Sandra Cortes Corrections

Chapter 1

1. **P23, L2: "samp" should read "sap"**

The typo was corrected.

2. **P23, L6 "which can serve as a substrate for growth and sooty moulds." Expand on how this could also attract ants (for example) who may then protect aphids from their natural predators and potentially exacerbate the infestation…**

As suggested, the following paragraph was added in P24, L11:

"Honeydew can mediate mutualistic interactions between aphids and certain ant species –a relationship known as trophobiosis (Delabie, 2001). In exchange for honeydew, ants protect aphids from fungal pathogens and parasitoids. For instance, the ant species Formica podzolica was observed to protect aphids from lethal fungal infections by grooming infective conidia from living aphids and rapidly removing the bodies of fungal-killed aphids, limiting the spread of infection within the aphid colony (Stadler & Dixon 2005). Similarly, the ant species Lasius turcicus was observed to aggressively attacked the parasitoid wasp Trioxys asiaticus, providing a protecting effect in the population growth rate of aphids (Sadeghi-Namaghi & Amiri-Jami, 2018)."

Chapter 2

1. **Context for using Ecoplates needs to be provided linked to a specific aim**

The context for using Ecoplates is explained in the last paragraph of the introduction, page 65, L3: "*Second, the metabolic activity of microbial communities was assessed, with the hypothesis that aphid-induced plant metabolites would influence microbial metabolic responses.*"

Furthermore, in the methods section, P75, L22, the purpose of using the Ecoplates is expanded:

"Biolog EcoPlatesTM (Biolog, Inc., Hayward, CA, United States) were used to test differences in the metabolic activity of microbial communities in the rhizosphere of plants under aphid herbivory, following the protocol by Mendes et al. (2019). These Ecoplates are commonly used as "fingerprints" of the metabolic potential of microbial communities and consist of 96-well plates containing three replicates of 31 carbon

sources (amino acids, carbohydrates, carboxylic acids, phenolic compounds, polymers, amides, and amides). A redox tetrazolium dye is present in each well, and changes in colour intensity indicate microbial respiration in the presence of each carbon substrate."

2. **P64 Soil needs more information. Soil characteristics eg sand/ silt/ clay % C and N and pH What was the moisture content used in the experiment? And how was this maintained during the experiment.**

Unfortunately, as changes in physical and chemical properties of the soil were outside the scope of this PhD, a chemical analysis was not performed in this soil. However, the available data on the soil properties was described in P66, L8:

"The soil used in this experiment was collected from the long-term bare fallow field at the Woburn Experimental Farm in Bedfordshire, UK (latitude: 52°00'04.3"N, longitude: 0°36'49.0"W). This well-draining sandy loam, classified as a Cambric Arenosol (FAO), has been maintained continuously free of vegetation for over 50 years."

Regarding the soil moisture content, there was no specific aim more than providing the plants the necessary moisture during their growth. Due to the aphid's presence in half of the plants in the experiment, it was difficult to maintain the same moisture level for plants, which meant checking the experiment continuously and watering when needed to avoid water deficit. An extra line was added in P67, L12:

"Plants were watered regularly to prevent water deficit, and the volume of water applied increased according with plant growth stage."

3. **'Define the treatment i.e. bare fallow soil mine'. Provide a rationale for using this soil**

P66, L12: The definition and rationale were included as follows:

"The use of this bare fallow soil was specifically chosen to minimise legacy effects from prior plant cultivation, which can significantly influence the composition and structure of soil microbial communities. By eliminating the residual impacts of previous crops or plant-derived inputs, this soil provides a relatively "neutral" microbial background, allowing for more controlled conditions to investigate the effect of aphid-plant interactions in soil microbial communities. Soil was collected from five field replicates, homogenised, sieved (5 mm), and stored at 4°C until the start of the experiment."

4. **P65 Provide an explanation for adding perlite and state the particle size of perlite used**

P67, L1, the explanation was included as follows:

"The substrate consisted of a 70:30 (v/v) mixture of unsterile soil and sterile perlite (particle size 3–5 mm). Perlite was added to improve aeration and drainage, prevent soil compaction, and create a more favourable environment for root penetration and growth."

5. **Please insert detail of the experimental design ie arrangement of pots in the CE room**

P67, L20: Added the requested information:

"To avoid cross-contamination between treatments, particularly the movement of aphids from infested to control plants, a simple experimental design was employed in which the two groups were physically separated by approximately 1.5 metres within the growth chamber. As a result, pots were not randomly distributed but grouped by treatment."

