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BRIEF REPORT

ENVIRONMENTAL MICROBIOLOGY



Investigating the inoculum dynamics of *Cladosporium* on the surface of raspberry fruits and in the air

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Abstract

Raspberry production is under threat from the emerging fungal pathogenic genus *Cladosporium*. We used amplicon-sequencing, coupled with qPCR, to investigate how fruit age, fruit location within a polytunnel, polytunnel location and sampling date affected the fruit epiphytic microbiome. Fruit age was the most important factor impacting the fungal microbiome, followed by sampling date and polytunnel location. In contrast, polytunnel location and fruit age were important factors impacting the bacterial microbiome composition, followed by the sampling date. The within-tunnel location had a small significant effect on the fungal microbiome and no effect on the bacterial microbiome. As fruit ripened, fungal diversity increased and the bacterial diversity decreased. *Cladosporium* was the most abundant fungus of the fruit epiphytic microbiome, accounting for nearly 44% of all fungal sequences. Rotorod air samplers were used to study how the concentration of airborne *Cladosporium* inoculum (quantified by qPCR) varied between location (inside and outside the polytunnel) and time (daytime vs. nighttime). Quantified *Cladosporium* DNA was significantly higher during the day than the night and inside the polytunnel than the outside. This study demonstrated the dynamic nature of epiphytic raspberry fruit microbiomes and airborne *Cladosporium* inoculum within polytunnels, which will impact disease risks on raspberry fruit.

INTRODUCTION

Raspberries (*Rubus ideaus*) contribute significantly to the UK economy, worth £147.5 million in 2021 (DEFRA, 2023), but are vulnerable to fungal pathogens such as *Botrytis*, *Rhizopus*, *Mucor*, *Cladosporium*, and bacterial pathogens such as *Erwinia* and *Agrobacterium* (Williamson et al., 1991). In recent years, UK raspberry production has shifted from open soil to coir-based substrate cultivation under polytunnels. However, there is a lack of knowledge on how the change in production systems may impact disease dynamics. *Cladosporium* is an opportunistic pathogen and has been reported as a problem for UK raspberry growers in recent years (Farwell et al., 2023). The most prevalent species found on UK raspberries was *Cladosporium cladosporioides* (Farwell et al., 2023); as this species is one of the most abundant in airborne spore samples (Damialis et al., 2017; Harvey, 1967) it is suspected to be an important primary inoculum source for colonizing raspberries. *Cladosporium* infections are suspected to occur more frequently in warm and humid environments (O'Neill et al., 2012), such as those conditions within polythene tunnels. *Cladosporium* is also frequently reported as being prevalent in plant tissues as an endophyte (Franco Ortega

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et al., 2020; Wicaksono et al., 2023), with the relative abundance being 37.8% in one study in homogenized strawberries (Abdelfattah et al., 2016). It is currently unknown, however, how dominant *Cladosporium* is within the epiphytic microbiome of fruit, where disease development likely begins for skin lesions.

Pathogenic fungi and bacteria have various mechanisms for dispersal, including aerial dispersal and insect vectors (West, 2014). There is a knowledge gap regarding the impact of horticultural environments, such as polytunnels, on airborne inoculum dispersal. Air movement was shown to be an important factor in dispersing *Botrytis* spores in glasshouses (Boulard et al., 2010). The spread of tomato powdery mildew (*Erysiphe neolycopersici*) in glasshouses may be facilitated by air movement due to workers moving down alleyways (Sokolidi et al., 2023). The concentration of plant material and warmer conditions may provide an more conducive to *Cladosporium* survival and sporulation inside polytunnels. Hence, understanding if these environmental factors impact the number and dynamics of airborne inoculum is vital for assessing the disease risk under polythene. A method frequently used to assess the airborne inoculum is the use of qPCRs (Clark et al., 2023), which can provide estimates of spore concentrations relatively quickly. The aerosol microbiome is known to have a complex relationship with temperature, relative humidity and rainfall (de Groot et al., 2021; Gusareva et al., 2020). Variation in airborne spores likely impacts the phyllosphere microbiome, as we would expect microbes present in the air to land on and colonize exposed plant tissues, potentially affecting disease risk. Variations in climatic conditions can occur on a local scale; for instance, temperature and humidity vary considerably within a polytunnel (Hall et al., 2020). The effect of polytunnels on both the inoculum load and the composition of resident microbial communities needs to be considered when developing and implementing disease management strategies for raspberry.

Survival and hence infectivity of plant pathogens, such as *Cladosporium*, may be greatly affected by the resident microbiome on plant tissue surfaces. *Cladosporium cladosporioides* was previously shown to only cause skin lesions on raspberries at the ripening and ripe stages, with green fruit showing no visible symptoms (Farwell et al., 2023); hence, the stages of plant development will also affect infectivity and potentially the loads of pathogens observed across ripening stages. As ripe fruits have a longer period of exposure to the environment and airborne microbes than immature fruits, they may have more complex communities of microorganisms. Fruit volatiles also change during ripening (Robertson et al., 1995), with microbes on the fruit surface contributing towards this complex cocktail of volatiles (Sangiorgio et al., 2022). Such plant-emitted volatiles can inhibit/induce recruitment, growth and survival of specific microbes, with some microbes adapted to exploit these plant defence volatiles for resource acquisition (Hammerbacher et al., 2019). To fully understand the ecology of pathogens on the fruit surface, we need to understand how they interact with the resident microbes within a given environmental niche.

The raspberry epicarp fruit microbiome compositions were observed to vary significantly across fruit ripening stages; a higher fungal diversity was found at the ripening stage of fruit development than on green and ripe fruit. However, as each raspberry development stage was sampled across different dates, the varying environmental conditions may have biased their observations (Jones et al., 2022). In soft fruits, particularly raspberries, fruit firmness decreases during ripening (Giongo et al., 2019), making fruit vulnerable to tissue damage. The Dipteran pest, *Drosophila suzukii*, also prefers ripe fruit due to a higher visual contrast with the surrounding foliage (Little et al., 2017), leading to higher oviposition damage on ripening fruit. This damage may release sugars and nutrients, affecting epiphytic communities and creating entry points for pathogens.

This study aims to (1) assess whether the extent of microbial communities on the raspberry fruit surface is influenced by fruit age, location within the polytunnel, polytunnel location, and sampling times using amplicon sequencing; and (2) investigate the diurnal pattern and within-day variations in the level of airborne *Cladosporium* inside and outside a polytunnel using qPCR.

EXPERIMENTAL PROCEDURES

Raspberry fruit epiphytic microbiome

Experimental design, sampling and fruit washing

In the first experiment, the effect of four factors on the raspberry epiphytic microbiome was investigated: fruit age (green fruit, ripening fruit that was turning pink, and ripe fruit), location within a polytunnel (the centre and the outer edge), polytunnel locations on a farm (four polytunnels at one farm) and sampling date (2 October 2021 and 9 October 2021). Raspberry fruits were collected from a commercial raspberry farm in Kent, England at the end of the growing season (October). Sampled polytunnels were separated by three buffer polytunnels. The same four polytunnels were sampled on each of the two sampling dates. Polytunnels were netted at the ends with no side skirts between tunnels.

Within each tunnel, four rows of raspberries were grown, with fruit samples taken from an approximately 10 m long area in the central two rows at (a) the outer

edge (both tunnel ends, ca. first 10 m) and (b) the middle of the tunnel (ca. 50 m from the tunnel entrance). For each outer edge sample, 20 fruits were collected for each of the three maturity stages; fruits from the two outer edges were then pooled into one sample. Similarly, 40 fruits were collected for each maturity stage from the central location.

In total, 48 samples were collected (two sampling dates × four polytunnels × two within-tunnel locations × three fruit ages). Fruits were placed into bleach-sterilized pipette boxes by snipping the tip of the petiole near the fruit with a sterilized pair of scissors to avoid cross-contamination. Collected samples were immediately placed into polystyrene boxes containing ice packs and taken to the lab for processing.

Each sample of 40 fruits was transferred into individual Ziplock bags with 100 mL of Maximum Recovery Diluent (07233, Sigma-Aldritch), sealed, surrounded horizontally by ice, and placed on a platform shaker set to 120 RPM for 1 h. All six samples from one tunnel collected on the same day were shaken concurrently. The bags were rotated 180° after 30 min of shaking. After shaking, the wash liquid from each sample was filtered from the Ziploc bags through a sterile muslin cloth into two sterile 50 mL falcon tubes to remove plant material and centrifuged at 3894 × *g* for 10 min to produce a pellet. Most of the supernatant was pipetted off (leaving approximately 0.5 mL), then the pellet was resuspended in the remaining liquid and transferred to a 2 mL Eppendorf tube. The Eppendorfs were then spun at 16,602 × *g* to produce the final pellet. Finally, the supernatant was removed, and samples were stored at −80°C until further processing.

DNA extraction, qPCR estimation of microbial communities and amplicon sequencing

DNA was extracted from the pellets using the TRI reagent protocol (93289, Sigma-Aldritch; TRI Reagent® Protocol, 2023) with an additional 75% ethanol precipitation step after the trisodium-citrate washes to help remove salt contaminants and increasing centrifugation speed to 16,000 × *g*. This method was used to maximize DNA yield as preliminary testing of DNA extraction kits containing spin columns gave very low DNA yields (data not shown). Samples were sent on dry ice to Novogene UK (Cambridge, UK) for library prep and 16S and ITS amplicon sequencing (primers detailed in Table A5) on the Illumina NovaSeq 6000 platform (San Diego, USA) to produce 250 nucleotide-paired reads.

The community size of bacteria and fungi in each sample was estimated with qPCR. The most abundant Amplicon Sequence Variants (ASVs) from the ITS and 16S sequences (see the bioinformatics section below) were synthesized into a synthetic gBlock™ gene (Integrated DNA Technologies INC.; Table A6), which were diluted and used as standard curves (in a 10-fold series dilution from 10^{-5} to 10^{-8}), and stored at −20°C. Samples were then run on 96 or 384 well plates with the standards, a non-template control and two dilutions of each sample (20× and 80× for ITS, 10× and 40× for 16S) with triplicates for each dilution. Each reaction was 10 μL total using SsoAdvanced Universal SYBR Green Supermix (1725270, Bio-Rad) with 2 μL of template DNA. The same primers were used as in the amplicon sequencing (Table A5), with the 16S V3-V4 primers totalling a 200 nM concentration and the ITS1F primers totalling a 100 nM concentration. The same thermocycler setting was used as in Papp-Rupar et al. (2022) for both ITS1F and 16S V3-V4: denaturation for 5 min at 94°C and 40 cycles of 60 s at 94°C, 10 s at 75°C, 30 s at 55°C, and 60 s at 72°C, and ending with a melt curve from 55 to 95°C.

Sample efficiencies were corrected using the same technique as Papp-Rupar et al. (2022); as the gBlocks™ were supplied with a known quantity of DNA and molecular weight, the copy number present in the reconstituted synthetic genes was calculated to estimate the efficiencies. Samples with efficiencies below 0.7 and above 1.3 were repeated with lower dilutions to reduce the effect of PCR inhibitors present in samples.

Bioinformatics and statistical data analyses

Paired-end amplicon sequence data from Novogene were used to generate Amplicon Sequence Variants (ASVs) and counts tables. The meta-barcoding data is available on the European Nucleotide Archive under project accession PRJEB71862. Primer sequences were removed from both filtered and unfiltered reads. Any read pairs with lengths less than 250 nucleotides in either of the reads, incorrect primer sequences, or adapters or primers within the sequence were removed. The remaining read pairs were then processed to produce reads for (1) ASV creation; and (2) frequency table creation. For generating ASVs, read pairs were merged with a maximum of five differences in the overlapping region for 16S and zero for ITS, and a minimum merged length of 400 (16S) and 185 (ITS). Reads were filtered for quality with a maximum expected error rate (Edgar & Flyvbjerg, 2015) of 0.5 (16S) and 0.1 (ITS). The UPARSE pipeline (v. 11.0; Edgar, 2013) was then used on the filtered reads to produce ASVs; the pipeline automatically identifies and removes any chimeral sequences. Taxonomic ranks were assigned to ASVs using the SINTAX algorithm (https://www.drive5.com/usearch/manual/sintax_algo.html), with bacterial sequences using the RDP Training Set 18 bacterial database (Cole et al., 2014) and fungal sequences using the Unite V8.3 database (Kõljalg et al., 2013). ASVs identified as originating from mitochondria or chloroplasts were removed before statistical analyses were performed.

In the bacterial samples, 12 samples were redacted (all samples from polytunnels 3 and 4 on 2 September 2021) from the analysis due to an experimental error. The bacterial and fungal abundances were normalized by the absolute (16S/ITS) biomass in each sample using the qPCR copy number. An ANOVA was performed to assess if fruit age, within-tunnel location, polytunnel location on the farm and sampling date affected the size of fungal and bacterial communities. The alpha diversity indices Chao1, Simpson, Shannon and Observed were calculated using the R package vegan v. 2.3-1 (Dixon, 2003). A permutation-based ANOVA was then performed on the rank of the diversity indices to determine the effects of sampling date, polytunnel, location within the tunnel and fruit age. The beta diversities were calculated as Bray–Curtis indices, then subjected to non-metric multidimensional scaling (NMDS) analysis with the R package vegan. Then, an ADONIS (a permutation MANOVA using *F*-tests based on the sequential sum of squares) was used to assess the importance of individual factors and their interactions in affecting Bray–Curtis indices. Principal components analysis (PCA) was also carried out to generate the first four PC scores, which were then subjected to ANOVA to determine the effects of individual factors and their interactions. A differential abundance analysis was used to assess the effects of within-tunnel location, fruit age and sampling date on the abundance of individual ASVs using the R package DESeq2 v. 1.34.0 (Love et al., 2014). In the differential analysis, we did not consider the three-way interactions between the three factors. *p* Values were adjusted using the Benjamini–Hochberg (BH) method (Benjamini & Hochberg, 1995) with the significance set at $p = 0.05$.

Fungal DNA and *Cladosporium* spores across time points and in polytunnels versus outdoors

Experimental design and sample collection

The effects of the time of day and the Rotorod sampler location (inside a polytunnel and in a neighbouring open field) on the concentration of *Cladosporium* airborne spores were investigated. Air samples were collected between 10 October 2022 and 31 October 2022 from the centre of a raspberry polytunnel at NIAB East Malling in Kent, England, and 100 m away from the tunnel in an open field. The polytunnels used were standard open-ended Spanish tunnels without side skirts. Samples were taken at the end of the growing season.

Rotorod air samplers (Lacey & West, 2006) were attached to a supported pole with rain covers attached at a height of 1.4 m, one inside the centremost pole of the polytunnel and one in the open field 100 m away. The Rotorod samplers were wired to an automatic timer system attached to a 12 V battery that was swapped for a freshly charged battery every 24 h to ensure the Rotorod samplers maintained maximum speed.

The air was sampled from 10 October 2022 to 21 October 2022 in four periods: 08:00–12:00 (morning), 12:00–16:00 (afternoon), 16:00–20:00 (evening) and 20:00–08:00 (nighttime), resulting in 83 samples (11 days × four time periods × two locations; excluding five samples where the Rotorod did not spin for the full sampling period). From 21 October 2022 to 31 October 2022, the air was sampled for two time periods: 08:00–20:00 (daytime) and 20:00–08:00 (nighttime), resulting in 40 samples (10 days × two time periods [daytime and nighttime] × two locations).

Before each sampling period, two plastic Rotorod arms (AS-10-04, Agri Samplers Ltd.) were placed into the Rotorod arm holders. The collecting edge was then coated with a thin film of Vaseline using a finger in a sterilized glove. The Rotorod was then run for the allotted time of collection, and the Rotorod arms were removed at the end of the designated sampling period with a pair of sterile tweezers. Both arms were placed into a single 2 mL screwcap tube and taken immediately to a −80°C freezer for storage.

