University of Reading

Multi-temporal Assessment of Diversity and Condition in UK Semi-natural Grasslands using Optical Reflectance

Doctor of Philosophy

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Declaration

I can confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

With 40% of the world's plants estimated to be under threat of extinction and ever lowering levels of ecological intactness of biological systems, the requirement to effectively monitor plant species and diversity has never been more pressing. Globally, natural, and semi-natural grassland ecosystems are at particular risk of degradation and conversion. Semi-natural grasslands in the UK currently make up about 1-2% of the permanent lowland grassland cover. Once degraded due to agricultural additions or inappropriate management, they can be difficult and costly to restore. As these systems display high levels of plant and invertebrate diversity, there is a need to safeguard their decline. However, there are currently significant challenges to providing the data needed to assess the condition of these systems. Remote sensing could contribute by providing information on herbaceous plant diversity and vegetation state across a wide range of spatial scales and time. Optical traits are a sub-set of plant traits that are detectable using reflectance data from leaf to canopy scales, dependent on the configuration of the sensor employed and can be linked to taxonomic diversity and condition of vegetation. Very high spatial resolution hyperspectral imaging technologies are, for the first time, enabling in-situ grassland plant phenotyping at the leaf, individual and high-resolution canopy scale. Analyses of these spectra have demonstrated promising results in application of mapping of taxonomic units and diversity metrics. However there is little evidence of the temporal stability of these observations. At the landscape scale, openly available, higher spatial resolution satellite data is also enabling examination of smaller field parcels, which are typical of UK fragmented landscapes. In this context, spectral time-series have the potential to be used to predict the condition of vegetation communities of conservation interest. In this thesis, the use of optical remote sensing data to further our understanding of semi-natural grasslands and to safeguard their decline, is examined, with a particular focus on the exploitation of multi-temporal sampling. Firstly, spectral variation in space, as a surrogate measure for species or community type diversity (also known as the spectral variation hypothesis), is assessed via a meta-analysis of existing studies. The results of the synthesis reveal some promise for the approach, but a large amount of variation between study outcomes is observed, suggesting that methodological approaches are important in the effectiveness of the proxy. Secondly, spectral data is collected alongside botanical and phenological diversity data at high spatial resolution over a growing season to test the stability of the spectral variation hypothesis over time. The results of these experiments show that the ability to detect biodiversity using this method is seasonally, and possibly, site dependent. Next, the suitability of hyperspectral leaf reflectance for distinguishing 17 herbaceous species growing within a calcareous grassland is examined. The application of machine learning classification models to multi-temporal leaf spectra show that although species are distinguishable at most sampling times within the year, the transferability of these models is very limited between sampling dates. Finally satellite time-series of vegetation indices are used to predict favourable or unfavourable vegetation condition criteria in calcareous fields across two years. A number of indices were successful in distinguishing between the different condition criteria but there was variation in results found between the two years sampled, due to differences in intra-annual vegetation phenology. Overall the results of this thesis, show promise for remote sensing of grassland biodiversity and condition. Both high spatial resolution hyperspectral data, as well as coarser resolution multi-spectral data sets, can be useful in evaluation of these systems. However, the dynamic nature of leaves and canopies over time, will require a multi-temporal approach to model building, which should be an integral part of developing these methods in the future.

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Contents

Glossary

Term	Acronym (if relevant)	Definition
Chlorophyll	CHL	The photosynthetically active pigment in plants that enables them to convert sunlight to energy
Coefficient of Variation	CoV	The ratio of the standard deviation to the mean. It shows the extent of variability in relation to the mean of the population.
Competitor-Ruderal-Stress-tolerator	CRS	A functional strategy framework developed by Grime which places plant species along three triangular vertices according to their adaptive responses to disturbance and stress
Equivalent Water Thickness	EWT	The fresh weight of leaves minus the dry weight divided by the leaf area; can also be described as the area weighted moisture content
Generalized Additive Models	GAM	A statistical model, where non-linear smooth functions are used instead of linear terms to describe the relationship between response and predictor variables
Global Sensitivity Analysis	GSA	A process of apportioning uncertainty to model outputs and their interactions when all input factors are varied simultaneously
Grasslands		Ecosystems defined by the dominance of herbaceous species; they include natural and cultural landscapes, maintained by agricultural practices
Hyperspectral		Reflectance data collected in very narrow contiguous bands.
Imaging spectrometer		An instrument that records hyperspectral reflectances as spatially contiguous pixels, analogous to Red/Green/Blue digital photography
Leaf Mass Area	LMA	A morphological leaf trait that describes the ratio between leaf dry mass and leaf area and that can be understood as the leaf-level cost of light interception
Leaf-clip		An attachment to a non-imaging spectrometer for measuring the hyperspectral reflectance of leaf material
Linear Mixed Models		A statistical model, with linear parameters, which contains both fixed and random effects, enabling modelling of known dependencies in the data set
Meta-analysis		A quantitative means of synthesizing results from independent studies using statistical methods
Multispectral		Reflectance data collected in discrete bands of varying width
N parameter	N	Mesophyll structure parameter in the PROSPECT model

