Isolation and Identification of Recalcitrant Materials in *Rhizopus* as Potential Sources of Sequestered Soil Carbon

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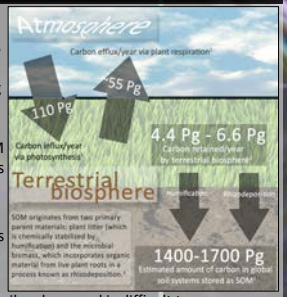
Abstract

Fungi are integral to carbon cycling due to their ubiquity in global soil systems as well as their typical ecosystem-dynamic role as decomposers. However, the overall contribution of fungi to the pool of sequestered soil organic matter (SOM) is unknown.[1] We sought to explore this question by determining whether fungi themselves are the source of recalcitrant macromolecules that contribute to the stabilization of the soil carbon pool. To do this we harvested cultures of *Rhizopus* wild-type fungi and subjected the tissue to a sequence of extractions (water and dichloromethane/methanol) and hydrolysis (hydrochloric acid). The remaining solids from each extraction/hydrolysis step were analyzed by Fourier-transform infrared spectroscopy (FTIR) and elemental analysis. Comparative FTIR analysis indicated that the amino acid and carbohydrate composition present in the raw sample changed only slightly after the water and DCM/MeOH extractions, but that both biomolecule classes were significantly altered following the HCl hydrolysis, leaving a residue containing no immediately identifiable macromolecules. As hypothesized, recalcitrant material was detectable in the hydrolysis residue spectrum. FTIR spectra indicated an increased CH_2:CH_3 ratio suggesting the presence longer of alkyl chains as well as C=O bonds.

Introduction



- Feedback from flux in soil system carbon dynamics has environmental implications in areas ranging from climate change to agriculture. However, the mechanisms by which SOM traps, stores, and releases carbon remain unclear.[2]
- Rhizodeposition accounts for a significant portion of below-ground carbon Input.[3] Its impact upon the soil carbon pool is difficult to quanitfy because organic exudates are rapidly consumed by soil microbes – inextricably linking the functioning of these microorganisms on a cellular level with the flow of carbon on a global level.

References and Acknowledgements

1. Lorenz, K.; Lal, R.; Preston, C. M.; Nierop, K. G. J., Strengthening the soil organic carbon pool by increasing contributions from recalcitrant aliphatic bio(macro)molecules. *Geoderma* **2007,** *142* (1-2), 1-10.
2. Dixon, R. K.; Turner, D. P., The Global Carbon-Cycle and Climate Change - Responses and Feedbacks from Belowground Systems. *Environ Pollut* **1991,** *73* (3-4), 245-262.
3. Kogel-Knabner, I., The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol Biochem* **2002,** *34* (2), 139-162.
4. Watanabe, Tsuneo. Pictorial Atlas of Soil and Seed Fungi. Morphologies of Cultured Fungi and Key to Species, 2nd Edition. CRC Press, 2002.

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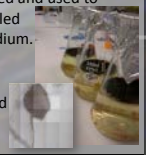
Hypothesis

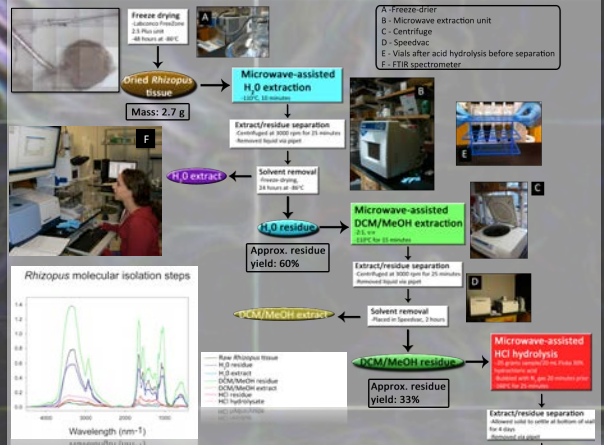
An organic fraction will be detectable in *Rhizopus* tissue residue after subjecting it to standard biomolecule extraction and isolation procedures.

Methods – Culturing

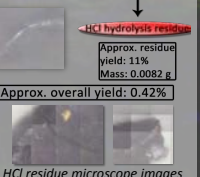
- Wild-type fungi were cultured from Chicago Botanic Garden's Dixon Prairie soil samples on a potato dextrose agar growth medium.
- Unique colonies were replated and used to inoculate Erlenmeyer flasks filled With a tryptic soy growth medium.
- Tissue was identified as *Rhizopus* via microscope analysis and hyphae/spore literature comparison.[4]
- Cultures were vacuum filtered and rinsed with DI water. Filtered material was then freeze dried.



