.crmicr.2025.100424).

## Data availability

Raw reads are deposited in the SRA database under BioProject PRJNA 1013064.

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