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**Assessing invasion risk based on leaf trait similarity in
Scotland: alien *Cotoneaster bullatus* compared to the
invasive *Rhododendron ponticum***

BY

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Abstract

Invasive alien plants (IAPs) pose a significant threat to ecosystems worldwide, and understanding the functional trait differences between them and native species is crucial for predicting invasion potential and developing effective management strategies. This study aimed to evaluate the variation in leaf traits (LT) between native, naturalised, and invasive woody angiosperms in Scotland, with a particular focus on comparing the alien *Cotoneaster bullatus* to the invasive *Rhododendron ponticum*. The results revealed distinct differences in LT profiles between the species groups: the invasive *R. ponticum* exhibited significantly higher values for key traits like leaf mass per area (LMA), leaf chlorophyll concentration (LCC), and photosynthetic rate (A) compared to native and naturalised species. These trait differences likely contribute to *R. ponticum*'s invasive success by enhancing its resource acquisition, carbon assimilation, and biomass allocation strategies. The alien *C. bullatus* showed a mixed pattern, with some traits similar to native species (A, leaf dry matter content, C/N ratio) and others resembling *R. ponticum* (g, LMA, LCC). This suggests that *C. bullatus* may have the potential to become invasive in Scotland, given its similarity to *R. ponticum* in the traits that most differentiate invasive from native species. The study highlights the importance of considering functional trait differences when assessing invasion risk and developing management strategies for non-native species. Future research should focus on incorporating a more comprehensive set of functional traits, including whole-plant characteristics and environmental factors, to gain a better understanding of the mechanisms underlying the invasion success.

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List of Abbreviations

ANOSIM	Analysis of Similarities
ANOVA	Analysis of Variance
CBD	Convention Biological Diversity
CHN	Carbon/Hydrogen/Nitrogen
CN	Carbon/Nitrogen
DBH	Diameter at Breast Height
dCO ₂	Delay CO ₂
DM	Dry Mass
HSD	Honestly Significant Difference
IAP	Invasive Alien Plant
IAS	Invasive Alien Species
LCC	Leaf Chlorophyll Content
LDMC	Leaf Dry Matter Concentration
LMA	Leaf Mass per Area
LSH	Limiting Similarity Hypothesis
LT	Leaf Trait
MP	Measuring Point
NABIF	New Atlas of British and Irish Flora
NMDS	Non-metric MultiDimensional Scaling
PAR	Photosynthetically Active Radiation
RBGE	Royal Botanic Gardens Edinburgh
SLA	Specific Leaf Area
WCA	Wildlife and Countryside Act
WM	Wet Mass
WUE	Water Use Efficiency

1. Introduction

1.1 Background and context

Invasive alien species (IAS) are considered one of the main drivers of biodiversity loss and inhibited ecosystem functioning, as well as economic activities worldwide (Ordonez *et al.*, 2010; Drenovsky *et al.*, 2012; Vilá and Hulma, 2017; Pyšek *et al.*, 2020). Their introduction and adverse effects have been a central point to conservation efforts - e.g., the 2002 Convention Biological Diversity (CBD) guidelines (CBD, 2002). The spread of IAS is expected to further increase with globalisation, urbanisation, and climate change (Seebens *et al.*, 2015; van Kleunen *et al.*, 2015). While the urgency of appropriate IAS management is apparent worldwide, and global collaborative action is addressing IAS, knowledge gaps hinder their effective management (Andersen *et al.*, 2004; Richardson and Rejmánek, 2011; Pyšek *et al.*, 2020; Traveset and Richardson, 2020).

Invasive alien plants (IAPs) are particularly concerning due to their ability to rapidly colonise and dominate novel ecosystems, outcompeting native species and altering ecosystem processes (Ordonez *et al.*, 2010; van Kleunen *et al.*, 2010; Pyšek *et al.*, 2012; Stace and Crawley, 2015; Abdallah *et al.*, 2022). The success of IAPs is attributed to their unique functional traits conferring advantages over native species in resource acquisition, growth, and reproduction (Ordonez *et al.*, 2010; van Kleunen *et al.*, 2010; Pyšek *et al.*, 2012; Stace and Crawley, 2015; Abdallah *et al.*, 2021, 2022). A combination of traits has been suggested to be the determinant of an alien species' invasion potential in a new habitat (Liu *et al.*, 2006; Herron *et al.*, 2007; Küster *et al.*, 2008; van Kleunen *et al.*, 2010; Bennet *et al.*, 2016; Liao *et al.*, 2021). Understanding these trait-invasiveness relationships is crucial for evaluating the drivers of invasiveness, predicting the invasion potential of non-native species, and developing effective management strategies (Andersen *et al.*, 2004; Catford *et al.*, 2010; Pyšek *et al.*, 2020; Traveset and Richardson, 2020). Comparative research elucidating the differences between native and invasive species is recommended (Küster *et al.*, 2008; Catford *et al.*, 2010; Ordonez *et al.*, 2010; Richardson, 2010; Lavoie *et al.*, 2016; Hulme and Bernard-Verdier, 2017; Traveset and Richardson, 2020; Kaushik *et al.*, 2022).

1.2 The importance of leaf traits

There is limited research in understanding the physiological mechanisms facilitating invasion success. Previous studies focused on various whole plant traits and performance measures (including growth rate, soil and tissue nutrient content, and carbon assimilation rate), with limited focus on leaf traits (LT) differences (Leishman *et al.*, 2007; Pyšek and Richardson, 2007; Küster *et al.*, 2008; Peñuelas *et al.*, 2009; van Kleunen *et al.*, 2010). LTs are particularly interesting as they determine the ability and effectiveness of critical physiological processes that determine

plant performance (Lavorel and Garnier, 2002; Wright *et al.*, 2004; Poorter and Bongers, 2006). Correspondingly, LTs can be reliable indicators of a plant's resource use strategies, stress tolerance, and competitive ability - all three essential to a plant's invasion success (Lavorel and Garnier, 2002; Wright *et al.*, 2004; Pyšek and Richardson, 2007; Peñuelas *et al.*, 2009; Ordonez *et al.*, 2010; Drenovsky *et al.*, 2012, Liao *et al.*, 2021, Kaushik *et al.*, 2022). Commonly studied LTs include leaf mass/area relationships (e.g. leaf mass per area, LMA, or its inverse, specific leaf area, SLA) and photosynthetic rate and capacity (Lavorel and Garnier, 2002; Wright *et al.*, 2004; Poorter and Bongers, 2006; Poorter *et al.*, 2009; Teixeira Oliveira *et al.*, 2014). These traits link to biomass and nutrient allocation, carbon assimilation, and water use efficiency (WUE) - key physiological processes that dictate plant establishment, growth, reproduction, and invasion potential (Lavorel and Garnier, 2002; Wright *et al.*, 2004; Poorter and Bongers, 2006; Poorter *et al.*, 2009).

IAPs often exhibit distinct LT values compared to their non-invasive counterparts, contributing to their success in novel environments - e.g. invasive species tend to possess traits with higher performance than native species (Leishman *et al.*, 2007; Pyšek and Richardson, 2007; Peñuelas *et al.*, 2009; van Kleunen *et al.*, 2010; Teixeira Oliveira *et al.*, 2014). Specifically, studies suggest increased photosynthetic rate (A) and higher SLA promote invasiveness (Pyšek and Richardson, 2007). Conversely, some studies have also found that IAPs show lower values for certain morphological LTs like LMA, while still finding higher values in physiological traits (including assimilation rate and WUE; Teixeira Oliveira *et al.*, 2014). The presence of these differences aligns with the Limiting Similarity Hypothesis (LSH), which predicts that invasive species are functionally dissimilar from their native counterparts (MacArthur and Levins, 1967). Quantifying such differences between native and invasive species has been a central goal of invasive science, and this dissimilarity of invasive species from natives is called phenotypic divergence: IAPs must be similar enough to the native flora to establish, but different enough to outcompete the native species and dominate in their environments (Catford *et al.*, 2010; Divíšek *et al.*, 2018). While some LTs have been researched extensively, the impacts of others (including chlorophyll content, WUE, and biomass allocation) on invasiveness remain understudied.

Many studies have focused on herbaceous IAPs or an overview of all IAPs thus far (Küster *et al.*, 2008; Pyšek *et al.*, 2008; Richardson and Rejmánek, 2011); however, only 20% of all IAP studies include woody species, which is concerning considering as the presence and negative effects of woody IAPs in novel ecosystems are increasing globally (Pyšek *et al.*, 2008; Richardson and Rejmánek, 2011). Understanding the invasiveness of woody species is increasingly important as their presence globally increases due to their economic significance

(Richardson and Rejmánek, 2011). Furthermore, as most studies on native-invasive LT dissimilarity have focused on non-temperate environments such as the Mediterranean and tropical ecosystems, research in temperate climates is scarce (Peñuelas *et al.*, 2009; Richardson and Rejmánek, 2011; Teixeira Oliveira *et al.*, 2014; Li *et al.*, 2018; Abdallah *et al.*, 2022). Notably, Dawson *et al.* (2017) showed opposing IAS richness patterns between tropical regions (where warmer areas harbour higher IAS richness) and temperate regions (where colder areas exhibit higher IAS richness). This highlights the importance of considering climatic context when evaluating plant invasion potential and the associated LT differences between native and invasive species.

1.3 Invasive species in Scotland

Given the scarcity of temperate climate information, evaluating native-invasive species differences is essential for accurate and effective IAP management, particularly in areas projected to harbour increasing numbers of IAS with continued climate change (Ordonez *et al.*, 2010; Dawson *et al.*, 2017; Li *et al.*, 2018). Globally, northern regions are expected to experience the highest IAS richness with continued climate change, and the UK currently exhibits the highest IAS richness in Europe (Dawson *et al.*, 2017). *Rhododendron ponticum* L. is particularly interesting in the UK, occurring in approximately 58% of grid cells (Preston *et al.*, 2002). Since its introduction, *R. ponticum* has established in and invaded the British Isles, mainly in acidic soils like heathlands (Dehnen-Schmutz *et al.*, 2004). This is particularly concerning for Scotland as most the country's soil is high in carbon and characterised as acidic (Paterson *et al.*, 2011) - consequently, Scotland represents the majority of *R. ponticum* accessions in the UK (Milne and Abbott, 2000). The country's moist climate and acidic soils represent ideal conditions for *R. ponticum* growth and domination across Scottish landscapes (Cross, 1975; Erfmeier and Bruelheide, 2009). *R. ponticum* already covers approximately 1.2% of all native woodlands and represents 65% of all non-native species in Scotland (Patterson *et al.*, 2013; Pyšek *et al.*, 2017). This invasion of UK habitats has led to the designation of *R. ponticum* as an IAP in Scotland, which retained the right to classify invasive species autonomously under the 2018 European Union (Withdrawal) Act and the 2020 Scottish Invasive Non-native Species Regulations Amendments.

Similar alien species, like *Cotoneaster bullatus* Bois, are classified as IAPs in England and Wales but not in Scotland under Schedule 9 of the Wildlife and Countryside Act (1981), which delineates species prohibited from sale, distribution, or propagation in the UK. Scotland's unique legislation allowing it to classify IAPs autonomously presents a unique opportunity for investigating common alien species for their invasion potential and acting with appropriate and preventative management. This study aims to determine the invasion potential of *C. bullatus* in

Scotland based on its leaf trait similarity to native and invasive species - the extent to which alien species vary from natives could illuminate their invasion potential and the magnitude of any consequent ecological and economic impacts (MacDougall *et al.*, 2009; Cleland, 2011).

1.4 Definitions and terminology

The terminology used in invasive species ecology varies between studies and other publications and should thus be clearly defined (Richardson *et al.*, 2000) - the terminology used hereafter is explained in Table 1 (adapted from Richardson *et al.*, 2000; Richardson and Pyšek, 2006; Stace and Crawley, 2015).

Table 1: Terminology used to describe invasive plant ecology relative to this study (adapted from Richardson *et al.*, 2000 and Richardson and Pyšek, 2006; Stace and Crawley, 2015).

Native plant species	Plant species native to the United Kingdom since immediately after the retreat of the ice sheets 10,000 years ago (Preston <i>et al.</i> , 2002; Stace and Crawley, 2015). The native species examined in this study were defined in the New Atlas of British and Irish Flora (NABIF; Preston <i>et al.</i> , 2002) and narrowed down to species native to Scotland by NatureScot (2011).
Alien plant species	Plant species not native to the UK and present due to human activity; either by accidental or purposeful introduction into the wild; also called non-native plant species (Stace and Crawley, 2015). The alien species examined in this study were defined by the Wildlife and Countryside Act (WCA; 1981) and Preston <i>et al.</i> (2002).
Naturalised plant species	Non-native plant species established (i.e. reproduce constantly and sustain populations on their own) in the UK following their introduction that represent no danger to the local ecology, economy, or human well-being (Stace and Crawley, 2015). The naturalised species examined in this study were defined by Preston <i>et al.</i> (2002) and the Woodland Trust (2019).
Invasive plant species	Non-native plant species that present a danger to the local ecology, economy, or human well-being following their introduction due to their rapid spread and reproduction, and the ability to outcompete the native species within their habitats (Stace and Crawley, 2015). The invasive species examined in this study were defined by the Wildlife and Countryside Act (1981), Preston <i>et al.</i> (2002), and narrowed down to species invasive to Scotland by NatureScot (2024).