DNA extraction and airborne spore estimation via qPCR

The MasterPure Yeast DNA Purification Kit (LGC Biosearch Technologies) was used to extract DNA from the Rotorod samples, where the Yeast Cell Lysis Solution was added to the screwcap tube containing the Rotorod arms. We used the same method as Fraaije et al. (2021), where 0.5 g of glass beads (0.425–0.600 mm) were added, and the tubes vortexed at maximum speed for 2 min before the MCP protein precipitation reagent was added. A standard curve was created using an isolate of *C. cladosporioides* (isolate #65 in Farwell et al., 2023); to generate the standard curve, DNA was extracted from 1 mL of a 5.7×10^7 spores/ml suspension of this specific strain and diluted four times in a 10-fold series dilution. These samples were included in each qPCR plate as standards (in triplicate). The slope and standard curve were calculated for each qPCR plate separately using these standards. For *Cladosporium* qPCRs the slopes ranged from −4.32 to −4.99 and the intercept ranged from 37.75 to 41.51. For ITS qPCRs, the slopes ranged from −3.70 to −4.22 and the intercept ranged from 25.46 to 31.43. In all qPCRs, the R^2 value was above 0.98. These curves were used to estimate ITS copy number and *Cladosporium* spore count in each sample using the following equation: spore number or copy number = 10^(Cq − Intercept)/(Slope).

Samples were then diluted 10× for the daytime-nighttime experiment and 5× for the 4-h and nighttime

experiment and ran with the same ITS1F primers and qPCR setup as detailed in Section 3.1.2. to estimate the overall fungal DNA present (Table A5). A pair of *Cladosporium* genus-specific primers (Zeng et al., 2006; Table A5) at a concentration of 400 nM were used to estimate the amount of *Cladosporium* spores in the samples. Thermocycler settings followed Zeng et al. (2006): denaturation set to 3 min at 95°C, 40 cycles of 95°C for 10 s and 68°C for 30 s, followed by a melt curve from 55 to 95°C.

Statistical analyses

To increase the number of samples in the daytime versus nighttime experiment, the estimated ITS copy number and *Cladosporium* spore counts from the 4-h periods collected from 10 October 2022 to 21 October 2022 were summed, and the 12-h nighttime estimates were included. Analyses were performed in R with the package glmmTMB v. 1.1.8 (Brooks et al., 2017). A Generalised Linear Mixed Model (GLMM) was fitted to the data to account for correlations between samples taken over time, with residuals assumed to follow a negative binomial distribution to account for overdispersion in the dataset. The model included the fixed effects of the Rotorod sampler location and the time of day. The day of sampling was treated as a random effect to account for temporal correlation. The significance of fixed effects was tested using a type 2 ANOVA. Post-hoc *z* tests were then performed with the R package emmeans v. 1.8.7. (Lenth, 2023) with *p*-values corrected by the Tukey tests for multiple comparisons. For the daytime 4-h samples, all data were expressed at the number of estimated spores per hour.

RESULTS

Raspberry fruit epiphytic microbiome

Microbial abundance (qPCR), overall sequencing and ASV generation

The abundance of epiphytic fungal microbiome (total fungal ITS copy numbers per 40 fruit) was significantly affected by sampling date, with higher ITS copy numbers found on fruit taken from 9 October 2021 than 2 October 2021 ($F(1) = 8.46$, $p < 0.01$; Figure A1). For the epiphytic bacterial microbiome abundance (total 16S copy numbers per 40 fruit) was significantly affected by fruit age, with significantly lower 16S copy numbers on ripening than ripe fruit ($F(2) = 3.45$, $p < 0.05$) and dates, with significantly higher 16S copy numbers on fruit collected on the 2 October 2021 than 9 October 2021 ($F(1) = 5.69$, $p < 0.05$; Figure A2).

For fungi, the number of raw reads per sample ranged from 47,360 to 112,216 (average reads = 88,089.6). Out of the total 227 fungal ASVs, the percentage that could be identified to the phylum, class, order, family, genus and species level with at least 80% confidence was 94.2%, 52.4%, 52.4%, 52.3%, 51.5% and 2.3%, respectively. The dominant phylum is ascomycetes across all fruit stages and within-tunnel locations (Figure 1A). The number of normalized reads per ASV ranged from 55.4 to 1,411,738, with an average of 18,903.5. The genus with the greatest abundance in the top 10 fungal ASVs was *Cladosporium*, accounting for 43.9% of the total number of fungal reads.

For bacteria, the number of raw reads per sample ranged from 55,492 to 103,520 (average reads = 85,933.3). Out of the total 679 bacterial ASVs, the percentage that could be identified to the phylum, class, order, family, and genus level with at least 80% confidence was 99.9%, 99.7%, 94.3%, 89.6% and 49.3%, respectively. As for fungi, the bacterial microbiome was dominated by ASVs from a single phylum—proteobacteria (Figure 1B). The number of normalized reads per ASV ranged from 34 to 601,765 (average = 4815.1). The *Erwinia* genus had the greatest abundance in the top 10 bacterial ASVs, accounting for 23.2% of the total number of bacterial reads.

For both fungal and bacterial sequences, rarefaction curves indicate that the sequencing depth was acceptable (Figure A3).

Alpha diversity of the raspberry fruit epiphytic microbiome

Fruit age significantly affected all fungal alpha diversity indices (Chao1, Simpson and Shannon; $p < 0.01$). Both the Shannon and Simpson indices were significantly higher on ripening and ripe fruit, indicating greater diversity on ripening and ripe fruit (Figure 2). Fruit age also significantly influenced the bacterial Shannon and Simpson diversity indices ($p < 0.05$); in contrast, higher diversity was observed on green fruit than on ripening and ripe fruit (Figure 3). Sampling date affected the Shannon and Simpson indices for fungi ($p < 0.01$) and a significant interaction was found between the polytunnel and the sampling date (Shannon $p < 0.01$, Simpson $p < 0.01$), with two of the four tunnels appearing to have lower diversity scores on the 9 October 2021 than the 2 October 2021. There was no significant effect of date on the bacterial diversity indices. Within-tunnel location only affected fungal species richness, with fruit from the outer edge having a higher number of ASVs than fruit from the centre (Chao1: $p < 0.05$). However, there was a significant interaction in the bacterial Shannon index between within-tunnel location and fruit age ($p < 0.05$): outer green fruit had a higher diversity than inner green

fruit, which was opposite to the diversity on the ripening and ripe fruit where the inner fruit has a higher diversity.

Beta diversity of the raspberry fruit epiphytic microbiome

Principal components analysis

For fungi, the first four principal components explained 18.7%, 12.1%, 9.1% and 6.4% of the total variance in the data, respectively (Figure A4A). The polytunnel location in the field accounted for the highest variance in the first four PCs, contributing 13.8% to the total variability (Table 1), particularly within PC3 and 4. Sampling date, fruit age and within-tunnel location also contributed 5.9%, 8.3% and 10.8% to the overall variability, respectively (Table 1).

For bacteria, the first four principal components explained 17.5%, 15.4%, 11.6% and 8.8% of the overall variance, respectively (Figure A4B). In contrast to fungi, fruit age was the most important factor driving epiphytic bacterial communities, accounting for 16.5% of the total variance (Table 1). Sampling date, polytunnel location within the field and within-tunnel location contributed 10.2%, 12.5% and 3.8%, respectively (Table 1).

Bray–Curtis indices

Samples were not distinctly separated for fungal composition, but there was a noticeable gradient of fruit age along the second dimension and a separation by within-tunnel locations (Figure 4A). ADONIS permutational analysis highlighted the importance of fruit age in shaping epiphytic fungal microbiomes. Fruit age explained 21.8% of the variation ($p < 0.001$), followed by sampling date (accounting for 14.2% of the variation, $p < 0.001$). Other factors contributed to the variation, but to a lesser extent. Polytunnel accounted for 7.4% ($p < 0.01$), and the within-tunnel location explained 2.4% ($p < 0.01$) of the variation in the

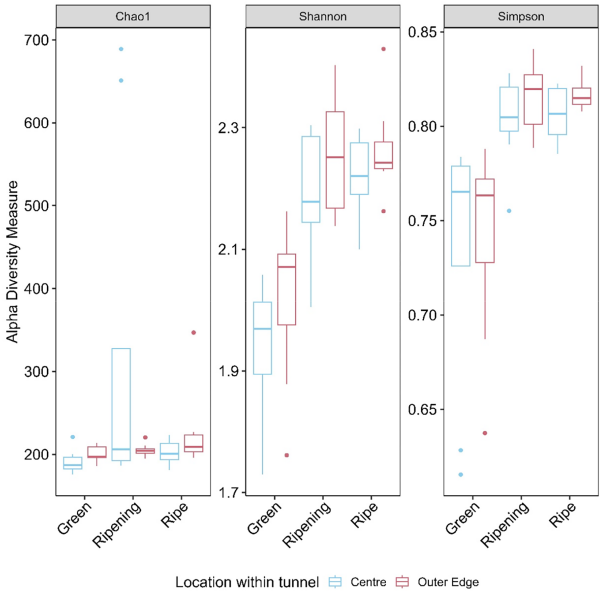


FIGURE 2 The alpha diversity measures for fungal Amplicon Sequence Variants (ASVs) of Chao1, Shannon and Simpson diversity indices. Four polytunnels were sampled across two repeats in time (2 October 2021 and 9 October 2021). The *x*-axis denotes fruit ripeness, and colour denotes location within the polytunnel.

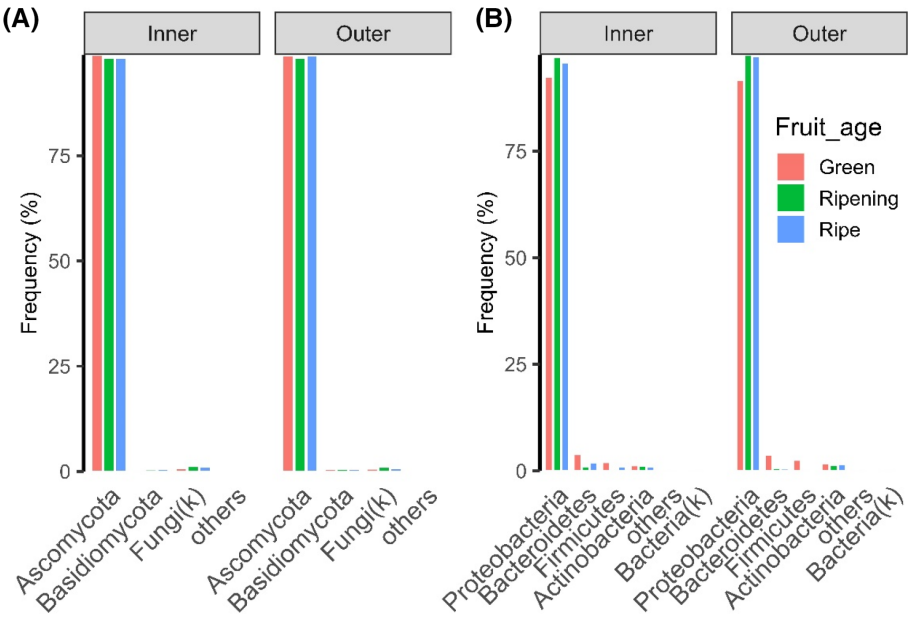


FIGURE 1 The percentage of (A) fungal and (B) bacterial ASVs assigned to each phylum at the 80% confidence level across fruit ages and within-tunnel locations.

Bray–Curtis indices (Table 1). None of the interaction terms were significant (Table 1).

In contrast to the fungal microbiome, the bacterial NMDS plot (Figure 4B) showed a clearer separation between sampling dates along the second dimension, explaining 5.3% of the variance ($p < 0.01$; Table 1). Fruit age accounted for 14.4% of the variability in the bacterial microbiome ($p < 0.001$), but there was no significant difference between the two locations within a tunnel. None of the interaction terms were significant (Table 1). Polytunnel location greatly impacted on the bacterial microbiome, explaining 14.5% of the variability ($p < 0.05$).

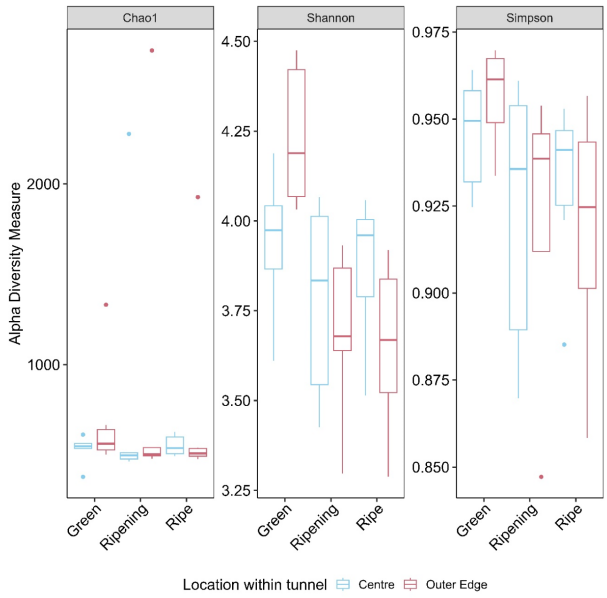


FIGURE 3 The alpha diversity measures for bacterial Amplicon Sequence Variants (ASVs) of Chao1, Shannon and Simpson diversity indices. Four polytunnels were sampled across two repeats in time (2 October 2021 and 9 October 2021). The *x*-axis denotes fruit ripeness, and the colour denotes location within the polytunnel.

Differential abundance analysis

A total of 58 fungal ASVs significantly differed among the fruit ripening stages (Table A1), 17 of which were identified at the species level. Of the 58 ASVs, 31 had a base mean larger than 20 (Table 2). Genera of interest include *Cladosporium*, which had a significantly higher abundance of green fruit than ripening and ripe fruit; the opposite was found for *Podosphaera* and *Epicoccum nigrum* (Table 2).

A total of 75 fungal ASVs significantly differed between the centre and the outer edge of tunnels (Table A2). Twenty-four ASVs had base means larger than 20 (Table 3), including three ASVs from *Cladosporium*, one *Botrytis* ASV and two *Alternaria* ASVs (Table 3), which were more abundant in the outer edge than in the central location.

Two hundred and eighty-seven bacterial ASVs differed in abundance among the three fruit ripening stages (Table A3). Among these ASVs, 205 could be identified at the genus level. A total of 86 of the 287 ASVs had a base mean greater than 100 (Table 4). Thirty-seven *Pseudomonas* ASVs were present, with some showing higher prevalence on green fruit and others on the ripening and ripe fruit (Table 4).

Forty bacterial ASVs had significant differences in abundance between the central location and the outer edge of the tunnels (Table A4). Only 16 of these ASVs had base means greater than 100. One *Bacillus* ASV was found to be more abundant in the centre of the tunnel, while two *Erwinia* ASVs exhibited higher levels on

TABLE 1 Percentage of variance accounted for by the factors date, polytunnel, fruit age and within-tunnel location and their interactions in the NMDS, and the associated *F* values for the first four principal components.

