Tracing single-cell scale chemical signaling between interacting soil fungi (INTERSPEC)

A Data Management Plan created using DMPRoadmap

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Project abstract:

Multiple species of fungi co-exist in soils and play an important role in biogeochemical cycles. To survive in a resource limited environment, they have developed the means for interspecific communication and warfare via an arsenal of secreted secondary metabolites. Specific ecological role of those metabolites and the extent to which they affect biogeochemical cycling during fungal interactions remains unknown. Because they are secreted and act at a single-cell scale, tracing them ‘then and there’ can aid in identifying potential triggers for their production and clarifying their function. Currently used methods have either insufficient resolution or are destructive, and are not suitable for such analyses. Here, I will use my expertise in spectroscopy techniques (1) to establish experimental protocols for the single-cell scale fungal secondary metabolite identification and characterization using surface-enhanced Raman scattering (SERS) microspectroscopy - a method that employs optical properties of gold nanoparticles for molecule specific sensing and that has been shown in biomedical research to have an extraordinary potential for studying microbial metabolic processes. I will combine it with microfluidics based soil chips, that provide visual access to and mimic real ecosystems via control over biotic and abiotic environment of soil microbes. I will then use the approach (2) to determine how interspecific fungal interactions under varying nutrient conditions affect the composition of their secondary metabolome and its functions. Additional transcriptome analysis (3) will reveal fungal genes involved in up- or downregulation of the metabolite biosynthesis, but also extracellular enzyme production for organic matter degradation and nutrient acquisition. Ultimately, the aim is to offer the community of soil fungal ecologists, and microbiologists in general, with a game-changing new tool to study ecosystem functions of secondary metabolites in more realistic settings.

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1. Data description

- collecting material or generate/produce data (e.g. interviews, measurement data)

The data (spectra) will be collected by performing surface-enahnced Raman microspectroscopy measurements on interacting soil fungi, fungal metabolite extractions or synthetic metabolite compounds deposited either on gold nanoparticle substrates or in microfluidic chips with the gold nanoparticles built in within. The nanoparticles themselves will be characterized using UV-VIS absorption spectroscopy (data type: spectra) and SEM/TEM microscopy (data type: images).
Additional data will be collected by taking time sequences of optical microscopy images of the fungal hyphae in the chips as well as photos of mycelium at different time points in fungal mono- and co-cultures growing in agar plates. Finally, liquid chromatography - mass spectrometry and nuclear magnetic resonance spectroscopy data (spectra) will be collected from fungal metabolite extractions obtained from those agar plates.

Digital microspectroscopy and optical microscopy data will be collected in data file formats defined by the commercial softwares connected with the instrumentation used: LabSpec 6 from Horiba for Raman microspectroscopy (file type .l6s), NX Studio from Nikon for optical microscopy (file type .nd2). In addition to the original data, the spectra will be converted to and stored in .txt file format for analysis with external analysis software (e.g. Quasar). Multispectral images (optical image and chemical image obtained after spectral processing) will also be stored in image format .png.
Optical microscopy images will be converted to .tif format and stored alongside original data. Mycelium diameter measurements will be made (1) using a ruler and documenting in an excel table (file formal .xlsx). Additionally, photos of the fungal cultures will be taken and stored in .tif format. Mycelium diamter measurements will then additionally be made (2) in the photos.
Lab notes will also be kept in physical notebooks.

- >1 TB

The spectral data does not require large storage capacity as typical file size is in the scale of kB. Images taken of the spectra collection points and photos of fungal cultures are in the scale of MB. The large storage is necessary for recording optical microscopy images of microfluidic soil chips where file size is in the scale of tens of GB.

2. Documentation and data quality

Metadata, such as data collection parameters during microspectroscopy (e.g., laser wavelength, spectral resolution (grating used), laser power (neutral density filter used), exposure time, objective used, etc.) and optical microscopy (objective used, exposure time, etc.) experiments, is documented and stored in original files created by the software, which will be stored together with the converted files (.txt or image file formats). Additionally, a README file will be created where the metadata, data structure in files and data collection conditions will be described. Other experimental conditions will also be documented in the physical lab notebook.
The data files will be named following the naming convention to clearly indicate the date the data was collected, sample name/type etc. and stored in data folders named according to the part of project/experiment.

Spectral data quality is described by signal-to-noise ratio, taking the collection method and sample type (singal-to-noise ratio can be increased by using higher laser powers when collecting spectral data, but biological samples burn and are destroyed under those conditions, so compromise needs to be made) into account. To increase spectral data quality, acquisition times as well as number of scans (single spectra from the same measurement point collected and averaged together) will be adjusted. For surface-enhanced Raman microspectroscopy measurements, the spectral quality also depends on the gold nanoparticle substrates. The preparation protocols of these substrates will therefore be adjusted accordingly to yield the best sensitivity and selectivity (when necessary).
For interpretation quality, data will be collected in replicates (different chip channels and interal replicated for different fungal hyphae and different chips as independent biological replicates). Biological replication will aslo be used for fungal mono- and cocultures in agar plates.

3. Storage and backup

The collected microspectroscopy data will be stored in the computer connected to the instruments and in a backup external storage device (hard drive), which will be created following each data collection event (experiment).
Optical microsopy data is directly collected to an external hard drive connceted to the computer that is connected to the microscope and will be stored there. Additionally, the data will be backed-up in another external hard drive, which will be stored in separate place from the originals.
Photos of fungal cultures will be stored in an external hard drive for backup (also stored separately for backup).
All the computers of the staff working within the project will furthermore be connected to the Lund Univeristy cloud service (OneDrive) for synchronized back up of all documents created (data analysis files, manuscript drafts, etc.)

No sensitive or personal data will be generated in this project, but access to data in the storage folders will be safeguarded via password.
Data sharing with collaborators, when necessary, will be achieved using LU Box, which can only be accessed by invitation from folder owner only.

4. Legal and ethical requirements

- No

Question not answered.

Question not answered.

- No, LU is the sole party.

5. Data sharing and long-term preservation

- Yes, both data and metadata

Upon publishing, all the data, both raw data and, when relevant, analysis algorithms (scripts), will also be made publicly available by uploading it to online repositories with links made available in the associated publication.
The spectral data, once converted to .txt files, has a tabular format, and its their structure will be described in the README file along with the metadata as described above. They will be both human- and machine-readable.
Any excel tables created (e.g. mycellium diameter records) and data sheets withing will be separately saved and published as .csv files.
The microscopy data will be published as images in .tif/.png format.

Upon publishing, all the data, both raw data and, when relevant, analysis algorithms (scripts), will be made publicaly available by uploading it to online repository, such as GitHub, or B2Share (EUDAT Collaborative Data Infrastructure), with links made availabe in the publication. The data will be shared under CC-BY license.

Yes, persistent identifyer will be assigned to all datasets published in respositories.

- My current assessment is that data should be kept.

6. Responsibilities and resources

The PI of the project is responsible for overall data management and long-term preservation. Data produced by project employees (postdocs, PhD students), project assistant or Master degree students working on projects related to this one will be asked to store data as described in this DMP, which will be accessible to the PI and other people potentially related to the project (following the description for data safety).

Lund University provides some data storage and sharing means, such as LU Box or OneDrive, which are free of charge. Some external storage devices (hard drives) will be additionally bought.