Summary:

Pg1 – NMDS with all sample types in separate

Pg2 – NMDS of bacteria and fungi without N or only-frass samples (has permanova stats on https://github.com/PedroBeschoren/shaphan\_ECA/blob/main/Results/Permanova\_output.txt)

Pg3 - heattree\_Bac\_pannel heattree\_Bac\_pannel. Taxonomies of ASVs that were different in pairwise comaprsions between treatments. Used as a follow-up of the permanovas, and describes some of the main differences between groups

Ps4: heattree\_Fun\_pannel : same as above, but for fungi

Ps5: heattree\_Bac\_source\_on\_CompostedUncomposted . separate composed and uncomposted samples, then look at the source effects one ach of them

Pg6: heattree\_BacFun\_Dose\_025 : compares dose 0 to 2 and 5. More meaningful for bacteria than fungi, absed on the output in page1

Pg6 heattree\_BacFun\_source\_NXY: compares source N to X and Y. More meaningful for Fungi than bacteria, absed on the output in page1

Pg7 Alpha diversity plots and testsNMDS\_**Bac**\_3\_sample\_types:

The results indicate a distinct separation of the N frass condition from all other conditions, with the frass dose being the major contributing factor to this differentiation between N and the other conditions. This differentiation is particularly evident in the rhizosphere samples. In terms of frass, it is evident that composting significantly increases the similarity of microbial communities. However, noticeable differences between sources X and Y are still discernible (Fig. 1).

Chart, scatter chart

Description automatically generated

**Fig. 1** Non-metric multidimensional scaling (NMDS) plot of the beta diversity of **bacterial** communities in the rhizosphere, bulk soil and frass data based on bray-Curtis' distances.

NMDS\_**Fun**\_3\_sample\_types:

The results demonstrate a distinct separation between the N frass condition and all other conditions, with the frass source being the primary factor contributing to this differentiation. This distinction is particularly pronounced in the rhizosphere samples. Regarding frass type, notable differences are observed between composted and uncomposted frass, although such differences are not apparent when comparing the different frass sources. It is worth noting that the differentiation among the microbial communities is less pronounced compared to the bacterial communities (Fig. 2).

Chart, scatter chart

Description automatically generated

**Fig. 2** Non-metric multidimensional scaling (NMDS) plot of the beta diversity of **fungal** communities in the rhizosphere, bulk soil and frass data based on bray-Curtis' distances.

NMDS\_BacFun\_BulkRhizo: Beta diversity of microbial communities.

Sginificant three-way interactions across the board indicate that all factors are important in defning microbial community structure and also interfere with each other. The most relevant two-way interaction (significant and with highest R2 across all sub-communities) is between frass\_treatment and Frass\_source. This means that composting will cause different microbial community shifts on the different sources. Frass source was the most important (highest R2) main effect for bulk soil bacteria; for the other subcommunities, frass\_treatment was the most important factor to separate microbial communities (Fig. 3).

The effect of dose on composted frass is more impactful in frass source Y than on X. This is clear on the rhizosphere samples. For the bulk soil samples, composting more strongly affects the fung of frass source Y and the bacteria of frass source X (Fig. 3).

Chart, scatter chart

Description automatically generated

**Fig. 3** Non-metric multidimensional scaling (NMDS) plot of the beta diversity of **bacterial** and **fungal** communities in the rhizosphere and bulk soil samples based on bray-Curtis' distances.

[To-do: add stats of permanovas into this fitures (R2, P; if it fits, add F values; include interactions]

[Pedro to-do: pariwse post-hoc tests for memanova]

Diagram

Description automatically generated

heattree\_Bac\_pannel heattree\_Bac\_pannel: These are the taxonomies of the ASVs classified as differentially abudnant in pairwise comparisons by the deseq2 algorithm. Composting clearly increased the abundance of firmicutes bacteria, in both bulk soil and rhizosphere.The paenibacillaceae group however was more abundant in the Uncomposted frass then on the composted frass. This indicates that composting is shifgin parts of the firmicutes communities from Paenibacillus to Bacillus. In addition, uncomposted frass has more and more diverse proteobacteria groups. It also shows more and more diverse bacteriodota, which includes Chitinophagaceae.