1.5 Research questions and hypotheses

Understanding the unique combination of LTs that confer the competitive advantage of invasive species over natives (their phenotypic divergence) defines their invasion potential, and is essential to predicting invasion potential, and developing appropriate management frameworks (Lavorel and Garnier, 2002; Andersen *et al.*, 2004; Catford *et al.*, 2010; Pyšek *et al.*, 2020; Traveset and Richardson, 2020). Consequently, an alien species that should have the potential to invade novel habitats must be similar enough to natives to establish but different enough to outcompete them in their own native habitats and invade (Catford *et al.*, 2010; Ordonez *et al.*,

2010; Divíšek *et al.*, 2018; Liao *et al.*, 2021). While most studies on native-invasive LT dissimilarity have focused on non-temperate environments and non-woody species, the effects of climate change on woody IAPs are likely to have opposite effects in temperate regions (Dawson *et al.*, 2017). This study aims to address the following research questions:

1. Does *R. ponticum*, Scotland's only woody IAP, differ from native species in its LTs?

Hypothesis: *R. ponticum* will exhibit higher LT values compared to native species, elucidating its establishment and invasion success in Scotland since its introduction, as predicted by the LSH (Macarthur and Levins, 1967; Leishman *et al.*, 2007; Peñuelas *et al.*, 2009; van Kleunen *et al.*, 2010; Teixeira Oliveira *et al.*, 2014; Divíšek *et al.*, 2018).

2. Can the dissimilarities in LTs between *R. ponticum* and native species be used to evaluate the invasion potential of an alien species, *Cotoneaster bullatus*, in Scotland?

Hypothesis: The degree of similarity in LT profiles between *C. bullatus* and native/invasive species will provide insights into its invasion potential in Scotland, based on the LSH (Macarthur and Levins, 1967; Leishman *et al.*, 2007; Peñuelas *et al.*, 2009; van Kleunen *et al.*, 2010; Teixeira Oliveira *et al.*, 2014; Divíšek *et al.*, 2018). If *C. bullatus* exhibits LT values similar to *R. ponticum* and distinct from native species, it may have a higher potential for invasion in Scotland, and conversely, if its LTs are similar to native species and distinct from *R. ponticum*, it might be more likely to naturalise in Scotland (Cleland, 2011; Divíšek *et al.*, 2018).

2. Materials and methods

2.1 Study site and environmental conditions

Leaf samples were collected from the Royal Botanic Gardens Edinburgh (RBGE), Scotland - see Figure 1a. The RBGE encompasses approximately 0.3 km² in North Edinburgh (NT 24586 75342; Figure 1b), which is characterised by sedimentary geology and temperate maritime climatic conditions (Köppen–Geiger climate classification: Cfb; Geiger, 1954; Kotek *et al.*, 2006; Trewin, 2008). The region's mean annual precipitation is 727.7 mm, and the mean annual temperatures range from 6.1°C to 13.0°C (Met Office, 2020). The soil at the site is predominantly light sandy loam, with a pH of around 6.5 (Brownless, personal communication). The abundance of species relevant to this study, and the consistent environmental conditions across the site, were critical factors in selecting the RBGE as the study site, as studies have repeatedly shown that LTs vary with environmental conditions (Givnish, 1979; Nicotra *et al.*, 2007; Poorter *et al.*, 2009, 2011; Wang *et al.*, 2022). Samples were collected during August and September 2023.

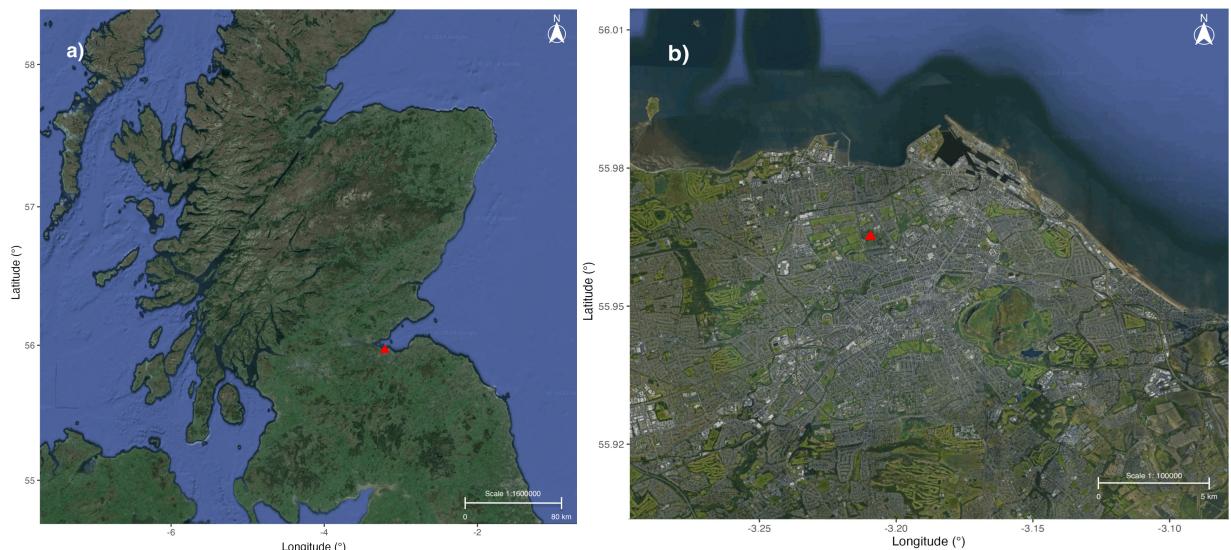


Figure 1a shows the study site location (RBGE) within Scotland, UK. **Figure 1b** shows the location of RBGE in North Edinburgh, Scotland, UK (NT 24586 75342). The maps were created using the ggmap package (Kahle and Wickham, 2013) in RStudio (version 4.3.2; R Core Team, 2022).

2.2 Species selection and sample collection

Species selection criteria mandated the classification of the woody angiosperm species as native, naturalised, or non-native by either the WCA (1981), the NABIF (Preston *et al.*, 2002), Woodland Trust (2019), or NatureScot (2011, 2024). Additional criteria included the selected species being present within the RBGE, their leaf area exceeding 3 cm² (to allow for standardised leaf trait measurements), and absence of herbivory damage, senescence, or disease. Consequently, 34 tree and shrub species were selected, comprising 22 native and 12

naturalised species in the UK. *R. ponticum*, the only woody species defined as an IAP in Scotland (WCA, 1981; Preston *et al.*, 2002; NatureScot, 2024), was chosen for comparison.

The selection of the alien species for comparison included cross-referencing non-native tree and shrub species lists (Preston *et al.*, 2002; Woodland Trust, 2019) with Schedule 9 of the WCA (1981). *C. bullatus*, the only species present on both lists and fulfilling the additional criteria, was examined for similarity to *R. ponticum*. Although *C. bullatus* is listed as an IAP in Schedule 9 of the WCA (1981), it is not classified as invasive in Scotland under the 2020 amendment of the Invasive Non-native Species (EU Exit) Regulations. Similarly, *Robinia pseudoacacia* ‘Semperflorens’, also listed as non-native in the UK (Preston *et al.*, 2002) and fulfilling additional selection criteria, was excluded from the study as an alien species for comparison because the WCA (1981) does not classify it as an IAP in the UK. For a full list of selected species, see Table 2.

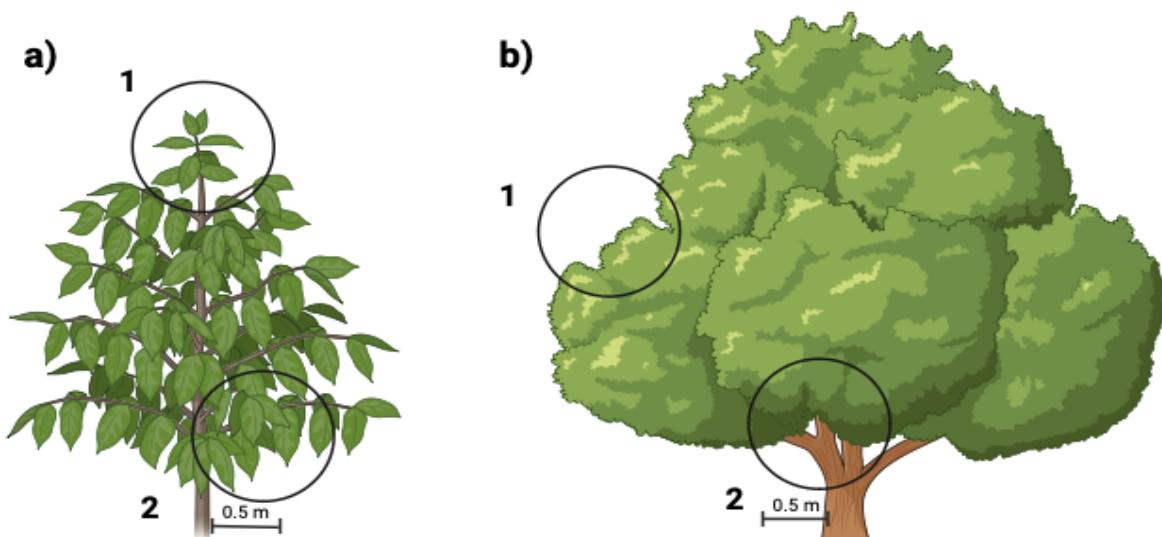
Table 2 shows the list of selected species and their invasion status.

Invasion status	Species	Invasion status	Species
Native	<i>Alnus glutinosa</i> (L.) Gaertn <i>Betula pendula</i> Roth <i>Betula pubescens</i> Ehrh. <i>Corylus avellana</i> L. <i>Crataegus monogyna</i> Jacq. <i>Euonymus europaeus</i> L. <i>Fraxinus excelsior</i> L. <i>Fagus sylvatica</i> L. <i>Ilex aquifolium</i> L. <i>Populus nigra</i> subsp. <i>betulifolia</i> L. <i>Populus tremula</i> L. <i>Prunus padus</i> L. <i>Quercus petraea</i> (Matt.) Liebl. <i>Quercus robur</i> L. <i>Rosa rubiginosa</i> L. <i>Rubus idaeus</i> L. <i>Sorbus aucuparia</i> L. <i>Sambucus nigra</i> L. <i>Tilia cordata</i> Mill. <i>Ulmus glabra</i> Huds.	Naturalised	<i>Acer platanoides</i> L. <i>Acer pseudoplatanus</i> L. <i>Castanea sativa</i> Mill. <i>Fagus sylvatica</i> ‘Atropurpurea’ L. <i>Malus x domestica</i> Mill. <i>Populus alba</i> L. <i>Pyrus communis</i> ‘Beth’ L. <i>Prunus cerasifera</i> ‘Diversifolia’ Ehrh. <i>Prunus cerasus</i> ‘Morello’ L. <i>Quercus cerris</i> L. <i>Quercus ilex</i> L. <i>Quercus rubra</i> L.
Alien	<i>Cotoneaster bullatus</i> Bois	Invasive	<i>Rhododendron ponticum</i> L.



Figure 2 shows the location of each selected species within the RBGE. The map was created using the ggmap package (Kahle and Wickham, 2013) in RStudio (version 4.3.2.; R Core Team, 2022).

For each species, two branches (one from the canopy edge and one within 0.5 m of the trunk to account for variations in solar radiation; per Pérez-Harguindeguy *et al.*, 2013) were collected - see Figures 3a and b. The severed branches were immediately submerged in water to prevent xylem cavitation and leaf dehydration, and transported to the lab for analysis on the same day (following procedures described by Pérez-Harguindeguy *et al.*, 2013). Three leaves/leaflets were selected randomly from each branch for analysis.



Figures 3a and b show the areas from which branches were collected from shrubs and trees, respectively: 1) from the outward-facing edge of the canopy and 2) from within 0.5 m of the trunk, facing inwards. Not to scale. Created with BioRender (2019).

Previous studies show that LTs vary interspecifically, with age and size of the plant, deciduous habit, and solar irradiation levels (Kappel and Flore, 1983; Reich *et al.*, 1991; Bassow and Bazzaz, 1998; Le Roux *et al.*, 2001; Niinemets *et al.*, 2003; Lee *et al.*, 2007; Poorter *et al.*, 2009; Thomas, 2010; Poorter *et al.*, 2011; Steppe *et al.*, 2011; Stace and Crawley, 2015; Bielczynski *et al.*, 2017; Li *et al.*, 2018; Xiong *et al.*, 2018; Asefa *et al.*, 2021; Mu and Cheng, 2021; Rawat *et al.*, 2021; Wang *et al.*, 2022). Additional data (species name, plant age, plant size (diameter at breast height (DBH, m), at 1.3 m height off the base of the trunk), deciduous habit, and variation in solar irradiation levels - defined as inside/outside the canopy) were collected; however, ultimately their analysis was outside the scope of this study.

