The Effects of Urbanization on Soil Microbiomes: An Analysis on Mycorrhizal Fungi Biology using Genome-Scale Metabolic Modelling

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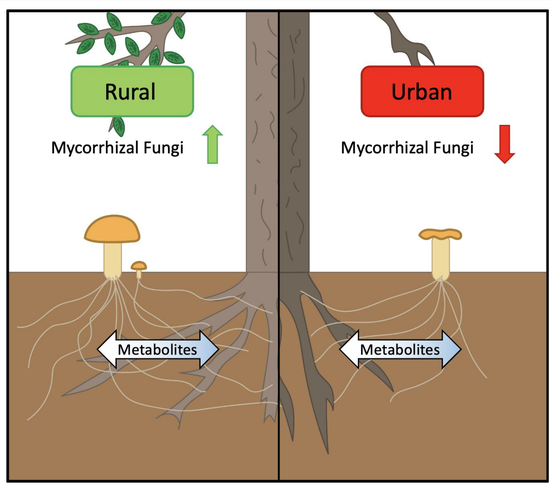
Introduction



- Urban areas are defined by areas of **high population densities**, **impervious surface area**, and **human land-use** [1].
- Urbanization often leads to **fragmented ecosystems** and disruptions to the **soil microbiome** (including **mycorrhizal fungi**), which, in turn, affects the growth of trees [2].
- Soil microbiomes and trees interact by **exchange of metabolites**, but the effects of urbanization on the metabolic activity of fungi are unknown.

Hypothesis

Because urbanization interrupts soil ecosystems, the metabolic activity and, thus, growth of **mycorrhizal fungi** will be **hampered** under urban stress.

Methods

Phase I: Modelling

Joint Genome Institute (JGI Data)

- Genomes and protein annotations from JGI were used [3].
- Fungi were selected based on previous network analyses; 6 fungal genera were found to play essential roles in soil microbiomes.

CarveMe for GSMM

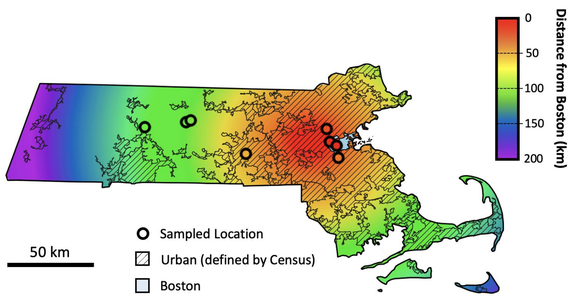
- Selected protein annotations from JGI were used to create **genome-scale metabolic models (GSMM)** in CarveMe [4].
- 2 types of models were created, **individual** and **community models**; individual models were for each fungi and community for all fungi
- Outputted matrix of metabolites and reactions.

CobraPy for FBA

- **Flux balance analysis (FBA)** utilizes an optimizes problem to calculate growth rates of organisms
- Here, FBA was run using **CobraPy** [5].
- Outputted **growth rates** for fungi in nutrients of interest (oxygen, nitrate, iron (II), manganese (II).

Phase II: Evaluation

Data Map



- To illustrate the validity of growth calculations with FBA, **relative growths** were compared to **bacterial abundances.**
- Samples were collected across the state of Massachusetts in **8 locations** (4 urban, 4 rural)
- Urban spots are on the far right; rural on left.

Results

Fungi from JGI	Maximum Relative Growth
Cortinarius sp. KIS3-TL2766	46.7409
Lactifluus cf. volemus	20.4884
Mortierella alpina	44.2076
Mortierella ambigua	23.9895
Mortierella elongata	17.9351
Mortierella gamsii	10.8546
Mortierella humilis	34.7980
Mortierella minutissima	44.7852
Piloderma byssinum	33.2001
Russula compacta	35.8777
Russula emetica	22.6740
Russula vinacea	26.6850

Table 1: List of Fungi Collected from the Joint Genome Institute (JGI). 12 fungi were selected from the genus *Cortinarius, Lactifluus, Mortierella, Piloderma, and Russula*. Metabolic models were created for each, and maximum relative growths in simulated media were determined using FBA.

