Project Report

I have been working at the Royal Botanic Gardens, Kew as a Centre for Forest Protection intern for the past 3 months on the *Climate variability of forest seed sources for landscape reforestation* project. I have had additional opportunities to develop both my field work skills and lab work skills, which are listed below.

Field work

During my time at the Royal Botanical Gardens, Kew I have had the opportunity to go on three field work trips. My first fieldwork trip was to collect data investigating invertebrate species composition in woodland areas of varying ages. This involved collecting 9 soil samples per $10m^2$ plot in different woodlands containing different tree species in three different age classes of trees, along with collecting a soil sample to measure soil chemistry.

Another field work trip I assisted with was for the project detecting mammalian presence in woodlands as part of the natural colonisation project. Current Defra protocol requires 9 soil samples per 10 m^2 plot to detect mammal presence. The aim of this soil sampling was to investigate whether the current recommendation of 9 soil samples per 10 m^2 is sufficient or whether Defra should update their policy to collect 18 or 32 samples per $10m^2$. Therefore, 9, 18 and 32 soil samples were collected in 4 mature woodlands and 4 naturally colonised woodlands. We also set up camera traps to provide a comparison with an alternative mammalian presence detection methodology.

My last field work trip was to find suitable sites for the genetic bottleneck project. This project aims to compare planted sites to tree populations from naturally colonised areas, with adjacent mature forests that have been identified as putative parental stands from which colonisers disperse. This project will test genetic diversity and potential genetic bottlenecks occurring in planted forests during seed collection, germination, nursing and planting stages. Potential sites were located using aerial photography. However, it is difficult to determine whether these sites have been naturally colonised or if they are planted woodland. Therefore, I attended the sites to determine their suitability and access. This involved guaranteeing that the naturally colonised sites had no areas of planted oak or birch, therefore ensuring that the naturally colonised sites had no rows of trees of similar age or tree guards present. Naturally colonised sites could still be used if the planted trees are too young to produce seeds, therefore I recorded an estimated height and age of planted trees in naturally colonised areas. Planted sites were also checked for suitability by identifying rows of trees in guards of similar ages and ensuring there are over 25 trees present per site. Oak and birch estimated counts were conducted to determine a high enough presence for sampling.

This fieldwork provided a break from the office and a chance to explore our native woodlands. In addition, it gave a chance to collaborate with a Centre for Forest Protection Intern and a research assistant both based at Forest Research.

Lab work

Due to an outsourcing issue with the DNA extractions as part of the acute oak decline (AOD) project, assistance with the internal DNA extraction of over 300 AOD infected and healthy oak leaves was required to meet a Defra deadline. The oak leaves were of low quality and highly degraded, therefore successful DNA extraction required following a lengthy 3 day protocol (Inglis, Resende and Grattapaglia., 2018). Briefly, approximately 30-50g of the greenest sections of the leaves were added to Eppendorf tubes with 2 ball bearings before being ground to a fine powder. Prior to using a high salt CTAB extraction protocol to remove the secondary metabolites present in the leaf tissue, samples were pre-wash step using a buffered sorbitol solution.

The Research Project: *Climate variability of forest seed sources for landscape reforestation*

In accordance with the UK Government's Net Zero greenhouse gas emissions target by 2050, the UK Government have pledged to increase UK forestry cover by a minimum of 4% by planting around 30 000 hectares annually. This equates to the afforestation of 90 to 120 million trees per year. Moreover, this feeds into the sustainable development goal 15, which by 2030 aims to "protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss".

Current UK Government policy requires tree planting schemes to use seeds sourced from registered areas called seed stands. Here, seeds are collected and are grown into samplings at nurseries before being distributed for planting. This enables the origin of trees to be traceable and discourages reforestation schemes from importing plants, therefore reducing the risk of spreading diseases and pests to UK native trees.

The resilience and future adaptive potential of planted UK woodlands is dependent on the diversity of the seed source. Therefore, it is paramount to assess the diversity of the seed stands used in tree planting. This was investigated by using extensive public data and high resolution regional climate data to explore the extent of environmental variability in seed sources. This involved processing and analysing a large Defra dataset (ArcGIS Dashboards), containing information regarding the location and species of 5 494 registered seed stands in the UK.