Near Infra Red	NIR	The part of the electromagnetic spectrum covering the wavelengths slightly longer than in the visible, between 700 and 1000nm
Non-imaging spectrometer		An instrument that records high accuracy hyperspectral reflectances as single measurements of targets, normally used for gathering ground truthing data for airborne imaging campaigns
Optical Remote Sensing		A type of remote sensing that detects solar radiation reflected from targets on the surface of the earth
Optical Traits		A sub-set of plant traits (at leaf or canopy scales) that can be detected by the use of optical remote sensing
Phenology		The seasonal occurrence of biological events
Pixel		The smallest spatial unit of measure of a satellite or aerially captured image
Proximal Remote Sensing		(also handheld) An instrument that collects reflectance data close to the surface of interest
Radiative Transfer Models	RTM	Physical models that represent how light interacts with leaf and canopy biophysical variables. Within the field of optical remote they aim to provide a mechanistic link between leaf/canopy traits and optical properties.
Reflectance		Light energy that is not absorbed or transmitted through an object
Satellite Image Time Series	SITS	A set of satellite images taken from the same scene at different times
Short Wave Infra Red	SWIR	The part of the electromagnetic spectrum covering the wavelengths longer than the NIR
Species diversity		A combined measure of both species richness and evenness in a given area
Species evenness		The relative abundance of species in a given area
Species richness		The number of species present in a given area
Spectral Variation Hypothesis	SVH	The idea that spectral variation in space could be a useful surrogate for plant species or habitat diversity
State and Transition Model	STM	Conceptual models that describe changes in plant communities and soil properties, the drivers of changes and the management required to prevent or revert processes
Unmanned Aerial Vehicle	UAV	(also drone) A small unmanned aircraft on which sensors are mounted used in near surface remote sensing
Vegetation Condition Assessment		A means of assessing vegetation change (usually degradation) based on a 'benchmark' state

Chapter 1

Introduction and literature review

1.1 Overview of the research aims

The aim of this research is to enhance the understanding and use of remotely sensed reflectance data in estimation of biodiversity and habitat quality variables within UK grassland systems of conservation interest. Recent advances such as high spatial resolution sensors mounted on satellites and unmanned aerial vehicles, have started to enable more detailed observation of grassland canopies, which were not previously possible. One important aspect of vegetation remote sensing (RS) is the impact of phenology on our observables. Temperate grasslands undergo periods of senescence and green-up in reaction to temperature and water availability, causing fluctuations in our RS observations. To potentially exploit these temporal fluctuations, it is vital we first unravel the drivers behind observed spatial and temporal variations in grassland reflectance. In this thesis I test the temporal stability, across the growing season, between field-based grassland condition variables and reflectance measurements at the spatial scales achievable by these new generation sensors at both the field and the landscape scale. The aim is to use existing knowledge frameworks, which describe the relationship between grassland condition and reflectance, to work towards a predictive framework whereby we can better understand the reasons why reflectance data may be a useful surrogate for grassland condition indicators such as taxonomic diversity, habitat heterogeneity and other system specific structural proxies of condition.

1.2 Grassland ecosystems and their decline

Grasslands are defined as vegetation systems, dominated by herbaceous (non-woody) species (Wilsey, 2018), with lower overall biomass and net primary productivity compared to forests. There are varying estimates of global grassland cover, depending on how grasslands are defined (Dixon et al. 2014). These estimates range between 20 to 45% (of the Earth's land surface (Buchhorn et al. 2020, Gibson 2008, Goldewijk et al. 2007, Latham et al. 2014). Within a global context, there are broadly speaking two main categories of naturally occurring grassland, tropical and temperate, distinguished by their distribution and annual water requirement. The traditional view is that both types occupy a 'climate envelope' where forest is not able to grow (Whittaker, 1975), however the simplicity of this view has been challenged (Bond, 2019). Instead, complex feedbacks between fire regimes (Beerling and Osborne 2006, Ratajczak et al. 2014) and wild grazing herbivores (Waldram et al. 2008) could be responsible for the persistence of grassland over large parts of the Earth, where forests could potentially grow but instead grasslands occur. An addition distinction can be made between naturally occurring biomes and manmade or maintained grassland systems, also termed ‘semi-natural’. These grasslands could be of considerable age or value for biodiversity but require human intervention, such as grazing with domesticated animals, to prevent their succession into woodland (Dixon et al. 2014).

The case has been made for the naturalness of some current day UK grasslands as relicts of the last glacial period (Bush 1993, Feurdean et al. 2018) or as expanded elements of the natural temperate forest glades (Vera 2002). However, the prevailing view is that grasslands within a UK context are ‘semi-natural’, having been created and maintained through human intervention, involving woody vegetation clearance, marsh drainage and systems of animal husbandry that involve grazing and cutting (Gibson, 2008). Irrespective of the origin of grassland habitats in the UK, man’s influence in shaping these systems is certainly substantial and long lived, beginning around 6,000 years ago, with the beginnings of Neolithic settled agriculture (Hejcman et al. 2013, Sheail et al. 1974). These human mediated grassland systems can therefore be thought of as ‘ancient’ displaying character traits and community composition acquired over long stable periods and under evolving management types (Ingrouille 1995). Modern day UK grasslands vary in their character and species composition, mainly due to elevational, edaphic, and weather conditions. They can be classified into lowland and upland types and those that occur on either acid, neutral or calcareous soil types (Blakesley and Buckley 2016, Rodwell 1992).

