

## INTRODUCTION

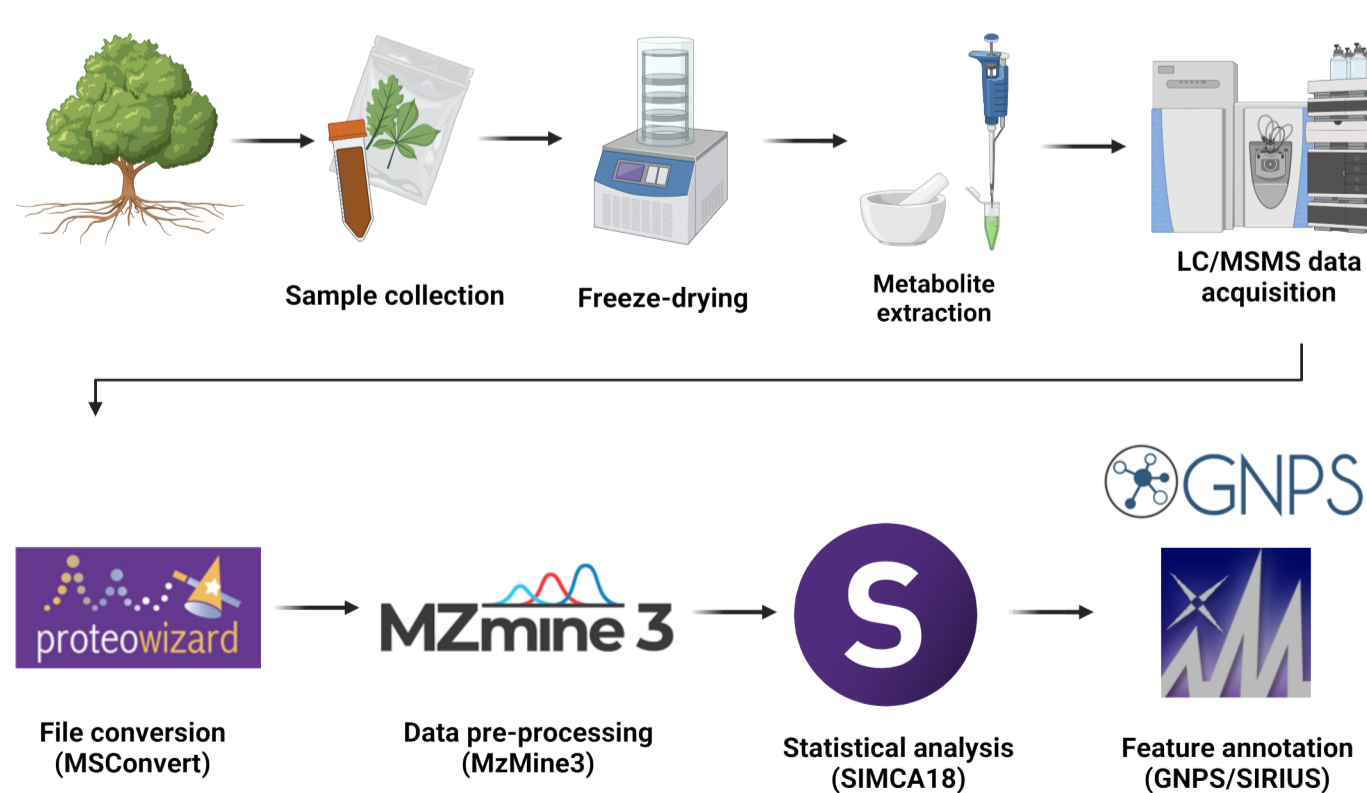
Forests play a vital role in supporting biodiversity, carbon storage, and climate regulation on a global scale. However, they are currently facing significant threats due to the rise in global temperatures and unpredictable climate conditions. These climatic changes have led to the proliferation of new pests and diseases, impacting forests both globally and within the UK.

## OBJECTIVES

This study seeks to analyze the metabolome of Oak and Horse Chestnut trees, specifically focusing on diseased trees. The aim is to understand their metabolic response and establish baselines for soil treatments. The study aims to investigate whether soil treatments such as mulch and phosphite root drench can enhance tree health.