For the source\_treatment effect, Source X had more firmicutes, Verrucomicrobiae and bacteriodota than source Y in both bulk soil and rhizosphere samples. The higher abundance of actinobacteria on source Y at bulk soils is not visible on the rhizosphere (the plant is equalizing these communities across frass source in the rhizosphere)

For frass dose, whch is affected by layers of interactions, we see higher populations of several common taxa at dose 5g, specially on the bulk soil when comapred to rhizopshere. Notably, genus Mucilaginibacter is more abundant on dose2 than dose 5

Diagram

Description automatically generated

heattree\_Fun\_pannel: Composting increase Ascomycota in both bulk soil and rhizosphere.. Uncomposte frass presented more Mortierella on the rhizosphere. Ascomycota is also more present on Frass source Y (in both bulk soils and rhizsphere). Mortirellomycota are more abundant in frass source X rhizosphere. Ascomycota are also more abudant in dose 5 than in dose 2

Diagram

Description automatically generated

heattree\_Bac\_source\_on\_CompostedUncomposted: : These are the taxonomies of the ASVs classified as differentially abudnant in pairwise comparisons by the deseq2 algorithm, comparing frass source X and Y, but on on-ly-composted or only-uncompsoted sampels sets. On bulk soils, alphaproteobacteria and actinobacteria are slightly more abundant on uncomposted X than Y, but composting clearly increases their abudannce in source Y. all the bacillaceae clade is more abandat in X than Y on uncomposted frass, ecept for paenibacllus which si more present in Y than X. When composted, source X still ahs more fimicutes and baciili, but source Y has more Bacialles and Bacilaceae. In Bacilaceae of composted frass, some genera are more abudant in Y and other are more abudnanr in X.

On the rhizosphere, X and Y differ in sub-groups of Proteobacteria, but these differences are not changed due to compsoting. Actinobacteriota, Verrucomicrobiota, and Bacteroidota are voerall more present in source X than Y, and this does not change much with composting. For source X presents overall more abudannce and diversity of firmicutes, but composting also causes Clostridia to become a major difference between X and Y in these brachnes of the taxonomy tree, with more Clostridia on X. [to-do: evluate these itneraction effects on the fungi plots, heattree\_Fun\_source\_on\_CompostedUncomposted]

Background pattern

Description automatically generated

heattree\_BacFun\_Dose\_025: comparisons of dose 0, 2 and 5, using only the ASVs hat havee differential abudancnce in pairwise comparisons of 0 VS 2 and 0 VS 5. Alpha and gamma proteobacteria are largely increased when frass isa dded to the soil, in addition to Chloroflexi, firmicutes groups. There is a very marked increase in Chitinophagaceae when frass is added. A few groups of gammaproteobacteria (Polycyclovorans, Arenimonas, Nitrosomonadaceae) that are more present in 0-frass of rhizosphere samples, as well as Nitrospira (algae).

For fungi… you might want to supress this aprt of the figure as dose wasy the key difference for 0-2-5 communities in the beta disversity plots, but source was the key differenciation for N-X-Y in fungi

Background pattern

Description automatically generated

heattree\_BacFun\_source\_NXY: on bulk soil, most fungal groups are alrgely supressed when frass is added (comparing N-X-Y). The same happens on the rhizospher, except that Orbiliaceae is more present in X, while Penicillium and Chaetomiaceae ASVs are more present in Y. for abcteria…. you might supress the top part for the same reason as above

Chart, scatter chart

Description automatically generated

alpha\_div\_3\_sample\_types: shannon diversity of all samples. In Bacteria, addition of frass does not heavely impact diversty in bulk and rhizosphere soil; maybe it’s a bit reduced by frass (pensing test). The diversity in Frass-only samples of source Y was quite smaller than that of frass X, but this difference disapears upon composting, which also increses diversity for both sources.

For fungin, frass is clearly decreasing the diversity of fungi in both bulk soil and rhizosphere. ITS amplicon sequencing extensively failed for uncompotes frass, possibly because the source samples had very low fungal biomass. Composting allowed for alpha diversity comparison, but indicates low diversity (which is similar between X and Y)

Chart, box and whisker chart

Description automatically generated

alpha\_div\_BacFun\_BulkRhizo: shannon diversity, which should account for the apha diversity tests thatw ere performed. Bac$bulk\_soil: Frass dose has a major influence, which depends on frass source and treatment. higher dose seems to reduce diversity, specially in composted frass. this difference is more evident in frass X than on frass Y. $Bac$rhizosphere: Frass source drives alpha diversity (X more diverse than Y), with interactions with frass treatment. composting decreases diversity of X\_2, but increases diversity of Y\_5. diversity of dose 2 was higher than that of dose 5. $Fun$bulk\_soil: frass source drives alpha diversity, with strong interaction with frass treatment. X is more diverse than Y, but composting reduces the diversity of Y while increasing diversity of X. high dose diminishes the diversity of composted frass, but not of uncomposted frass. $Fun$rhizosphere: onyl frass treatment affects alpha diversity. compoting frass reduces fungal diversity. [TO-DO: incorporate pairwise comparisons] overall, there are interactions and dependencies across the board. higher dose has a tendency to reduce diversity. composting accentuates the differences between the frass sources in bulk soil, but reduces such difference in teh rhizosphere