There is evidence that the condition of natural and semi-natural grasslands is globally in decline (Bond 2016). However, compared to forests they are poorly studied and defined (Dixon et al. 2014, Dudley et al. 2020), so their status is not well understood, and conservation efforts are not well co-ordinated (Bardgett et al. 2021). At a European level, over half of all grassland habitats are believed to be threatened to some degree (Janssen et al. 2016), one of the highest percentages of all vegetation types.

Today, grassland cover in the UK is stable at around 40% of the total land mass (Nafilyan 2015). However, over the past 100 years, there has been a dramatic shift in the character of this land cover type. Prior to the two world wars at the beginning of the 20th century, most of this grassland retained regional variability and was rich in native plants and associated fauna. Due to the ‘green revolution’ (Brassley et al. 2021) and the associated advances in mechanisation, a large area of these grasslands has either been converted to arable, re-seeded to a few productive grass species with rapid biomass accumulation, or ‘improved’ via fertilizer addition (Fuller 1987). Other areas have been abandoned and have developed into secondary woodland (Cornish, 1954). This leaves approximately 100,000 hectares of unimproved semi-natural grassland in the UK (as opposed to the 4.5 million hectares of improved), about 2% of the total grassland cover. Estimates based on ecosystem service assessments, indicate that 90% of these grasslands are in some way degraded (Bullock et al. 2011). The patch size and connectivity of these landscape parcels has also been shown to be severely reduced over the last century (Hooftman and Bullock 2012). During the latter half of the 20th century, habitat loss through conversion has dramatically slowed (Ridding et al. 2015) however, threats to quality and long-term viability of the remnant habitat remain (Haines-Young et al. 2013, Norton et al. 1998, Ridding et al. 2020).

1.3 Biodiversity loss: causes and implications

The current Earth epoch has been labelled the Anthropocene (Lewis and Maslin 2015, Mahli 2017) characterised by an unprecedented increase in human numbers and their influence. Effects on the biosphere include high taxonomic extinction rates (Johnson et al. 2017), a reduction in the abundance of wild organisms (Pereira et al. 2012), plus dramatic changes in species distributions (McGill et al. 2015) and community homogenization (Daru et al. 2021). Within a UK context, habitat indicator species abundances have declined by between 13 and 22% during the period 1970 to 2016 (Hayhow et al. 2019). The UK has some of the largest and longest established biological recording schemes in the world which enable temporal trends to be detected for some taxonomic groups (Gaul et al. 2020). Using data collected from these schemes, the biggest species losses have been recorded in vascular plants (Hodgson et al. 2004) and invertebrates with specialist habitat requirements (Brereton et al. 2011). Two hundred and six of the most endangered organisms in the UK, noted under the UK Biodiversity Action Plan, are associated with semi-natural grasslands (Webb et al. 2010).

Species losses are mainly driven by the movement towards intensive management of agricultural land (Burns et al. 2016). However, losses have been compounded by lack of appropriate grazing pressures (Weiss and Jeltsch 2015), invasions by more vigorous (Bobbink, 1987) or invasive plant species (Seabloom et al. 2015), encroachment of woody vegetation (Svenning 2002), atmospheric nitrogen deposition (Stevens et al. 2010) and habitat fragmentation (Botham et al. 2011).

Benefits of correctly managing these systems and maximising their potential for biodiversity conservation is likely to ameliorate broader scale environmental ills. For example, the presence of species-rich semi-natural grasslands within intensive agricultural matrices has been shown to boost wild pollination to domesticated crops, while simultaneously improving soil stability and sequestering greenhouse gases (Bengtsson et al. 2019). Local plant species loss has also been linked more generally to a decline in ecosystems services (Hooper et al. 2012) in particular, ecosystem productivity (Tilman, Reich and Isbell 2012).

The ability of natural habitats to aid in climate mitigation has received much interest of late (Giradin et al. 2021). UK semi-natural grasslands are estimated to store approximated 0.09 Gtonnes of Carbon (Field et al. 2020). In particular, the organic soil component would be more stable if we maintained these systems in good condition. It is even thought that under optimal conditions the soils could become a carbon net sink. This coincides with the need to quantify semi-natural grassland condition for natural capital accounting within an ecosystem approach, going beyond their biodiversity value per se, to include, provision of foods, biofuels, genetic resources, climate regulation, air and water quality regulation, recreation and tourism, aesthetic values, cultural heritage, spiritual values, education, sense of place and health benefits (Brown et al. 2011).