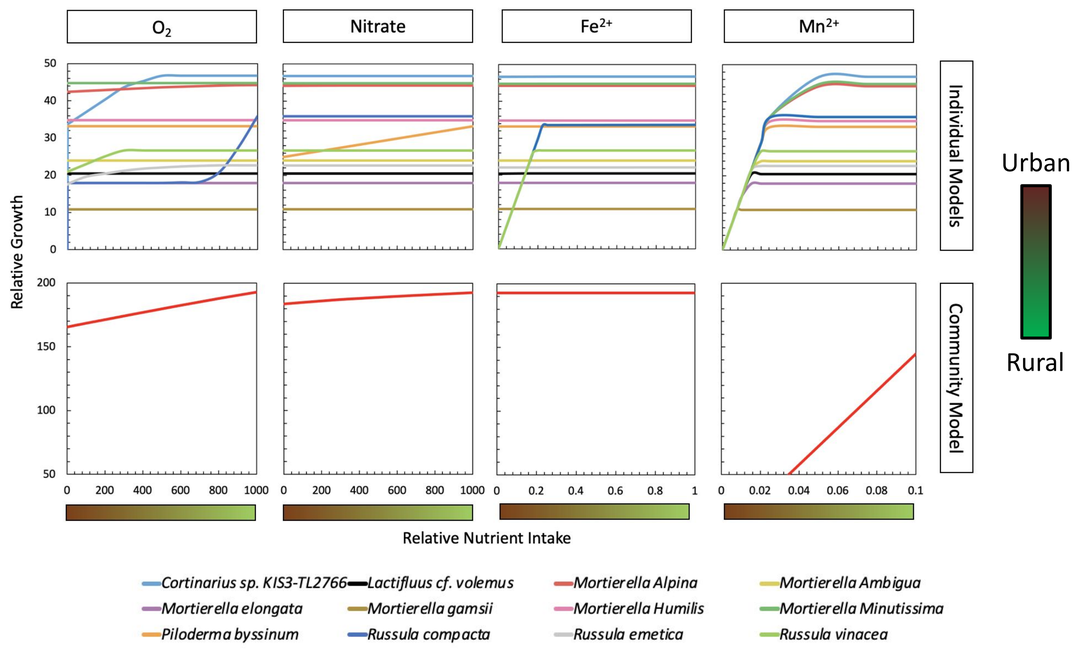


Figure 1: FBA Illustrated Overall Fungal Growth Increases in Rural and Decreases in Urban Conditions. Using both individual and community models, growth in different nutrient intakes were measured for oxygen, nitrate, iron (II), and manganese (II). Urban conditions are represented by lower nutrient intakes for all nutrients, while rural counterparts are simulated by higher intakes.