2.3 Description of selected traits

The selected leaf traits for the study are shown in Table 3 - adapted after Pérez-Harguindeguy *et al.* (2013). Each trait is classified into physiological, structural, or chemical leaf traits - after Niinemets (2020). All LTs are related to plant functioning efficiency, reflective of plant survival strategies, and essential to maximising productivity (Kaushik *et al.*, 2022).

Table 3 shows the selected traits, the units in which they are measured, and their functional roles (after Niinemets, 2020). The table is adapted after Pérez-Harguindeguy *et al.* (2013).

Leaf trait	Unit	Trait type	Functional role
Photosynthetic rate (A)	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	Physiological	Metabolic capacity, carbon uptake efficiency, plant growth rate (Pérez-Harguindeguy <i>et al.</i> , 2013; Niinemets <i>et al.</i> , 2014).
Transpiration rate (E)	$\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$	Physiological	Water exchange efficiency (Pérez-Harguindeguy <i>et al.</i> , 2013).
Dark respiration rate (R_{leaf})	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	Physiological	Leaf metabolic rate and basal respiratory efficiency (Pérez-Harguindeguy <i>et al.</i> , 2013).
Stomatal conductance (g)	$\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$	Physiological	Leaf gas and water exchange efficiency (Pérez-Harguindeguy <i>et al.</i> , 2013).
Leaf mass-per-area (LMA)	g cm^{-2}	Structural	Light capture, plant growth rate and biomass allocation, and ecological strategy (Poorter <i>et al.</i> , 2009; Pérez-Harguindeguy <i>et al.</i> , 2013).
Leaf dry matter concentration (LDMC)	g g^{-1}	Structural	Plant growth rate, resistance to herbivory, structural integrity (Pérez-Harguindeguy <i>et al.</i> , 2013).
Leaf chlorophyll content (LCC)	SPAD	Chemical	Photosynthetic capacity, carbon assimilation efficiency (Richardson <i>et al.</i> , 2002).
Leaf carbon-nitrogen content (C/N ratio)	No unit	Chemical	Leaf metabolic activity and photosynthetic capacity (Pérez-Harguindeguy <i>et al.</i> , 2013).

2.4 Measurement of physiological leaf traits

A portable gas-exchange system (GFS-3000; Walz, Effeltrich, Germany) was used with standardised settings (see Table 4 in Appendix 1) to ensure consistency in measuring physiological leaf traits. Each leaf was clipped into the 8 cm² GFS-3000 leaf cuvette and analysed for A, E, R_{leaf}, and g. For leaves with an area smaller than the leaf cuvette (< 8 cm²), the leaf area was measured using ImageJ (version 1.54i; Schneider *et al.*, 2012) and the physiological rates subsequently adjusted by the method shown in Equation 1. One physiological measurement was performed on each leaf (n = 202; n = 118, 72, 6, and 6 for native, naturalised, invasive, and alien species, respectively). Two samples were not measured for any traits as they were dead following their transport to the laboratory, deduced from their A values < 0 µmol CO₂ m⁻² s⁻¹ (both *A. glutinosa*).

$$\text{adjusted rate } (\mu\text{mol m}^{-2} \text{s}^{-1}) = \text{initial obtained rate } (\mu\text{mol m}^{-2} \text{s}^{-1}) * \frac{\text{leaf area (cm}^2\text{)}}{8 \text{ cm}^2} \quad \text{Eq. 1}$$

2.5 Measurement of structural leaf traits

Following the physiological trait measurements, three leaf discs (area = 1.34 cm²) were cut from each leaf/leaflet and weighed to obtain the wet mass (WM, in g) - see Figures 4a and b. The leaf discs were dried in an oven at 60°C for 72 hours, or until constant dry mass was achieved, and weighed to obtain dry mass (DM, in g). For LMA and LDMC calculations, see Equations 2 and 3, respectively (n = 202; n = 118, 72, 6, and 6 for native, naturalised, invasive, and alien species, respectively).

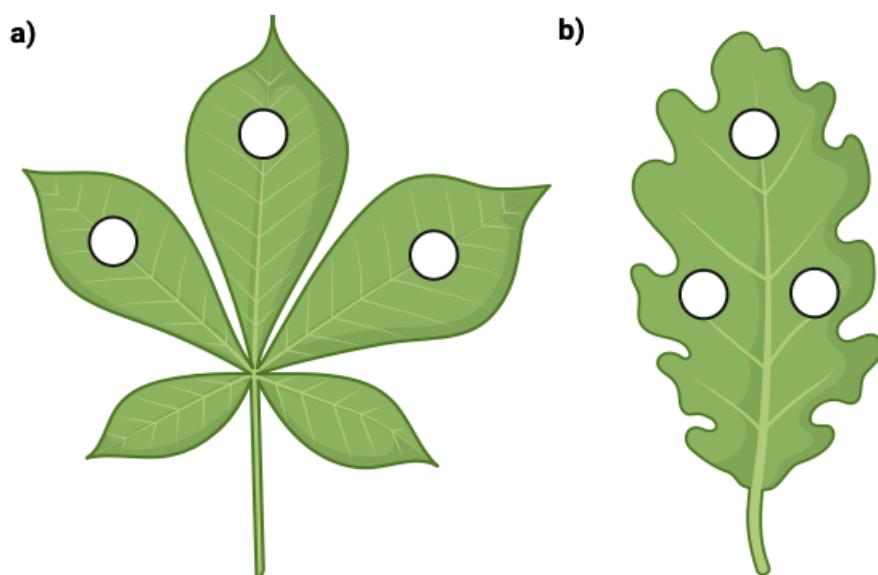


Figure 4 shows how leaf discs were collected from various leaf samples: **a)** from separate leaflets for compound leaves and **b)** from one leaf for simple leaves. Created with BioRender (2019).

$$LMA \left(\text{g cm}^{-2} \right) = \frac{\text{leaf dry mass(g)}}{1.34 \text{ cm}^2} \quad \text{Eq. 2}$$

$$LDMC \left(\text{g g}^{-1} \right) = \frac{\text{leaf DM(g)}}{\text{leaf WM (g)}} \quad \text{Eq. 3}$$

2.6 Measurement of chemical leaf traits

LCC was estimated using a SPAD-502 plus metre (SPAD units; Konica Minolta Optics, Japan) on fresh leaf discs before drying them as described in Section 2.5 ($n = 202$; $n = 118$, 72, 6, and 6 for native, naturalised, invasive, and alien species, respectively). SPAD values are directly proportional to LCC (Shibaeva *et al.*, 2020). The dried leaf discs were ground to a homogenous texture using a ball mill (Retsch, Germany) following the structural LT measurements. The ball mill sample container was rinsed with 70% ethanol between samples to prevent sample carryover. The ground samples were oven-dried again at 55°C for 24 hours, after which 3.5 mg of each sample was analysed using the Flash SMART 2000 CHNS/O analyser (Thermo Fisher Scientific, Massachusetts, USA) following the parameters in Table 5 (Appendix 2). One sample from each species and canopy location was selected initially for C/N ratio analysis ($n = 64$). The lab technician randomly selected the remaining 35 slots (out of 99 for the instrument). C/N ratios were analysed for $n = 99$, $n = 54$, 39, 3, and 3 for native, naturalised, invasive, and alien samples, respectively.

2.7 Statistical analysis

All data was transcribed using Microsoft Excel, with visualisations and analyses conducted in RStudio version 4.3.2. (R Core Team, 2022) - the code used can be found in Appendix 3. The significance threshold for all tests was set at $p = 0.05$. Non-metric multidimensional scaling (NMDS) using Euclidean distance was employed to visualise differences in LT profiles between the four species groups. A two-dimensional NMDS was chosen after analysing a stress plot, where the stress value decreased substantially between 1 and 2 dimensions, but the reductions in stress were negligible when adding more dimensions (see Figure 5 in Appendix 4). The 2-dimensional NMDS achieved a stress value of 0.073, indicating a good representation of the original dissimilarity matrix in the ordination space (Kruskal, 1964). The goodness of fit of the 2D NMDS was further assessed using a scree plot, which provided non-metric and linear R^2 values of 0.95 and 0.98, respectively, indicating a good representation of the original dissimilarity matrix in ordination space (see Figure 6 in Appendix 4). An analysis of similarities (ANOSIM) was performed on the NMDS ordination to assess the statistical significance and the magnitude of the observed differences.

The relationships between plant invasion status (native, naturalised, invasive, and alien) and individual LTs were initially analysed using a one-way analysis of variance (ANOVA). Normality and homogeneity of variances (homoscedasticity) of residuals were evaluated visually using diagnostic plots and mathematically using the Shapiro-Wilk and Bartlett tests, respectively. When either assumption was violated, a Box-Cox maximum likelihood transformation was attempted. The goal of the analysis was to perform post-hoc multiple comparisons to identify

significant differences among the invasion status groups if the overall test was significant. The preferred post-hoc test following an ANOVA is Tukey's Honestly Significant Difference (HSD) test; however, it assumes equal sample sizes across all groups (Tukey, 1949). This study's sample sizes for each species group were highly uneven, violating this assumption. To account for the unequal sample sizes across the species groups and ensure valid statistical inferences, the non-parametric Kruskal-Wallis test was employed in place of the ANOVA, as it does not require the assumptions of normality and homoscedasticity and can account for unequal sample sizes (Hollander *et al.*, 2014). If the Kruskal-Wallis test indicated a significant effect of invasion status on an LT, post-hoc multiple comparisons were conducted using the Dunn test, which also does not require equal sample sizes (Dinno, 2015). Boxplots were used to visualise the relationships between LTs and the four invasion status groups.

2.8 Estimation of invasion potential

To assess the similarity of *C. bullatus* to native and invasive species based on LTs, pairwise comparisons of z-scaled LT means were conducted between the alien species and native/invasive groups on LTs that showed significant overall differences. Smaller differences indicated greater trait similarity between the alien species and that respective group. The distributions of native-alien and invasive-alien LT differences were compared using a Wilcoxon rank-sum test.

3. Results

3.1 Spatial analysis using NMDS

The species groups showed high overlap in their LT distributions in 2D NMDS, though with significant differences across the 8 LTs (ANOSIM, $R = 0.095$, $p < 0.05$, on 9999 permutations; Figure 7). Native and naturalised species exhibited substantial overlap, indicating similarity in their LT profiles. Conversely, the invasive *R. ponticum* and alien *C. bullatus* exhibit some separation from the native and naturalised groups, suggesting some dissimilarity, but overlap with each other, implying similar LT profiles between them. Variations in LMA, g, and LCC appear to be the main drivers of the differences between the four groups, followed by C/N ratio and A. LDMC, E, and R_{leaf} did not affect the variation (see Table 6). Trait relationships showed negative correlations of A and LMA, E and g, and E and R_{leaf} . Positive correlations can be seen between A, and R_{leaf} , and LMA, LDMC, LCC, C/N ratio, g. LCC and A, C/N ratio and R_{leaf} , and LDMC and R_{leaf} show no correlation.