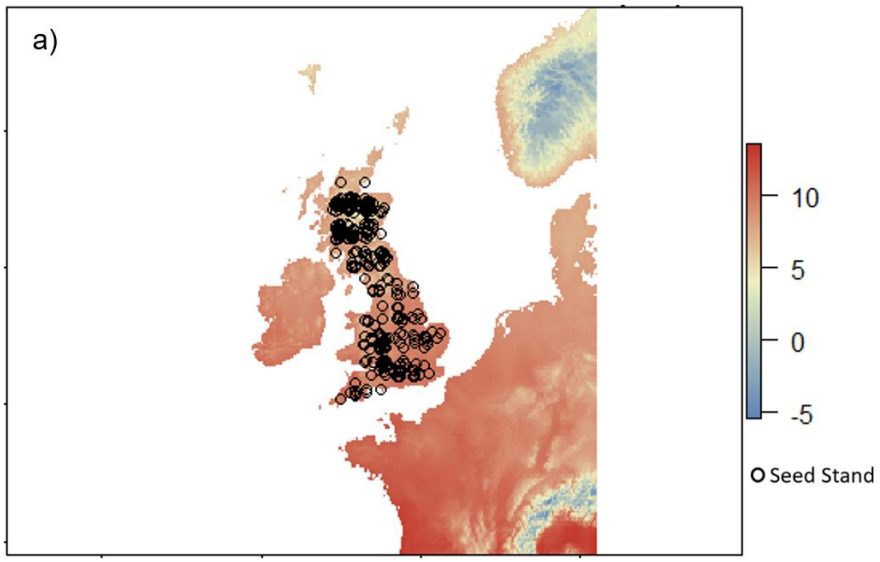
To begin investigating this, time was initially spent formatting the seed stand dataset to be R compatible. This included separating data into columns by species, latitude coordinate, longitude coordinate as well as formatting all coordinate data into decimal degrees. Next, seed stands outside of the UK latitude (50.10319 to 60.15456) and longitude (-7.64133 to 1.75159) coordinate range were removed. This step removed 581 of the total 5 494 total seed stands, leaving 4 913 seeds stands. Following the initial filtering process, seed stands plotted in the sea were also removed from downstream analysis. This removed another 1 904 seed stands, leaving a total of 3 009 seed stands for downstream analysis. The removed seed stands were subsetted in order to explore the coordinate data to determine potential formatting errors that lead to their exclusion.

To determine the UK tree diversity represented by the seed stands, 1 000 randomly naturally distributed *Quercus robur* (oak) trees across the UK were extracted from the Global Biodiversity Information Facility (GBIF) database. Next, 8 climate variables from the Worldclim database on average monthly temperature and precipitation values at a spatial resolution of 1 square kilometre (Table 1) were used to determine environmental diversity.

Table 1: Climate variables from WorldClim data used to create PCA plots and map seed stands.

Environmental Variable	Description
WC_bio1	Annual Mean Temperature
WC_bio3	Isothermality (BIO2/BIO7) (×100)
WC_bio4	Temperature Seasonality (standard deviation ×100)
WC_bio5	Max Temperature of Warmest Month
WC_bio6	Min Temperature of Coldest Month
WC_bio7	Temperature Annual Range (BIO5-BIO6)
WC_bio12	Annual Precipitation
WC_bio15	Precipitation Seasonality (Coefficient of Variation)

A map of the UK was created, representing the WC_bio1 annual mean temperature data with all registered seed stand locations plotted across the UK (Figure 1). This figure reveals high seed stand clustering, particularly in Scotland.



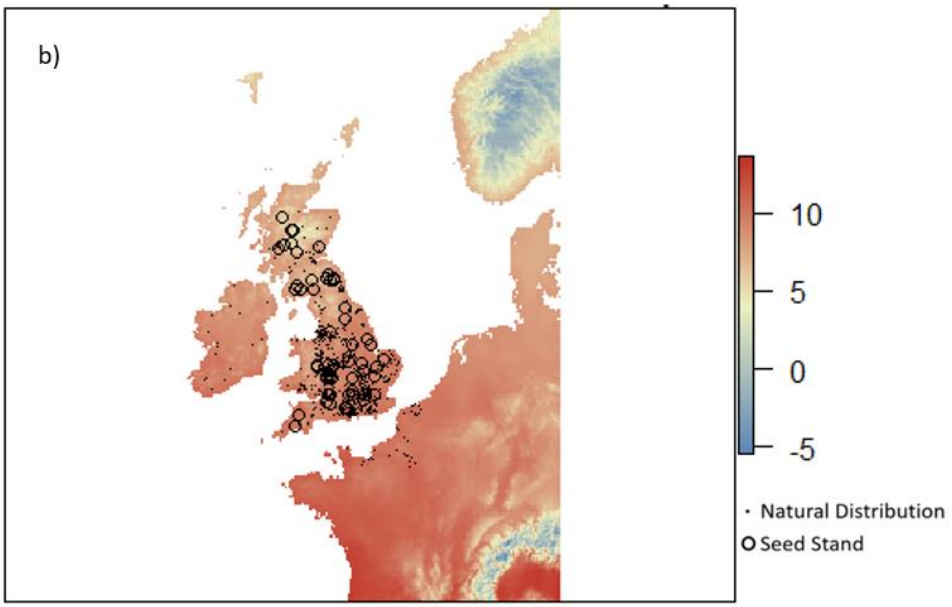


Figure 1: The study area with the annual mean air temperature (WC_Bio1) in the background with (a) all UK registered seed stands mapped and (b) naturally distributed oak trees and oak tree seed stands plotted.