Methods – Isolation and Analysis

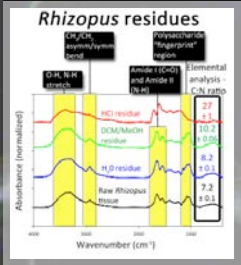


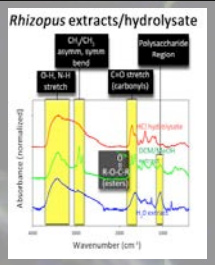
- Fourier-transform Infrared (FTIR) spectra were collected of the solids obtained at each circled step. Absorption measurements were recorded from KBr pellets containing ~3% sample by weight.
- Each absorption peak corresponds to a characteristic functional group absorption such as hydroxyls or amines.
- Peaks were assigned based upon comparison with literature values, and changes in sample composition were deduced through variations in peak locations and intensities.
- Elemental analysis was run on residues from each step to determine C:N ratios.



HCl residue microscope images

Results





Rhizopus Residue Principle Peaks

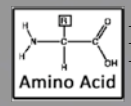
Raw *Rhizopus* tissue	H_2O residue	DCM/MeOH residue	HCl residue
~3350	~3370	~3380	~3400
~3280	~3280	~3260	~3200
2961	2958		
2930	2927	2930	2921
2873 shoulder	2873 shoulder		
2851 shoulder	2855	2876 shoulder	2852
			1707
1654	1650	1658	
			1607
1549	1547	1548	
			1400
~1220	1260	1240	1210
1149	1151	1151	
			~1120 shoulder
1079	1078	1077	
1041	1039	1039	

Rhizopus Extract Principle Peaks

H_2O extract	DCM/MeOH extract	HCl hydrolysate
~3370	~3380	~3400
~3280	~3260	~3300
2958		
2927	2930	2921
2873 shoulder		
2855	2878 shoulder	2852
		1707
1650	1658	
		1607
1547	1548	
		1400
1260	1240	1210
1151	1151	
		~1120 shoulder
1078	1077	
1039	1039	

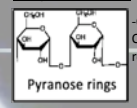
Major biomolecules detectable in raw material through DCM/MeOH residue:

- Proteins (amino acids)



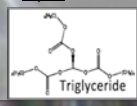
-N-H bonds (amide I)
-C-O bonds (amide II)
-Carboxylic acids (-COOH)

- Carbohydrates (Polysaccharides)



-C-O, C-H, O-H overlap (fingerprint region)

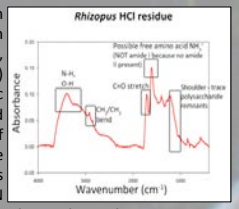
- Lipids



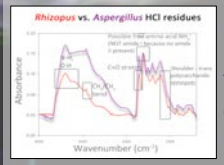
-Alkyl chains (CH_2)
-Esters

Discussion and Conclusions

The presence of certain functional group absorptions in the HCl residue such as CH_2, CH_3, and C=O (carbonyls) indicate that some organic material was retained throughout the series of extractions. The disappearance of the amide bands is supported by the increased C:N elemental analysis ratio in the HCl residue, suggesting polypeptide-containing amino acids were removed in the hydrolysis step.

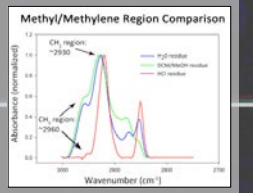


The peak height ratio of CH_2:CH_3 (as measured by the peaks at ~2930 and ~2960) decreased from 1.9 to 1.6 following the DCM/MeOH extraction step due to dissociation of fatty acid alkyl chains. However, this ratio rose to 2.5 following the HCl hydrolysis step. This indicates that the recalcitrant material present in the residue might contain longer alkyl chains.



Analysis of *Aspergillus* (an ascomycete fungus) using the same extraction method yielded a similar HCl residue spectrum to that of *Rhizopus* (a deuteromycete). This cross-phylogenetic comparison indicates that these results are not unique to just one species.

The presence of hyphae in the HCl residue indicate that these structures contain a non-protein structural component that withstood hydrolysis. Therefore, our hypothesis that fungal tissue is a source of recalcitrant organic material is correct.



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