## METHODS

Samples were collected at two timepoints: late spring (May 2022) and late summer (September 2022). Oak leaves were collected from two locations, Wyre forest and Heart of England forest (HEF). Horse chestnut leaves were collected from one location (Sunny Hill Park, London). The samples were flash frozen and processed further as described in **Figure 1**.

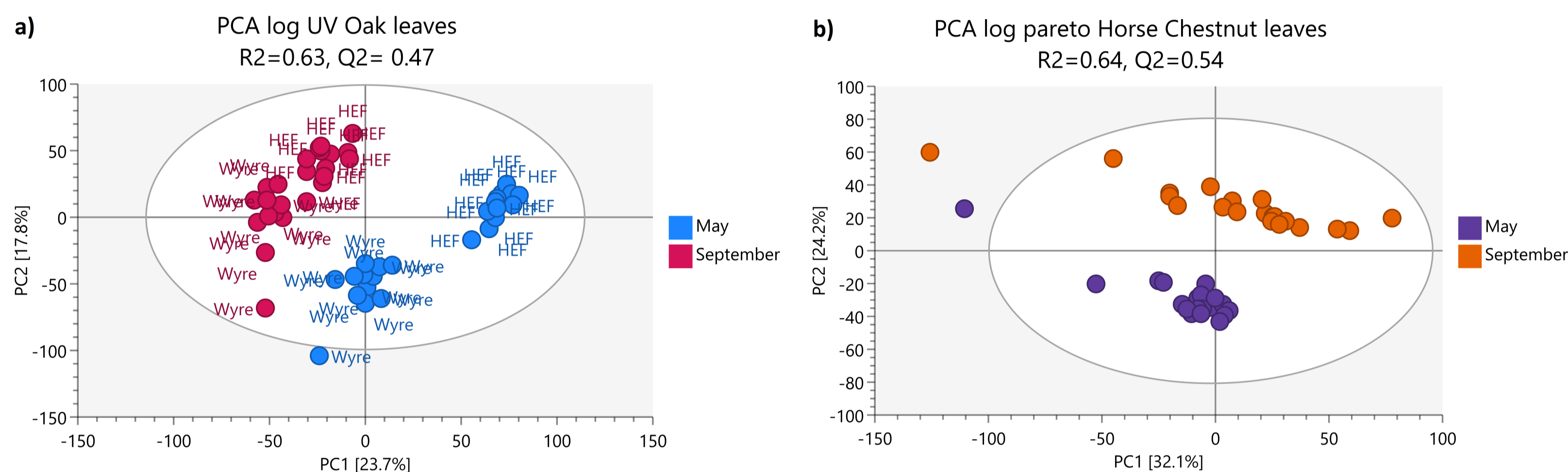


**Figure 1.** Metabolomics workflow: sample collection, sample pre-processing, metabolite extraction, data acquisition, data pre-processing, statistical analysis, feature identification and annotation.

## RESULTS

**Oak metabolites separate into 4 groups based on tree location and season.** The PCA plot of Oak samples (**Fig. 2a**) shows the presence of four groups: HEF-September, Wyre-September, HEF-May, Wyre-May.