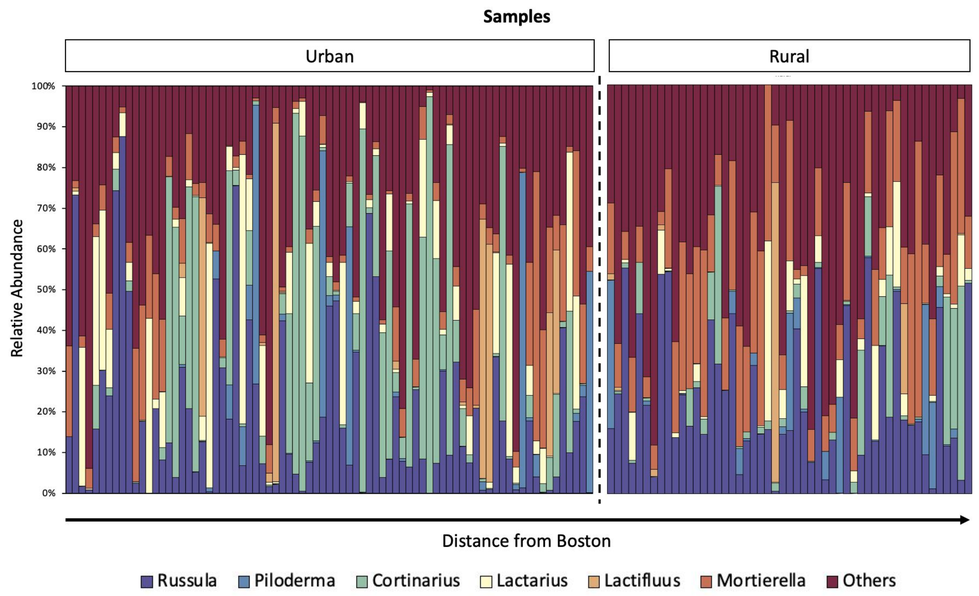


Figure 2: Relative Abundance of Key Fungal Genus in Urban vs. Rural. Relative abundances were created and sorted increasingly by distance from Boston.

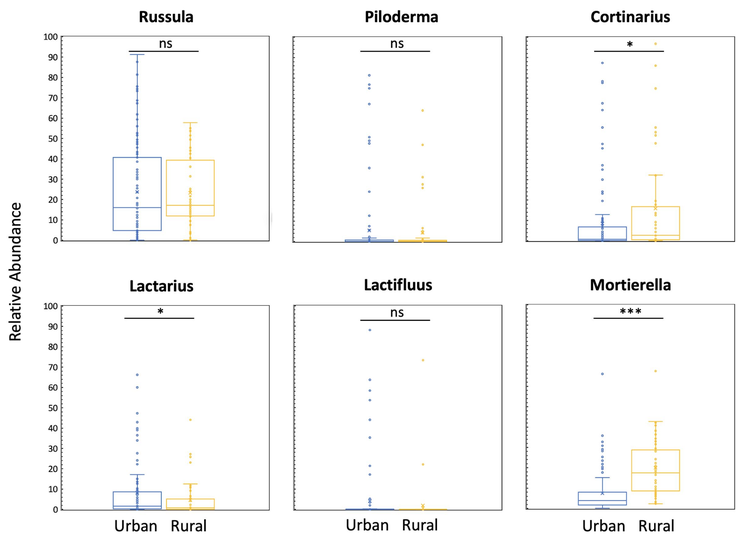


Figure 3: Significant Differences in Urban vs. Rural Conditions for 3 Genera
Using relative abundances from Figure 2, urban and rural samples of each genera were compared. Significance was indicated in Cortinarius, Lactarius, and Mortierella, but no significance (ns) was found in the other three. The p-values of <0.05 (*), <0.01 (**), and <0.001 (**) were considered significant.

Discussion/Conclusion

Analysis:

Using FBA, it was determined that (Figure 1):

- The growth of Russula and Cortinarius genera were impeded by lower O_2 intakes.
- The growth of the Piloderma genus was impeded by lower nitrate intakes.
- The growth of the Russula genus was impeded by lower Fe^{2+} intakes.
- The growth of all genera were impeded by lower Mn^{2+} intakes.

However, comparison of relative abundances across genera in urban vs. rural demonstrated only significant differences in the genera of Cortinarius, Lactarius and Mortierella – all of which were not significantly affected by urban nutrients (Figure 3).

- It is possible that the four nutrient intakes tested were not the only major nutrients affected by urbanization; other nutrients need to be tested, such as mannitol [6].
- It is also possible that there limitations in fungal genomes from JGI, as shown by Lactarius not included in the fungal list (Table 1).
- Another possibility is that the differences between urban and rural areas are not clear-cut; hence, relative abundances deemed as rural or urban are also ambiguous [7].
- A probable reason for differences in urban vs. rural for Cortinarius is that their growth is the greatest, making it more prone to disturbance (Table 1) differences in rural vs. urban of fungi did

Key Conclusions:

- Model data showed that urban nutrients are not conducive for fungal growth as found in lower nutrient (oxygen, nitrate, iron (II), manganese (II)) intake conditions
- Growths in model did not match relative abundance differences; further work needs to be done to investigate this discrepancy.

Future Works:

- Instead of relative growths, standardized units of growth can be derived by sampling metabolites in soil samples *in vivo* [8].
- Complete metabolic pathway models can be derived from biomass fluxes of fungi.
- Metabolites can be simulated to determine effects on trees using analytical tools such as MetaboAnalyst [9].

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