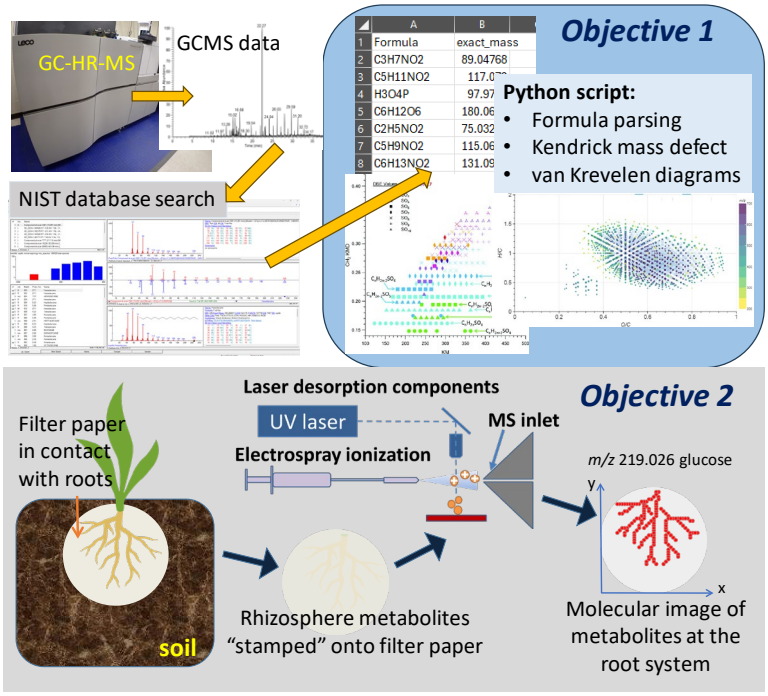
i. **Visualizing molecular complexity: analysis of coal-derived samples and rhizosphere by High-Resolution (HR)-Mass Spectrometry**

ii. **PI**: Franco Basile (Chemistry, CEPS)
co-PIs: Maohong Fan (Chemical Engineering, CEPS), Cynthia Weinig (Botany, AG).

iii. **Total amount requested:** $23,990

iv. **Description of the research problem:**

This proposal addresses the chemical analysis of highly complex and difficult to analyze samples, specifically, the characterization at the molecular level of coal-derived samples and rhizosphere metabolites. Coal is a highly complex and crosslinked mixture of hydrocarbons, polyaromatic hydrocarbons, organic acids, etc. that easily overwhelms the selectivity of analytical techniques, making compound identification strategies highly inaccurate and often futile (e.g., dodecane $C_{12}H_{26}$ has 58 isomers!). Equally challenging is the detection of plant root exudates, which are metabolite molecules, secreted by the plant root system to interact with the surrounding soil microbiome. These exudates lie within a millimeter from the surface of the root tissue and are surrounded by soil background, making the specific sampling of these molecules very challenging (i.e., excluding soil background molecules or molecules from damaged root tissue). To overcome the shortcomings in these analyses, this proposal applies High-Resolution (HR) Mass Spectrometry (MS) to visualize the molecular composition. To achieve this goal, two objectives will be pursued:



Objective 1) generate Kendrick mass defect and van Krevelen diagrams to visualize the molecular diversity in coal-derived samples, and **Objective 2)** spatially map the metabolite (root exudate) composition of the root system via imaging-Mass Spectrometry. In both applications, the complexity of the sample is reduced into an information-rich image conveying the molecular composition of the sample (**Figure 1**).

Proposed long-term benefits: The proposed work, when completed, will result in a suite of methodologies and techniques for the analysis of complex samples. These techniques are applicable to many areas of research at UW and provide opportunities to seek new funding in areas of Chemical Engineering (coal processing, biofuels, biopolymers), Chemistry (recycled materials, environmental analysis), Geology (biogeochemistry) and fundamental Biology (plant rhizosphere).

v. Short-term objectives and proposed research.

Objective 1: Develop methodology and computational tools to generate Kendrick mass defect plots and van Krevelen diagrams (O/C vs. H/C elemental ratios) of coal-derived samples.

Specific study 1: *Develop GC(HR)MS methodology to analyze coal derived products.* Based on previous work by the PI on GCMS analysis of coal (Mahat et al. 2014) and available GC(HR)MS instrumentation, work will focus on the analysis of highly non-polar residues from the extraction of coal samples provided by co-PI Prof. Fan (Wang et al. 2020). The proposed methodology will be validated with standards and expected to obtain high resolution mass spectral data (<1 ppm mass accuracy, 0.02 amu).

Specific study 2: *Generate Kendrick mass defect plots and van Krevelen diagrams (O/C vs. H/C elemental ratios) derived from GC(HR)MS data***.** We propose developing Python-based computational tools to generate the Kendrick and van Krevelen diagrams for coal-derived samples. The algorithm will parse molecular formulas (obtained via mass spectral NIST database search) to generate the diagrams. Once tested and validated with control data and samples, the algorithm will be applied to characterize coal-derived samples provided by the co-PI Prof. Fan.

Objective 2: Develop Imaging-MS methodology for the spatial mapping of metabolites in plant root systems (rhizosphere).

Specific study 1: *Develop a membrane blotting transfer (MBT) technique for root exudate sampling.* Based on preliminary data in collaboration with co-PI Prof. Weinig (UW-Botany), metabolites at the surface of the root system will be sampled via blotting onto a membrane.