	Fungi					Bacteria				
		Principal components					Principal components			
	NMDS	PC1	PC2	PC3	PC4	NMDS	PC1	PC2	PC3	PC4
Date [A]	14.2***	52.26***	2.37	2.33	17.58***	5.3**	<0.01	18.87***	3.17	37.96***
Polytunnel [B]	7.4**	2.77	0.94	7.62	12.15*	14.5*	2.1	4.19	1.89	8.75
Fruit age [C]	21.8***	17.17***	1.2	0.87	14.68***	14.4***	13.83***	1.6	1.58	4.05*
Within-tunnel location [D]	2.4**	15.31*	1.21	18.03*	9.95	5.3	1.07	3.56	0.02	<0.01
[A] × [B]	3.3	1.21	0.42	4.51*	0.88	2.2	0.02	0.26	<0.01	0.05
[A] × [C]	4.2	0.93	0.47	2.66	0.19	2.3	0.93	0.22	0.18	0.4
[A] × [D]	1.9	0.4	1.06	1.51	0.77	1.4	0.02	0.65	0.01	0.42
[C] × [D]	2.4	3.43*	0.9	0.8	0.11	5.8	4.24*	2.6	0.16	0.42
[A] × [C] × [D]	2.1	1.94	0.63	0.67	0.02	3.2	<0.01	1.17	0.05	0.38
Residual	40.4					40.6				

*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

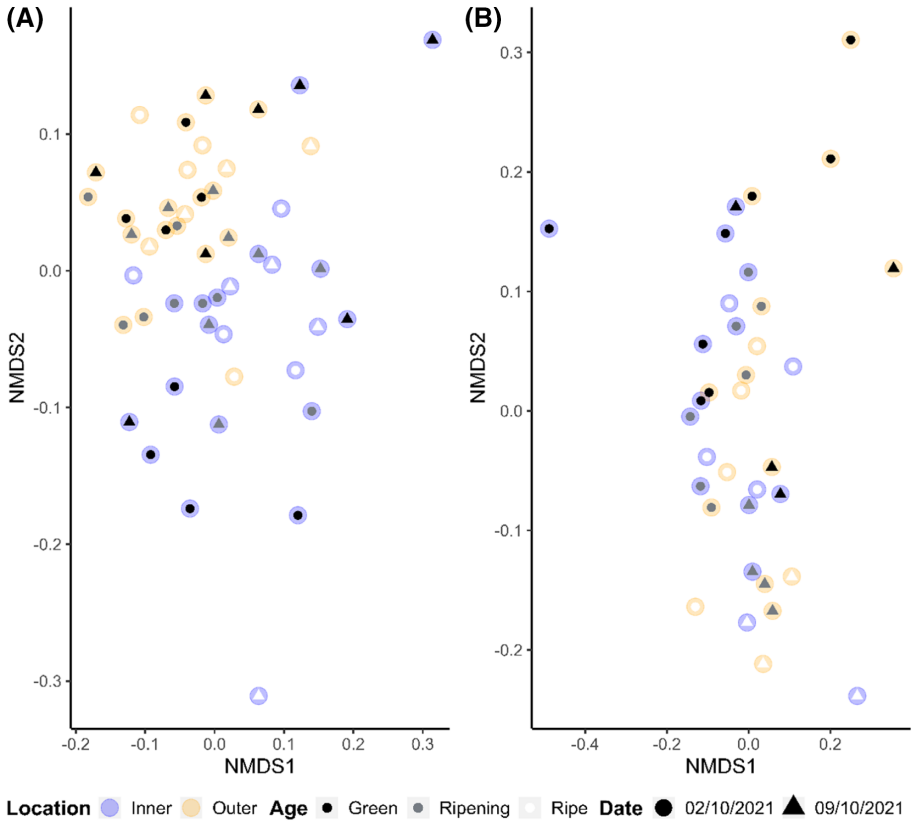


FIGURE 4 The first two dimensions of the NMDS analysis based on Bray–Curtis indices for (A) fungi and (B) bacteria from the surface of raspberries collected on two dates (2 October 2021 and 9 October 2021), from two locations within polytunnels (inner vs. outer) and across three ripening stages (green, ripening and ripe).

the outer edge (Table 5). Four *Pseudomonas* ASVs had higher abundance in the outer edge; the opposite was true for one other *Pseudomonas* ASV (Table 5).

Variations in airborne fungal inoculum

Estimated ITS copy numbers and *Cladosporium* spore loads during the daytime and nighttime

The factor 'time' significantly affected the estimated ITS copy number, with higher copy numbers in the nighttime samples ($\chi^2(1) = 4.90$, $p < 0.05$). No significant difference was found between the two Rotorod locations ($p = 0.23$). There was a significant interaction between 'time' and 'location' ($\chi^2(1) = 5.18$, $p < 0.05$), with higher copy numbers in the nighttime than in the daytime for the outside location only (Figure A5).

For *Cladosporium*, both 'time' ($\chi^2(1) = 67.12$, $p < 0.001$) and 'location' ($\chi^2(1) = 68.70$, $p < 0.001$) impacted spore concentrations. In contrast to the ITS copy number, a higher number of *Cladosporium* spores were trapped during the daytime than during the nighttime, and inside the tunnel than in an open field (Figure 5A). There was a significant interaction between 'time' and 'location' ($\chi^2(1) = 5.17$, $p < 0.05$), with more *Cladosporium* spores being trapped inside the polytunnel than compared to the open field across the day and night (Figure 5B).

Estimated ITS copy numbers and *Cladosporium* spore loads and across time periods

The estimated ITS copy numbers were significantly affected by the location of the Rotorod sampler ($\chi^2(1) = 6.97$, $p < 0.01$); more ITS copies were found in the inside polytunnel samples (Figure A6). The omnibus test (ANOVA) indicated the interaction between the Rotorod location and time of day was close to significant ($\chi^2(2) = 5.87$, $p = 0.12$); post hoc tests revealed that there were significantly less fungal (ITS) spores outside the tunnel than inside the tunnel in the afternoon ($z = 3.21$, $p < 0.01$) (Figure A6).

Estimated *Cladosporium* spore counts differed among the four sampling periods of a given day ($\chi^2(3) = 83.72$, $p < 0.001$) and the Rotorod sampler location ($\chi^2(1) = 77.70$, $p < 0.001$), but no significant interaction was found between the two factors ($p = 0.14$). Similar to the ITS copy numbers, more *Cladosporium* spores were found inside the polytunnel than in the outdoor air (Figure 6). The number of *Cladosporium* spores was

TABLE 2 Fungal ASVs significantly differed ($p < 0.05$, BH adjusted) across raspberry fruit ages with a base mean read count of 20 or above.

Factor comparison	ASV ID	Taxonomy	Base mean read count	Log_2 fold change
Green vs. Ripening	ASV57	*Ascomycota* (p)	27.56	0.91
	ASV1	*Cladosporium* (g)	29,411.20	0.56
	ASV2	*Ascomycota* (p)	23,509.46	−0.51
	ASV3	*Ascomycota* (p)	6959.27	−0.57
	ASV5	*Ascomycota* (p)	3877.30	−0.57
	ASV17	*Epicoccum nigrum* (s)	269.26	−0.66
	ASV32	*Ascomycota* (p)	42.66	−0.80
	ASV39	*Hypocreales* (o)	33.68	−0.80
	ASV40	*Hypocreales* (o)	31.10	−0.83
	ASV55	*Pithomyces chartarum* (s)	27.49	−0.84
	ASV46	*Vishniacozyma carnescens* (s)	27.67	−0.87
	ASV54	*Epicoccum nigrum* (s)	28.97	−0.91
	ASV58	*Sarocladium strictum* (s)	20.57	−0.93
	ASV37	*Vishniacozyma victoriae* (s)	42.90	−0.95
	ASV20	*Fungi* (k)	199.86	−1.02
	ASV18	*Fungi* (k)	246.23	−1.08
	ASV22	*Wallemia sebi* (s)	99.72	−1.12
	ASV24	*Fungi* (k)	84.37	−1.14
	ASV48	*Podosphaera* (g)	28.47	−1.40
Green vs. Ripe	ASV57	*Ascomycota* (p)	27.56	1.10
	ASV1	*Cladosporium* (g)	29,411.20	0.50
	ASV40	*Hypocreales* (o)	31.10	−0.65
	ASV39	*Hypocreales* (o)	33.68	−0.82
	ASV58	*Sarocladium strictum* (s)	20.57	−1.12
	ASV48	*Podosphaera* (g)	28.47	−1.40
Ripening vs. Ripe	ASV54	*Epicoccum nigrum* (s)	28.97	0.95
	ASV22	*Wallemia sebi* (s)	99.72	0.93
	ASV24	*Fungi* (k)	84.37	0.91
	ASV20	*Fungi* (k)	199.86	0.82
	ASV18	*Fungi* (k)	246.23	0.71
	ASV31	*Entyloma dahlia* (s)	79.64	−1.42

Note: A positive log_2 fold change indicates a higher relative abundance in the factor listed first in the comparison. (k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family, (g) denotes genus and (s) denotes species.

higher in the morning, afternoon and evening than the nighttime period (Morning vs. Nighttime, $z = 7.26$, $p < 0.001$), (Afternoon vs. Nighttime, $z = 8.34$, $p < 0.001$), (Evening vs. Nighttime, $z = 5.56$, $p < 0.001$) and higher in the afternoon than evening ($z = 2.73$, $p < 0.001$; Figure 6).

DISCUSSION

The present results demonstrate that as raspberries ripen, there is a notable shift in microbial communities on the fruit surface, with a decrease in bacterial diversity and an increase in fungal diversity. The presence of microbial genera, such as *Cladosporium*, *Pseudomonas*, *Epiccocum*, *Rouxiella* and *Bacillus*, varied with fruit development stages. Fruit epiphytes varied with the location of sampled polytunnels and sampling dates but were not much affected by the within-tunnel location (centre vs. the outer edge). Polytunnel cover has impacted airborne *Cladosporium* inoculum concentrations and total fungal community size: higher inside the polytunnel than in the open field. There was also a diurnal pattern of trapped *Cladosporium* spores, with more spores trapped during the daytime.

Raspberry fruit epiphytic microbiome

As the fruit ripened, the bacterial diversity on the fruit surface decreased, whereas the fungal diversity

TABLE 3 Fungal ASVs that significantly differed ($p < 0.05$, BH adjusted) across locations within a polytunnel with a base mean read count of 20 or above.

ASV ID	Taxonomy	Base mean read count	Log_2 fold change
ASV48	*Podosphaera* (g)	28.47	1.31
ASV53	*Neoerysiphe galeopsidis* (s)	30.95	1.3
ASV74	*Corynespora cassiicola* (s)	27.79	1.24
ASV80	*Didymellaceae* (f)	25.11	1.08
ASV24	*Fungi* (k)	84.37	0.52
ASV20	*Fungi* (k)	199.9	0.33
ASV13	*Botrytis* (g)	466.8	−0.39
ASV7	*Cladosporium ramotenellum* (s)	2746	−0.45
ASV15	*Cladosporium* (g)	255.1	−0.55
ASV44	*Mycosphaerellaceae* (f)	30.24	−0.55
ASV58	*Sarocladium strictum* (s)	20.57	−0.58
ASV29	*Golovinomyces orontii* (s)	78.16	−0.6
ASV42	*Sarocladium kiliense* (s)	39.68	−0.89
ASV11	*Cladosporium iridis* (s)	610.8	−1
ASV37	*Vishniacozyma victoriae* (s)	42.9	−1.04
ASV35	*Pichia terricola* (s)	30	−1.16
ASV46	*Vishniacozyma carnescens* (s)	27.67	−1.23
ASV28	*Pichia terricola* (s)	44.68	−1.27
ASV45	*Sporidiobolus* (g)	29.79	−1.27
ASV69	*Alternaria* (g)	23.01	−1.41
ASV17	*Epicoccum nigrum* (s)	269.3	−1.46
ASV54	*Epicoccum nigrum* (s)	28.97	−1.52
ASV36	*Alternaria* (g)	96.73	−1.67
ASV55	*Pithomyces chartarum* (s)	27.49	−1.95

Note: A positive log_2 fold change indicates a higher relative abundance in the centre of the polytunnel than in the outer edge. (k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family, (g) denotes genus and (s) denotes species.

increased. Previous research on the raspberry epicarp found fungal phylotypes were more diverse in the middle ripening stages than on ripe fruit (Jones et al., 2022). However, they sampled each ripening stage on different dates; thus, the fruit age effect was confounded with the sampling dates, which was shown to have significant effects on fruit epiphyte communities in the present study. There are conflicting findings on the compositions of microbiomes on the surface of ripening fruit. For instance, one study found a higher bacterial microbial diversity on ripe strawberries, but no difference in the fungal diversity (Olimi et al., 2022). However, another study found a significant difference in the fungal community between immature and mature strawberries (Abdelfattah et al., 2016). The results from all these studies highlight the need for repeats of these experiments to elucidate what factors interact with fruit ripening to alter fruit microbial communities. The microbiome from the skin of blueberries was significantly different in composition and species diversity to the fruit pulp (Szymanski et al., 2023), further indicating that more research is required on the epiphytic communities of fruits, where many diseases enter. Investigating if the endophytic microbial communities display a similar dynamic pattern as has been found in the present study is needed. The contrast in the dynamic of bacterial and fungal alpha diversity indices during fruit ripening may have resulted from multiple causes. On the fruit surface, fungi may have more opportunities to colonize and survive than bacteria due to dispersal via airborne spores. Previous research on *C. cladosporioides* found that it could cause skin lesions at the ripening and ripe stages of development and not on green fruit, perhaps due to the fruit being less firm and more prone to abrasions allowing *Cladosporium* to colonize (Farwell et al., 2023); similar effects may have occurred with other fungal genera in this study. Bacteria may have less opportunity to disperse under polythene as there are no rain splashes to disperse bacteria (although some water from condensation did drip from the polytunnel roof onto plants; these areas were infrequent and not sampled from). It is feasible that under polythene, bacteria are more likely to colonize the plant surface from earlier stages of development. This transfer of microorganisms as the plant develops was demonstrated to occur in strawberries (Olimi et al., 2022). It

TABLE 4 Bacterial ASVs that significantly differed ($p < 0.05$, BH adjusted) across raspberry fruit ages with a base mean read count of 100 or above.

Factor comparison	ASV ID	Taxonomy	Base mean read count	Log_2 fold change
Green vs. Ripening	ASV80	*Pedobacter* (g)	108.5	4.6
	ASV40	*Janthinobacterium* (g)	315.1	3.49
	ASV95	*Sphingobacterium* (g)	100.5	3.31
	ASV61	*Paenibacillus* (g)	143.9	3.23
	ASV47	*Paenibacillus* (g)	231.1	3.22
	ASV49	*Pseudomonas* (g)	199	3.04
	ASV54	*Pedobacter* (g)	212.98	2.83
	ASV43	*Pseudomonas* (g)	202.8	2.32
	ASV74	*Bacillus* (g)	205.1	2.24
	ASV38	*Enterobacterales* (o)	258.2	1.72
	ASV32	*Ewingella* (g)	421.7	1.67
	ASV36	*Pseudomonas* (g)	516.8	−0.79
	ASV8	*Pseudomonas* (g)	3286	−0.83
	ASV29	*Pseudomonas* (g)	493.1	−1.11
	ASV108	*Pseudomonas* (g)	114.5	−1.12
	ASV800	*Pseudomonadaceae* (f)	103.9	−1.31
	ASV31	*Pseudomonas* (g)	562.1	−1.46
	ASV806	*Gammaproteobacteria* (c)	104.8	−1.49
	ASV2	*Pseudomonas* (g)	5081	−1.56
	ASV21	*Rouxiella* (g)	655.1	−1.71
	ASV24	*Rouxiella* (g)	686.3	−2.13
	ASV48	*Rouxiella* (g)	279	−3.24
Green vs. Ripe	ASV80	*Pedobacter* (g)	108.5	6.75
	ASV54	*Pedobacter* (g)	213	4.92
	ASV62	*Janthinobacterium* (g)	145.7	4.72
	ASV95	*Sphingobacterium* (g)	100.5	4.58
	ASV77	*Pseudomonas* (g)	110.9	3.9
	ASV74	*Bacillus* (g)	205.1	3.24
	ASV40	*Janthinobacterium* (g)	315.1	2.8
	ASV43	*Pseudomonas* (g)	202.8	2.56
	ASV49	*Pseudomonas* (g)	199	2.34
	ASV41	*Pseudomonas* (g)	293.7	2.27
	ASV42	*Brucella* (g)	151.8	2.06
	ASV71	*Pseudomonas* (g)	111.5	1.98
	ASV15	*Stenotrophomonas* (g)	1055	1.96
	ASV52	*Pseudomonas* (g)	298.7	1.84
	ASV12	*Pseudomonas* (g)	2272	1.7
	ASV38	*Enterobacterales* (o)	258.2	1.7
	ASV64	*Pseudomonas* (g)	195.5	1.66
	ASV32	*Ewingella* (g)	421.7	1.56
	ASV743	*Pseudomonas* (g)	106.9	1.48
	ASV629	*Pseudomonas* (g)	124.5	1.47
	ASV18	*Luteibacter* (g)	662.7	1.45
	ASV526	*Pseudomonas* (g)	157.1	1.45
	ASV619	*Pseudomonadales* (o)	114.2	1.26
	ASV63	*Pseudomonas* (g)	524.2	1.22

