**Microbial Diversity, Abundance and Community Composition in Frass-Treated Bulk Soil and Rhizosphere**

**Results**

**Hierarchical heat tree of bacterial taxa abundance in bulk soil and rhizosphere: composted and uncomposted frass amendment**

Key findings:

* Composted frass clearly increased the abundance of firmicutes (including bacilli and paenibacilli), in both bulk soil and rhizosphere
* The paenibacilli, however, was more abundant in the uncomposted frass than in the composted frass
* Frass dose, influenced by complex interactions, showed elevated populations of common taxa (including bacillaceae, chitinophagacaea, etc.) at 5 g/kg, particularly in bulk soil as opposed to rhizosphere.

**Hierarchical Heat Tree of Fungal Taxa Abundance in Bulk Soil and Rhizosphere: Composted and Uncomposted Frass Amendment**

Key findings:

* Composted frass (frass treatment) increased the abundance of Ascomycota in both the bulk soil and rhizosphere
* Frass source Y exhibited a higher abundance of Ascomycota compared to frass source X in both bulk soils and rhizosphere
* Uncomposted frass showed a higher abundance of Mortierellomycetes (including Mortierella) in the rhizosphere than composted frass
* Higher dose 5 promotes Ascomycota abundance more than dose 2, in both bulk soils and the rhizosphere

**Heat tree analysis of bacterial taxa abundance across frass dose levels (0, 2, and 5 g/kg)**

Key findings:

* Adding frass to the soil at 5 g/kg led to a higher abundance of bacteria groups such as alpha and gamma proteobacteria, Chloroflexi and firmicutes (including bacilli) than 0 (no-frass) and 2 g/kg.
* Chitinophagaceae showed a marked increase in response to frass supplementation

**Heat tree analysis of fungal taxa abundance across frass dose levels (0, 2, and 5 g/kg)**

Key findings:

* Frass dose was not the primary factor influencing fungal communities in both bulk soil and rhizosphere samples, as shown in the NMDS (beta diversity) analysis

**Heat tree analysis: bacterial taxa abundance in bulk soil, rhizosphere and frass sources (X, Y)**

Key findings:

* Adding frass (X and Y) significantly increased the abundance of bacterial taxa in both bulk soil and rhizosphere compared to no-frass (N)
* Bacillaceae and Chitinophagaceae are among the major groups that are stimulated
* Differences in the abundance of bacterial taxa between frass sources X and Y are visible

**Heat tree analysis: fungal taxa abundance in bulk soil, rhizosphere and frass sources (X, Y)**

Key findings:

* Adding frass (X and Y) markedly reduced the abundance of fungal taxa in both bulk soil and rhizosphere compared to no-frass (N)

**Shannon diversity index analysis of bulk soil, frass and rhizosphere bacterial and fungal microbiome: comparison between frass treatment (C, U) and source (N-nofrass, X, Y)**

Key findings:

* Frass application significantly decreased fungal diversity in both bulk soil and rhizosphere samples
* Frass-only (frass) samples had lower bacterial and fungal diversity than bulk soil and rhizosphere samples

**NMDS plot: bacterial community similarities/dissimilarities**

Key findings:

* Distinct separation of the no-frass (N) from the frass amendments (X, Y) (see Fig. A and B)
* Frass dose (2 and 5 g/kg) is the main contributing factor to this separation
* Composting frass significantly increased the similarity of bacterial communities (see Fig. C).
* However, noticeable dissimilarities between sources X and Y are still discernible

**NMDS plot: fungal community similarities/dissimilarities**

Key findings:

* Distinct separation of the no-frass (N) from the frass amendments (X, Y)
* Frass source (N, X, Y) is the main contributing factor to this separation
* Notable dissimilarities between composted and uncomposted frass
* However, such dissimilarities are not apparent when comparing the different frass sources.
* differentiation among the fungal communities is less pronounced compared to the bacterial communities

**Microbial taxa impacting *Brassica rapa* plant biomass prediction: random forest feature importance analysis**

Key findings:

* In both bulk soil and rhizosphere, the presence of Bacilli taxa (including Bacillus and Paenibacillus) emerges as a significant predictor of plant biomass in *B. rapa*
* Among the beneficial fungi in both bulk soil and rhizosphere, Sordariomycetes (including Metarhizium), Eurotiomycetes (Penicillium), and Mortierellomycetes (Mortierella) emerge as important predictors of plant biomass

**Comparative predictive performance of random forest models for bacterial and fungal data in plant biomass prediction across bulk soil and rhizosphere**

Key findings:

* Random Forest (RF) models demonstrate superior predictive performance for bacterial data (high R2, low RMSE values) compared to fungal data in relation to plant biomass
* Particularly, the models exhibit higher efficacy when applied to bulk soil samples (bacteria)

**Summary of the main message**

**Bacterial Community Results:**

* Composted frass increased the abundance of firmicutes (bacilli and paenibacilli) in bulk soil and rhizosphere.
* Paenibacilli were more abundant in uncomposted frass.
* Frass dose influenced bacterial populations, with higher doses (5 g/kg) promoting specific taxa (e.g., bacilli).
* Frass supplementation increased bacterial abundance in bulk soil and rhizosphere, with notable differences between frass sources X and Y.
* Distinct separation of bacterial communities was observed between no-frass and frass-amended samples, with **frass dose** as the main contributing factor.

**Fungal Community Results:**

* Composted frass increased Ascomycota abundance in both bulk soil and rhizosphere.
* Frass source Y had a higher abundance of Ascomycota than source X.
* Uncomposted frass had a higher abundance of Mortierellomycetes (Mortierella) in the rhizosphere.
* Frass dose had a less pronounced impact on fungal communities compared to bacteria.
* Distinct separation of fungal communities was observed between no-frass and frass-amended samples, with **frass source** as the main contributing factor.

**Plant Biomass Prediction:**

* Bacilli taxa, including Bacillus and Paenibacillus, were significant predictors of plant biomass in both bulk soil and rhizosphere.
* Beneficial fungi, including Sordariomycetes (Metarhizium), Eurotiomycetes (Penicillium), and Mortierellomycetes (Mortierella), were important predictors of plant biomass.
* Random Forest models performed better for bacterial data than fungal data in predicting plant biomass, especially in bulk soil.

This study highlights the impact of frass amendments on bacterial and fungal communities in soil, with distinct effects on different taxa and their role in influencing plant biomass.