Specific study 2: *Develop a HR-MS methodology for the spatial detection (i.e., imaging-MS) of root exudates directly from the sampling membrane*. Using instrumentation already developed and tested in the PI's lab, imaging-MS of the metabolites adsorbed/absorbed onto the filter paper will be mapped (imaged), making possible the differentiation of root exudates from soil background molecules.

vi. Potential future partnerships

The development of methodology for generating van Krevelen diagrams can be used by any investigator working in the energy sector, especially those focusing on research of coal-derived products (Li-Oakey), biofuels (Belmont), biopolymers (Sabino) and other biogeochemical samples (Geology). The development of imaging-MS technology can be used by investigators involved in fundamental Biology (UW), physiology (UW) and biomaterials (UW). The PI plans to give a seminar at the college level to highlight the capabilities of this technology and methodology.

vii. Future funding sources

The exudate work is part of a proposal to be submitted to the NSF-DBI program this Spring 2024, focusing on the imaging-MS of root exudates and IR-laser upgrade. The Kendrick mass defect and van Krevelen diagram methodologies are applicable to projects in Chemical Engineering, Chemistry and Geology. The PI will actively seek collaborations to submit proposals to the NSF Division of Materials Research and/or DoE's Office Fossil Energy and Carbon Management.

References cited

Mahat RK, Rodgers W, Basile F (2014) Microwave Radiation Heating in Pressurized Vessels for the Rapid Extraction of Coal Samples for Broad Spectrum GC–MS Analysis. Energy & Fuels 28:6326–6335. https://doi.org/10.1021/ef501659h

Wang T, He X, Gong W, et al (2020) Flexible carbon nanofibers for high-performance free-standing supercapacitor electrodes derived from Powder River Basin coal. Fuel 278:117985. https://doi.org/10.1016/j.fuel.2020.117985

Budget

2024-2025 CEPS Interdisciplinary-Collaborative Seed Grant				
Project: Visualizing molecular complexity: analysis of coal-derived samples and rhizosphere by High-Resolution (HR)-Mass Spectrometry				
description	**quantity**	**unit cost**	**total**	**link/part number**
column rtx-5ms (50 m)	1	$ 1,169.00	$ 1,169.00	https://www.agilent.com/store/productDetail.jsp?catalogId=122-5052&
guard column Z-Guard (10 m)	2	$ 266.00	$ 532.00	https://www.phenomenex.com/products/zebron-gc-columns/zebron-zb
helium	1	$ 297.73	$ 297.73	stockroom
syringe	2	$ 76.95	$ 153.90	https://www.agilent.com/store/en_US/Prod-5181-3354/5181-3354
GC liners	8	$ 122.00	$ 976.00	https://www.restek.com/p/23302
MSTFA 10x1mL	6	$ 195.00	$ 1,170.00	https://www.sigmaaldrich.com/US/en/product/sial/69479
MeOX	1	$ 47.80	$ 47.80	https://www.sigmaaldrich.com/US/en/substance/methoxyaminehydroc
PVDF Durapore membrane	1	$ 250.00	$ 250.00	https://www.emdmillipore.com/US/en/product/Durapore-Membrane-Fi
MeOH Case of 4	1	$ 621.00	$ 621.00	https://www.fishersci.com/shop/products/methanol-optima-lc-ms-grad
H2O Optima HPLC	4	$ 98.16	$ 392.64	stockroom
MTBE HPLC	1	$ 324.76	$ 324.76	stockroom
Acetone 4L hplc	2	$ 69.72	$ 139.44	stockroom
Hexanes 4L optima	2	$ 111.44	$ 222.88	stockroom
gloves	10	$ 10.92	$ 109.20	stockroom
GC vials 1000pk	1	$ 241.00	$ 241.00	https://www.restek.com/p/21141
GC vials insert pack 500pk	3	$ 495.00	$ 1,485.00	https://www.restek.com/p/22434
GC caps 1000pk	2	$ 285.00	$ 570.00	https://www.restek.com/p/24486
LC vials 1000pk	1	$ 126.98	$ 126.98	https://www.thermofisher.com/order/catalog/product/6ASC9ST1
LC vials inserts 100pk	11	$ 56.77	$ 624.42	https://www.thermofisher.com/order/catalog/product/C4012-529L?SID
LC caps 100pk	11	$ 34.53	$ 379.83	https://www.thermofisher.com/order/catalog/product/6ASC9ST1
NIST 2023 mass spectral database (upgrade)			$ 1,897.56	https://www.sisweb.com/software/ms/nist.htm
		total supplies:	$ 11,731.14	
S&H	2%		$ 234.62	
Salary				
PI summer salary	0.75	month	$ 8,534.00	
Fringe	40.90%		$ 3,490.41	
Proposal total requested			**$ 23,990.17**	

Budget justification:

GC(HR)MS of coal-derived samples: a new 50 meters GC column and column guards are requested to analyze the complex mixtures in coal samples (in addition to the high-resolution mass analysis). Funds are requested to upgrade to the NIST2023 mass spectral database with spectra from 347,100, and 40,000 more than the current version used in the Basile lab (NIST2020). *Note: this NIST2023 database will also benefit Objective 2 of this proposal*. Also, GC consumables like helium gas, injector liners, derivatizing agents, extraction solvents and vials are requested for method development and sample analysis. Coal-derived samples will be analyzed also with the LC-High Resolution Orbitrap-MS system for potential polar compounds**.**
Imaging-MS of root exudates: supplies are requested for the blotting PVDF filters, extraction solvents, and LC-MS/MS analysis (for metabolite identification confirmation).
Salary: A ¾ month summer salary and benefits are requested for the PI Franco Basile who will perform duties of project management, data analysis and Python software development.