(Continues)

TABLE 4 (Continued)

Factor comparison	ASV ID	Taxonomy	Base mean read count	Log_2 fold change
	ASV3	*Pseudomonas* (g)	3831	1.21
	ASV113	*Pseudomonas* (g)	278.6	1.15
	ASV1207	*Gammaproteobacteria* (c)	165	0.9
	ASV55	*Pseudomonas* (g)	211.6	0.86
	ASV1230	*Gammaproteobacteria* (c)	207.2	0.85
	ASV22	*Pseudomonas* (g)	1015	−1.12
	ASV800	*Pseudomonadaceae* (f)	103.9	−1.15
	ASV2	*Pseudomonas* (g)	5081	−1.38
	ASV39	*Rhizobiaceae* (f)	175.1	−1.69
Ripening vs. Ripe	ASV48	*Rouxiella* (g)	279	3.4
	ASV89	*Pseudomonas* (g)	113.7	2.24
	ASV15	*Stenotrophomonas* (g)	1055	2.12
	ASV54	*Pedobacter* (g)	213	2.09
	ASV77	*Pseudomonas* (g)	110.9	2.08
	ASV927	*Gammaproteobacteria* (c)	129.3	1.77
	ASV743	*Pseudomonas* (g)	106.9	1.68
	ASV113	*Pseudomonas* (g)	278.6	1.55
	ASV42	*Brucella* (g)	151.8	1.4
	ASV17	*Yersiniaceae* (f)	1088	1.35
	ASV5	*Yersiniaceae* (f)	3013	1.34
	ASV699	*Gammaproteobacteria* (c)	188.1	1.29
	ASV6	*Pseudomonas* (g)	2987	1.27
	ASV747	*Gammaproteobacteria* (c)	300.9	1.23
	ASV63	*Pseudomonas* (g)	524.2	1.21
	ASV8	*Pseudomonas* (g)	3286	1.14
	ASV619	*Pseudomonadales* (o)	114.2	1.14
	ASV593	*Pseudomonas* (g)	143.9	1.1
	ASV772	*Gammaproteobacteria* (c)	144.8	1.08
	ASV29	*Pseudomonas* (g)	493.1	1.06
	ASV55	*Pseudomonas* (g)	211.6	1.05
	ASV108	*Pseudomonas* (g)	114.5	1.05
	ASV762	*Gammaproteobacteria* (c)	173.4	1.03
	ASV879	*Gammaproteobacteria* (c)	229.5	1
	ASV23	*Rahnella* (g)	735.4	0.92
	ASV1207	*Gammaproteobacteria* (c)	165	0.83
	ASV36	*Pseudomonas* (g)	516.8	0.82
	ASV1230	*Gammaproteobacteria* (c)	207.2	0.79
	ASV25	*Microbacteriaceae* (f)	693.7	0.72
	ASV47	*Paenibacillus* (g)	231.1	−1.87
	ASV61	*Paenibacillus* (g)	143.9	−2.24

Note: A positive log2 fold change indicates a higher relative abundance in the factor listed first. (k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family and (g) denotes genus.

may therefore be necessary to investigate if a comparable pattern arises in other horticultural crops grown under polythene protection. Fungi may also be better adapted to surviving on the exposed fruit surface over time than bacterial species. For instance, many fungal species have thick spore coats to aid in spore survival during dispersal. The nutrient and water availability during ripening or surface abrasions may also provide more niches suitable for fungi than bacteria.

Cladosporium spp. were more abundant on green fruit than on ripening and ripe fruit. As *Cladosporium* can be abundant in the airborne inoculum

TABLE 5 Bacterial ASVs that significantly differed ($p < 0.05$, BH adjusted) across locations within a polytunnel with a base mean read count of 100 or above.

ASV ID	Taxonomy	Base mean read count	Log_2 fold change
ASV54	*Pedobacter* (g)	213	1.94
ASV74	*Bacillus* (g)	205.1	1.47
ASV33	*Pseudomonas* (g)	314.3	1.46
ASV67	*Erwiniaceae* (f)	127.1	1.17
ASV18	*Luteibacter* (g)	662.7	0.96
ASV25	*Microbacteriaceae* (f)	693.7	−0.89
ASV339	*Pseudomonas* (g)	129.4	−1.14
ASV800	*Pseudomonadaceae* (f)	103.9	−1.15
ASV56	*Erwiniaceae* (f)	332.4	−1.18
ASV2	*Pseudomonas* (g)	5081	−1.32
ASV20	*Pantoea* (g)	960.6	−1.38
ASV57	*Pantoea* (g)	300.6	−1.54
ASV89	*Pseudomonas* (g)	113.7	−1.62
ASV46	*Erwiniaceae* (f)	279.3	−1.7
ASV66	*Erwiniaceae* (f)	116.6	−1.74
ASV44	*Pseudomonas* (g)	262.4	−2

Note: A positive log_2 fold change indicates a higher relative abundance in the centre of the polytunnel vs. the outer edge. (k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family and (g) denotes genus.

(as demonstrated by the Rotorod sampling data), it is perhaps surprising that *Cladosporium* was not more abundant on ripe fruit as more spores could land on the fruit surface over time. However, *Cladosporium* lesions develop more readily when the fruit surface has been damaged (Swett et al., 2019), hence if fruit surface abrasions are minimal *Cladosporium* may not establish or could be quickly outcompeted. Despite the high inoculum load on the fruit surface, *Cladosporium* development on ripe fruit in the studied tunnels was very limited, indicating the importance of avoiding fruit wounding to reduce *Cladosporium* development. *Drosophila suzukii* can cause wounds on the fruit surface during ovipositioning, providing entry sites and nutritionally facilitating fungal infections such as *Cladosporium* (Swett et al., 2019). At the site sampled, recommended commercial pest and disease management were adopted to control pests (including *D. suzukii*) and diseases. Other organisms may have outcompeted or inhibited *Cladosporium* in the later stages of fruit development when there was limited fruit wounding. Further research is needed to study the survival of *Cladosporium* and its competition with other common fungal groups on the fruit surface.

Many bacterial and fungal epiphytic genera varied with fruit ages in their abundance. Several *Pseudomonas* ASVs were more prevalent earlier in fruit ripening and others later. *Pseudomonas* species have varying roles, with some *P. syringae* strains pathogenic to raspberry (Ivanović et al., 2023) and *P. fluorescens* as a biological control agent against *Didymella applanate* (spur blight) on raspberry (Shternshis et al., 2016). *Epiccocum* and *Rouxiella* spp. were more prevalent at the later fruit development stages, and *Bacillus* more prevalent on green fruit. All these genera contain species with strains that have been tested as biocontrol organisms against raspberry pathogens (Christova & Slavov, 2021; Shternshis et al., 2016). Strains in the *Bacillus* genus have been frequently used in commercial biopesticide development on soft fruits. Of particular interest is the genus *Rouxiella*; an in vitro study showed that specific *Rouxiella* strains successfully controlled common pathogens of strawberries (Morales-Cedeño et al., 2021). This genus was more prevalent on the later fruit development stages (when *Cladosporium* was noted to decrease); hence, further investigations into the antagonism of this genus against *Cladosporium* may be warranted. The use of the 16S primers in this study meant bacteria could only be identified at the genus level. Future work with more species-specific primers may allow further investigations into the abundance of beneficial and pathogenic bacterial species to understand their importance for fruit health.

In addition to fruit age, sampling date and the polytunnel location were also important factors significantly impacting raspberry epiphytic microbial communities. The polytunnel location explained nearly twice as much of the variability in the bacterial microbiome when compared to the fungal microbiome. In contrast, the sampling date explained over double the variability in the fungal microbiome when compared to the bacterial microbiome. For both of the factors, we speculate that differences in the micro-climate and the surrounding

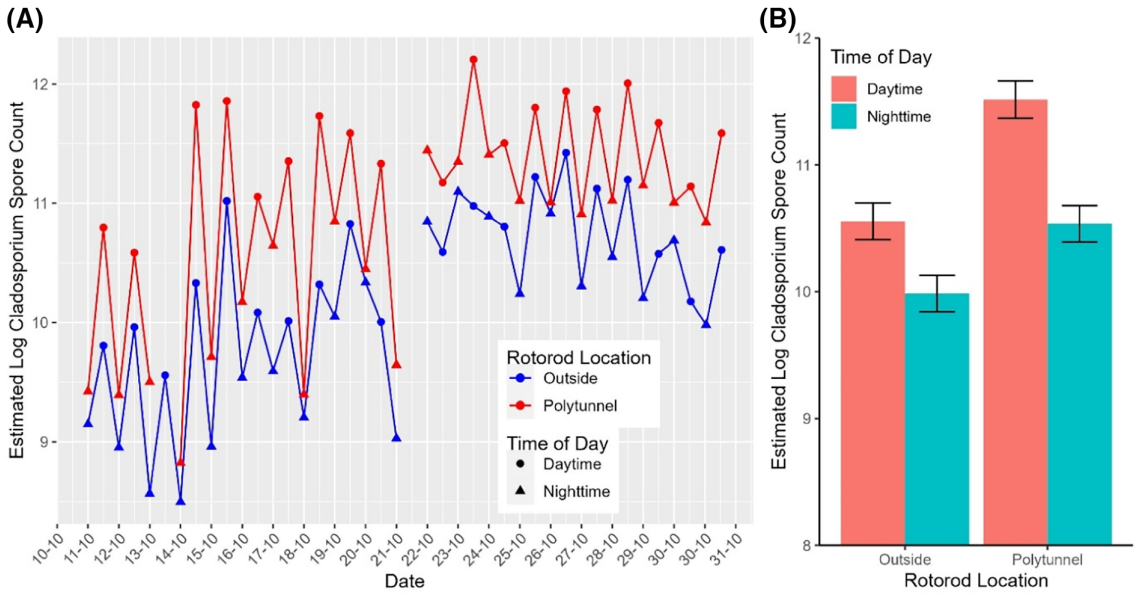


FIGURE 5 (A) The estimated Log *Cladosporium* spore count and (B) the estimated Log *Cladosporium* spore count averaged over location and time of day from spores collected using a Rotorod sampler from the centre of a polytunnel and in an open field over the day (08:00–20:00) and night (20:00–08:00) across 21 days in October 2022.

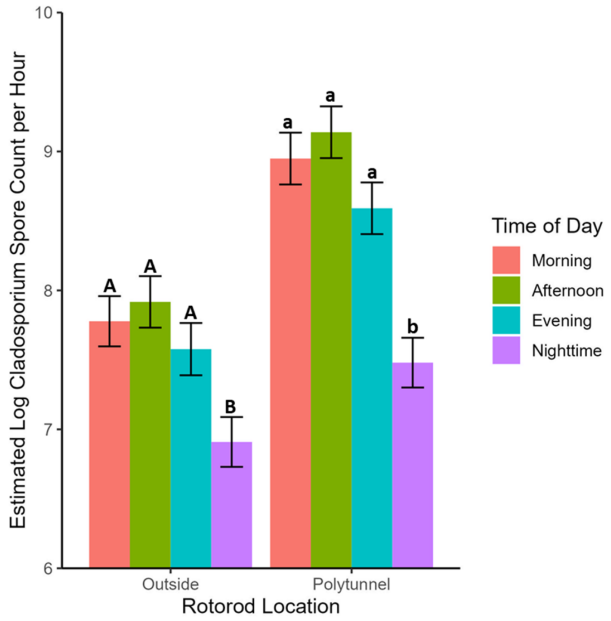


FIGURE 6 The estimated *Cladosporium* spore counts for samples collected from Rotorod samplers inside a polytunnel and in an open field across the day (morning 08:00–12:00, afternoon 12:00–16:00, evening 16:00–20:00) and the night (20:00–08:00) for 10 days. Letters indicate significant differences between factor levels ($p < 0.05$).

vegetation (including debris/decaying materials) contribute towards variation in microbes on the fruit surface. A larger effect of sampling time would have been observed had sampling spanned over a period longer than 1 week. It is also important to note that this study was only conducted across 1 year, and while enough samples were taken to ensure suitable replication, further research would benefit by sampling over multiple growing seasons. Larger scale distances on farms may facilitate greater climatic and vegetational differences that impact bacteria more than fungi, with fungi potentially being more evenly present due to their airborne spore movement. Geographical location significantly affects the microbiome of multiple fruit crops (Abdelfattah et al., 2021; Mezzasalma et al., 2018; Wicaksono et al., 2023). Therefore, future research on raspberry microbiomes may benefit from including more than one geographical location to understand how large an impact it has on the microbiome composition compared to other factors.

Of all the factors investigated, the one having the smallest effect on the fruit microbiome was the within-tunnel location, only explaining a small amount of the variability in the fungal microbiome. The architecture of raspberry fruits has many crevices that may allow transpiring water to collect around the fruit surface, perhaps creating a more stable microclimate around the fruits than other plant organs, partially explaining why within-tunnel location is of less importance than other factors. It is, however, important to note that during the sampling periods, there was far less difference in temperature and humidity between the two locations within the tunnel than in the early growing season (Figure A7 and Figure A8). Hence, more samples across the entire season may have resulted in large differences between locations within a tunnel. Nevertheless, there were significant differences in the abundances of several putative pathogenic and beneficial genera between the two within-tunnel locations. Three *Cladosporium* ASVs

were more frequent on the outer edge of the tunnels than in the centre, which could be due to more air movement at the open ends of the tunnel, indicating the importance of external sources for these microbes. It is also important to note that one end of the polytunnels faced a wheat field that had been harvested prior to sampling, and the decaying material left over may have provided a source for saprophytic fungi such as *Cladosporium* to thrive. Hedgerows were also present at the end of the tunnels. A meta-analysis found higher numbers of *D. suzukii* taking refuge in these non-cropping habitats (Buck et al., 2023), which may also contribute towards higher wounding of the fruits and thus, the differential abundance of epiphytes. The genus *Bacillus* was in higher abundance at the centre of the tunnel, potentially favouring warmer and more humid conditions.

Airborne fungal DNA and *Cladosporium* spores

The number of *Cladosporium* spores was higher within the polytunnel than in the open field conditions. For the total fungal community (estimated by the total number of ITS copy numbers), a similar trend was found in the hourly numbers across the day, but not in the day versus night samples. In the day versus night experiment, *Cladosporium* spores were higher in the day than night; the opposite being true for ITS. This is likely due to *Cladosporium* passively releasing spores which occurs with wind movement in drier air during the day than compared to other fungi which require higher humidities to actively disperse their spores. Potentially, the concentration of plant material combined with a polythene covering allowed spores to concentrate inside the polytunnel as opposed to dispersing with air movement. As sampling was performed later in the growing season, dead branches and fruit receptacles were abundant within the raspberry canopy, which may have provided substrate where *Cladosporium* could colonize and sporulate. Previous studies have found a negative correlation between relative humidity and the number of *Cladosporium* airborne spores (Ballero et al., 1992; Grinn-Gofroń & Strzelczak, 2013; Kurkela, 1997). In the present study, the average humidity was 10. 7% lower inside the polytunnel than outdoors (Figure A9), and the inside of the polytunnel was warmer by 0.3°C (Figure A10). During sampling, it was noted that dew formed on the grass in the open field, whereas less dew formed inside the polytunnel where the alleyways were mowed. The low humidity may have contributed towards spore release within the polytunnel but would reduce *Cladosporium* germination and mycelial growth. Future experiments need to investigate if venting practices alter the number of *Cladosporium* spores in the air. Reducing *Cladosporium* inoculum in the air may be important for reducing post-harvest development of *Cladosporium* if the spores are capable of surviving for extended periods on the fruit surface. Removing dead material within the canopy and the polytunnel will reduce the potential material for *Cladosporium* to colonize and sporulate, which may be another useful management strategy.

