## Mycorrhizal colonisation

* Methodology
  + New biochemical and molecular tools are useful but costly, time consuming to train in and time consuming
  + Staining techniques aren’t the standard any more in favour of new quantitative and qualitative molecular methods, but was done here because of practical reasons
  + Staining techniques are (in theory) more time efficient, but existing techniques are mainly optimised for lab-ground homogenous samples
  + There are many possible staining techniques, but trypan blue was chosen here because of expertise in the Crop Science Centre, and the (in theory) small learning curve
  + Trypan blue binds most strongly to phenolic-like materials in hyphal walls but also stain plant cell walls with lignin, and any residual cell contents
  + There are some AMF taxa which aren’t responsive to staining such as *Acaulospora* and *Paraglomus* species (Morton, 1985), which we weren’t able to consider
  + Didn’t use ink because of time restraints
* Sample selection
  + Mycorrhizal colonisation data
    - From Cedar Creek Ecosystem Science Reserve
    - From five functional guilds, a root sample from the species with the highest coverage from each guild were randomly chosen and dug up
    - Lignified and too thick roots were excluded due to difficulties in clearing and staining
    - This resulted in 52 samples, of which 33 were possible to be stained with the available methods and equipment
* Sample preparation
  + 12x1cm root sections of correct diameter randomly cut from root stock
* Clearing
  + Experimented, two weeks of pilot tests in KOH from 15mins to 1hour 10% KOH at 96C
  + Lignified roots, didn’t have time to selectively treat with hydrogen peroxide
  + Didn’t work so switched to dataset with less lignified roots
  + More pilot studies, settled on 48h 10% KOH at room temperature, which seemed to work for most species
  + Washed with dH20 and HCl (30%) before moving onto staining
* Staining
  + 10/12 root sections were chosen
  + Roots were stained with 0.05% trypan blue, and incubated in 96C for 8mins
  + Removed and placed in acidic glycerol (50% glycerol, 50% HCl) for 48h
* Microscopy
  + Mounted on slides
  + Light microscope at 40X
  + If staining/clearing unsuccessful, recorded why and moved on
  + If staining successful, ten equally spaced focal areas, identification of six types of fungal structures were recorded
  + Ten measurements per root section, 10 root sections per sample

Chart, funnel chart

Description automatically generated

## Root Traits

* Physiological traits
* Scanned using a Canon flatbed scanner
* Analysis on RhizoVision software to obtain physiological root traits (average diameter, volume, and total root length)
* Root Mass
* Mass measurements taken, but had to exclude samples that had already taken root samples from for mycorrhizal analysis
* Using both measurements, calculated SRL (total root length / dry mass), and RTD (root volume / dry mass)

## Environmental Data

* Obtained data from Cedar Creek Ecosystem Reserve publications on soil nitrogen and phosphorous (2021), herbaceous biomass and light availability (2021) and herbaceous species cover (2021)
* Though this wasn’t from the exact year that the mycorrhizal colonisation data was taken, it was the best we could do

## Statistical Analysis

* Processed and combined data using R
* Constructed a full model
* Test correlation between