There are 2 types of enzymes that are assayed for: (1) hydrolytic enzymes and (2) oxidative enzymes. Hydrolytic enzyme activities are assayed using fluorimetric methods and oxidative enzyme activities are assayed using absorbance after reacting with pyrogallol and hydrogen peroxide. Methods, theory, and calculations follow from German et al 2011, cited below.

Hydrolytic enzymes (black plates, fluorescence)

Activities of hydrolytic enzymes 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 only assays for a single enzyme (please refer to the ***2020-11-24-Plate-Layouts.xlsx*** spreadsheet for the plate layout). There are 7 hydrolytic enzymes that are assayed for, and they are assayed for in columns 1-7 where each column contains only a single substrate and so only assays for a single enzyme, and substrate concentrations decrease as you go down each column. Columns 1-7 in buffer plates (more on buffer vs sample plates below) serve as substrate controls (more on controls below). Columns 8-10 serve other 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 an enzyme's activity. Columns 8 & 9, which contain standard solutions, will also fluoresce.

Each assay round has 2 types of plates: (1) a buffer plate, which will be used for control purposes, and (2) at least 1 sample plate. As stated above, there are 7 substrates in each assay round. Each assay round also uses 2 standards in columns 8 & 9 with each standard having a column to itself. Water will be in column 10. Both plate types will contain all of the above (substrates in columns 1-7, standards in columns 8 & 9, and water in column 10); however, while a sample plate will be plated with a homogenized leaf litter sample while a buffer plate will be plated with be plated with a 6.0 pH 25 mM maleate buffer. In each assay round, a serial dilution is carried out for each of the 7 substrates where they are sequentially diluted by half. Each assay round uses the same dilution. 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 in columns 8 & 9, and water in column 10; leaf litter from another plot is homogenized and then plated into another plate with substrates in columns 1-7 and standard solutions in columns 8 & 9, and water in column 10, and so on, so forth.

There are 3 controls: (1) substrate control, (2) quench control, and (3) homogenate control. These substrate controls are figured into enzyme activity calculations using the equations in the paper cited below. Substrates will fluoresce by themselves, and the substrate control controls for that; if not controlled for, then the substrate control will artificially increase fluorescence, making analysts think that the activity of an enzyme is higher than it actually is. 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, 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.

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.

Enzyme assays were conducted following the theory and methods outlined in this paper.