The amount of airborne fungal DNA, including *Cladosporium*, varied with sampling time within 24 h. The amount of fungal DNA was higher in the nighttime periods than in the day, but when sampled over the daytime, no difference was detected in the hourly average ITS copy number, which corroborates with previous research investigating the total fungal spore load in outdoor environments in Dublin, Ireland (O'Gorman & Fuller, 2008). In contrast, the average number of airborne *Cladosporium* spores was higher in samples taken during the day than the night, and with a higher number of spores in the morning and afternoon periods than in the nighttime period. This finding agrees with previous studies (Kurkela, 1997; O'Connor et al., 2014; Stephen et al., 1990). The spore number predictions used for allergen warnings may, therefore, also be useful to predict *Cladosporium* within agricultural environments, but need to take into account closed or semi-closed environments such as polytunnels. This experiment only covered the very end of the growing season. Hence, it warrants further investigation into the difference in spore numbers across the entire growing season.

Understanding how air movement impacts the risk of disease within polytunnels is complex. If fungal spores are concentrated within a polytunnel due to increased crop debris, air movement may allow for better dispersal of such spores to land and colonize new areas within the crop canopy. Polytunnels, however, are designed to aid air movement to help decrease the humidity within them. Future research should aim to understand how managing air movement within polytunnels affects the spore load within the air, and hence, how this relates to the pathogen load and disease development on the fruit.

Conclusions

The raspberry epiphytic microbiome was significantly affected by fruit age, particularly the fungal microbiome composition. During fruit ripening, fungal diversity increased while bacterial diversity decreased. The polytunnel location and the sampling date were also important, affecting both fungal and bacterial microbiome composition on the raspberry fruit surface. Within-tunnel location had a small effect on the composition of the raspberry epiphytic microbiome. *Cladosporium* was the most abundant fungal genera on the surface of raspberries and more prevalent on green

fruit and on fruit at the outer edge of tunnels. Polytunnels had a higher number of *Cladosporium* spores compared to an open field, but numbers still followed a diurnal pattern with higher spore counts during the day than at night.

AUTHOR CONTRIBUTIONS

Lauren Helen Farwell: Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; software; formal analysis; project administration; data curation; resources. **Matevz Papp-Rupar:** Writing – review and editing; methodology; supervision. **Greg Deakin:** Methodology; writing – review and editing; software; formal analysis; data curation; supervision; visualization. **Naresh Magan:** Conceptualization; supervision; methodology; funding acquisition. **Xiangming Xu:** Conceptualization; funding acquisition; writing – review and editing; supervision; methodology; visualization; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The meta-barcoding data that support the findings of this study are openly available in the European Nucleotide Archive, reference number PRJEB71862: https://www.ebi.ac.uk/ena/browser/view/PRJEB71862. The data used for the airborne spore experiments are accessible in the Supporting Information.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX

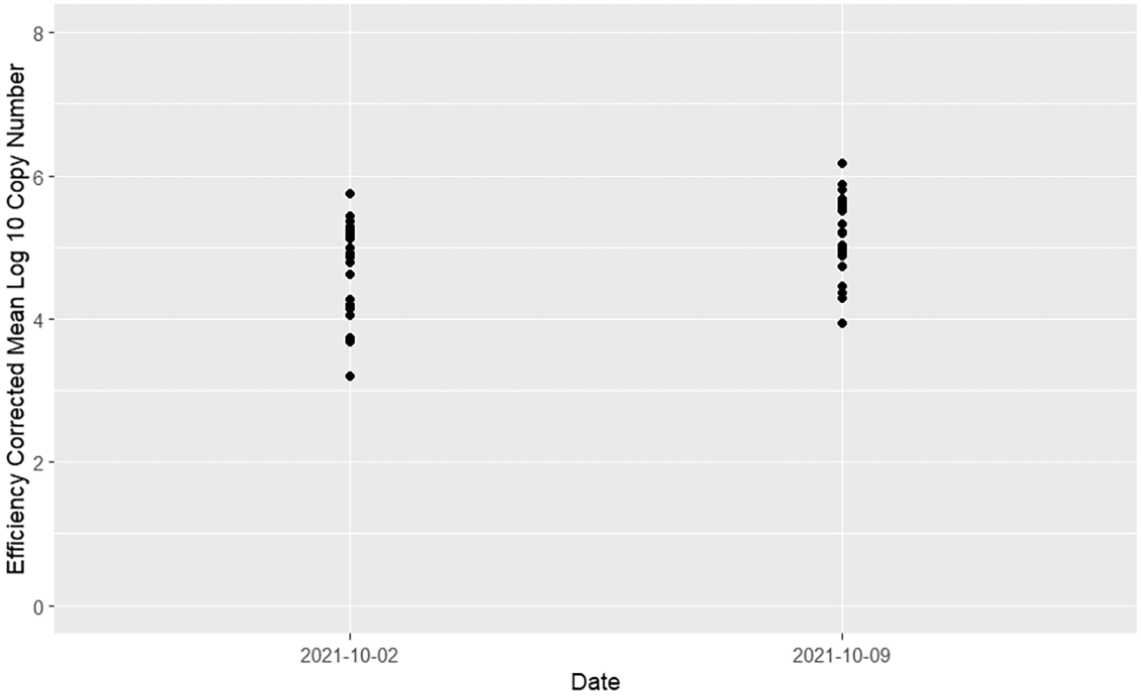


FIGURE A1 The efficiency corrected mean Log10 copy number quantitative PCR results for the ITS of fungal epiphytes on the surface of raspberries across the two sampling dates.

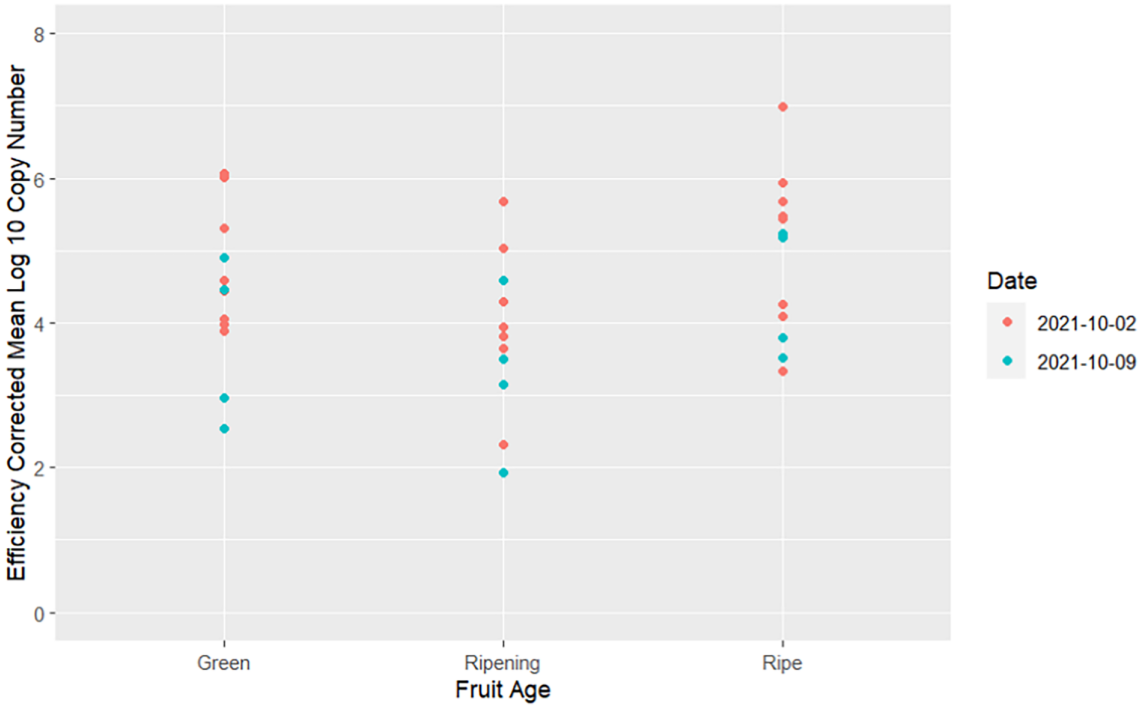


FIGURE A2 The efficiency corrected mean Log10 copy number quantitative PCR results for the 16S of bacterial epiphytes on the surface of raspberries across fruit ages and two sampling dates.

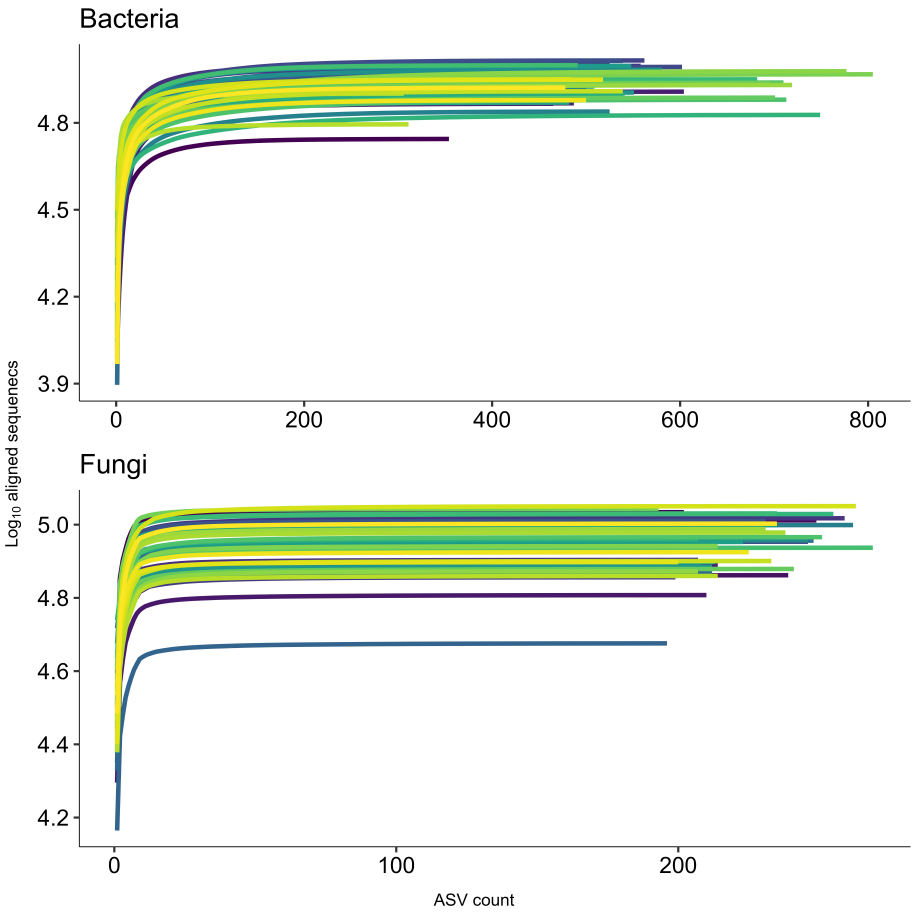


FIGURE A3 Rarefaction curves of $\log_{10}$ ASV counts of fungal and bacterial ASVs.

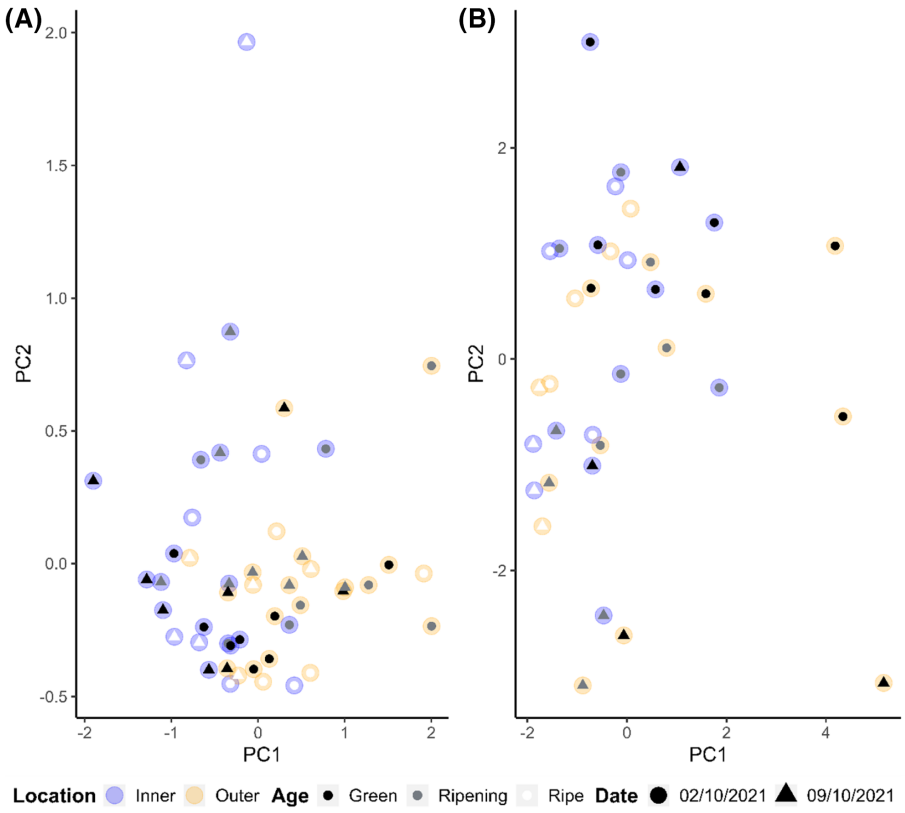


FIGURE A4 The first two principal components for the (A) fungal and (B) bacterial communities on raspberry fruits that were sampled at two time points (2 October 2021 and 9 October 2021), in two locations within polytunnels (centre vs. outer edge) and across three fruit development stages (green, ripening and ripe).

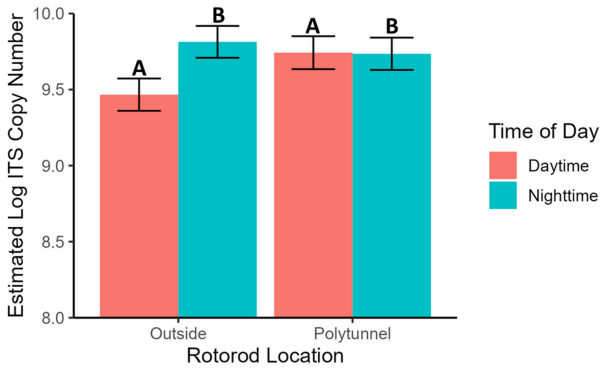


FIGURE A5 The estimated Log ITS copy number from airborne inoculum collected using a Rotorod sampler from the centre of a polytunnel and in an open field over the day (08:00–20:00) and night (20:00–08:00) across 21 days. Letters indicate significant differences between factor levels ($p < 0.05$).

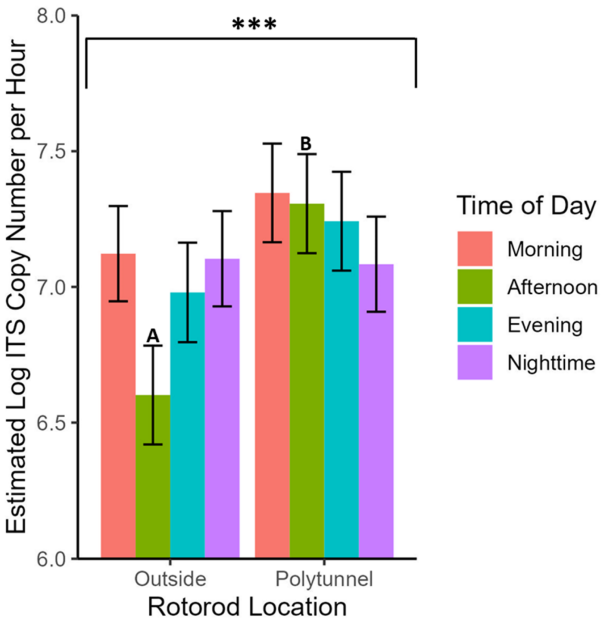


FIGURE A6 The estimated ITS copy number for samples collected from Rotorod samplers inside a polytunnel and in an open field across the day (morning 08:00–12:00, afternoon 12:00–16:00, evening 16:00–20:00) and the night (20:00–08:00) for 10 days. Letters indicate significant differences between factor levels ($p < 0.05$). The asterisk '***' indicates significant differences between factor levels ($p < 0.001$).