In addition to conserving remaining fragments of semi-natural grasslands, attempts have been made to extend these systems through restoration. Formerly improved or ploughed fields have undergone re-seeding and top-soil removal (Martin et al. 2021). While attempts at improving species richness of grasslands by changing restoration practices has had varying levels of success (Woodcock et al. 2008, Pallett et al. 2016), there is evidence that the more valuable examples of these systems require long periods of consistent recovery time to achieve prior levels of taxonomic complexity and conservation value (Horrocks et al. 2016, Woodcock et al. 2012).

1.4 Current approaches to conservation in UK grasslands

In the middle of the last century, the UK government recognised the need to prevent further loss of semi-natural grassland extent by the creation of Sites of Special Scientific Interest (SSSIs) (Spash and Simpson 1992). This process started in 1949, and today around 100,000 hectares are protected under this legislation (Christie and Rayment, 2012). More recently, the establishment of biodiversity action plan priority habitats, under the European Union Habitats Directive, has furthered legal protection. Protected areas are an essential component of supporting biodiversity into the future and helping the UK to achieve the 20 Aichi Biodiversity Targets laid out by the Convention on Biological Diversity (Starnes et al. 2021). The cost-effectiveness to the UK government in investing in SSSIs has been demonstrated within a payment for ecosystems framework (Christie and Rayment 2012). However, the lack of commercial value within an agricultural context of these systems compared to improved grassland is at the root of their neglect (Sienkiewicz-Paderewska et al. 2021). Semi-natural grasslands owned by conservation agencies or Non-Government Organisations have shown to be less at risk of conversion (Ridding et al. 2015), although there is no guarantee that positive results are always achieved by these organisations. Environmental stewardship payments as a type of farm subsidy were established as a mechanism to encourage better practices, however, there is conflicting evidence that these schemes offer good value for money for the taxpayer (Batáry et al. 2015, Wheeler and Wilson 2016). One important issue is that payments are action orientated and not results based. This has led to outcomes being reported in terms of 'actions' taken (i.e., the number of farmers who have taken up schemes), rather than the resulting effects on biodiversity (Burton and Schwartz 2013).

1.5 Habitat quality metrics and their use in species-rich grasslands

Lack of long-term and continuous time-series data on the condition of European protected systems is an ongoing challenge for policy makers and environmental groups that wish to encourage and implement conservation actions (Henle et al. 2013). Estimation of habitat quality within natural systems can take several approaches. One direction is to use a measure of taxonomic richness, often linked with a measure of species abundance (Gotelli and Colwell 2011). From these observations, various types of 'species diversity' metrics have been developed (Maurer and McGill 2011), which form the backbone of much biodiversity research. They are used to characterise biomes (Mannion et al. 2014), to inform conservation planning (Fleishman et al. 2006), and to determine species distributions at the landscape scale (Jones 2011).

However, there are inherent issues with using these metrics alone as a proxy for habitat or ecosystem quality (Lamb et al. 2009). Their reliability as an indicator of biodiversity, is affected by species or group level detection rate (Moore et al. 2011), the size of the propagule pool (Dickson and Foster 2008) and habitat fragmentation (Matthews et al. 2014), and their values can be elevated in disturbed systems of lower conservation value (Boch et al. 2013). Some alternative conceptual approaches have been proposed, such as 'optimal biodiversity' (Bukvareva 2018), which evaluates richness for a specific system, depending on the environmental stability and the amount of available resource. Although these metrics have been criticised by some in favour of measures such as endemism, intra-specific genetic diversity and presence of species that fulfil ecosystem functions (keystone species), in the realistic, sub-optimal world of conservation planning, they may still provide us with some of the best guidance on conservation planning priorities (Meir et al. 2004).

Regular acquisition of species diversity data is time-prohibitive in any biome, even where detection rate for a group is good, such as in the higher plants. Some have proposed that focusing only on these easier to detect groups (such as plants) is a good enough proxy for over-all species diversity (Williams and Gaston 1994). An extension of this idea is using certain plant species as habitat quality indicators. These indicator species must be carefully chosen to ensure they are appropriate for the system, and species lists have been developed for UK semi-natural grasslands (Wagner et al. 2019).

Although plant species lists may be correlated to positive grassland condition in some systems there are also alternative approaches involving the collection of structural vegetation traits. The approach is used globally for routine ecological assessment and is the principal medium through which governments record, regulate and fund land management for biodiversity. Vegetation structure and composition may be one of the best options for assessing the successful restoration of habitat condition and is recommended under the 'build it and they will come' philosophy, asserting that well-structured habitats are the basis of conservation practice and restoration (Palmer et al. 1997). For example, where biomass accumulation is high, grassland management may have been abandoned, a sign that vegetation may be shifting away from a state of high plant biodiversity to one of lower diversity dominated by grasses. Increasing cover of woody species within the sward, also indicates insufficient grazing or mowing pressures required to maintain the herbaceous state of the vegetation. Mitchley and Xofis (2005) predicted grassland condition, defined as the number of indicator species present in a land parcel, by use of management information and landscape spatial characteristics. They found that grazing management was one of the biggest drivers of condition.