6. **P65 What was the rationale for adding 6.5 g NPK ie what was the nutrient status of the soil before and after addition?**

As the experiment did not aim to investigate changes in soil nutrient status, chemical analyses were not performed before and after the experiment. Instead, nutrient supplementation followed established recommendations based on previous work with this soil type, which is widely used in studies on soil microbiome structure by the Molecular Microbial Ecology Group at Rothamsted Research.

Accordingly, the following sentence was added for clarification in P67, L4:

"Prior to planting, each pot was supplemented with 6.5 g of slow-release NPK fertilizer granules (Osmocote; 15% N, 9% P_2O_5, 11% K_2O, 2% MgO), following recommendations established in previous experiments using this soil type."

7. **P65 Add details of the temperature, light and humidity regime in the CE cabinet**

The details were added in P67, L9:

“A total of 25 replicates were placed in a controlled environmental chamber for the duration of the experiment (21°C, 16 h light/8 h dark photoperiod, 65% relative humidity).”

8. **P65 after how many days were aphids added? Ie when was third leaf stage. These aspects need more explanation in the text**

P67, L14: Added the plants age as follows:

“Once the seedlings reached the third-leaf stage (3-weeks old plants), 20 pots were randomly assigned to one of the two groups (10 each): aphid herbivory (treatment) and no herbivory (control).”

9. **Origin of the bird cherry oats should be provided eg how they were reared and their age**

P65, L15: Added the following paragraphs to include the requested information:

“The aphids used in this study were bird cherry-oat aphids (Rhopalosiphum padi L.) obtained from long-term laboratory cultures maintained at Rothamsted Research (Hertfordshire, UK; coordinates: 51°48'36.8" N, 0°22'34.4" W). These colonies are reared on wheat (Triticum aestivum cv. Solstice; Advanta Seeds UK Ltd) in ventilated Perspex cages maintained under controlled environmental conditions: 20 °C, 60–70% relative humidity, and a 16 h:8 h light:dark photoperiod. To ensure a stable population of non-winged (apterous) aphids, host plants are replaced every two weeks by the Rothamsted Insectary team.

Two weeks prior to the start of the experiment, approximately 20 aphids were transferred from the main colony to initiate a new sub-culture, maintained under identical conditions. This sub-culture served as the source of aphids for the experimental treatments. On the day of infestation, aphids were gently collected using a fine-haired brush and placed in Petri dishes containing fresh wheat leaves. They were then transported to the controlled environment chamber where the experiment was conducted.”

10. **P66 add in method for removing soil from roots prior to dry weight measurement eg sequential washing with deionised water?**

P69, L5: Added the following sentence to include the requested information:

"After each sampling, the plant root dry weight was measured by washing the roots with tap water to remove the soil from plant roots and drying them in the oven at 70°C for 96 hours, before weighing."

11. **P69 Add volume injected onto the GC**

P71, L2: Added the requested information:

"Chemical analyses were performed using gas chromatography (GC). Four microlitres (4 µL) of the concentrated VOCs samples were injected into an Agilent 6890A GC equipped with a non-polar HP-1 column of 50 m length, 0.32 mm inner diameter and 0.52 µm film thickness (J&W Scientific)."

12. **P83 there was no significant difference in root biomass of herbivore and control treatment- this text needs to be revised to make this point clear**

P86, L4: Rewrote the sentence for clarity about statistical significance:

"Regarding root biomass production, Figure 2.8 shows a non-significant but noticeable decrease in root dry weight in plants subjected to aphid herbivory, with the effect becoming more pronounced after four weeks of infestation (p = 0.056). Although not statistically significant, these results suggest that aphid feeding progressively reduced biomass production in the roots."

13. **P84 Legend of Fig 2.5 add narrative of what's being shown ie bar and whisker plot, state what data points, error bars and horizontal bars represent,**

According to the description, it seems like the figure referred to corresponds to Figure 2.8. The legend was modified to add the required information in P87:

"Figure 2.8. Root dry weight of wheat plants under aphid herbivory at two sampling time points. Dry weight was measured after oven-drying roots at 70 °C for 96 hours. Boxplots display the mean (horizontal bar) and individual data points for each of the five biological replicates per treatment group. Statistical comparisons between treatments were conducted using the Wilcoxon rank-sum test (n = 5)."