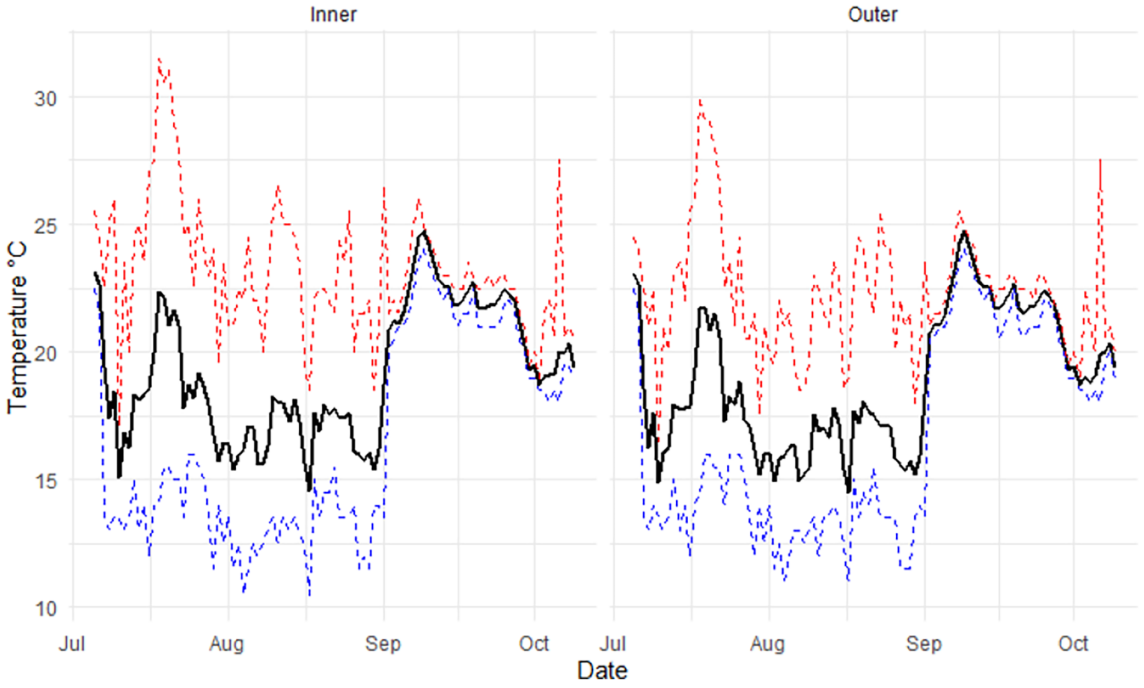


FIGURE A7 The average weekly temperature from the outer edge and centre of a polytunnel, the first samples were taken on 2 October 2021 and the second set of samples were taken on 10 October 2021.

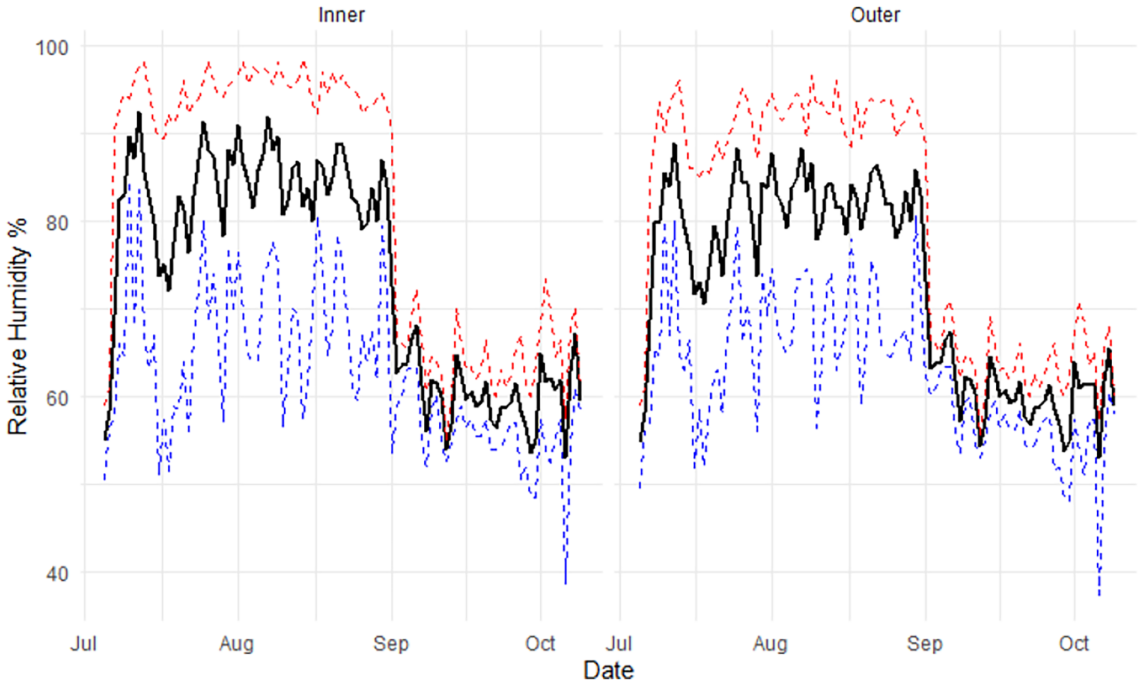


FIGURE A8 The average weekly humidity from the outer edge and centre of a polytunnel, the first samples were taken on 2 October 2021 and the second set of samples were taken on 10 October 2021.

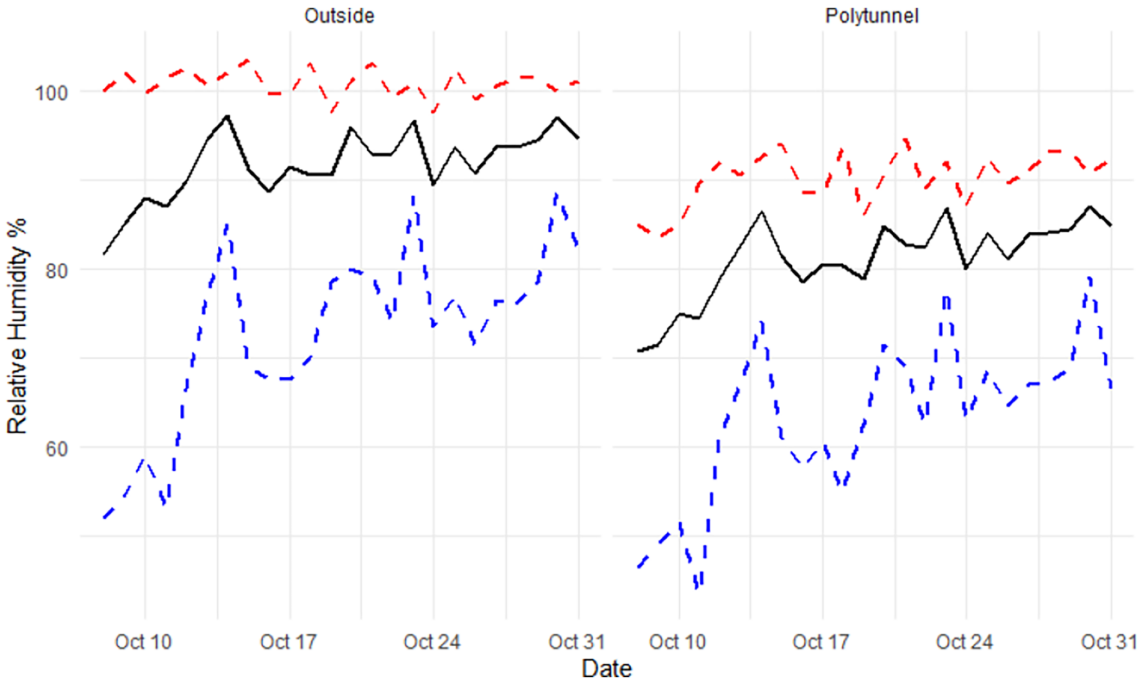


FIGURE A9 The average (black line), maximum (red line) and minimum (blue line) relative humidity from inside a polytunnel and in the outdoor air collected from dataloggers near the Rotorod samplers.

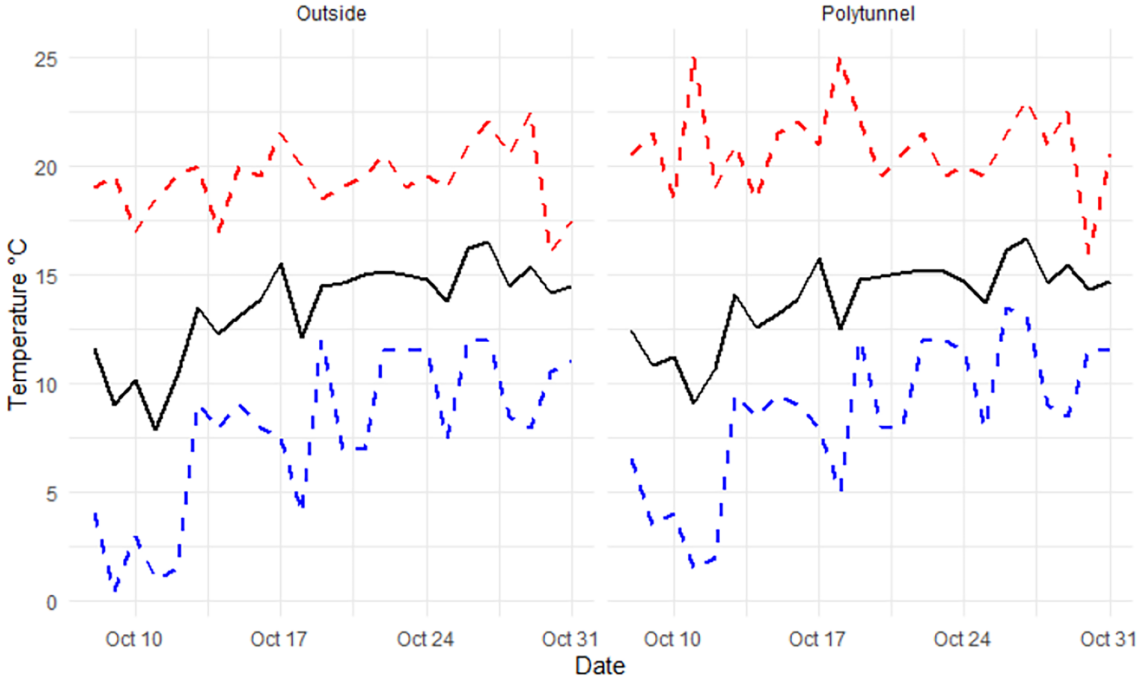


FIGURE A10 The average (black line), maximum (red line) and minimum (blue line) temperatures (°C) from inside a polytunnel and in the outdoor air collected from dataloggers near the Rotorod samplers.

TABLE A1 Fungal ASVs that significantly differ ($p < 0.05$, Benjamini–Hochberg [BH] adjusted) across raspberry fruit ages.

Factor comparison	ASV ID	Taxonomy	Base mean	Log_2 fold change
Green vs. Ripening	ASV121	*Basidiomycota* (p)	5.68	2.26
	ASV166	*Ascomycota* (p)	3.27	1.52
	ASV57	*Ascomycota* (p)	27.56	0.91
	ASV1	*Cladosporium* (g)	29,411	0.56
	ASV2	*Ascomycota* (p)	23,509	−0.51
	ASV3	*Ascomycota* (p)	6959	−0.57
	ASV5	*Ascomycota* (p)	3877	−0.57
	ASV51	*Ascomycota* (p)	19.63	−0.6
	ASV17	*Epicoccum nigrum* (s)	269.3	−0.66
	ASV56	*Ascomycota* (p)	17.78	−0.66
	ASV78	*Ascomycota* (p)	17.24	−0.75
	ASV32	*Ascomycota* (p)	42.66	−0.8
	ASV39	*Hypocreales* (o)	33.68	−0.8
	ASV40	*Hypocreales* (o)	31.1	−0.83
	ASV55	*Pithomyces chartarum* (s)	27.49	−0.84
	ASV46	*Vishniacozyma carnescens* (s)	27.67	−0.87
	ASV54	*Epicoccum nigrum* (s)	28.97	−0.91
	ASV58	*Sarocladium strictum* (s)	20.57	−0.93
	ASV37	*Vishniacozyma victoriae* (s)	42.9	−0.95
	ASV20	*Fungi* (k)	199.86	−1.02
	ASV18	*Fungi* (k)	246.23	−1.08
	ASV22	*Wallemia sebi* (s)	99.72	−1.12
	ASV24	*Fungi* (k)	84.37	−1.14
	ASV131	*Peniophora* (g)	4.44	−1.25
	ASV112	*Vishniacozyma victoriae* (s)	4.76	−1.37
	ASV48	*Podosphaera* (g)	28.47	−1.4
	ASV61	*Tremellales* (o)	15.04	−1.4
	ASV83	*Tremellomycetes* (c)	7.22	−1.56
	ASV153	*Nectriaceae* (f)	2.2	−1.65
	ASV110	*Vishniacozyma victoriae* (s)	5.12	−1.67
	ASV77	*Saccharomycetes* (c)	6.55	−1.68
	ASV143	*Dioszegia hungarica* (s)	1.69	−1.7
	ASV111	*Vishniacozyma tephrensis* (s)	4.56	−1.75
	ASV81	*Plectosphaerellaceae* (f)	8.45	−2.24
	ASV104	*Mucor mucedo* (s)	11.02	−2.87
Green vs. Ripe	ASV168	*Ascomycota* (p)	1.96	2.78
	ASV102	*Basidiomycota* (p)	8.84	2.44
	ASV121	*Basidiomycota* (p)	5.68	2.2
	ASV57	*Ascomycota* (p)	27.56	1.1
	ASV1	*Cladosporium* (g)	29,411.2	0.5
	ASV40	*Hypocreales* (o)	31.1	−0.65
	ASV39	*Hypocreales* (o)	33.68	−0.82
	ASV58	*Sarocladium strictum* (s)	20.57	−1.12
	ASV72	*Plectosphaerella* (g)	11.54	−1.26
	ASV48	*Podosphaera* (g)	28.47	−1.4
	ASV81	*Plectosphaerellaceae* (f)	8.45	−1.85
	ASV77	*Saccharomycetes* (c)	6.55	−2.1
	ASV119	*Plectosphaerella* (g)	3.52	−2.2
	ASV182	*Leotiomycetes* (c)	2.27	−3.77

TABLE A1 (Continued)

Factor comparison	ASV ID	Taxonomy	Base mean	Log_2 fold change
Ripening vs. Ripe	ASV104	*Mucor mucedo* (s)	11.02	2.81
	ASV54	*Epicoccum nigrum* (s)	28.97	0.95
	ASV22	*Wallemia sebi* (s)	99.72	0.93
	ASV24	*Fungi* (k)	84.37	0.91
	ASV20	*Fungi* (k)	199.86	0.82
	ASV51	*Ascomycota* (p)	19.63	0.81
	ASV18	*Fungi* (k)	246.2	0.71
	ASV31	*Entyloma dahlia* (s)	79.64	−1.42
	ASV119	*Plectosphaerella* (g)	3.52	−2.8

Note: A positive log_2 fold change indicates a higher relative abundance in the factor listed first. (k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family, (g) denotes genus and (s) denotes species.