However, establishment of appropriate baselines, in biodiverse man-made or degraded natural systems is challenging (Fuller et al. 2017). The scope of these approaches, in conserving or reverting land to 'idealised' conditions, perhaps brought about by financial subsidies is also problematic and probably unsustainable in the long term (Fischer et al. 2012). This is particularly true when we consider biodiversity conservation in the light of more ambitious targets such as those found in the 're-wilding' debate (Nogués-Bravo et al. 2016).

Some have proposed combining taxonomic and biophysical habitat variables to indicate habitat value and suitability (Dumont et al. 2022). This is the main approach used for assessment of UK sites with national or international recognition. These habitats are expected to be monitored under what is more broadly known as 'common standards monitoring' (Williams 2006). Under the European Union's Habitat Directive, each member state is obliged to report on the conservation of habitats and species under Annexes 1 and 2 of the directive every 6 years. This monitoring is carried out using 'rapid assessments' (Robertson and Jefferson 2000), which result in a categorical criterium from favourable to destroyed depending on the state of certain components of the habitat and require knowledge of a reference community, which is described under the UK Biodiversity Action Plan (JNCC, 2011). These schemes are supported by more detailed data, such as detection and abundance estimates of target species (DEFRA 2020).

The reporting of common standards has been less than optimal since its introduction, mainly due to a lack of investment in staff and infrastructure (House of Commons Audit Committee, 2021). The delivery and inclusivity of common standard's monitoring is currently under review, because of the lack of success of the historical system and the need to incorporate more technologically advanced approaches. There has been a recognition that these processes will require landscape scale observations over time, which are not currently being achieved via traditional field methods.

1.6 An introduction to optical remote sensing

One of the ways that we can monitor the earth's surface and relieve the financial and time burden of ground surveys is to utilise 'remotely sensed' data. Remote sensing can be defined as: 'the art, science, and technology of obtaining reliable information about physical objects and the environment, through the process of recording, measuring and interpreting energy patterns derived from non-contact sensor systems' (Jenson 2007).

Optical remote sensing (also known as passive remote sensing) is where the reflectance of light from surfaces is used to determine their type or quality. Like our eyes, optical remote sensing instruments are able to detect signals from the visible part of the electromagnetic spectrum, but are often designed to also capture reflectance within the Near and Short Wave Infra-red. The shortest wavelengths that are practical to detect are the ultraviolet or UV. Most UV light is absorbed by the earth's ozone layer and is therefore not present at the earth's surface. UV-A and B light (315-400nm) does travel to the earth's surface and vegetation interacts with light of these wavelengths. However, this part of the spectrum is not often used for studies of vegetation, (although there are ambitions to use the UV for plant phenotyping and disease detection, see Brugger et al. 2019 as an example). Currently, the most commonly used parts of the spectrum are, the visible (covering approximately 400nm-780nm), the Near-Infra-Red (NIR: 780-1100nm, and the Short-Wave-Infra-Red, (SWIR: 1100-2500nm).

Figure 1.1 is a representation of the reflectance of light from a leaf as a percentage of incoming radiation from the sun, at wavelengths from the visible to SWIR parts of the spectrum. In order to calculate reflectance values we require both a measure of incoming radiation, which is often described as down-welling radiation and a measure of outgoing radiation, also referred to as up-welling radiation. The ratio of these two values (upwelling as a proportion of downwelling) is termed percentage or absolute reflectance. When using satellite data products, most of the time these calculations are made for us by the data provider, but when capturing data using other types of instrumentation (aerial or handheld), these calculations must be made by whomever collects the data.