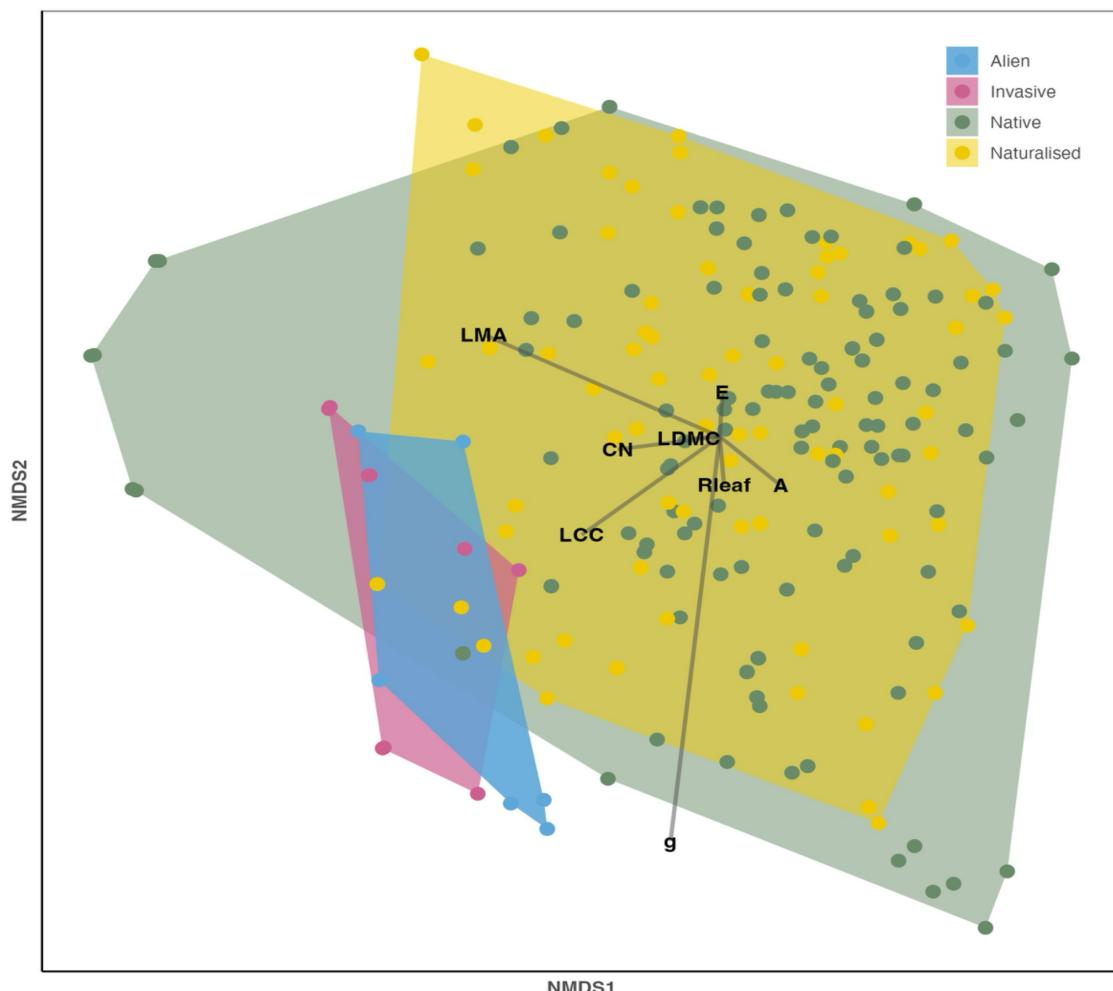


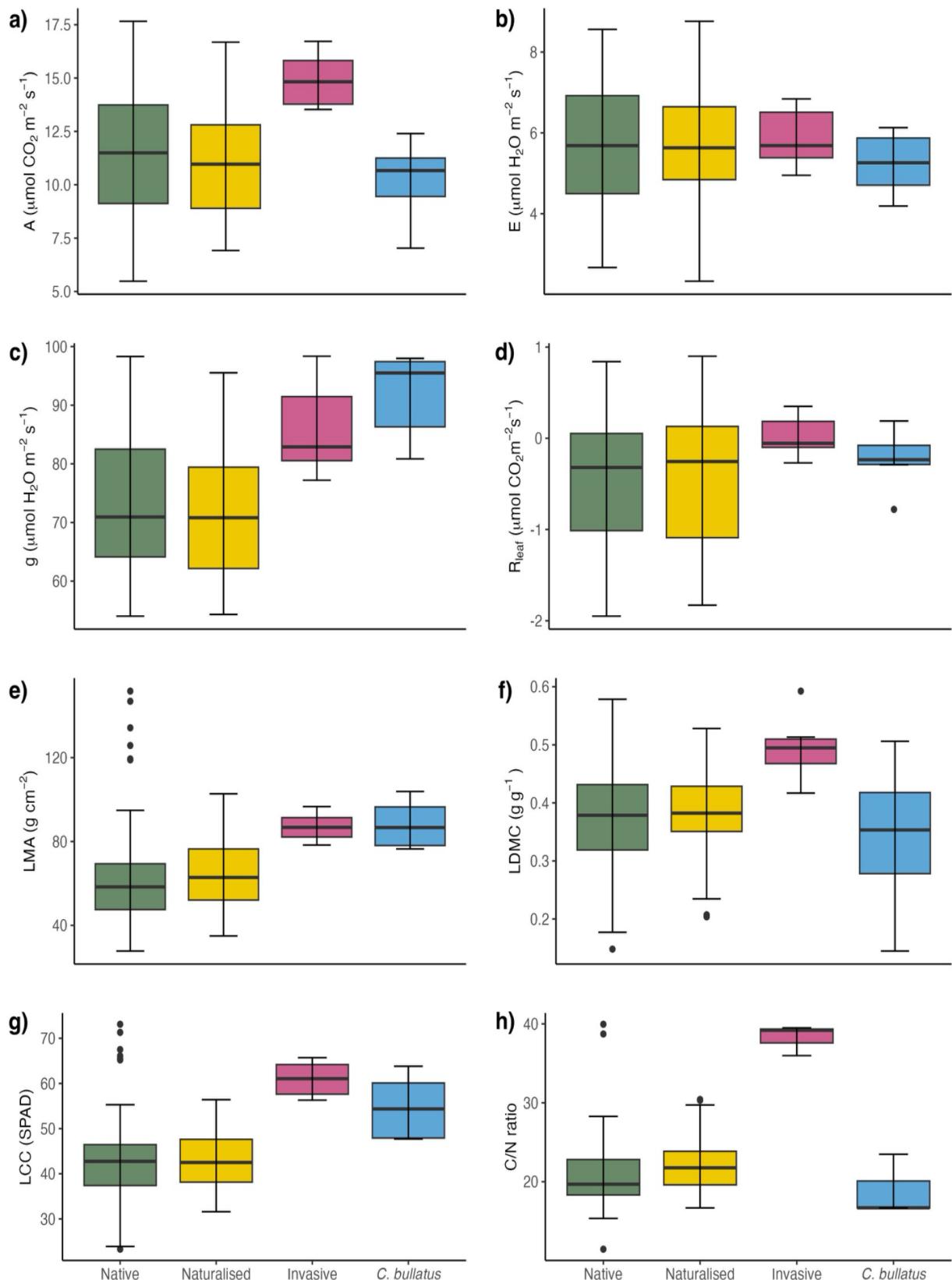
Figure 7 shows the NMDS plots for a) native, naturalised, and invasive species ($n = 196$ in total; 118, 72, and 6 for native, naturalised species, and invasive, respectively) and b) for all four species groups ($n = 202$ in total; 118, 72, 6, and 6 for native, naturalised species, invasive, and alien species respectively). The plots are colour-coded to represent the species groups. The dots represent the individual species data, and the polygons connect the outermost points. The lines and lengths represent each LT's gradient and strength in ordination space, respectively.

Table 6 shows the significance of each LT on the differences between the four species groups in the 2-dimensional NMDS. Significance levels: n.s. for not significant, * for $0.05 > p > 0.01$; ** for $0.01 > p > 0.001$, and *** for $p < 0.001$. Significant values are shown in bold.

Leaf trait	R ²	p-value	Significance
A	0.0760	< 0.05	***
E	0.0114	> 0.05	n.s.
R _{leaf}	0.0137	> 0.05	n.s.
g	0.9815	< 0.05	***
LMA	0.9896	< 0.05	***
LDMC	0.0158	> 0.05	n.s.
LCC	0.3746	< 0.05	***
C/N ratio	0.1747	< 0.05	***

3.2 Effects of invasion type on each LT

The differences between the species groups in each LT are shown in Figures 8a - h. *R. ponticum* exhibits higher average LT values than native and naturalised species for all traits. *C. bullatus* shows the highest mean trait values for LMA and g among all groups. The groups differ significantly in their trait values for A, g, LMA, LDMC, C/N ratio, and LCC, whereas E and R_{leaf} show no significant differences between the groups (Table 7). LMA, C/N ratio, and LCC appear to be the strongest differentiators between the groups, whereas A and LDMC display a slightly less significant effect (Table 7). Interestingly, g shows lower power in differentiating the groups individually than the spatial analysis, while the C/N ratio shows a stronger effect (see effect sizes in Table 7 compared to the R² values in Table 6).



Figures 8a - 8h show the distribution of LT values for each species group: A, E, g, R_{leaf} , LMA, LDMC, LCC, and C/N ratio, respectively ($n = 202$ for plots a-g; 118, 72, and 6 for native, naturalised, invasive species, and *C. bullatus*, respectively. For 9h: $n = 99$; 54, 39, 3, and 3 for native, naturalised, and invasive, and *C. bullatus*, respectively). The boxplots show the minimum, first quartile, median, third quartile, and maximum LT value for each trait; the points show the outliers.

Table 7 shows the effect size and results obtained for the effect of invasion status on each LT using a Kruskal-Wallis test. Significance levels: n.s. for not significant, * for $0.05 > p > 0.01$; ** for $0.01 > p > 0.001$, and *** for $p < 0.001$. Significant values are shown in bold.

Trait	Sample size	Degrees of freedom	Test statistic	Effect size and magnitude	p-value	Significance
A	202	3	15.6	0.0639; moderate	< 0.05	**
E	202	3	1.81	-0.00600; small	> 0.05	n.s.
g	202	3	18.4	0.0777; moderate	< 0.05	***
R _{leaf}	202	3	2.72	-0.00142; small	> 0.05	n.s.
LMA	202	3	24.6	0.109; moderate	< 0.05	***
LDMC	202	3	12.1	0.0459; small	< 0.05	**
LCC	202	3	25.1	0.112; moderate	< 0.05	***
C/N ratio	99	3	15.0	0.126; moderate	< 0.05	**

3.3 Multiple comparison tests for each LT

To determine how the groups differed from one another, each significant effect (section 3.2) was further examined using the Dunn post-hoc test (see Table 8). *R. ponticum* differs significantly from native species in all LTs except g. *C. bullatus* differs significantly from native species (and does not differ significantly from the invasive species) in g, LMA, LCC, and C/N ratio, indicating similarity to the invasive species. However, *C. bullatus* also shows significant differences from *R. ponticum* in its values for A, C/N ratio, and LDMC.

Table 8 shows the results of the Dunn test performed on LTs that showed a significant overall effect of plant invasion type on each LT (Table 9). Significance levels: n.s. for not significant, * for $0.05 > p > 0.01$; ** for $0.01 > p > 0.001$, and *** for $p < 0.001$. Significant values are shown in bold.

Trait	Comparison	Test statistic	Adjusted p-value	Significance
A	Native - Naturalised	-2.03	> 0.05	n.s.
	Naturalised - Invasive	-3.58	< 0.05	**
	Native - Invasive	-2.91	< 0.05	*
	Native - <i>C. bullatus</i>	1.27	> 0.05	n.s.
	Naturalised - <i>C. bullatus</i>	0.532	> 0.05	n.s.
	Invasive - <i>C. bullatus</i>	3.02	< 0.05	*
g	Native – Naturalised	-0.737	> 0.05	n.s.
	Naturalised - Invasive	-2.70	< 0.05	*
	Native - Invasive	-2.47	> 0.05	n.s.

Trait	Comparison	Test statistic	Adjusted p-value	Significance
g	Native - <i>C. bullatus</i>	-3.26	< 0.05	**
	Naturalised - <i>C. bullatus</i>	-3.48	< 0.05	**
	Invasive - <i>C. bullatus</i>	-0.573	> 0.05	n.s.
LMA	Native - Naturalised	2.13	> 0.05	n.s.
	Naturalised - Invasive	-2.69	< 0.05	*
	Native - Invasive	-3.49	< 0.05	**
	Native - <i>C. bullatus</i>	-3.45	< 0.05	**
	Naturalised - <i>C. bullatus</i>	-2.65	< 0.05	**
	Invasive - <i>C. bullatus</i>	0.0247	> 0.05	n.s.
LDMC	Native - Naturalised	0.737	> 0.05	n.s.
	Naturalised - Invasive	-3.09	< 0.05	*
	Native - Invasive	-3.40	< 0.05	**
	Native - <i>C. bullatus</i>	0.480	> 0.05	n.s.
	Naturalised - <i>C. bullatus</i>	0.732	> 0.05	n.s.
	Invasive - <i>C. bullatus</i>	2.81	< 0.05	*
LCC	Native - Naturalised	-0.620	> 0.05	n.s.
	Naturalised - Invasive	-3.99	< 0.05	***
	Native - Invasive	-3.83	< 0.05	***
	Native - <i>C. bullatus</i>	-3.03	< 0.05	*
	Naturalised - <i>C. bullatus</i>	-3.20	< 0.05	**
	Invasive - <i>C. bullatus</i>	0.583	> 0.05	n.s.
C/N ratio	Native - Naturalised	2.35	> 0.05	n.s.
	Naturalised - Invasive	-2.28	> 0.05	n.s.
	Native - Invasive	-3.14	< 0.05	*
	Native - <i>C. bullatus</i>	0.774	> 0.05	n.s.
	Naturalised - <i>C. bullatus</i>	1.59	> 0.05	n.s.
	Invasive - <i>C. bullatus</i>	2.84	< 0.05	*

3.4 Pairwise LT differences

The z-scaled mean differences between native/invasive and *C. bullatus* for each LT are shown in Table 9 - smaller difference values indicate higher similarity for each LT. *C. bullatus* differs more from the invasive species in A, LDMC, and C/N ratio, and more from native species in g, LMA, and LCC - similarly to the previous findings in post-hoc tests and spatial NMDS analysis (sections 3.1 and 3.3, respectively). The Wilcoxon tests conducted on these pairwise differences show the same degree of statistical significance ($V = 21$, $p = 0.031$), suggesting *C. bullatus* exhibits a mixed pattern of similarity to both native and invasive species across different LTs.

Table 9 shows the scaled mean differences between native/*C. bullatus* and invasive/*C. bullatus* LT absolute difference between the two. The values are rounded to 4 significant figures.