14. **Note that experimental design is a little uneven, 4 replicates in Fig 2.7, 5 in Fig 2.8 and 2.9, 4 for the no herbivory treatment in 2.10, 3 for data in 2.11, 4 in Fig 2.21; methods mentions replicates in places p77, 74, 3 replicates; but design included 5 replicates (fig 2.1). Overall more detail on this needs to be provided eg what happened when only 4 replicates are shown?**

Added the following sentences and paragraph explaining the number of replicates used in each section:

P76, L20:

“For this test, three replicates (n=3) per condition were used in each sampling time due to cost constraints.”

P77, L14:

“Due to cost constraints, four (n=4) biological replicates per condition were selected for DNA extraction and sequencing of soil samples in this analysis.”

P78, L22:

“It is important to note that during visual inspection of the chromatograms for aboveground volatile organic compounds (VOCs), two samples—one from each condition—were excluded due to contamination. As a result, the analysis of aboveground VOCs was conducted using four biological replicates per group. Similarly, for the belowground VOCs, one sample from the control group was excluded for the same reason, resulting in five replicates in the herbivory treatment and four in the control group.”

15. **Fig 2.7 193 peaks were detected- presumably only those that were significantly different in the 2 treatments are shown. Please clarify in the text. It would be useful to add the full dataset to the appendix ie so that those metabolites which are not affected by aphids can be seen. Similarly for the root VOC on p84 please be more definitive about the 18 metabolites associated with herbivory- presumably only these were significantly different between the 2 treatments? Same with the non volatile metabolites described on P86; more details on the number of significantly different metabolites would be helpful here.**

P105: According to the comments, the heatmap including all VOCs collected during weeks 2 and 4 was added in Supplementary Figure 2. and is mentioned in the main text in P85, L2.

P106: According to the comments, a heatmap showing the rhizosphere VOCs collected after two weeks of aphid herbivory was included in Supplementary Figure 2.2, and is mentioned in the main text (P87, L10). Furthermore, more details were added about the statistical analysis of the selected peaks (P87, L5):

"After processing belowground VOCs, a total of 117 peaks (metabolites) were detected, of which 111 were retained for statistical analysis after removal of near-constant peaks. As observed in Figure 2.9a, VOCs samples were clustered based on condition (aphid herbivory and control plants), which was confirmed by the PERMANOVA test ($F= 6.48$, $p < 0.05$). The VOCs were inspected to identify metabolites uniquely present in samples under aphid herbivory (Supplementary Figure 2.2), as performed for aboveground VOCs. This time, visual inspection of the heatmap revealed a subset of 18 metabolites with a higher peak area associated with samples from the rhizosphere of plants under aphid herbivory. However, these differences were not statistically significant after correction for multiple testing (padj > 0.05), likely due to limited statistical power. Tentative annotation was possible for 7 metabolites, with some belonging to green leaf volatiles, like hexanal, (Z)-3-hexenal and (E)-2-pentenal. Notably, these metabolites were different from VOCs released aboveground (Figure 2.10)."

P107: According to the comments, a heatmap for non-volatile metabolites in the rhizosphere of plants under two weeks of aphid feeding was included in Supplementary Figure 2.3. The figure is mentioned in P89, L16. Furthermore, to add information about this dataset, the following lines were added in P89, L5:

"Samples from control plants showed higher variability, which is evident in the PCA and was confirmed by the lack of statistical significance in the PERMANOVA test ($F=2.28$, $p=0.13$). As mentioned before, a subset of these samples was selected for tentative annotation of metabolites by reanalysing them using LC-MS/MS in Data-Independent Acquisition mode. Following the approach used for belowground VOCs, visual inspection of the data revealed a subset of metabolites that were exclusively present in the rhizosphere of plants under aphid herbivory. Although these metabolites did not reach statistical significance after adjustment for multiple testing (padj > 0.05), their peak areas were significantly increased under aphid herbivory. For clarity, only the top 24 metabolites are displayed in Figure 2.11, while the complete set of metabolites is available in Supplementary Figure 2.3."

16. **Fig 2.12 what are the large symbols on the figure- average? Need to state this in the legend**

P92: According to the comment, the figure legend was modified as follows:

"Figure 2.12. Principal Component Analysis (PCA) of microbial carbon source utilisation profiles based on Biolog™ EcoPlates. Data represent the Average Well Colour Development (AWCD) across 31 carbon sources after 72 hours of incubation. Technical replicates were averaged to obtain three biological replicates per condition (n = 3). Larger symbols indicate the centroids of each group."