TABLE A2 Fungal ASVs that significantly differ ($p < 0.05$, Benjamini–Hochberg [BH] adjusted) across locations within a polytunnel.

ASV ID	Taxonomy	Base mean	Log_2 fold change
ASV197	*Podosphaera* (g)	1.61	1.52
ASV63	*Bullera* (g)	14.35	1.5
ASV48	*Podosphaera* (g)	28.47	1.31
ASV53	*Neoerysiphe galeopsidis* (s)	30.95	1.3
ASV74	*Corynespora cassiicola* (s)	27.79	1.24
ASV80	*Didymellaceae* (f)	25.11	1.08
ASV97	*Penicillium adametzioides* (s)	11.84	0.68
ASV24	*Fungi* (k)	84.37	0.52
ASV20	*Fungi* (k)	199.9	0.33
ASV13	*Botrytis* (g)	466.8	−0.39
ASV7	*Cladosporium ramotenellum* (s)	2746	−0.45
ASV60	*Cladosporium* (g)	14.63	−0.54
ASV15	*Cladosporium* (g)	255.1	−0.55
ASV44	*Mycosphaerellaceae* (f)	30.24	−0.55
ASV58	*Sarocladium strictum* (s)	20.57	−0.58
ASV29	*Golovinomyces orontii* (s)	78.16	−0.6
ASV67	*Saccharomycetes* (c)	16.52	−0.69
ASV42	*Sarocladium kiliense* (s)	39.68	−0.89
ASV117	*Hypocreales* (o)	4.89	−0.89
ASV251	*Dissoconium aciculare* (s)	5.6	−0.9
ASV92	*Vishniacozyma victoriae* (s)	7.08	−0.91
ASV11	*Cladosporium iridis* (s)	610.8	−1
ASV99	*Ramularia collo-cygni* (s)	5.61	−1.02
ASV37	*Vishniacozyma victoriae* (s)	42.9	−1.04
ASV166	*Ascomycota* (p)	3.27	−1.06
ASV118	*Ascomycota* (p)	3.38	−1.13
ASV35	*Pichia terricola* (s)	30	−1.16
ASV114	*Sordariomycetes* (c)	3.59	−1.18
ASV132	*Podosphaera fuliginea* (s)	3.84	−1.18
ASV75	*Ramularia* (g)	10.28	−1.19
ASV111	*Vishniacozyma tephrensis* (s)	4.56	−1.19
ASV112	*Vishniacozyma victoriae* (s)	4.76	−1.21
ASV46	*Vishniacozyma carnescens* (s)	27.67	−1.23
ASV198	*Mycosphaerellaceae* (f)	2.66	−1.23
ASV28	*Pichia terricola* (s)	44.68	−1.27
ASV45	*Sporidiobolus* (g)	29.79	−1.27
ASV145	*Blumeria graminis* (s)	3.35	−1.27
ASV69	*Alternaria* (g)	23.01	−1.41
ASV17	*Epicoccum nigrum* (s)	269.3	−1.46
ASV124	*Golovinomyces* (g)	4.23	−1.48
ASV126	*Hypocreales* (o)	3.3	−1.48
ASV175	*Tremellomycetes* (c)	1.44	−1.51
ASV54	*Epicoccum nigrum* (s)	28.97	−1.52
ASV169	*Itersonilia perplexans* (s)	2.13	−1.54
ASV173	*Didymella* (g)	3.86	−1.56
ASV83	*Tremellomycetes* (c)	7.22	−1.57
ASV177	*Vishniacozyma victoriae* (s)	1.35	−1.63
ASV61	*Tremellales* (o)	15.04	−1.66

TABLE A2 (Continued)

ASV ID	Taxonomy	Base mean	Log_2 fold change
ASV85	*Vishniacozyma victoriae* (s)	8.89	−1.66
ASV190	*Sarocladium implicatum* (s)	1.77	−1.66
ASV36	*Alternaria* (g)	96.73	−1.67
ASV244	*Ramularia* (g)	3.25	−1.67
ASV259	*Peniophora* (g)	2.62	−1.69
ASV91	*Ascomycota* (p)	4.32	−1.74
ASV141	*Stemphylium vesicarium* (s)	3.03	−1.8
ASV161	*Mycosphaerellaceae* (f)	2.36	−1.8
ASV130	*Tremellomycetes* (c)	2.67	−1.83
ASV98	*Sporobolomyces ruberrimus* (s)	5.64	−1.89
ASV167	*Vishniacozyma victoriae* (s)	2.26	−1.91
ASV86	*Vishniacozyma victoriae* (s)	7.74	−1.93
ASV93	*Alternaria metachromatica* (s)	11.94	−1.93
ASV55	*Pithomyces chartarum* (s)	27.49	−1.95
ASV100	*Tremellales* (o)	4.32	−1.99
ASV157	*Saccharomycetales* (o)	2.1	−2.02
ASV184	*Fusarium cerealis* (s)	1.5	−2.04
ASV170	*Cystofilobasidium macerans* (s)	1.64	−2.13
ASV189	*Rhodotorula babjevae* (s)	1.98	−2.13
ASV108	*Vishniacozyma carnescens* (s)	4.89	−2.15
ASV115	*Tremellales* (o)	3.16	−2.27
ASV140	*Ascomycota* (p)	2.1	−2.28
ASV195	*Monilinia fructigena* (s)	1.19	−2.34
ASV201	*Vishniacozyma victoriae* (s)	1.37	−2.4
ASV104	*Mucor mucedo* (s)	11.02	−2.61
ASV136	*Dothideomycetes* (c)	2.22	−2.64
ASV77	*Saccharomycetes* (c)	6.55	−2.8

Note: A positive log_2 fold change indicates a higher relative abundance in the inside of the polytunnel vs. the outer edge. (k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family, (g) denotes genus and (s) denotes species.

TABLE A3 Bacterial ASVs that significantly differ ($p < 0.05$, Benjamini–Hochberg [BH] adjusted) across raspberry fruit ages.

Factor comparison	ASV ID	Taxonomy	Base mean	Log_2 fold change
Green vs. Ripening	ASV62	*Janthinobacterium* (g)	145.7	5.06
	ASV208	*Sphingobacterium* (g)	18.33	4.68
	ASV80	*Pedobacter* (g)	108.5	4.6
	ASV141	*Paenibacillus* (g)	40.31	4.17
	ASV158	*Janthinobacterium* (g)	25.47	3.98
	ASV409	*Sphingobacterium* (g)	8.36	3.95
	ASV368	*Janthinobacterium* (g)	6.52	3.8
	ASV213	*Paenibacillus* (g)	23.26	3.75
	ASV321	*Carnobacterium* (g)	9.03	3.59
	ASV258	*Janthinobacterium* (g)	12.75	3.55
	ASV40	*Janthinobacterium* (g)	315.1	3.49
	ASV306	*Sphingobacteriaceae* (f)	16.79	3.48
	ASV96	*Listeria* (g)	58.76	3.35
	ASV103	*Janthinobacterium* (g)	50.62	3.35
	ASV95	*Sphingobacterium* (g)	100.5	3.31
	ASV61	*Paenibacillus* (g)	143.9	3.23
	ASV47	*Paenibacillus* (g)	231.1	3.22
	ASV49	*Pseudomonas* (g)	199	3.04
	ASV301	*Pedobacter* (g)	13.06	3
	ASV352	*Pedobacter* (g)	7.71	3
	ASV319	*Pedobacter* (g)	8.78	2.96
	ASV452	*Janthinobacterium* (g)	6.1	2.91
	ASV345	*Sphingobacteriaceae* (f)	8.88	2.9
	ASV54	*Pedobacter* (g)	212.98	2.83
	ASV166	*Erwinia* (g)	27.34	2.77
	ASV322	*Chryseobacterium* (g)	9.87	2.76
	ASV125	*Pseudomonas* (g)	80.9	2.7
	ASV313	*Massilia* (g)	13.05	2.65
	ASV153	*Janthinobacterium* (g)	26.53	2.54
	ASV184	*Pedobacter* (g)	28.85	2.34
	ASV43	*Pseudomonas* (g)	202.8	2.32
	ASV76	*Pedobacter* (g)	77.41	2.25
	ASV74	*Bacillus* (g)	205.1	2.24
	ASV200	*Rhizobiales* (o)	12.43	2.19
	ASV259	*Comamonadaceae* (f)	9.46	2.19
	ASV239	*Bacillus* (g)	44.65	2.15
	ASV132	*Chryseobacterium* (g)	39.27	2.1
	ASV217	*Duganella* (g)	14.88	2.1
	ASV157	*Pedobacter* (g)	39.34	2
	ASV106	*Pedobacter* (g)	56.21	1.78
	ASV115	*Devosia* (g)	18.49	1.78
	ASV122	*Comamonadaceae* (f)	46.48	1.76
	ASV38	*Enterobacterales* (o)	258.2	1.72
	ASV32	*Ewingella* (g)	421.7	1.67
	ASV422	*Pseudomonas* (g)	19.45	1.55
	ASV150	*Duganella* (g)	30.42	1.42
	ASV924	*Pseudomonas* (g)	22	1.39
	ASV110	*Duganella* (g)	60.44	1.33

TABLE A3 (Continued)

Factor comparison	ASV ID	Taxonomy	Base mean	Log_2 fold change
	ASV136	*Duganella* (g)	43.95	1.3
	ASV787	*Erwiniaceae* (f)	42.56	1.04
	ASV1134	*Pseudomonas* (g)	24.15	1.03
	ASV36	*Pseudomonas* (g)	516.8	−0.79
	ASV8	*Pseudomonas* (g)	3286	−0.83
	ASV1081	*Pseudomonas* (g)	70.19	−0.87
	ASV365	*Pseudomonas* (g)	15.63	−1.08
	ASV29	*Pseudomonas* (g)	493.1	−1.11
	ASV108	*Pseudomonas* (g)	114.5	−1.12
	ASV800	*Pseudomonadaceae* (f)	103.9	−1.31
	ASV1115	*Yersiniaceae* (f)	84.51	−1.33
	ASV31	*Pseudomonas* (g)	562.1	−1.46
	ASV806	*Gammaproteobacteria* (c)	104.8	−1.49
	ASV2	*Pseudomonas* (g)	5081	−1.56
	ASV21	*Rouxiella* (g)	655.1	−1.71
	ASV165	*Phyllobacteriaceae* (f)	17.84	−1.9
	ASV1205	*Enterobacterales* (o)	47.15	−2.06
	ASV24	*Rouxiella* (g)	686.3	−2.13
	ASV130	*Pantoea* (g)	52.99	−2.15
	ASV195	*Yersiniaceae* (f)	26.55	−2.65
	ASV503	*Rickettsia* (g)	4.63	−3.2
	ASV48	*Rouxiella* (g)	279	−3.24
	ASV140	*Bacteria* (k)	11.01	−3.91
Green vs. Ripe	ASV280	*Bacteria* (k)	3.59	13.83
	ASV1045	*Pedobacter* (g)	2.21	10.55
	ASV80	*Pedobacter* (g)	108.5	6.75
	ASV141	*Paenibacillus* (g)	40.31	5.45
	ASV54	*Pedobacter* (g)	213	4.92
	ASV166	*Erwinia* (g)	27.34	4.87
	ASV96	*Listeria* (g)	58.76	4.8
	ASV62	*Janthinobacterium* (g)	145.7	4.72
	ASV306	*Sphingobacteriaceae* (f)	16.79	4.67
	ASV68	*Sphingobacterium* (g)	63.58	4.66
	ASV208	*Sphingobacterium* (g)	18.33	4.6
	ASV95	*Sphingobacterium* (g)	100.5	4.58
	ASV76	*Pedobacter* (g)	77.41	4.53
	ASV322	*Chryseobacterium* (g)	9.87	4.28
	ASV821	*Alkalihalobacillus* (g)	7.56	4.06
	ASV409	*Sphingobacterium* (g)	8.36	4.05
	ASV239	*Bacillus* (g)	44.65	4.03
	ASV189	*Buttiauxella* (g)	18.05	4.01
	ASV559	*Carnobacterium* (g)	4.6	3.99
	ASV158	*Janthinobacterium* (g)	25.47	3.94
	ASV258	*Janthinobacterium* (g)	12.75	3.93
	ASV103	*Janthinobacterium* (g)	50.62	3.92
	ASV77	*Pseudomonas* (g)	110.9	3.9
	ASV321	*Carnobacterium* (g)	9.03	3.85
	ASV171	*Sphingobacterium* (g)	20.33	3.79

(Continues)

TABLE A3 (Continued)

Factor comparison	ASV ID	Taxonomy	Base mean	Log_2 fold change
	ASV125	*Pseudomonas* (g)	80.9	3.56
	ASV232	*Erwinia* (g)	15.14	3.55
	ASV192	*Pedobacter* (g)	9.43	3.46
	ASV150	*Duganella* (g)	30.42	3.43
	ASV313	*Massilia* (g)	13.05	3.42
	ASV592	*Mucilaginibacter* (g)	2.61	3.39
	ASV184	*Pedobacter* (g)	28.85	3.33
	ASV555	*Massilia* (g)	4.42	3.31
	ASV666	*Pedobacter* (g)	8.17	3.31
	ASV301	*Pedobacter* (g)	13.06	3.29
	ASV74	*Bacillus* (g)	205.1	3.24
	ASV505	*Massilia* (g)	3.35	3.16
	ASV259	*Comamonadaceae* (f)	9.46	3.15
	ASV145	*Pedobacter* (g)	7.21	3.07
	ASV284	*Pedobacter* (g)	13.97	3.03
	ASV122	*Comamonadaceae* (f)	46.48	3
	ASV615	*Microbacteriaceae* (f)	2.54	2.96
	ASV620	*Microbacteriaceae* (f)	2.91	2.9
	ASV514	*Bacillaceae* (f)	5.46	2.89
	ASV452	*Janthinobacterium* (g)	6.1	2.88
	ASV722	*Pseudomonas* (g)	21.94	2.88
	ASV586	*Pedobacter* (g)	8.17	2.87
	ASV684	*Alkalihalobacillus* (g)	4.22	2.84
	ASV40	*Janthinobacterium* (g)	315.1	2.8
	ASV319	*Pedobacter* (g)	8.78	2.76
	ASV667	*Massilia* (g)	2.61	2.63
	ASV43	*Pseudomonas* (g)	202.8	2.56
	ASV157	*Pedobacter* (g)	39.34	2.39
	ASV950	*Stenotrophomonas* (g)	49.2	2.37
	ASV49	*Pseudomonas* (g)	199	2.34
	ASV41	*Pseudomonas* (g)	293.7	2.27
	ASV115	*Devosia* (g)	18.49	2.27
	ASV686	*Pseudomonas* (g)	8.73	2.21
	ASV193	*Enterobacterales* (o)	22.71	2.2
	ASV491	*Pseudomonas* (g)	7.45	2.19
	ASV179	*Pedobacter* (g)	12.83	2.18
	ASV760	*Erwinia* (g)	7.81	2.16
	ASV217	*Duganella* (g)	14.88	2.13
	ASV250	*Enterobacteriaceae* (f)	16.88	2.08
	ASV1214	*Enterobacterales* (o)	12.23	2.08
	ASV42	*Brucella* (g)	151.8	2.06
	ASV902	*Pseudomonas* (g)	3.17	2.06
	ASV71	*Pseudomonas* (g)	111.5	1.98
	ASV15	*Stenotrophomonas* (g)	1055	1.96
	ASV1134	*Pseudomonas* (g)	24.15	1.96
	ASV898	*Pseudomonadaceae* (f)	4.65	1.94
	ASV376	*Massilia* (g)	6.24	1.87

TABLE A3 (Continued)