Trait	Native - <i>C. bullatus</i>	Invasive - <i>C. bullatus</i>	Absolute difference
A	0.5437	1.708	1.164
g	1.712	0.6646	1.047
LMA	1.173	0.2102	0.9628
LDMC	0.0307	1.457	1.426
LCC	1.577	0.5760	1.001
C/N ratio	0.3448	3.129	2.784

4. Discussion

4.1 Study findings

The study aimed to evaluate the LT similarities and differences between native, naturalised, and invasive plant species in Scotland, with a particular focus on comparing the alien *C. bullatus* to the notoriously invasive *R. ponticum*. The findings revealed distinct differences between the groups, highlighting the LTs contributing to the invasiveness of *R. ponticum* in Scotland and providing insights into the invasion potential of *C. bullatus* based on its similarity to the highly successful invader. Consistent with findings from tropical and Mediterranean regions (Peñuelas *et al.*, 2009; Richardson and Rejmánek, 2011; Teixeira Oliveira *et al.*, 2014; Li *et al.*, 2018; Abdallah *et al.*, 2022), invasive species in temperate Scotland exhibited significant functional dissimilarity from native species. The key LTs driving these differences were LMA, g, LCC, A, and C/N ratio, all closely related to biomass allocation, carbon assimilation, and resource acquisition strategies (Lavorel and Garnier, 2002; Wright *et al.*, 2004; Poorter and Bongers, 2006; Poorter *et al.*, 2009). The invasive *R. ponticum* showed higher values for these traits than native species, likely enhancing its productivity and competitive ability (Pyšek and Richardson, 2007). The alien *C. bullatus* exhibited a similar trait profile to *R. ponticum* in the traits differentiating the invasive species from natives, suggesting potential for invasion over naturalisation in Scotland (MacDougall *et al.*, 2009; Cleland, 2011). These findings underscore the importance of considering functional LT differences when assessing invasion risk and developing management strategies for non-native species.

4.1.1 Overall trait differences

The NMDS analysis revealed significant differences in LT profiles among the species groups (Figure 7). Native and naturalised species showed high overlap, indicating similarity, whereas the invasive *R. ponticum* appears dissimilar from both groups in ordination space. This aligns with the LSH, which predicts that invasive species should exhibit functional dissimilarity from native species to facilitate their establishment and domination in novel environments (MacArthur and Levins, 1967). These findings are also in accordance with the study by Divíšek *et al.* (2018), who conducted a trait-based comparison in temperate Central European species and found that invasive species in the area generally exhibit higher trait values, which distinguish them from the native species, whereas the traits of native and naturalised species are similar statistically. *C. bullatus* showed a similar pattern in ordination space to *R. ponticum*, suggesting similarity in LTs to the invasive species. Such similarity to invasive species may suggest a higher potential for invasion over naturalisation of *C. bullatus* in Scotland (MacDougall *et al.*, 2009; Cleland, 2011; Divíšek *et al.*, 2018).

The key LTs driving the observed differences were LMA, g, and LCC, followed by A and C/N ratio. These traits are crucial determinants of plant performance and invasiveness due to their role in plant biomass allocation, carbon assimilation, and resource acquisition strategies (Lavorel and Garnier, 2002; Wright *et al.*, 2004; Poorter and Bongers, 2006; Leishman *et al.*, 2007; Pyšek and Richardson, 2007; Peñuelas *et al.*, 2009; van Kleunen *et al.*, 2010; Teixeira Oliveira *et al.*, 2014). LDMC, E and R_{leaf} showed no effect in differentiating the species groups - this is particularly interesting, as LDMC is also an LT closely linked with biomass allocation strategies (like LMA, with which LDMC is positively correlated in orientation space; Poorter *et al.*, 2011).

Further, all the key LTs driving the differences between the species showed positive correlations with one another, indicating a combination of these traits drives the differences between native and invasive (and potentially invasive alien) species. LMA, for example, is positively correlated with investment in leaf structural components and negatively correlated with mass-based nitrogen concentration, reflecting a trade-off between resource acquisition and conservation strategies (Poorter *et al.*, 2009, 2011). Higher LMA values, as observed in *R. ponticum*, are associated with increased resource-use efficiency and longer leaf lifespan, conferring advantages in resource-limited environments (Pyšek and Richardson, 2007; Poorter *et al.*, 2009, 2011). This is particularly interesting, as most successful plant invasions are associated with habitat disturbance, and the ability of non-native species to outcompete their native counterparts is essential to their establishment (Peñuelas *et al.*, 2009; Poorter *et al.*, 2009; Liao *et al.*, 2021).

The high LMA values and their trade off with N-investment is also reflected by the leaf C/N ratio, which represents the balance between C and N allocation in the leaf, with higher values suggesting greater investment in carbon-rich structural compounds versus nitrogen-rich photosynthetic systems (Niinemets *et al.*, 2003; Pyšek and Richardson, 2007). Furthermore, the higher photosynthetic rate/capacity (A) and water use efficiency (g) both promote invasiveness (Pyšek and Richardson, 2007). Finally, LCC is linked to the amount of carbon invested in structural and defensive compounds, with higher values potentially enhancing stress tolerance and herbivory resistance - this result is intriguing, as *R. ponticum* has been shown to release leaf and root allelopathic chemicals which deter herbivory and decrease the N content of the soil (Cross, 1975; Niinemets *et al.*, 2003; Peñuelas *et al.*, 2009).

The LTs driving the dissimilarity between *R. ponticum* and the native species reflect the competitive advantage of the IAP over the native and naturalised species, particularly in terms of survival strategies, including biomass allocation and carbon assimilation.

4.1.2 Comparisons between groups

The groups exhibit statistically significant differences in six out of eight measured LTs (Table 7), with *R. ponticum* showing higher mean LT values than native and naturalised species for all 8 traits. The higher LT values are consistent with previous findings (e.g. Pyšek and Richardson, 2007; Divíšek *et al.*, 2018), highlighting the competitive advantage that enables *R. ponticum* to be a successful invader in Scotland. Interestingly, the only LTs that show no differences between the species groups are R_{leaf} and E. This suggests that WUE (E; Niinemets *et al.*, 2014), and the metabolic cost of maintaining leaf tissue (R_{leaf} ; Daley and Phillips, 2006) are similar across all species groups. While water acquisition and loss strategies appear similar between the groups, *R. ponticum* demonstrates a greater ability to regulate gas exchange (as indicated by its higher g values; Figure 8c). This trait correlation is supported by the spatial analysis, where the efficient gas exchange regulation abilities of *R. ponticum* correspond to its higher A and C/N ratio values (Table 8), indicating a greater potential for carbon assimilation via photosynthesis compared to native species.

The lack of a significant relationship in E implies that water regulation strategies are consistent across the groups and that the higher g values in *R. ponticum* are not necessarily linked to water use efficiency in distinguishing between native and invasive species. This decoupling of g and E suggests that *R. ponticum* may have more efficient water use strategies than native species - the higher g values allow *R. ponticum* to assimilate C more rapidly via photosynthesis while maintaining similar E rates to the native species. However, g is the only LT where *R. ponticum* does not significantly differ from native species, although it does from naturalised species (Table 8). This could be explained by the wider range of g values seen in native species (Figure 8c), indicating that native species exhibit a wider variety of gas exchange strategies. The invasive *R. ponticum* shows a much narrower range at the higher end of the spectrum, suggesting it favours higher g values that facilitate rapid C uptake for photosynthesis. However, the alien *C. bullatus* shows a significant difference in g from native and naturalised species, which aligns with the expected pattern for invasive species (Pyšek and Richardson, 2007; Divíšek *et al.*, 2018).

Table 8 provides further insights into the specific LT differences between the groups. Notably, *R. ponticum* differs significantly from native species in A, LMA, LDMC, LCC, and C/N ratios. These differences suggest that *R. ponticum* may possess a more efficient resource acquisition and utilisation strategy than native species, potentially contributing to its invasive success (Pyšek and Richardson, 2007). Higher values in these traits indicate greater investment in leaf structural components, more efficient carbon assimilation, and a more conservative nutrient use strategy, which allows *R. ponticum* to outcompete the native species by rapidly taking up

resources and using them for reproduction and spread (MacArthur and Levins, 1967; Pyšek and Richardson, 2007; Catford *et al.*, 2010). These findings are aligned with the LSH, which posits that invasive species should exhibit LT values associated with a "fast" return on investment strategy characterised by rapid resource acquisition and growth (MacArthur and Levins, 1967; Peñuelas *et al.*, 2009). This contrasts with the "slow" strategy typically observed in native species, prioritising resource conservation and stress tolerance (Peñuelas *et al.*, 2009). Additionally, invasive species like *R. ponticum* are known for their stress tolerance in novel habitats, often outperforming native species in low-resource habitats (Peñuelas *et al.*, 2009; van Kleunen *et al.*, 2010).

Furthermore, the significant differences between the species groups in LDMC (Figure 8g and Table 8), but the lack of an effect in the ordination space (Figure 7), provide insights into the WUE of the species. While LDMC does not appear to drive the overall differences between the groups, it does differ significantly between them, whereas leaf mass per area (LMA) does contribute to the observed differences. This suggests that the differences in LDMC between the species groups are not related to their overall resource acquisition and carbon assimilation strategies, which are more strongly influenced by traits like LMA, g, and A. Instead, the differences in LDMC may be more closely linked to WUE and drought tolerance strategies, which are not the primary drivers of the observed functional dissimilarities between the species groups in this study system. This, and the decoupling of g and E, suggests that the invasive *R. ponticum* has evolved more efficient water-use strategies compared to the native species, allowing it to maximise carbon assimilation while minimising water loss, contributing to its competitive advantage and invasive success in Scotland (Pyšek and Richardson, 2007).

Interestingly, the alien *C. bullatus* exhibited a mixed pattern of similarity and dissimilarity to native and invasive species across different LTs, suggesting *C. bullatus* may possess a combination of strategies. For traits like A, LDMC, and C/N ratio, *C. bullatus* exhibits values similar to native species (Table 8), potentially indicating a more conservative resource-use strategy (Peñuelas *et al.*, 2009). However, for traits like g, LMA, and LCC, *C. bullatus* showed greater similarity to the invasive *R. ponticum*, suggesting a more acquisitive strategy (Peñuelas *et al.*, 2009). This mixed strategy may be an adaptive response to the environmental conditions in the study area, allowing *C. bullatus* to exploit resources efficiently while maintaining some stress tolerance (van Kleunen *et al.*, 2010; Stace and Crawley, 2015). On the other hand, the similarity in some LTs to *R. ponticum* may indicate the potential for *C. bullatus* to become invasive in the future, as it shares key functional characteristics with a successful invader (Pyšek and Richardson, 2007; van Kleunen *et al.*, 2010; Divíšek *et al.*, 2018).

This mixed pattern of trait similarities and differences between *C. bullatus* and the native/invasive groups is further supported by the scaled mean difference analysis (Table 9). The Wilcoxon rank-sum test revealed no significant difference between the distributions in native/alien and invasive/alien LT differences, potentially due to the evenness of the dissimilarity in traits (3 similar and 3 different to *R. ponticum*). With an equal number of LTs similar to native and invasive species, the overall dissimilarity between *C. bullatus* and the two groups is comparable, however, the similarity of *C. bullatus* to *R. ponticum* in the three traits differentiating the IAP from native species should be noted.

4.3 Study implications

The focus on the study of an invasive species in temperate climates has filled in a specific knowledge gap, as the data on such species in this context is scarce (Peñuelas *et al.*, 2009; Richardson and Rejmánek, 2011; Teixeira Oliveira *et al.*, 2014; Li *et al.*, 2018; Abdallah *et al.*, 2022). Similar studies investigating functional traits in temperate climatic conditions have been performed on either all species present in the area (e.g. Divíšek *et al.*, 2018 investigated the flora of Central Europe) or with a specific focus on herbaceous species (Küster *et al.*, 2008) and found similar trends - invasive species generally show higher trait values than natives, elucidating their ability to outcompete the native species in their habitats (Pyšek and Richardson, 2007). Understanding the phenotypic divergence of invasive species from native species is essential to predicting the invasion potential of alien species (Catford *et al.*, 2010; Ordóñez *et al.*, 2010; Drenovsky *et al.*, 2012; Stace and Crawley, 2015; Lavoie *et al.*, 2016; Divíšek *et al.*, 2018). This is particularly important to IAP management as only a small number of introduced alien species will invade: most alien species naturalise in the new habitat, as is quantified by the ‘rule of tens’ where one out of every 10 alien species will become invasive in the UK (Williamson, 1993; Lavoie *et al.*, 2016).

The observed LT differences between native and invasive species, particularly in traits like LMA, LCC, and C/N ratio, suggest that invasive species like *R. ponticum* may possess a more efficient resource acquisition and utilisation strategy - supported by their higher photosynthetic rates. This could contribute to their competitive advantage over native species, facilitating their establishment, growth, and spread in novel environments (Lavorel and Garnier, 2002; Wright *et al.*, 2004; Pyšek and Richardson, 2007; Peñuelas *et al.*, 2009; Ordóñez *et al.*, 2010; Drenovsky *et al.*, 2012; Stace and Crawley, 2015; Pyšek *et al.*, 2017; Liao *et al.*, 2021; Kaushik *et al.*, 2022).