Following this, a Principal Component Analysis (PCA) plot was created to visualise the environmental variability (Table 1) surrounding naturally distributed oak trees compared to the oak tree seed stands (Figure 2). Axis 1 explains 49.32% of the environmental variability with axis 2 explains 23.4% of the environmental variability. Most of the naturally distributed oak trees are highly concentrated in the central area of the PCA plot, with the oak tree seed stands thinly covering a larger spatial area.

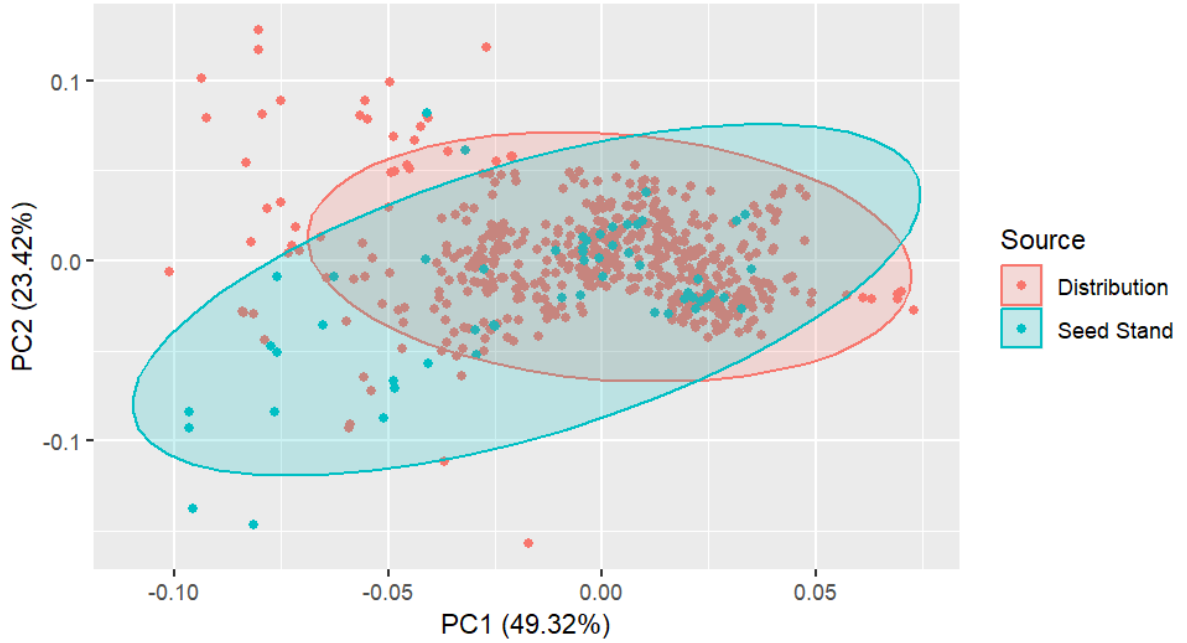


Figure 2: Principal Component Analysis (PCA) plot for climate variables (Table 1) clustered by oak tree natural distribution and oak tree seed stand location across the UK.

Despite considerable overlap between the seed stands and the naturally distributed trees there is some divergence between the two sets of points as show in the plot (Figure 2). A group of naturally distributed oaks, with both a low PC1 value and a high PC2 value, are not covered by the oak seed stands. Therefore, the environmental factors that these trees are adapted for, and their associated genetic diversity are perhaps not represented by the seeds collected from the registered seed stands. Thus, this diversity will not be present in new planted woodlands, whose saplings are solely grown from seed stands.

By only using collected seeds from a small selection of registered seed stands, there is a risk of underrepresenting the broad ecological niches and environmental variability that naturally distributed trees across the UK have evolved to thrive in. This potential bottleneck in diversity could reduce future forest resilience and tree survival rate. This will become increasingly problematic due to their need to survive in an ever-changing environment with extreme weather events induced by anthropogenically accelerated climate change. Moreover, a decline in successful woodland establishment will have wider negative biodiversity implications, resulting in a net loss of the ecosystem services that humans are highly dependant on. Further research is required to assess the full extent of seed stand diversity across all UK native tree species and the implications of the potential diversity loss in planting schemes on future forest resilience.

References

Inglis, P.W., Pappas, M.C.R., Resende, L.V. and Grattapaglia, D. (2018) Fast and inexpensive protocols for consistent extraction of high quality DNA and RNA from challenging plant and fungal samples for highthroughput SNP genotyping and sequencing applications. *PLoS ONE,* 13(10): e0206085.