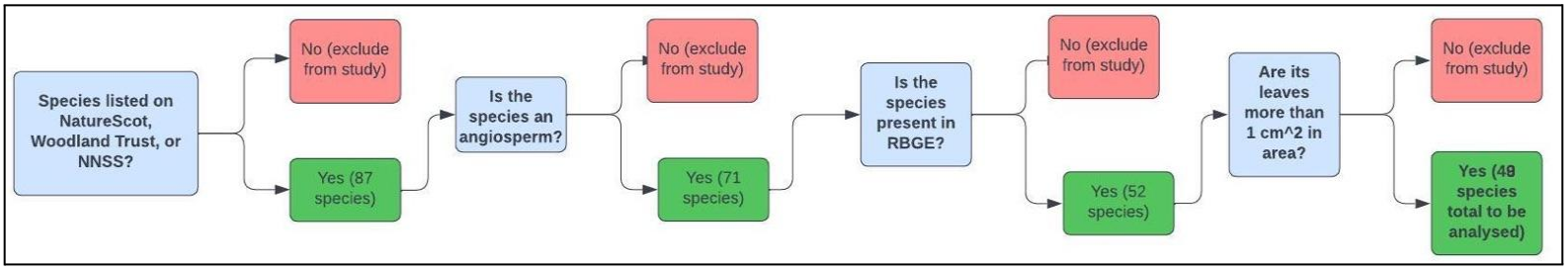
Title: Evaluating the invasion potential of 6 UK non-native woody species in Scotland based on their leaf traits.

Introduction: Invasive plant species are considered one of the main threats to global biodiversity, and have a specifically intense impact on islands as they are more susceptible to invasion (Abdallah *et al*., 2022). Studies show that invasive species often outcompete natives in a wide variety of traits, most notably morphological (e.g. leaf mass-per-area, LMA) and physiological (e.g. CO2 assimilation rates) traits relating to photosynthetic efficiency (Abdallah *et al*., 2022). These traits may play an important role in favouring the invasive character of non-native species, as observed in the study conducted by Abdallah *et al*. (2022) on a Mediterranean island. Additionally, is has also been shown that the degree of similarity of traits between native, naturalised, and invasive species influences their behaviour in an environment - similarity to native species facilitates naturalisation, and differences enhance invasion success, as demonstrated in central European species (Divíšek *et al*., 2018), however, no such studies have been performed in the UK, Europe's largest island. Furthermore, the majority of morphological and physiological leaf trait data originates from tropical regions, with a limited representation of plants present in temperate climates like the UK (Li *et al*., 2018).

This study will aim to determine whether similar trends can be observed in native, naturalised, and invasive angiosperm woody species in Scotland. If the same pattern is detected, it will then be used to infer the invasion potential of 6 alien tree and shrub species not yet classified as invasive or naturalised. The traits of native and naturalised species will be compared to those of *Rhododendron ponticum* - an extremely invasive shrub that has spread all over the UK. By examining the photosynthetic and photosynthesis-related traits exhibited by *R. ponticum*, we hope to gain insight into the traits that enable successful invasion and subsequent economic and ecological damage brought on by alien species. We expect to observe similar trends between native, invasive, and naturalised species in Scotland to those in central Europe (Divíšek *et al*., 2018) and that the alien species more similar to *R. ponticum* will exhibit a higher invasion potential than those more similar to the Scottish native species.

Methods: The native species for analysis were obtained from the list provided by NatureScot (2011) and the study by Sjölund *et al.* (2017) which identified beech (*Fagus sylvatica*) as a native species in Scotland and the UK. The list of naturalised species was obtained from the Woodland Trust (n.d.). Currently, Scotland officially recognises only one invasive woody species: *Rhododendron ponticum* (NatureScot, 2011) - the traits of alien species will be compared to those of *R. ponticum*, which will serve as a reference species for characters that facilitate invasion in Scotland. The alien species were selected from the list in section 14 of the Wildlife and Countryside Act of 1981 and the Non-Native Species Secretariat (NNSS, n.d.) - see the Appendix for a list of all 48 species to be analysed. The species selected for the study are only those woody angiosperm species that are present within the Royal Botanic Garden Edinburgh (RBGE), as their shared environment will standardise the external influencing factors on photosynthetic and photosynthesis-related parameters we are measuring (e.g. soil nutrient availability influences LMA; Chatzistathis and Therios (2013)). The omission of woody gymnosperm species is acknowledged as a limitation of the study due to the time constraints, as the measurement techniques for certain parameters (e.g. LMA) differ greatly between the two clades due to leaf structure variations. Additionally, angiosperm species with needle-like leaves (e.g. *Ulex europaeus*) will be excluded, as will species with blade area not exceeding 1 cm² (the area of the leaf puncturer):



We will analyse 2 morphological and 4 physiological traits, all relating to photosynthesis - see Table 1. The distinctions between morphological and physiological will be further revised or divided into subcategories (e.g. chemical, functional) to fully encapsulate the role and function of each trait as necessary.

Table 1 shows the indications and expectations of each trait that will bemeasured.