The success of *R. ponticum* in Scotland represents a significant management challenge - it is a consistent and widespread participant in Scottish habitats, growing across a variety of habitat

boundaries (including heaths, bogs, forests, and open grasslands) and is virtually unremovable once established (Cross, 1975). *R. ponticum* is resistant to most herbicides and other management techniques (e.g. burning) and often shows increased success following human intervention (Cross, 1975). The continued presence (and often domination) of *R. ponticum* in Scottish habitats due to its ability to outcompete native species through fast reproduction and establishment in specific habitats represents an immense challenge to its management and restriction (Cross, 1975; Milne and Abbot, 2000, Erfmeier and Bruelheide, 2009; Stace and Crawley, 2015). The ability to predict the invasion potential of alien species based on their similarity to successful IAPs in a given habitat is essential to preventing their establishment and domination, particularly in rare and endangered habitats, as the proliferation of non-native species often results in a decrease of local biodiversity (Ordonez *et al.*, 2010; Stace and Crawley, 2015; Vilá and Hulma, 2017; Pyšek *et al.*, 2020). The goal of this study was to investigate the invasion potential of the alien *C. bullatus* and determine whether this alien species could present similar challenges as *R. ponticum* if established in Scotland.

The mixed pattern of LT similarities and differences exhibited by *C. bullatus* raises interesting questions about its potential invasiveness - while some traits align with native species, others resemble the invasive *R. ponticum*, indicating a potential for invasiveness under certain environmental conditions. The study results indicate that *C. bullatus* has the potential to invade Scottish habitats, particularly based on LTs like g, LMA, and LCC - three traits which drive the differences between the groups observed in ordination space. Conversely, *C. bullatus* appears more similar to native species in A, LD_{MC}, and C/N ratio - three traits which showed a lesser effect in driving the differences between native and invasive species. As *C. bullatus* appears similar to *R. ponticum* in the three traits which separate them most from native and naturalised species, *C. bullatus* has high invasion potential in Scotland. Its combined dissimilarity from native and naturalised species in ordination space (Figure 7) and the insignificant mean differences between it and *R. ponticum* in the three leading LTs (Table 8) suggest *C. bullatus* to be similar to *R. ponticum* in its LTs. Furthermore, as *R. ponticum* is the only woody IAP in Scotland, and its overwhelming success in invading native Scottish habitats (Patterson *et al.*, 2013), *C. bullatus* could represent a significant threat as a potentially invasive species. Further research is needed to understand the exact LT combinations and environmental factors that may trigger or facilitate the invasiveness of alien species like *C. bullatus*.

4.3 Study limitations

While the study showcased valuable insights into the differences between native and invasive woody species in Scotland regarding their leaf traits, various limitations are associated with it. Namely, the limited environmental and geographical scope - as the sampling was limited to the

RBGE and the species present within it, the species selected for this study may not fully represent the environmental heterogeneity across Scotland. Environmental and ecological dynamics (including herbivory, competition, and microclimate variations), which were limited or equalised across species in this study system, have been shown to influence LTs (Küster *et al.*, 2008; Nicotra *et al.*, 2011; Stace and Crawley, 2015; Bennet *et al.*, 2016; Hulme and Bernard-Verdier, 2017; Liao *et al.*, 2021). The narrow focus on woody angiosperm species further reduces the scope of species this study analysed. Scotland is known for its immense plant diversity, including rare species confined to few specific habitats and a wide variety of native gymnosperm woody species representing a high proportion of Scotland's woodland area (Patterson *et al.*, 2013). Past studies have also illuminated that invasion patterns may not be discernible from single spatial scale studies (Pyšek and Hulme 2005; Stace and Crawley, 2015), and the effects of a given trait may vary at various scales (including spatial, climatic, and habitat; Pyšek and Richardson, 2007; Stace and Crawley, 2015).

Furthermore, due to the same limitation to the RBGE (which standardised the environmental conditions that these species grow under), the environmental conditions are not representative of Scotland in its entirety - the country spans four Köppen climate classifications (Geiger, 1954; Kottek *et al.*, 2006), and exhibits extensive variation in topography (Trewin, 2008) - both of which have been shown to influence invasion (Stace and Crawley, 2015; Hulme and Bernard-Verdier, 2017; Wang *et al.* 2022) - the vast combinations of both could not be captured in this study. Still, the limitation of the study system to the RBGE provided valuable insights into the functioning of these species in similar environmental conditions. Moreover, while the RBGE holds an extensive living collection (RBGE, 2021), the species selection process illuminated that most selected species were only present once and each species selected for this study could thus only be sampled once - as LTs have been shown to vary interspecifically (Bassow and Bazzaz, 1998; Le Roux *et al.*, 2001; Poorter *et al.*, 2009; Poorter *et al.*, 2011; Steppe *et al.*, 2011; Xiong *et al.*, 2018), this is an important limitation of the study. It has been shown that LTs vary extensively with plant age and size, both of which this study could not account for (Reich *et al.*, 1991; Lee *et al.*, 2007; Poorter *et al.*, 2009; Thomas, 2010; Steppe *et al.*, 2011; Stace and Crawley, 2015; Bielczynski *et al.*, 2017; Asefa *et al.*, 2021). Furthermore, as *R. ponticum* is designated as Scotland's only woody invasive species, the uneven sample sizes represent statistical challenges, which we aimed to combat using non-parametric test alternatives (however, these often have lower statistical power; Nahm, 2016).

While a wide range of additional data was collected, including whether a species is evergreen or deciduous, their analysis was beyond the scope of this study. Studies like Poorter *et al.* (2011) and Wang *et al.* (2022) have shown that LTs vary between evergreen and deciduous species

and along climate gradients, which is particularly interesting when investigating *R. ponticum*. The evergreen IAP showed higher mean trait values than native and naturalised species for all 8 LTs (Figure 9). Notably, both the invasive and alien species were evergreen, while most native and naturalised species (except *I. aquifolium*) were deciduous. Interestingly, Niinemets *et al.* (2003) compared the LTs of both *R. ponticum* and *I. aquifolium* and found that the invasive *R. ponticum* shows higher photosynthetic efficiency and investment of N into LCC and overall higher leaf plasticity, suggesting these differences may be the drivers behind the success of *R. ponticum*, and deciduous habit alone may not play a significant role in determining invasion success. Similar differences in LTs can be observed when solar irradiation levels, tree size and age, and interspecific variation are considered drivers of the variation, none of which could be analysed in this study (Kappel and Flore, 1983; Reich *et al.*, 1991; Bassow and Bazzaz, 1998; Le Roux *et al.*, 2001; Niinemets *et al.*, 2003; Lee *et al.*, 2007; Poorter *et al.*, 2009; Thomas, 2010; Poorter *et al.*, 2011; Steppe *et al.*, 2011; Stace and Crawley, 2015; Bielczynski *et al.*, 2017; Li *et al.*, 2018; Xiong *et al.*, 2018; Asefa *et al.*, 2021; Mu and Cheng, 2021; Rawat *et al.*, 2021; Wang *et al.*, 2022).

Finally, this study focused solely on LTs, which are representative of plant function (Lavorel and Garnier, 2002; Wright *et al.*, 2004; Poorter and Bongers, 2006). However, this approach is limited in scope as invasion potential depends on a variety of traits that are not directly linked to LTs, including reproduction mode (namely the ability to reproduce vegetatively), flowering time, root traits, and their response to the environment and stress (Mejías *et al.*, 2002; Liu *et al.*, 2006; Pyšek and Richardson, 2007; van Kleunen *et al.*, 2010; Stace and Crawley, 2015; Bennet *et al.*, 2016; Liao *et al.*, 2021). Particularly in the case of *R. ponticum*, studies suggest its competitive advantage over native species is at least partially due to its allelopathic leaf and root systems - *R. ponticum* secretes herbivore-deterrant chemicals, which increase its competitive advantage over native species without such deterrents (Niinemets *et al.*, 2003). Furthermore, *R. ponticum* has been shown to release nitrate reductase (an allelopathic chemical) via its roots, limiting the nitrogen content of the soil in the area, disadvantaging the other species present (Cross, 1975; Niinemets *et al.*, 2003). This, coupled with the extensive endotrophic mycorrhizal network of *R. ponticum* (Cross, 1975), makes for an efficient growth strategy post-establishment. By focusing specifically on LTs, this study may have overlooked important aspects of invasion potential, such as reproductive traits, root characteristics, and allelopathic effects, all of which may confer a competitive advantage of the non-native species over their native counterparts.

4.4 Future directions

In the future, a combined focus on LTs and other plant functional traits that may contribute to the future invasion success of a species is necessary. When investigating the similarity of alien species like *C. bullatus* to an invasive species, such as *R. ponticum*, the inclusion of additional trait data beyond LTs (e.g. the ability to reproduce vegetatively and release allelopathic chemicals; Cross, 1975; Niinemets *et al.*, 2003) is essential. Incorporating such whole-plant functional traits in addition to LTs will provide a more comprehensive understanding of the invasion potential of alien species. Furthermore, including diversity data is essential - the invisibility of a habitat depends greatly on its community composition (Catford *et al.*, 2010).

Developing an index that focuses on functional traits and community composition to accurately place the alien species within their ecological context is crucial. Most studies have focused on functional diversity indices, but it is clear that invasion potential is determined by a combination of both. For instance, Küster *et al.* (2008) found that trait interactions help explain invasion success in German flora. Similarly, Divíšek *et al.* (2018) found that functional traits like plant height at maturity are essential for differentiating invasive species from their native counterparts in Central Europe. Further, Kaushik *et al.* (2022) highlighted the importance of considering functional traits and community structure when assessing invasion risk. It is thus critical to develop a combined framework for evaluating invasiveness that includes whole-plant traits, LTs, and functional diversity. Such an attempt has been made for gymnosperm species (Rejmánek and Richardson, 1996), however, a similar comprehensive index is still lacking for angiosperms.

Future research should focus on creating a standardised framework incorporating multiple facets of invasion potential, including LTs, functional traits, community composition, and environmental factors. The inclusion of the latter is essential, as IAPs show a variety of trends that are habitat- and environment-specific, meaning the framework would need to account for that (Pyšek and Richardson, 2007; Küster *et al.*, 2008; van Kleunen *et al.*, 2010; Stace and Crawley, 2015; Bennet *et al.*, 2016; Hulme and Bernard-Verdier, 2017). Such a framework will enable more accurate predictions of the invasion risk posed by alien species and inform targeted management strategies to prevent the establishment and spread of invasive species and their associated economic and ecological costs.

Conclusions

The study reveals distinct differences in LTs between native, naturalised, and invasive species in Scotland, with the invasive *R. ponticum* exhibiting higher values for key traits like LMA, g, LCC, A, and C/N ratio compared to native species. Conversely, naturalised species do not differ significantly from native species. This highlights the phenotypic divergence of invasive species from native and naturalised species in a novel area - invasive species are similar enough to establish but different enough to outcompete the natives in their own habitat. These differences likely contribute to *R. ponticum*'s invasive success by enhancing its resource acquisition, carbon assimilation, and biomass allocation strategies. The alien *C. bullatus* showed a mixed pattern, with some traits similar to native species (A, LDMC, C/N ratio) and others similar to *R. ponticum* (g, LMA, LCC). This suggests *C. bullatus* may have the potential to become invasive in Scotland, particularly given its similarity to *R. ponticum* in the latter three traits, which most differentiate invasive from native species. However, the study was limited in geographic and functional scope, investigating only the woody angiosperms present in the RBGE. Further research is needed to assess the invasion potential of *C. bullatus* across a broader range of environmental conditions in Scotland, as well as across a wider range of traits.