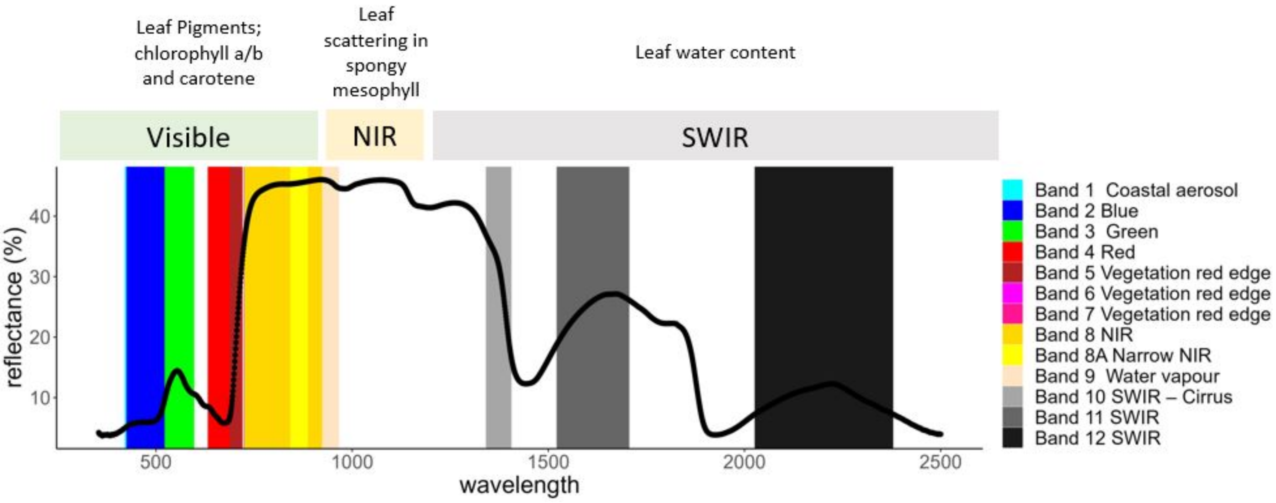

Figure 1.1 An example of the hyperspectral reflectance of a leaf surface (black line). Coloured regions show the spectral bands captured by the multi-spectral imager on board the Sentinel-2 satellite mission. The main spectral regions, visible, NIR and SWIR, are labelled, with the corresponding leaf level traits that make up the principal drivers of variance in reflection within these regions.

When data is collected throughout the visible, NIR and SWIR parts of the spectrum in very narrow contiguous band, we call this ‘hyperspectral’ reflectance. Data of this type is represented by the black line in Figure 1.1. This method is also widely known as ‘imaging spectroscopy’ or ’spectral imaging’. When only discrete parts of the spectrum are sampled we call this ‘multi-spectral’ reflectance. These bands can be narrow, covering only a few wavelengths, or broad, covering larger parts of the spectrum. Also shown in Figure 1.1 are examples of multi-spectral bands. The coloured bands are representative of the 12 channels from the multi-spectral sensor mounted on the Sentinel-2 satellites. Also labelled on Figure 1.1 is the main spectral regions and the biochemical and biophysical drivers of the variability in reflectance within these different spectral regions (see section 1.11 for more details). In general terms, the visible part of the spectrum is associated with pigments, the NIR with leaf structure and the SWIR with water content.

The field of optical remote sensing is especially useful for detection or evaluation of vegetation as plant material reacts with radiation from the sun in a very particular way. The chemical compound in leaves, chlorophyll, strongly absorbs incoming radiation in the blue and red wavelengths but reflects green wavelengths. This is why leaves appear greenest to the human eye in the summer when chlorophyll content is at its highest. In autumn or during drought, there is less absorption and more reflectance of red wavelengths, making leaves appear red/orange/yellow. The internal structure of healthy leaves are excellent diffuse reflectors of NIR wavelengths. This strong light absorption in the visible and reflection in the NIR characterises the spectral ‘signature’ of vegetation and represents our ability to use remote sensing for determination of photosynthetically active material over other surfaces (see Figure 1.2 for an example of how the hyperspectral signature of non-photosynthetically active plant parts differs from other parts of plants).

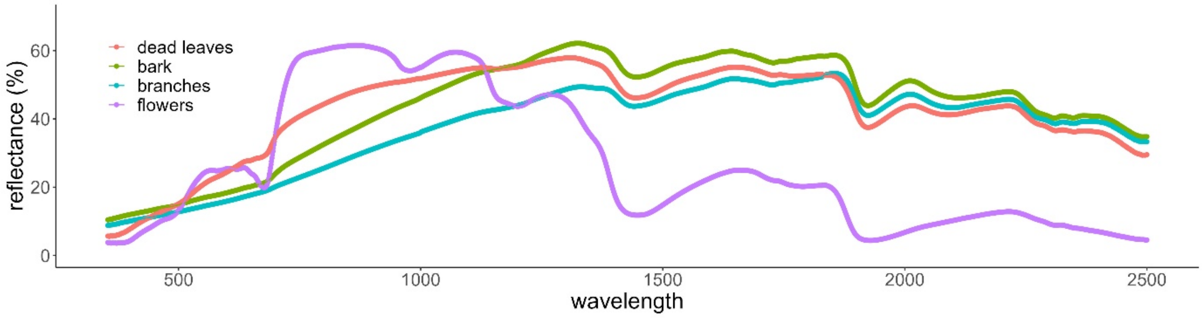

Figure 1.2 Examples of the hyperspectral reflectance of non-photosynthetically active plant material. Spectra sourced from the ECOSpeclib NASA library https://speclib.jpl.nasa.gov/ (Meerdink et al. 2019)

Certain functional groups of plants can also be distinguished using their leaf optical properties. For example, surface reflectance, the thickness of the spongy mesophyll and water content differences enables distinctions to be made between the leaves of succulent plants, broad leaf trees and conifer species (see Figure 1.3 for examples of these spectra).

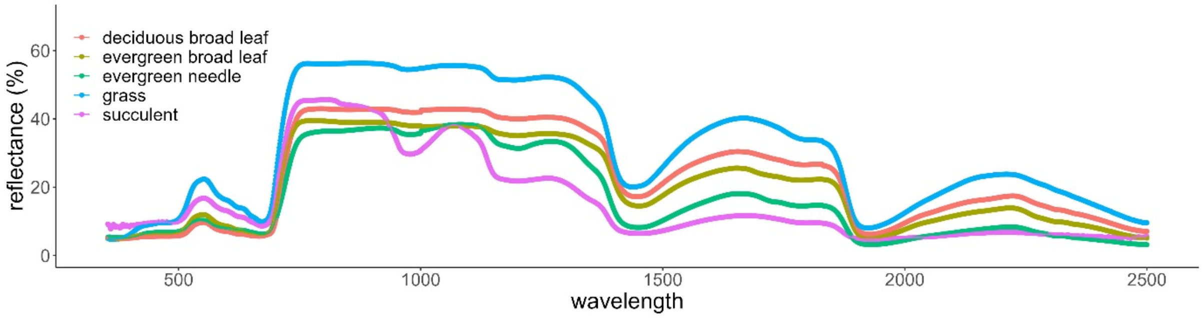

Figure 1.3 Different functional groups of vegetation have differing optical properties which can be observed via their leaf-level hyperspectral reflectance. Spectra sourced from the ECOSpeclib NASA library.