17. **P88 state which substrates didn't get metabolised**

P92-L4: Added the following sentence for clarification:

"The five carbon substrates that were not metabolised corresponded to a- Cyclodextrin, Glycogen, a-D-Lactose, 2-Hydroxy Benzoic Acid, and a-Keto Butyric Acid."

18. **P89 text dealing with differences in substrates metabolised between the treatments is a bit confusing- state which 8 substrates are different. Text describing asparagine, hydrxy benzoic acid and tween is confusing- these are not significantly different, and this point should be made clearler**

P93, L1: The text was modified to include the carbon sources where microbial activity was statistically significant. Furthermore, as asparagine, hydroxy benzoic acid and tween were not statistically significant, the sentence was removed to avoid confusion.

"A detailed analysis of individual carbon source use (Figure 2.13b) shows that the AWCD was statistically significant in seven carbon sources (padj < 0.05), which include D-Galacturonic Acid, D- Glucosaminic Acid, D-Mannitol, D-Xylose, Glucose-1- Phosphate, N-Acetyl-D- Glucosamine, and β-Methyl-D- Glucoside, with N-acetyl D-Glucosamine displaying the highest statistical significance (p < 0.001). Detail of statistical analyses can be found in Supplementary Table 2.4. These results show that the EcoplatesTM were able to capture differences in metabolic rate of consumption of carbon sources after two weeks of aphid herbivory."

19. **P90 rationale for a 50 % cut off would be helpful; is this a suitable threshold for commonness? How does this relate to literature thresholds?**

The choice of a 50% presence threshold to define common taxa was based on a combination of empirical testing and guidance from recent literature. As highlighted by Cao et al. (2021) , "most filtering approaches are based on rules of thumb, which vary from lab to lab," highlighting the lack of a universally accepted standard. However, (Risely et al., 2021) recommended a maximum prevalence threshold of 70% for

amplicon sequencing data, as the authors showed that diversity metrics are significantly affected above this level.

To determine a suitable cut-off for this dataset, a range of prevalence thresholds was tested (30%, 50%, and 70%) to assess their impact on downstream diversity analyses. A 50% threshold was ultimately selected as it retained enough taxa to meaningfully describe community structure while removing low prevalence features likely to represent noise or sampling artifacts. This threshold falls within the 70%, as suggested by Risely et al. (2021), and it reflects a compromise informed by both the literature and dataset-specific considerations. The aim was to include taxa present in at least half of the samples, which was considered a reasonable definition of "common" within this context.

20. **P90 Add P levels for bacterial phyla differences, and more narrative about aphid treated vs control treatments ie were there actually any differences at the phylum level? Clarify what the labels on Fig 2.14 c are.**

There were no significant differences at the Phylum level according to the statistical analysis, which is the reason why no statistical significance is included in the graph. Only the analysis at the most specific level (ASVs) showed significant differences, with the results described in detail in the differential abundance analysis found in Chapter 2, Section 2.3.4, P97.

The labels were corrected in the legend of Fig 2.14, as follows:

> *"Figure 2.1. Taxonomic diversity and richness of bacterial communities during the experiment. a) Observed and b) Shannon diversity index at the ASV level (n=4) Significance is shown by post-hoc Wilcoxon comparison (FDR < 0.05). c) Rank abundance plot (abundance >1%) at the class level. Four biological replicates per sample were used for the analyses. BS= Bulk soil, NH = Rhizosphere before herbivory, RH= Rhizosphere after two weeks under herbivory; RN= Rhizosphere of healthy plants after two weeks; TH= Rhizosphere after 4 weeks under herbivory; TN= Rhizosphere of healthy plants after 4 weeks."*

21. **P93 were the ASV unique to the herbivory treatment the same at 2 and 4 weeks? Is there any indication of the identity of these?**

According to the bioinformatic analysis, 95.6% ASVs were shared between rhizosphere samples from plants under aphid herbivory at weeks 2 and 4, indicating that the

majority of ASVs in the herbivory treatment were shared across both time points. The main difference observed between these timepoints was an overall increase in ASV richness –higher number of ASVs- by Week 4, as mentioned on P97, L7:

"This pattern further supports the observation that bacterial diversity in the wheat rhizosphere increased with plant age."

The identities of these ASVs can be observed in the bar plot of relative abundances shown in Fig. 2.14c, P95. Individual ASV identities were not listed separately in the text, as most of the overlapping ASVs correspond to those shown in the bar plot for wheat plants after two weeks of aphid herbivory.

22. P94 Similarly what were the ASV that were significantly enriched in the herbivory treatment after 4 weeks and were there any overlaps with those seen at 2 weeks?