References

- Abdallah, M., Hervías-Parejo, S. and Traveset, A. (2021). Low Pollinator Sharing Between Coexisting Native and Non-native Plant Pairs: The Effect of Corolla Length and Flower Abundance. *Frontiers in Ecology and Evolution*, 9.
- Abdallah, M., Douthe, C. and Flexas, J. (2022). Leaf morpho-physiological comparison between native and non-native plant species in a Mediterranean island. *Biological Invasions*.
- Andersen, M.C., Adams, H., Hope, B. and Powell, M. (2004). Risk Assessment for Invasive Species. *Risk Analysis*, 24(4), pp.787–793.
- Bassow, S.L. and Bazzaz, F.A. (1998). How Environmental Conditions Affect Canopy Leaf-Level Photosynthesis in Four Deciduous Tree Species. *Ecology*, 79(8), pp.2660–2675.
- Bennett, J.A., Riibak, K., Tamme, R., Lewis, R.J. and Pärtel, M. (2016). The reciprocal relationship between competition and intraspecific trait variation. *Journal of Ecology*, 104(5), pp.1410–1420.
- BioRender (2019). BioRender. Available at: <https://biorender.com/>. [Accessed 19 March 2024].
- Brownless, P. (2024). Interviewed by Zoja Manček Páli, 19 March, Edinburgh, UK.
- Catford, J.A., Vesk, P.A., Richardson, D.M. and Pyšek, P. (2011). Quantifying levels of biological invasion: towards the objective classification of invaded and invasible ecosystems. *Global Change Biology*, 18(1), pp.44–62.
- CBD (2002). *Alien species: guiding principles for the prevention, introduction and mitigation of impacts*. www.cbd.int. Available at: <https://www.cbd.int/kb/record/recommendation/7021?Event=SBSTTA-05> [Accessed 16 Apr. 2024].
- Cleland, E.E. (2011). Trait divergence and the ecosystem impacts of invading species. *New Phytologist*, 189(3), pp.649–652.
- Cross, J.R. (1975). Rhododendron Ponticum L. *The Journal of Ecology*, 63(1), p.345.
- Daley, M.J. and Phillips, N.G. (2006). Interspecific variation in nighttime transpiration and stomatal conductance in a mixed New England deciduous forest. *Tree Physiology*, 26(4), pp.411–419.
- Dawson, W., Moser, D., van Kleunen, M., Kreft, H., Pergl, J., Pyšek, P., Weigelt, P., Winter, M., Lenzner, B., Blackburn, T.M., Dyer, E.E., Cassey, P., Scrivens, S.L., Economo, E.P., Guénard, B., Capinha, C., Seebens, H., García-Díaz, P., Nentwig, W. and García-Berthou, E. (2017). Global hotspots and correlates of alien species richness across taxonomic groups. *Nature Ecology & Evolution*, 1(7).
- Dehnen-Schmutz, K., Perrings, C. and Williamson, M. (2004). Controlling Rhododendron ponticum in the British Isles: an economic analysis. *Journal of Environmental Management*, 70(4), pp.323–332.
- Dinno, A. (2015). *Nonparametric pairwise multiple comparisons in independent groups using Dunn's test*. *The Stata Journal*, 15(1), 292-300
- Divíšek, J., Chytrý, M., Beckage, B., Gotelli, N.J., Lososová, Z., Pyšek, P., Richardson, D.M. and Molofsky, J. (2018). Similarity of introduced plant species to native ones facilitates naturalization, but differences enhance invasion success. *Nature Communications*, 9(1).
- Drenovsky, R.E., Grewell, B.J., D'Antonio, C.M., Funk, J.L., James, J.J., Molinari, N., Parker, I.M. and Richards, C.L. (2012). A functional trait perspective on plant invasion. *Annals of Botany*, 110(1), pp.141–153.
- Erfmeier, A. and Bruelheide, H. (2009). Invasibility or invasiveness? Effects of habitat, genotype, and their interaction on invasive Rhododendron ponticum populations. *Biological Invasions*, 12(3), pp.657–676.

European Union (Withdrawal) Act 2018. Available at:
<https://www.legislation.gov.uk/ukpga/2018/16/contents/enacted> [Accessed 21 March 2024]

Geiger, R. (1954). "Klassifikation der Klimate nach W. Köppen" [Classification of climates after W. Köppen]. *Landolt-Börnstein – Zahlenwerte und Funktionen aus Physik, Chemie, Astronomie, Geophysik und Technik, alte Serien*. Vol. 3. Berlin: Springer. pp. 603–607.

Givnish, T. (1979). On the Adaptive Significance of Leaf Form. *Topics in Plant Population Biology*, pp.375–407.

Heinz Walz GmbH. GFS-3000 [Apparatus and software]. Available at:
https://www.walz.com/products/gas_exchange/gfs-3000/introduction.html [Accessed 14 September 2023].

Herron, P.M., Martine, C.T., Latimer, A.M. and Leicht-Young, S.A. (2007). Invasive plants and their ecological strategies: prediction and explanation of woody plant invasion in New England. *Diversity and Distributions*, 13(5), pp.633–644.

Hollander, M., Wolfe, D. A. and Chicken, E. (2014). *Nonparametric statistical methods* (3rd ed.). John Wiley & Sons

Hulme, P.E. and Bernard-Verdier, M. (2017). Comparing traits of native and alien plants: Can we do better? *Functional Ecology*, 32(1), pp.117–125.

Kahle, D. and Wickham, H. (2013) ggmap: Spatial Visualization with ggplot2. *The R Journal*, 5(1), 144–161. Available at: <http://journal.r-project.org/archive/2013-1/kahle-wickham.pdf>.

Kappel, F. and Flore, J.A. (1983). Effect of Shade on Photosynthesis, Specific Leaf Weight, Leaf Chlorophyll Content, and Morphology of Young Peach Trees. *Journal of the American Society for Horticultural Science*, 108(4), pp.541–544.

Kaushik, P., Pati, P.K., Khan, M.L. and Khare, P.K. (2022). Plant functional traits best explain invasive species' performance within a dynamic ecosystem - A review. *Trees, Forests and People*, p.100260.

Konica Minolta Optics. SPAD 502+ Chlorophyll meter [Apparatus]. Available at:
<https://www.konicaminolta.eu/eu-en/hardware/measuring-instruments/colour-measurement/chlorophyll-meter/spad-502plus#specifications> [Accessed August 21 2023]

Kotttek, M., Grieser, J., Beck, C., Rudolf, B. and Rubel, F. (2006). World Map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, 15(3), pp.259–263.

Küster, E.C., Kühn, I., Bruelheide, H. and Klotz, S. (2008). Trait interactions help explain plant invasion success in the German flora. *Journal of Ecology*, 96(5), pp.860–868.

Le Roux, X., Walcroft, A.S., Daudet, F.A., Sinoquet, H., Chaves, M.M., Rodrigues, A. and Osorio, L. (2001). Photosynthetic light acclimation in peach leaves: importance of changes in mass:area ratio, nitrogen concentration, and leaf nitrogen partitioning. *Tree Physiology*, 21(6), pp.377–386.

Kruskal, J.B. (1964). Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika*, 29(1), pp.1–27.

Lavorel, S. and Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*, 16(5), pp.545–556.

Lee, S.-H., Tewari, R.K., Hahn, E.-J. and Paek, K.-Y. (2007). Photon flux density and light quality induce changes in growth, stomatal development, photosynthesis and transpiration of *Withania Somnifera* (L.) Dunal. plantlets. *Plant Cell, Tissue and Organ Culture*, 90(2), pp.141–151.

Leishman, M.R., Haslehurst, T., Ares, A. and Baruch, Z. (2007). Leaf trait relationships of native and invasive plants: community- and global-scale comparisons. *New Phytologist*, 176(3), pp.635–643.

Li, Y., He, N., Hou, J., Xu, L., Liu, C., Zhang, J., Wang, Q., Zhang, X. and Wu, X. (2018). Factors Influencing Leaf Chlorophyll Content in Natural Forests at the Biome Scale. *Frontiers in Ecology and Evolution*, 6(64).

Liao, H., Pal, R.W., Niinemets, Ü., Bahn, M., Cerabolini, B.E.L. and Peng, S. (2021). Different functional characteristics can explain different dimensions of plant invasion success. *Journal of Ecology*, 109(3), pp.1524–1536.

Liu, J., Dong, M., Miao, S.L., Li, Z.Y., Song, M.H. and Wang, R.Q. (2006). Invasive alien plants in China: role of clonality and geographical origin. *Biological Invasions*, 8(7), pp.1461–1470.

MacArthur, R. and Levins, R. (1967). The Limiting Similarity, Convergence, and Divergence of Coexisting Species. *The American Naturalist*, 101(921), pp.377–385.

MacDougall, A.S., Gilbert, B. and Levine, J.M. (2009). Plant invasions and the niche. *Journal of Ecology*, 97(4), pp.609–615.

Mejías, J.A., Arroyo, J. and Ojeda, F. (2002). Reproductive ecology of Rhododendron ponticum (Ericaceae) in relict Mediterranean populations. *Botanical Journal of the Linnean Society*, 140(3), pp.297–311.

Milne, R.I. and Abbott, R.J. (2000). Origin and evolution of invasive naturalized material of Rhododendron ponticum L. in the British Isles. *Molecular Ecology*, 9(5), pp.541–556.

Met Office. (2020). *UK climate averages*. Available at:

<https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-climate-averages/gfj1d6wgf> [Accessed 8 November 2023].

Nahm, F.S. (2016). Nonparametric Statistical Tests for the Continuous data: the Basic Concept and the Practical Use. *Korean Journal of Anesthesiology*, 69(1), pp.8–14.

NatureScot (2024). *Invasive non-native plants*. NatureScot. Available at:

<https://www.nature.scot/professional-advice/protected-areas-and-species/protected-species/invasive-non-native-species/invasive-non-native-plants>. [Accessed 13 March 2024]

NatureScot (2011). *Native versus non-native woodland*. NatureScot. Available at:

<https://www.nature.scot/professional-advice/land-and-sea-management/managing-land/forests-and-woodlands/native-versus-non-native-woodland>. [Accessed 13 March 2024]

Nicotra, A.B., Leigh, A., Boyce, C.K., Jones, C.S., Niklas, K.J., Royer, D.L. and Tsukaya, H. (2011). The evolution and functional significance of leaf shape in the angiosperms. *Functional Plant Biology*, 38(7), p.535.

Niinemets, Ü., Keenan, T.F. and Hallik, L. (2014). A worldwide analysis of within-canopy variations in leaf structural, chemical and physiological traits across plant functional types. *New Phytologist*, 205(3), pp.973–993.

Niinemets, Ü., Valladares, F. and Ceulemans, R. (2003). Leaf-level phenotypic variability and plasticity of invasive Rhododendron ponticum and non-invasive *Ilex aquifolium* co-occurring at two contrasting European sites. *Plant, Cell and Environment*, 26(6), pp.941–956.

Niinemets, Ü. (2020). Leaf Trait Plasticity and Evolution in Different Plant Functional Types. *Annual Plant Reviews online*, pp.473–522.

Ordonez, A., Wright, I.J. and Olff, H. (2010). Functional differences between native and alien species: a global-scale comparison. *Functional Ecology*, 24(6), pp.1353–1361.

Paterson, E., Land, M., Campbell, C., Coull, M. and Shand, C. (2011). *AI content, mineral soils Geochemical Atlas for Scottish Topsoils Frequency Concentration intervals, mg/kg AI content, organic soils*. James Hutton Institute.

Patterson, G., Nelson, D., Robertson, P. and Tullis, J. (2013). *Scotland's Native Woodlands*. Forestry Commission Scotland.

Peñuelas, J., Sardans, J., Llusià, J., Owen, S.M., Carnicer, J., Giambelluca, T.W., Rezende, E.L., Waite, M. and Niinemets, Ü. (2009). Faster returns on 'leaf economics' and different biogeochemical niche in invasive compared with native plant species. *Global Change Biology*, 16(8), pp.2171–2185.

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P.B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., de Vos, A.C. and Buchmann, N. (2013). New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, 61(3), p.167.

Poorter, L. and Bongers, F. (2006). Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology*, 87(7), pp.1733–1743.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J. and Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist*, 182(3), pp.565–588.

Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P. and Mommer, L. (2011). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist*, 193(1), pp.30–50.

Preston, C.D., Pearman, D.A. and Dines, T.D. (2002). *New atlas of the British & Irish flora*. Oxford: Oxford Univ. Press.

Pyšek, P. and Hulme, P.E. (2005). Spatio-temporal dynamics of plant invasions: Linking pattern to process. *Écoscience*, 12(3), pp.302–315.

Pyšek, P. and Richardson, D.M. (2007). Traits associated with invasiveness in alien plants: where do we stand? *Biological invasions*, pp. 97–125.

Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., Carlton, J.T., Dawson, W., Essl, F., Foxcroft, L.C., Genovesi, P., Jeschke, J.M., Kühn, I., Liebhold, A.M., Mandrak, N.E., Meyerson, L.A., Pauchard, A., Pergl, J., Roy, H.E., Seebens, H. and Kleunen, M. (2020). Scientists' warning on invasive alien species. *Biological Reviews*, 95(6), pp.1511–1534.

Pyšek, P., Jarošík, V., Hulme, P.E., Pergl, J., Hejda, M., Schaffner, U. and Vilà, M. (2012). A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. *Global Change Biology*, 18(5), pp.1725–1737.

Pyšek, P., Pergl, J., Essl, F., Lenzner, B., Dawson, W., Kreft, H., Weigelt, P., Winter, M., Kartesz, J., Nishino, M., Antonova, L.A., Barcelona, J.F., Cabesaz, F.J., Cárdenas, D., Cárdenas-Toro, J., Castaño, N., Chacón, E., Chatelain, C., Dullinger, S. and Ebel, A.L. (2017). Naturalized alien flora of the world. *Preslia*, 89(3), pp.203–274.

Pyšek, P., Richardson, D.M., Pergl, J., Jarošík, V., Sixtová, Z. and Weber, E. (2008). Geographical and taxonomic biases in invasion ecology. *Trends in Ecology & Evolution*, 23(5), pp.237–244.

R Core Team (2022). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. Available at: <https://www.R-project.org/>.

Rawat, M., Arunachalam, K., Arunachalam, A., Alatalo, J.M. and Pandey, R. (2021). Assessment of leaf morphological, physiological, chemical and stoichiometry functional traits for understanding the functioning of Himalayan temperate forest ecosystem. *Scientific Reports*, 11(1), p.23807.