Using reflectance data we can make observations about vegetation properties at both the leaf and the canopy scale depending on the chosen instrumentation and the scale of the observations. Optical data displays differences in quality in several different ways, broadly referred to as the spatial, temporal, or spectral resolution of the data (see sections 1.8 and 1.9 for more detail) but a graphical overview is available in Lechner et al. 2020. Optical data is collected at coarser spatial resolution by satellite borne sensors. The larger of these satellite missions require huge amounts of investment (for example the latest Landsat mission cost around 150 million US dollars). The outputs from such missions are generally high quality, with near-global to global coverage and frequent re-visit times. They are also open source (Belward and Skoien 2015, Radočaj et al. 2020). Higher spatial resolution satellites are also available, but they are not free of charge and mostly do not have global coverage (Singh et al. 2020). The highest spatial resolution data is collected by small aircraft, also known as unmanned aerial vehicles or drones (Aasen et al. 2018). Research data in the field of remote sensing is often collected at high resolution, in order to design data products that can be collected in the future at lower resolutions during automated satellite missions.

1.7 Overview of remote sensing of grassland habitats and biodiversity

Our increasing need to quantity and monitor ecological processes and responses to change, has fuelled an interest in monitoring of biodiversity using remote sensing. The research field has undergone an explosion over the last few decades. In one review of the subject, Wang and Gamon (2019) searched for articles containing the terms ‘Remote Sensing’ and ‘Biodiversity’ between 1990 and 2018. They found that before 2000, only around 65 papers had been published containing these search terms, but from 2000 to 2018, the number per year had risen steadily, resulting in a cumulative total of around 1500 research outputs. Satellite remote sensing is so attractive to ecologists, governments, and land-managers, as it offers the opportunity to expand observations across time and space.

One of the prime applications of satellite remote sensing data is in land-cover classification (Latifovic et al. 2004), and this approach has been extended to that of habitat mapping (Henrys and Jarvis 2019). Most historical work has occurred in forested systems where individual plants are larger in size and individual plant canopies can be more reliably mapped. Grassland systems have principally been evaluated in terms of fodder quantity and quality for commercial agriculture (Punalekar et al. 2018, Punalekar et al. 2021) and the use intensity of fields (Griffiths et al. 2020). Although there is now a growing interest in how we can expand vegetation assessment to herbaceous species, for example, in pursuit of fine scale habitat discrimination (Tarantino et al. 2021).

Biodiversity gradients have also been estimated in grasslands using RS methods. An early study by Levin et al. (2007) showed that reflectance from coarse scale satellite data could be used to map the diversity of mountain habitats verified using ground-truthed botanical data from large plots. Despite the promise of RS methods, the use of traditional species diversity measures in the ground truthing element of remote sensing studies, is problematic. Species incidence (richness), which includes all species equally despite their spatial dominance, is likely to be poorly predicted by remote sensing technologies. Dominance centred indices, such as the Shannon index or the reciprocal of the Simpson's Index, instead of richness centred indices, may be more useful (Lopes et al. 2017b, Wang et al. 2018a, Oldeland et al. 2010).

1.8 Policy perspective on the use of remote sensing for biodiversity and habitat monitoring

The European Union, alongside other countries, has agreed to develop a global biodiversity monitoring network as part of the Group on Earth Observations Biodiversity Observation Network (GEO BON) (Walters and Scholes 2016). This network seeks to establish priorities of biodiversity monitoring based on topical priorities (which species and habitats have higher importance than others) and spatial priorities (the locations in which these species / habitats should be monitored) (Henle et al. 2013). A list of essential biodiversity variables (EBV) with remote sensing technologies in mind has now been established by the group (Skidmore et al. 2021). The recommendations highlighted that only a small subset of EBVs, such as primary productivity or phenological cycles, were likely to be detectable via Earth observation, and the most likely classes of variables were those related to ecosystem structure or function (i.e., landscape characteristics that can be observed at grosser spatial scales). They did not consider the possibilities of remote sensing technologies associated with airborne, unmanned aerial vehicle or terrestrial sensors, where data could be captured at very high spatial resolution.