P99, L3: The information about this analysis was included as follows:

"Due to the high resolution of ASVs, there was no overlap between individual ASVs that were significantly enriched after two and four weeks of aphid herbivory. However, when examined at the genus level, some consistent patterns emerged. Specifically, Acidothermus and Singulisphaera showed increased abundance under herbivory at both time points, while ASVs from Burkholderia and Conexibacter were reduced, showing a consistency in the attraction of bacterial genera to the rhizosphere of plants under aphid herbivory at different plant growth stages. Overall, bacterial communities showed significant changes and differentially abundant taxa, but differences between control plants and those under aphid herbivory were more evident after two weeks of herbivory."

23. Page 96 interesting to discuss what 'turgid' means. The effect on root biomass wasn't significant, so this needs more careful wording.

The use of the term "turgid" in this case refers to the observation of more wilted leaves in the plants after four weeks of aphid herbivory when compared with the leaves of healthy plants. According to this recommendation, the term "turgid" was replaced by "wilted" as follows:

P100, L20: "This hypothesis is supported by visual observations as plants survived four weeks of aphid herbivory; however, their leaves appeared more wilted compared to those of healthy controls. Future studies should focus on the developmental stage-

specific impacts of aphid herbivory and the potential trade-offs between growth and development involved."

24. **Was any data on survival of aphids collected? Ie were they all still alive after 4 weeks?**

Unfortunately, due to the fast reproduction rate of the aphids it was difficult to count the aphids at the end of the experiment. For that reason, the plant dry weight was taken as a measurement of the impact of aphid herbivory on plant growth and development.

Chapter 3

1. **I don't think replication is described, and there is no detail on spatial arrangement of the treatments in the growth room eg were cages randomised or arranged in a block?**

As this experiment was performed following the methods described in Chapter 2, details of the plant and soil sample preparation can be found in Sections 2.2.1 and 2.2.2. However, to clarify the experimental design in this second experiment, the following paragraph was added in P121, L12:

"Briefly, 10 pots containing 10 wheat plants each were grown in a controlled environment room (21°C, 16 h light/8 h dark photoperiod). Once plants reached the third-leaf stage (3-weeks old plants) the pots were randomly assigned to one of two groups: aphid herbivory treatment and no herbivory (control), for a total of five biological replicates (pots) per group. Five additional pots containing soil were added as negative control. Similar to the experiment described in Section 2.2.2, pots from each treatment were physically separated to avoid cross contamination. As an additional step in this experiment, individual pots were kept in mesh cages to prevent any aphid from going to the untreated control plants (Figure 3.1)."

2. **State more clearly that the PDMS tube was under the water?**

The sentence "under water" was added in P123, L7:

"These flasks also contained a 10 cm sterile Polydimethylsiloxane (PDMS) tube (internal diameter 1,00 mm, external diameter 1,80 mm, wall thickness 0,40 mm, VWR

INTERNATIONAL LTD), placed at the bottom, to capture root exudate volatile organic compounds (VOCs) under water."

3. **Fig 3.12 discuss inclusion of metabolites at p<0.1 significance.**

Due to the limited sample size (n = 5) in this experiment, combined with the high dimensionality of the untargeted metabolomics dataset, the statistical power of the analysis was low. As a result, the p-value adjustment method may have been overly conservative, potentially masking biologically meaningful differences. To avoid overlooking relevant metabolites, we applied a more relaxed FDR threshold of 0.1. This broader cutoff allowed for the inclusion and annotation of metabolites that were initially significant before correction. However, as this is an exploratory study, the biological relevance of these metabolites requires further validation in follow-up experiments.

4. **Line 146 ' potential root derived response to aphid attack; or transport of this to the root from aboveground? Clarify the possibilities**

As suggested, a sentence was added to clarify in P154, L3:

> *"Manual inspection of all samples confirmed that this compound was exclusively detected in samples exposed to aphid herbivory, suggesting that this metabolite was either transported from aboveground tissue or synthesized in plant roots as a response to aphid attack."*

5. **Line 153 para 1 first sentence needs rewriting**

The paragraph was modified in P160, L3, as follows:

> *"The effect of aboveground aphid herbivory on jasmonic acid signalling pathway has been observed to vary depending on the species involved in the aphid-plant interactions. For instance, Karssemeijer et al. (2020) showed that 72 hours of aboveground herbivory by the aphid Brevicoryne brassicae on cabbage plants did not affect jasmonic acid concentration on plant primary roots, nor had any impact in the performance of a root herbivore. Nevertheless, other types of oxylipins can be impacted under aphid herbivory, as has been shown previously in maize leaves (Tzin et al., 2015) and is suggested by the results in this chapter."*