Factor comparison	ASV ID	Taxonomy	Base mean	Log_2 fold change
	ASV136	*Duganella* (g)	43.95	1.85
	ASV52	*Pseudomonas* (g)	298.7	1.84
	ASV127	*Enterobacteriaceae* (f)	34.94	1.84
	ASV924	*Pseudomonas* (g)	22	1.84
	ASV93	*Pedobacter* (g)	59.94	1.83
	ASV456	*Pedobacter* (g)	9.11	1.82
	ASV780	*Stenotrophomonas* (g)	20.29	1.82
	ASV106	*Pedobacter* (g)	56.21	1.8
	ASV225	*Pigmentiphaga* (g)	12.68	1.77
	ASV1100	*Betaproteobacteria* (c)	45.62	1.75
	ASV12	*Pseudomonas* (g)	2272	1.7
	ASV38	*Enterobacterales* (o)	258.2	1.7
	ASV1070	*Pseudomonas* (g)	17.53	1.69
	ASV64	*Pseudomonas* (g)	195.5	1.66
	ASV422	*Pseudomonas* (g)	19.45	1.66
	ASV1063	*Pseudomonas* (g)	50.32	1.66
	ASV110	*Duganella* (g)	60.44	1.64
	ASV116	*Sanguibacter* (g)	49.5	1.63
	ASV275	*Sanguibacter* (g)	17.5	1.63
	ASV552	*Enterobacteriaceae* (f)	9.6	1.6
	ASV32	*Ewingella* (g)	421.7	1.56
	ASV773	*Gammaproteobacteria* (c)	20.4	1.49
	ASV743	*Pseudomonas* (g)	106.9	1.48
	ASV629	*Pseudomonas* (g)	124.5	1.47
	ASV18	*Luteibacter* (g)	662.7	1.45
	ASV526	*Pseudomonas* (g)	157.1	1.45
	ASV938	*Gammaproteobacteria* (c)	76.17	1.44
	ASV128	*Pseudomonas* (g)	42.6	1.4
	ASV873	*Gammaproteobacteria* (c)	27.99	1.33
	ASV744	*Pseudomonas* (g)	72.59	1.31
	ASV1033	*Xanthomonadales* (o)	83.13	1.3
	ASV619	*Pseudomonadales* (o)	114.2	1.26
	ASV1225	*Pseudomonas* (g)	49.01	1.23
	ASV63	*Pseudomonas* (g)	524.2	1.22
	ASV3	*Pseudomonas* (g)	3831	1.21
	ASV859	*Pseudomonas* (g)	21.16	1.2
	ASV830	*Pseudomonas* (g)	14.59	1.18
	ASV113	*Pseudomonas* (g)	278.6	1.15
	ASV727	*Pseudomonadales* (o)	72.6	1.13
	ASV750	*Xanthomonadales* (o)	47.94	1.13
	ASV1200	*Yersiniaceae* (f)	74.1	1.06
	ASV1193	*Pseudomonas* (g)	24.43	1
	ASV146	*Microbacteriaceae* (f)	34.96	0.97
	ASV892	*Pseudomonas* (g)	50.94	0.96
	ASV1207	*Gammaproteobacteria* (c)	165	0.9
	ASV787	*Erwiniaceae* (f)	42.56	0.87
	ASV55	*Pseudomonas* (g)	211.6	0.86
	ASV1084	*Yersiniaceae* (f)	85.98	0.85

(Continues)

TABLE A3 (Continued)

Factor comparison	ASV ID	Taxonomy	Base mean	Log_2 fold change
	ASV1230	*Gammaproteobacteria* (c)	207.2	0.85
	ASV1162	*Yersiniaceae* (f)	37.79	0.82
	ASV22	*Pseudomonas* (g)	1015	−1.12
	ASV800	*Pseudomonadaceae* (f)	103.9	−1.15
	ASV286	*Pseudomonas* (g)	24.7	−1.31
	ASV2	*Pseudomonas* (g)	5081	−1.38
	ASV70	*Rhizobium* (g)	67.71	−1.63
	ASV39	*Rhizobiaceae* (f)	175.1	−1.69
	ASV126	*Gluconobacter* (g)	26.33	−1.89
	ASV297	*Alphaproteobacteria* (c)	6.26	−2.1
	ASV87	*Pseudomonas* (g)	68.39	−2.22
	ASV263	*Enterobacterales* (o)	14.68	−2.3
	ASV214	*Bosea* (g)	11.78	−2.52
	ASV51	*Yersiniaceae* (f)	41.47	−3.51
Ripening vs. Ripe	ASV171	*Sphingobacterium* (g)	20.33	4.59
	ASV232	*Erwinia* (g)	15.14	4.38
	ASV48	*Rouxiella* (g)	279	3.4
	ASV68	*Sphingobacterium* (g)	63.58	3.3
	ASV102	*Erwinia* (g)	67.51	3.24
	ASV237	*Erwiniaceae* (f)	14.92	3.13
	ASV950	*Stenotrophomonas* (g)	49.2	3.1
	ASV780	*Stenotrophomonas* (g)	20.29	2.87
	ASV149	*Stenotrophomonas* (g)	19.03	2.65
	ASV195	*Yersiniaceae* (f)	26.55	2.4
	ASV666	*Pedobacter* (g)	8.17	2.34
	ASV192	*Pedobacter* (g)	9.43	2.33
	ASV76	*Pedobacter* (g)	77.41	2.28
	ASV773	*Gammaproteobacteria* (c)	20.4	2.25
	ASV89	*Pseudomonas* (g)	113.7	2.24
	ASV15	*Stenotrophomonas* (g)	1055	2.12
	ASV54	*Pedobacter* (g)	213	2.09
	ASV77	*Pseudomonas* (g)	110.9	2.08
	ASV1214	*Enterobacterales* (o)	12.23	2.08
	ASV150	*Duganella* (g)	30.42	2.01
	ASV128	*Pseudomonas* (g)	42.6	2
	ASV130	*Pantoea* (g)	52.99	1.97
	ASV1115	*Yersiniaceae* (f)	84.51	1.91
	ASV938	*Gammaproteobacteria* (c)	76.17	1.9
	ASV239	*Bacillus* (g)	44.65	1.88
	ASV830	*Pseudomonas* (g)	14.59	1.82
	ASV1225	*Pseudomonas* (g)	49.01	1.81
	ASV456	*Pedobacter* (g)	9.11	1.8
	ASV226	*Pseudomonas* (g)	68.41	1.79
	ASV927	*Gammaproteobacteria* (c)	129.3	1.77
	ASV548	*Pseudomonas* (g)	32.78	1.75
	ASV744	*Pseudomonas* (g)	72.59	1.72
	ASV743	*Pseudomonas* (g)	106.9	1.68
	ASV123	*Stenotrophomonas* (g)	69.84	1.64
	ASV1159	*Pseudomonas* (g)	90.05	1.62

TABLE A3 (Continued)

Factor comparison	ASV ID	Taxonomy	Base mean	Log_2 fold change
	ASV873	*Gammaproteobacteria* (c)	27.99	1.57
	ASV1231	*Pseudomonas* (g)	14.65	1.56
	ASV113	*Pseudomonas* (g)	278.6	1.55
	ASV1153	*Xanthomonadaceae* (f)	52.03	1.51
	ASV985	*Pseudomonadales* (o)	42.63	1.48
	ASV262	*Microbacteriaceae* (f)	14.32	1.45
	ASV1200	*Yersiniaceae* (f)	74.1	1.45
	ASV42	*Brucella* (g)	151.8	1.4
	ASV577	*Yersiniaceae* (f)	16.06	1.4
	ASV1059	*Pseudomonas* (g)	49.79	1.38
	ASV1033	*Xanthomonadales* (o)	83.13	1.36
	ASV17	*Yersiniaceae* (f)	1088	1.35
	ASV5	*Yersiniaceae* (f)	3013	1.34
	ASV1205	*Enterobacterales* (o)	47.15	1.33
	ASV699	*Gammaproteobacteria* (c)	188.1	1.29
	ASV275	*Sanguibacter* (g)	17.5	1.28
	ASV6	*Pseudomonas* (g)	2987	1.27
	ASV747	*Gammaproteobacteria* (c)	300.9	1.23
	ASV1144	*Xanthomonadaceae* (f)	32.12	1.23
	ASV63	*Pseudomonas* (g)	524.2	1.21
	ASV8	*Pseudomonas* (g)	3286	1.14
	ASV619	*Pseudomonadales* (o)	114.2	1.14
	ASV593	*Pseudomonas* (g)	143.9	1.1
	ASV772	*Gammaproteobacteria* (c)	144.8	1.08
	ASV29	*Pseudomonas* (g)	493.1	1.06
	ASV736	*Pseudomonadaceae* (f)	53.23	1.06
	ASV55	*Pseudomonas* (g)	211.6	1.05
	ASV108	*Pseudomonas* (g)	114.5	1.05
	ASV895	*Xanthomonadales* (o)	98.63	1.04
	ASV762	*Gammaproteobacteria* (c)	173.4	1.03
	ASV879	*Gammaproteobacteria* (c)	229.5	1
	ASV727	*Pseudomonadales* (o)	72.6	0.97
	ASV1193	*Pseudomonas* (g)	24.43	0.97
	ASV146	*Microbacteriaceae* (f)	34.96	0.96
	ASV1178	*Enterobacterales* (o)	27.79	0.96
	ASV23	*Rahnella* (g)	735.4	0.92
	ASV633	*Pseudomonas* (g)	55.85	0.9
	ASV1055	*Pseudomonas* (g)	58.18	0.88
	ASV1207	*Gammaproteobacteria* (c)	165	0.83
	ASV36	*Pseudomonas* (g)	516.8	0.82
	ASV1230	*Gammaproteobacteria* (c)	207.2	0.79
	ASV1162	*Yersiniaceae* (f)	37.79	0.77
	ASV25	*Microbacteriaceae* (f)	693.7	0.72
	ASV107	*Chryseobacterium* (g)	69.8	−1.78
	ASV47	*Paenibacillus* (g)	231.1	−1.87
	ASV61	*Paenibacillus* (g)	143.9	−2.24
	ASV78	*Acetobacteraceae* (f)	65.31	−2.97

Note: A positive log_2 fold change indicates a higher relative abundance in the factor listed first. (k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family and (g) denotes genus.

TABLE A4 Bacterial ASVs that significantly differ ($p < 0.05$, Benjamini–Hochberg adjusted) across locations within a polytunnel.

ASV ID	Taxonomy	Base mean	Log_2 fold change
ASV142	*Chryseobacterium* (g)	48.6	2.33
ASV117	*Paenibacillus* (g)	43.29	2.31
ASV179	*Pedobacter* (g)	12.83	2.17
ASV222	*Pedobacter* (g)	25.66	1.98
ASV54	*Pedobacter* (g)	213	1.94
ASV90	*Enterobacterales* (o)	72.97	1.74
ASV76	*Pedobacter* (g)	77.41	1.64
ASV74	*Bacillus* (g)	205.1	1.47
ASV33	*Pseudomonas* (g)	314.3	1.46
ASV251	*Rathayibacter* (g)	13.44	1.46
ASV239	*Bacillus* (g)	44.65	1.44
ASV67	*Erwiniaceae* (f)	127.1	1.17
ASV18	*Luteibacter* (g)	662.7	0.96
ASV340	*Erwiniaceae* (f)	19.49	0.94
ASV136	*Duganella* (g)	43.95	0.89
ASV25	*Microbacteriaceae* (f)	693.7	−0.89
ASV365	*Pseudomonas* (g)	15.63	−1
ASV534	*Pseudomonas* (g)	9.68	−1.05
ASV339	*Pseudomonas* (g)	129.4	−1.14
ASV800	*Pseudomonadaceae* (f)	103.9	−1.15
ASV56	*Erwiniaceae* (f)	332.4	−1.18
ASV118	*Methylobacterium* (g)	35.92	−1.3
ASV2	*Pseudomonas* (g)	5081	−1.32
ASV116	*Sanguibacter* (g)	49.5	−1.32
ASV20	*Pantoea* (g)	960.6	−1.38
ASV242	*Bacteria* (k)	9.91	−1.43
ASV57	*Pantoea* (g)	300.6	−1.54
ASV89	*Pseudomonas* (g)	113.7	−1.62
ASV46	*Erwiniaceae* (f)	279.3	−1.7
ASV66	*Erwiniaceae* (f)	116.6	−1.74
ASV273	*Erwiniaceae* (f)	14.25	−1.8
ASV102	*Erwinia* (g)	67.51	−1.9
ASV44	*Pseudomonas* (g)	262.4	−2
ASV351	*Erwiniaceae* (f)	12.2	−2.13
ASV623	*Frondihabitans* (g)	3.5	−2.22
ASV87	*Pseudomonas* (g)	68.39	−2.31
ASV420	*Acetobacteraceae* (f)	5.35	−2.31
ASV215	*Erwiniaceae* (f)	19.1	−2.75
ASV92	*Erwinia* (g)	81.37	−2.8
ASV324	*Brachybacterium* (g)	9.96	−2.85

Note: A positive log_2 fold change indicates a higher relative abundance in the inside of polytunnel vs. the outer edge. (k) denotes kingdom, (p) denotes phylum, (c) denotes class, (o) denotes order, (f) denotes family and (g) denotes genus.

TABLE A5 The primer sequences used in Amplicon Metagenomic Sequencing (sourced from Novogene U.K.) and *Cladosporium* genus qPCRs.

Types	Region	Fragment length	Primer	Primer sequence 5′-3′
Bacterial 16S	V3-V4	466 bp	341F	CCTAYGGGRBGCASCAG
			806R	GGACTACNNGGGTATCTAAT
Fungal ITS	ITS1-1F	321 bp	ITS1-1F-F	CTTGGTCATTTAGAGGAAGTAA
			ITS1-1F-R	GCTGCGTTCTTCATCGATGC
Cladosporium Genus Primer	mt SSU rDNA	87 bp	Clado-SYBRG-PF	TACTCCAATGGTTCTAATATTTTCCTCTC
			Clado-SYBRG-PR	GGGTACCTAGACAGTATTTCTAGCCT

TABLE A6 The most abundant ASVs present in the samples for the 16S and ITS regions used to create synthetic genes (gBlocks) for use as a qPCR standard for estimating microbial community mass.

Metabarcoding region	Sequence
16S	CCTACGGGGTGCAGCAGCAGCAGTG GGGAATATTGCACAATGGGCGCAAG CCTGATGCAGCCATGCCGCGTGTAT GAAGAAGGCCTTCGGGTTGTAAAGT ACTTTCAGCGGGGAGGAAGGCGATA AGGTTAATAACCTTGTCGATTGACG TTACCCGCAGAAGAAGCACCGGCTA ACTCCGTGCCAGCAGCCGCGGTAAT ACGGAGGGTGCAAGCGTTAATCGGA ATTACTGGGCGTAAAGCGCACGCAG GCGGTCTGTCAAGTCAGATGTGAAA TCCCCGGGCTTAACCTGGGAACTGC ATTTGAAACTGGCAGGCTAGAGTCT TGTAGAGGGGGGTAGAATTCCAGGT GTAGCGGTGAAATGCGTAGAGATCT GGAGGAATACCGGTGGCGAAGGCGG CCCCCTGGACAAAGACTGACGCTCA GGTGCGAAAGCGTGGGGAGCAAACA GGATTAGAATTAGATACCCGAGTAG TCC
ITS	CTTGGTCATTTAGAGGAAGTAAAAG TAAAAGTCGTAACAAGGTCTCCGTA GGTGAACCTGCGGAGGGATCATTAC AAGTGACCCCGGTCTAACCACCGGG ATGTTCATAACCCTTTGTTGTCCGA CTCTGTTGCCTCCGGGGCGACCCTG CCTTCGGGCGGGGGCTCCGGGTGGA CACTTCAAACTCTTGCGTAACTTTG CAGTCTGAGTAAACTTAATTAATAA ATTAAAACTTTTAACAACGGATCTC TTGGTTCTGGCATCGGCATCGATGA AGAACGCAGC



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Investigating the inoculum dynamics of Cladosporium on the surface of raspberry fruits and in the air

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