RBGE (2021). Responding to the Biodiversity Crisis and Climate Emergency. Royal Botanic Garden Edinburgh. Available at: <https://www.rbge.org.uk/media/dt3plrw/corporate-plan-2021-2026.pdf> [Accessed 2 April 2024].

Reich, P.B., Walters, M.B. and Ellsworth, D.S. (1991). Leaf age and season influence the relationships between leaf nitrogen, leaf mass per area and photosynthesis in maple and oak trees. *Plant, Cell and Environment*, 14(3), pp.251–259.

Rejmánek, M. and Richardson, D.M. (1996). What Attributes Make Some Plant Species More Invasive? *Ecology*, 77(6), pp.1655–1661.

Retsch GmbH. Mixer Mill MM 400 [Apparatus]. Available at: https://www.retsch.com/products/milling/ball-mills/mixer-mill-mm-400/?gad_source=1&gclid=Cj0KCQjwq86wBhDiARIsAJhuphIEvqJEDLMFdtMfKa6hJotq2QqzPjCMsu9PoloSlvja0KwOHCLFugaArqXEALw_wCB [Accessed October 7 2023]

Richardson, D.J. (2010). Invasion Science: The Roads Travelled and the Roads Ahead. *Wiley-Blackwell eBooks*, pp.396–407.

Richardson, D.M. and Pyšek, P. (2006). Plant invasions: merging the concepts of species invasiveness and community invasibility. *Progress in Physical Geography: Earth and Environment*, 30(3), pp.409–431.

Richardson, D.M. and Rejmánek, M. (2011). Trees and shrubs as invasive alien species - a global review. *Diversity and Distributions*, 17(5), pp.788–809.

Richardson, D.M., Pyšek, P., Rejmánek, M., Barbour, M.G., Panetta, F.D. and West, C.J. (2000). Naturalization and invasion of alien plants: concepts and definitions. *Diversity Distributions*, 6(2), pp.93–107.

Richardson, A.D., Duigan, S.P. and Berlyn, G.P. (2002). An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytologist*, 153(1), pp.185–194.

Shibaeva, T.G., Mamaev, A.V. and Sherudilo, E.G. (2020). Evaluation of a SPAD-502 Plus Chlorophyll Meter to Estimate Chlorophyll Content in Leaves with Interveinal Chlorosis. *Russian Journal of Plant Physiology*, 67(4), pp.690–696.

Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675.

Stace, C.A. and Crawley, M.J. (2015). *Alien plants*. London: William Collins.

Seebens, H., Essl, F., Dawson, W., Fuentes, N., Moser, D., Pergl, J., Pyšek, P., van Kleunen, M., Weber, E., Winter, M. and Blasius, B. (2015). Global trade will accelerate plant invasions in emerging economies under climate change. *Global Change Biology*, 21(11), pp.4128–4140.

Steppe, K., Niinemets, Ü. and Teskey, R.O. (2011). Tree Size- and Age-Related Changes in Leaf Physiology and Their Influence on Carbon Gain. *Tree Physiology*, pp.235–253.

Teixeira Oliveira, M., Matzek, V., Dias Medeiros, C., Rivas, R., Marinho Falcão, H. and Santos, M.G. (2014). Stress Tolerance and Ecophysiological Ability of an Invader and a Native Species in a Seasonally Dry Tropical Forest. *PLoS ONE*, 9(8), p.e105514.

The Invasive Non-native Species (EU Exit) (Scotland) (Amendment etc.) Regulations 2020. Available at: <https://www.legislation.gov.uk/ssi/2020/473/contents/made> [Accessed 21 March 2024].

Thermo Fisher Scientific. Flash SMART 2000 [Apparatus]. Available at: <https://www.thermofisher.com/order/catalog/product/11230245> [Accessed 8 April 2024]

Thomas, S.C. (2010). Photosynthetic capacity peaks at intermediate size in temperate deciduous trees. *Tree Physiology*, 30(5), pp.555–573.

Traveset, A. and Richardson, D.J. (2020). Plant invasions: the role of biotic interactions - an overview. *CABI eBooks*, pp.1–25.

Trewin, N.H. (2008). *The geology of Scotland*. London Geological Society.

Tukey, J. W. (1949). Comparing individual means in the analysis of variance. *Biometrics*, 5(2), 99–114.

van Kleunen, M., Dawson, W., Essl, F., Pergl, J., Winter, M., Weber, E., Kreft, H., Weigelt, P., Kartesz, J., Nishino, M., Antonova, L.A., Barcelona, J.F., Cabezas, F.J., Cárdenas, D., Cárdenas-Toro, J., Castaño, N., Chacón, E., Chatelain, C., Ebel, A.L. and Figueiredo, E. (2015). Global exchange and accumulation of non-native plants. *Nature*, 525(7567), pp.100–103.

van Kleunen, M., Weber, E. and Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*, 13(2), pp.235–245.

Vilá, M. and Hulme, P. (2017). *Impact of Biological Invasions on Ecosystem Services*. Cham: Springer International Publishing.

Wang, H., Wang, R., Harrison, S.P. and Prentice, I.C. (2022). Leaf morphological traits as adaptations to multiple climate gradients. *Journal of Ecology*, 110(6), pp.1344–1355.

Wildlife and Countryside Act 1981, c14. Available at: <https://www.legislation.gov.uk/ukpga/1981/69> [Accessed 24 May 2023].

Williamson, M. (1993). Invaders, weeds and the risk from genetically manipulated organisms. *Experientia*, 49(3), pp.219–224.

Woodland Trust (2019). A-Z of British Trees. *Woodland Trust*. Available at: <https://www.woodlandtrust.org.uk/trees-woods-and-wildlife/british-trees/a-z-of-british-trees/>.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C. and Midgley, J.J. (2004). The worldwide leaf economics spectrum. *Nature*, 428(6985), pp.821–827.

Xiong, D., Douthe, C. and Flexas, J. (2018). Differential coordination of stomatal conductance, mesophyll conductance, and leaf hydraulic conductance in response to changing light across species. *Plant, Cell & Environment*, 41(2), pp.436–450.

Appendices

Appendix 1: The table of GFS-3000 settings

Table 4 specifies the parameters used on the GFS-3000 for the physiological leaf trait measurements. Parameters not included in this table were not standardised for this study.

Parameter	Value	Unit	Additional comments
Flow	750	µmol/min	
Light (PARtop)	750	µmol m ⁻² s ⁻¹	Light source only from the top of the cuvette. The light was turned off (PARtop = 0) for measuring R _{leaf} .
Temperature (Tcuv)	20	°C	
Impeller	7	steps	
dCO ₂ MP	0.462 - 0.672	ppm	Re-measured after every 10 leaf samples for consistency

Appendix 2: CN measurement parameters

Table 5 specifies the parameters used on the Flash SMART 2000 instrument for measuring the leaf C/N ratio. Parameters courtesy of Joseph Casillo (lab technician).

Column information	
Column	CN/CHN Separation column, 2m, Stainless steel
Manufacturer, Part #, Lot #	Elemental Microanalysis, E3023, BN293467
Reaction tube information	
Type	CN/CHN Prepacked Quartz Reaction Tube
Manufacturer, Part #	Elmtex, PXR1000
Instrument programme	
Furnace temperature	950°C
Oven temperature	60°C
Carrier gas (He) flow	140 mL min ⁻¹
Oxygen flow	250
Reference gas (He) flow	140
Run time	220 seconds
Sampling delay	12 seconds
Oxygen injection end	5 seconds
Detector	TCD
Detector gain	1 gain

Appendix 3: Code used for data analysis.

To access the code used for the data analysis see link to the dissertation repository -
<https://github.com/zmancekpali/UOE-Dissertation-2024>.

Appendix 4: NMDS diagnostic plots

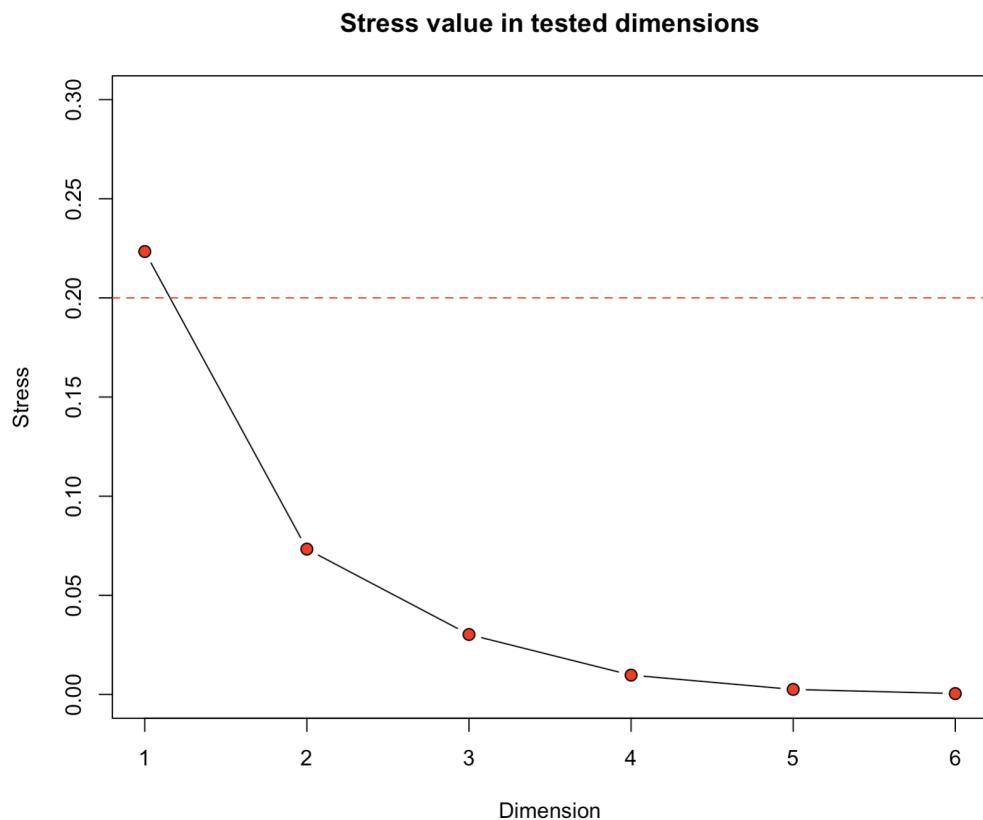


Figure 5 shows the scree plot used to determine the appropriate number of dimensions for spatial analysis.

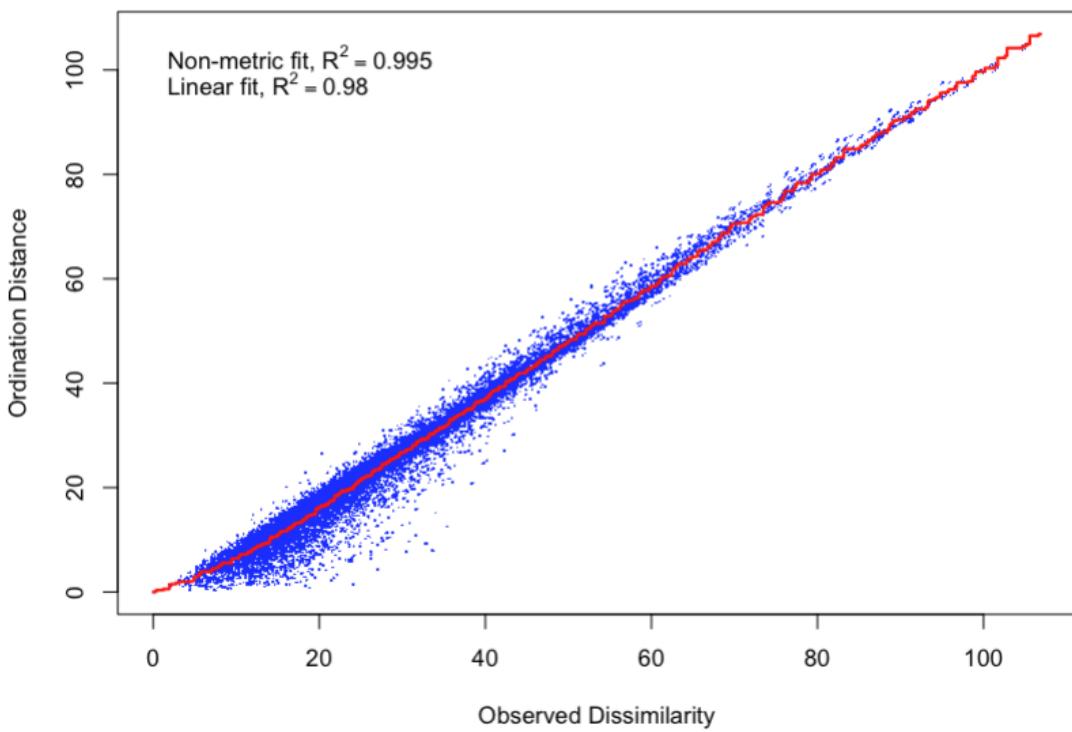


Figure 6 shows the stress plot used to assess the NMDS goodness of fit.