Chapter 4

1. **Line 173 why does the change in bacterial community have to represent a 'potential benefit' and also question the hypothesis that plant roots use exudates to attract bacterial communities- cause and effect issue. Please provide more rationale.**

P80, L5: The sentence was modified for clarity, as follows:

> *"These findings suggest that aphid herbivory alters plant chemistry, thereby influencing interactions with soil microbial communities and leading to an increased relative abundance of bacteria from this taxon."*

2. **P174 Check methods for rhizosphere soil collection and root collection- seems different here to Chapter 2? Was there root washing?**

The collection of rhizosphere soil was performed following the same protocol described in Chapter 2. This is stated in P182, L6:

"Rhizosphere soil collections were made after carefully removing the wheat plants from the pots, following the protocol described in Chapter 2, Section 2.2.2"

3. **Clarify what MMEG is**

The definition of MMEG can be found in page 183, line 7:

"The sequencing depth for soil samples was set to 30k, a standard depth used in the Molecular Microbial Ecology Group (MMEG) to ensure adequate coverage."

4. **ASV were not agglomerated for analysis- Chapter 2 doesn't mention this so please clarify there**.

A sentence was added in Chapter 2, P83, L12 to include the information requested:

"A significance threshold of α = 0.05 was applied, with p-values adjusted for multiple comparisons using the Holm method. ASVs were not agglomerated for this analysis."

5. **P180- ISP-3 is presumably for Actinomycetes rather than Actonomyces?**

This misspell was corrected in P187, L3.

6. **P180 state size of PCR product**

The size of the PCR product was added in P188, L12, as follows:

"Five microlitres of PCR products (~1300 bp) were loaded onto a 2% agarose gel and gel electrophoresis was performed at 100 V for 45 min. The gel was then stained with RedGel for 30 min, de-stained in 1× TAE buffer for 15 min, and visualized under UV light. Finally, PCR products were sent to Eurofins Genomics for purification and Sanger sequencing."

7. **P187 text describing herbivory effects on bacterial abundance - mention the significance value used is P<0.1**

The significance was added as $p_{adj} < 0.1$ as follows:

P194, L12: *"In the rhizosphere, aphid herbivory was associated with significant increases in bacterial ASVs belonging to the genera Paenibacillus and Frauteria, as well as several ASVs that could not be assigned to a genus (padj < 0.1). In contrast, ASVs that decreased in abundance were mostly unclassified at the genus level but were taxonomically assigned to the Alphaproteobacteria and Acidobacteriae classes (Figure 4.4a)."*

P194, L18: "In plant roots, most ASVs that increased in abundance under aphid herbivory belonged to the Actinobacteria class, with the highest enrichment in the genera Marmoricola, Devosia, and Streptacidiphilus (Figure 4.4b) (padj < 0.01). These shifts suggest that aphid herbivory may selectively influence specific bacterial taxa, particularly Actinobacteria, within the root microbiome. Further details on statistical results and taxonomic classifications are available in Supplementary Table 4.3 and 4.4."

8. **P193 please be more explicit about what the PCR limitations were- no amplification?**

The limitations for the PCR were added on P200, L6:

"A total of 183 bacterial isolates were selected for 16S rRNA gene amplicon sequencing. Some of the isolated bacteria were excluded from sequencing as they did not grow when transferred from ISP-3 medium to TSA, while others failed to yield PCR amplification."

9. **P196 clarify how sporulation was it observed/ measured**

P203, L3: Added the following sentence:

"Sporulation was observed by comparing colony morphology on control media versus plates supplemented with the selected compounds, with the presence of powdery colonies indicating sporulation."

References

Cao, Q., Sun, X., Rajesh, K., Chalasani, N., Gelow, K., Katz, B., Shah, V. H., Sanyal, A. J., & Smirnova, E. (2021). Effects of Rare Microbiome Taxa Filtering on Statistical Analysis. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.607325

Risely, A., Gillingham, M. A. F., Béchet, A., Brändel, S., Heni, A. C., Heurich, M., Menke, S., Manser, M. B., Tschapka, M., Wasimuddin, M., & Sommer, S. (2021). Phylogeny- and Abundance-Based Metrics Allow for the Consistent Comparison of Core Gut Microbiome Diversity Indices Across Host Species. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.659918