**Data description**

Enzyme activity data is in the titular “Enzyme activity” data folder. Enzyme assays were conducted by diluting 7 substrates for hydrolytic enzymes and 1 substrate for oxidative enzymes. The litter from each sample is homogenized and then plated with varying concentrations of each substrates and left to incubate and then read in the old plate reader (BioTek Synergy 4). There are 2 types of plates, each for a different type of enzymes: black plates for hydrolytic enzymes, and clear plates for oxidative enzymes. Hydrolytic enzyme activity was obtained by reading the fluorescence from each well in a sample black plate while oxidative enzyme activity was obtained from reading the absorbance from a sample clear plate. Please refer to the 2020-11-24-Plate-Layouts.xlsx spreadsheet as a reference.

Enzyme assays follow principles and recommendations described in the paper cited below. Let me briefly describe the assays for hydrolytic enzymes. As mentioned above, their activities are measured via fluorescence in black 96-well plates that only allow light to enter and leave through the opening of each well, as opposed to clear 96-well plates that allow light to pass through all directions. According to the plate layout for black plates, each column is denoted by a number and assays for a single enzyme. Columns 1-7 are the hydrolytic enzymes that are assayed for, and substrate concentrations decrease as you go down each column. Columns 8-10 serve as control purposes and contain standard solutions and water. Each substrate contains a group that, once reacted, will cause the well it is in to fluoresce, and it is this fluorescence that I use to measure their activity. Columns 8 & 9, which contain standard solutions, will also fluoresce. In each assay round, leaf litter from different plots are homogenized and then plated with the same number of sample plates as the number of plots. That means that leaf litter from a single plot is homogenized and plated into one plate with substrates in columns 1-7 and standard solutions and water in columns 8-10; leaf litter from another plot is homogenized and then plated into another plate with substrates in columns 1-7 and standard solutions and water in columns 8-10, and so on, so forth. Also, in each assay round, a buffer plate is plated along with all of the sample plates where instead of containing homogenized leaf litter, the buffer plate is plated with a 6.0 pH 25 mM maleate buffer solution with substrates, standard solutions, and water. There are 3 controls: substrate control, quench control, and homogenate control. The substrate control controls for the fluorescence from the substrates. For each assay round, the fluorescence values of columns 1-7 of the BUFFER plate will be used as the substrate control for all samples in that round. The quench control controls for any “quenching”, “masking”, or “dimming” caused by the suspended particles in the homogenized leaf litter blocking some of the fluorescence; if not controlled for and reduced, it can artificially reduce fluorescence and, as an extension, cause an underestimation of enzyme activity. Each sample plate will have its own quench control values in the fluorescence values in the plate’s own columns 8 & 9. In each sample plate, columns 1-5 and 7 will use the standard MUB (column 9) as their quench control while column 6 will use the standard AMC (column 8) as its quench control. The homogenate control controls for any fluorescence that the homogenate might give off; if not controlled for, fluorescence from the homogenate can cause an overestimation of enzyme activity. Each sample plate will have its own homogenate control in its column 10; column 10 of the buffer plate in each assay round will not be used, as these values are essentially meaningless. Columns 8-9 of the buffer plate in each assay round will be used to aid in quench control for that round.

Enzyme activity data are .txt files. A data file with “Black-Plates” in its title only contains data of hydrolytic enzymes, while a data file with “Clear-Plates” in its title only contains data of oxidative enzymes. In addition, each of these .txt files also contain the timepoint in its title (T0, T3, T5, or T6).

**Columns description (in order from left to right in a data file)**

Well ID: the identifier of a particular well; all wells are plated with the same sample, but each well has a unique concentration of a substrate.

* Unitless

Plate ID: the identifier of a particular sample and plate. Each plate only contains one sample, and each plate has 96 wells, so for a particular sample, there should be 96 rows with unique Well IDs. Each of these 96 rows will have an identical Plate ID as each other. This column contains the following useful pieces of information: (1) the date the assay was performed in YYMMDD, (2) a unique sample identifier that is described in the descriptive metadata file (3) and if the file is a “Black-Plates” file, a letter denoting the plate type (*B* for buffer plates and no letter if the plate is a sample plate).

* Unitless

Fluorescence or absorbance reading: this is the raw fluorescence (for “Black-Plates” files) or absorbance (for “Clear-Plates” files) reading for each well.

* Fluorescence/absorbance units

**Association metadata files**

2020-11-10-Litter-bag-codes.xlsx: this association metadata file describes the unique sample identifier in the Plate ID column in the raw enzyme activity and data files. This is available under the “Metadata” folder.

2020-11-10-Dry-weight.xlsx: this file describes the month and year (YY) of each time point in which litterbags were picked up from the field. Although this file contains metadata, it is primarily a data file, hence its location under the “Dry weights” folder.

2020-11-24-Plate-Layouts.xlsx: this describes the plate layout of black plates (which assays for hydrolytic enzymes) and clear plates (which assays for oxidative enzymes). Included is the highest concentration of each substrate. Each substrate is diluted serially by half with the top row having the highest substrate concentration while the bottom row has the lowest substrate concentration. This file is available under the “Metadata” folder.

**File/data owner**

Steven Allison

**Who is permitted to access**

All project personnel

**Permission to update/edit/use data**

Please contact the data owner for permission to change or analyze the data.

**Works cited**

German, Donovan P. et al. “Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies.” *Soil Biology & Biogeochemistry* vol. 43, no. 7, 2011, pp. 1387-1397.