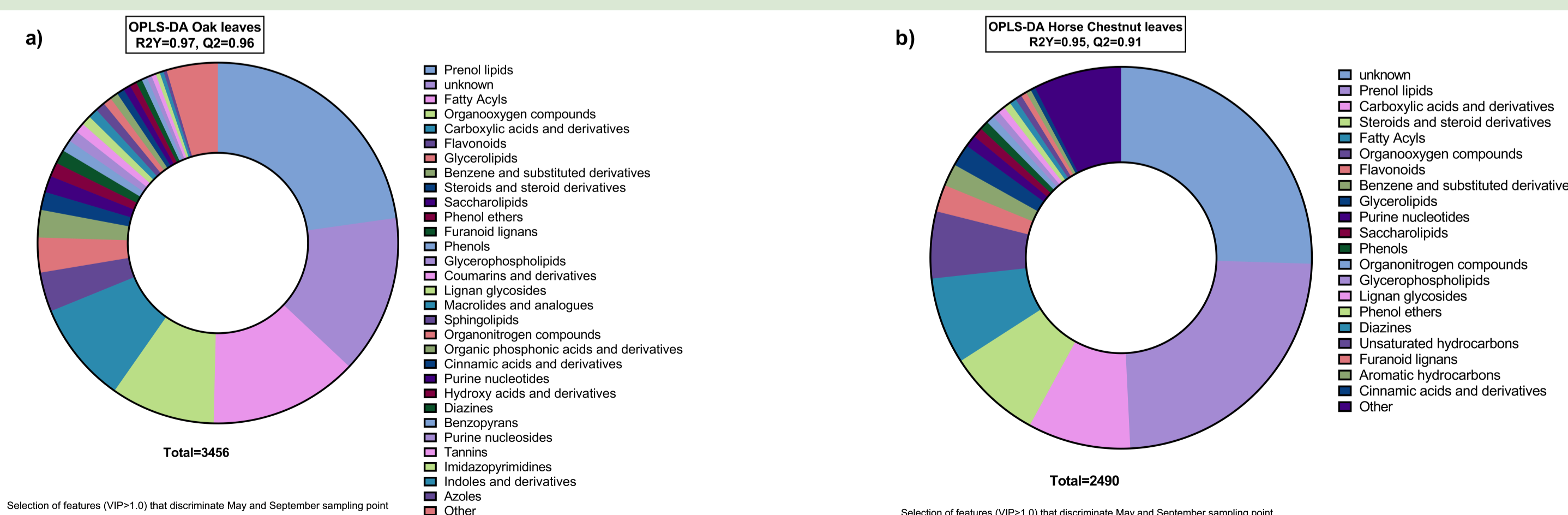
**Horse Chestnut metabolites separate into 2 groups based on season.** The PCA plot of Horse Chestnut samples (**Fig. 2b**) shows the presence of two groups: May and September, with two outliers.



**Figure 2.** **a)** PCA of Oak leaf metabolites. Each point represents an individual sample colored by the month of sampling, and labeled by the location of the trees (Wyre forest, Heart of England forest). **b)** PCA of Horse Chestnut leaf metabolites.

**A large proportion of the metabolites remains unidentified after feature annotation.** In **Figure 3**, many of the molecules could not be sorted into a specific molecular class.

However, we are still able to analyze the data and fit statistical models that allow us to discriminate specific groups within the samples. OPLS-DA models allow for selection of molecules, identified to class level, that are responsible for sorting the samples of both Oaks (**Fig. 3a**) and Horse Chestnuts (**Fig. 3b**) in two distinct groups based on the season of sampling.



**Figure 3.** Molecular class of metabolites selected through the OPLS-DA analysis (VIP > 1) that discriminate the **a)** Oak, and **b)** Horse Chestnut leaf metabolome during the two different seasons (May and September).

## CONCLUSION

There are significant differences in the metabolomic profiles of trees in late spring (May) and late summer (September). Additionally, with Oaks we see a difference between the two locations. This difference is more pronounced in Spring, while by the end of summer these two groups are more similar.

Seasonal changes in the tree leaf metabolome are expected due to the significant role of secondary metabolites in plant-environment interactions. Climate factors like water availability and temperature have a known impact on the production of various classes of secondary metabolites.

Untargeted metabolomics generates extensive datasets, but a considerable number of extracted metabolites remain unidentified at the compound level. Nevertheless, one of the notable advantages of this approach is the ability to analyze differences in metabolomic profiles, even in the absence of compound annotation.

## FUTURE DIRECTIONS

Our research is centered around the study of bacterial tree diseases, specifically Acute Oak Decline and Bleeding Canker of Horse Chestnut.

To investigate potential solutions, we have implemented soil treatments involving organic mulch and phosphite root drench on both symptomatic and non-symptomatic trees. Over the next two years, our focus will be on monitoring the trees' response to the diseases and soil treatments, with a particular emphasis on evaluating these responses through the lens of the metabolome. Additionally, we will examine the soil and leaf microbiome of the trees under investigation.