Trait	Indication	Expectations	Measurement method
Leaf mass-per-area (LMA), morphological	Indicative of the plant's ability to intercept photosynthetically-active radiation as well as resource-use efficiency.	We expect a higher LMA in invasive/potentially invasive species.	Obtain area (LA) and dry weight (DW; drying in oven at 60°C for 72 hours), then calculate by: LMA = DM / LA
Leaf dry matter content (LDMC), morphological	Indicative of plant growth rate and C assimilation as well as leaf structural integrity and resilience to stress.	We expect a lower LDMC in invasive/potentially invasive species.	Measure lamina thickness (LT) of dry leaves and then calculate: LDMC = DW / (LA x LT)
Leaf chlorophyll content, physiological	Indicative of leaf photosynthetic capacity and C assimilation efficiency.	We expect higher leaf chlorophyll content in invasive/potentially invasive species.	Dissolve leaf matter in DMSO and analyse absorbance by spectrophotometer; then use calculations to convert to concentrations
Photosynthetic rate (A), physiological	Indicative of C assimilation efficiency and growth	We expect a higher photosynthetic rate in invasive/potentially invasive species.	Use an infra-red gas analyser (IRGA) on fresh leaves
Transpiration rate (E), physiological	Indicative of water exchange efficiency,	We expect higher transpiration rates in invasive/potentially invasive species.	Use an IRGA on fresh leaves
Stomatal conductance (Gs), physiological	Indicative of gas exchange and water exchange efficiency.	We expect higher stomatal conductance in invasive/potentially invasive species.	Use an IRGA on fresh leaves

Results and analysis: To elucidate the differences between native and invasive/potentially invasive species, the expectations for each trait are outlined in Table 1 (see above). Additionally, we expect to find native and naturalised species in Scotland exhibit significant differences, similar to the findings of Divíšek *et al*. (2018) for species in central Europe. A comparative analysis will be conducted (by a t-test/single-way ANOVA) to compare the mean trait values of alien species with those of native species (the significant level will be reported at $p = 0.05$). If there is no significant difference in the majority (>3) of the traits, it can be inferred that the species has the potential to naturalise in Scotland; conversely, if a significant difference is detected in the majority of the traits, the species may be classified as potentially invasive. The data will be presented visually using boxplots, with separate plots created for each alien species or for each trait (as seen below), enabling a comprehensive understanding of trait variation among the species. We will also attempt to construct an invasion matrix to assess the intensity of invasion potential for each species. The index will be validated through the aforementioned hypothesis testing (t-test/ANOVA) and the use of z-scores. The matrix will consider the differences in trait values between each alien species and *R. ponticum,* incorporating appropriate weights based on further analysis of traits driving invasion success from literature. A higher index value will indicate a greater invasion potential compared to *R. ponticum*, while a lower index value suggests a lower invasion potential.

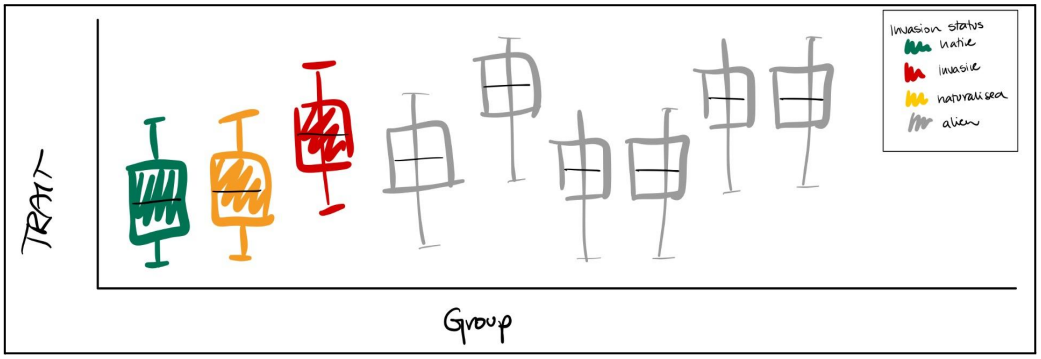


Figure 1 shows a potential representation of the data for a trait.

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Appendix:

Native species: *Alnus glutinosa, Betula pendula, Betula pubescens, Corylus avellana, Crataegus monogyna, Euonymous europaeus, Fagus sylvatica, Fraxinus excelsior, Ilex aquifolium, Populus nigra var. Betulifolia, Populus tremula, Prunus avium, Prunus padus, Prunus spinosa, Quercus petraea, Quercus robur, Ribes rubrum, Rosa canina, Rosa rubiginosa, Rubus idaeus, Salix aurita x repens, Salix caprea x repens, Salix cinerea, Salix myrsinifolia, Salix pentandra, Sambucus nigra, Sorbus aucuparia, Tilia cordata, Ulmus glabra, Vibernum opulus.*

Naturalised species: *Acer platanoides, Acer pseudoplatanus, Aesculus hippocastanum, Castanea sativa, Fagus sylvatica* (Atropurpurea group), *Malus pumila, Populus alba, Prunus cerasifera 'Diversifolia', Prunus cerasus 'Morello', Pyrus communis 'Beth', Quercus rubra*

Invasive species: *Rhododendron ponticum*

Alien species: *Cotoneaster bullatus, Cotoneaster horizontalis, Quercus cerris, Quercus ilex, Robinia pseudoacacia semperflorens, Symphoricarpos albus*