Similar considerations have also been made within a UK context. In 2009 the UK Joint Nature Conservation Committee (JNCC) published an approach to tackling the challenge of biodiversity monitoring called 'The UK Terrestrial Biodiversity Surveillance Strategy' (JNCC, 2008). The main gaps in monitoring identified by the report included the following:

- Proven methods for repeated measurement of habitats at the landscape scale (e.g., area, patch-size, pattern, conversion rates).
- Repeated representative surveillance within each habitat type and widespread validated rapid assessment methods for condition.
- More sensitive (in time) surveillance of a more balanced set of species.

The principal message is that there is a need for biodiversity observations that are repeatable and reliable over time, both at the landscape scale, (to monitor changes in extent and condition of known habitat types), and more detailed observations at the field level. The report emphasised the role that remote sensing could play in these objectives.

Following on from this, the project 'Making Earth Observation Work for UK Biodiversity Conservation' (Medcalf et al. 2014a, 2014b), produced the 'Crick' framework to identify how well Earth observation could identify priority habitats within a UK context. Later, recommendations were made to develop a system specific suite of remote sensing variables for monitoring habitat condition (Gerard et al. 2015), and for estimating the likelihood of being able to monitor systems using different remote sensing instruments and approaches. The findings of these reports showed that semi-natural grasslands will be some of the most challenging systems to monitor, due to small size of individual plants and the spectral similarity of different types of grasslands at larger pixel sizes.

1.9 Approaches to remote sensing at different scales

Acquisition of remotely sensed variables involves sensor-based trade-offs associated with spatial resolution, spectral resolution, spectral range, and temporal resolution. Spatial resolution refers to the 'ground sample distance' of the instrument and is largely determined by the distance of a sensor from the surface of observation. Spectral resolution refers to the detail at which a sensor can resolve the electromagnetic spectrum. For example, in hyperspectral sensors, data is collected at many narrow contiguous bands, whereas in multi-spectral applications the bands are spread apart and can vary in the number of wavelengths they cover. Spectral range refers to the part of the electromagnetic spectrum a sensor can measure. Temporal resolution refers to re-visit time (i.e., the time elapsed between repeat observations) and for space borne sensors this mainly depends upon the satellites orbit. Sun-synchronous orbits give us the most frequent global revisit times, while geo-stationary orbits continuously observe regional areas. As signal strength reduces with the narrowing of spectral bandwidth, spectral and spatial resolution are often inversely related, due to sensor technological limitations.

Until recently, high spectral resolution alongside high spatial resolution data were achievable principally through airborne missions. Limiting factors were the cost and therefore the difficulty, in creating time-series data sets through repeat sampling. The advance in Unmanned Aerial Vehicle (UAV) technology over the last decade has seen their increased use for vegetation mapping, especially at the detailed site level, and offers exciting opportunities for research and monitoring (Anderson and Gaston 2013). The technique has the potential to provide plant level information and indicators of grassland condition, such as species diversity and community stability, and to produce data sets on species specific phenology, abundance, and distribution. Drone-based sampling campaigns are currently still limited by pixel size for very small plants (Hu et al. 2019), hyper- or multi- spectral sensor affordability (Reynolds et al. 2019), spatial accuracy (Hugenholtz et al. 2016), image correction (orthorectification) (Habib et al. 2016), plus the ability of image stitching algorithms to produce spatially accurate maps of complex canopies (Ma et al. 2021). However, sensor capabilities are advancing at pace, becoming less expensive and smaller. The technique has the potential to provide plant level information and indicators of grassland condition, such as species diversity and community stability, and to produce data sets on species specific phenology, abundance, and distribution. However, the drone-based approach still requires more effort and expense, as data collection, pre and post -processing and distribution is not yet fully automated and standardised like satellite data through multi-billion-dollar space programmes. Therefore, this approach may, at the moment, only be useful for research programmes or occasional sampling, rather than ‘real-time’ field to landscape scale observations. The scalability of the drone-based approach will depend on how the drone technologies and drone data pre-processing capacities are developed and deployed in the future.

In 2015, the European Space Agency (ESA), launched Sentinel-2, a multi-spectral sensor with a ground sample size of 10m^2 within the visible and Near Infra-Red parts of the spectrum, and a ground sample size of $20m^2$ in the Short Wave Infra-Red. This mission offers unprecedented coverage and medium pixel size for monitoring of grassland parcels at the field and landscape scale. Habitat based measures are appealing as they can more readily be assessed using satellite imagery, which is convenient and at times free to use (for example Landsat and ESA Sentinel missions). However, both spectral and spatial resolution is compromised in space borne sensors. The pixel size is in almost all situations larger than the individual plants in question (the exception being large tree canopies). The pixel is also often 'mixed' and the reflectance signal challenging to interpret or classify. However, perhaps counter-intuitively, a similar problem is encountered with very high-resolution images with small pixel sizes. In this case several pixels can represent a single plant, leading to high intra-class variability in the spectral signature, influenced by for example illuminated and shaded and/or green and brown plant parts. This observation is probably most relevant to data gathered using aerially mounted instruments but could also apply to remote sensing of medium to large tree canopies using more recent satellite sensors (for example Sentinel at $10m^2$ resolution) (Rocchini et al. 2007).

Managed, temperate semi-natural grasslands have been demonstrated to contain global plant species maxima at small spatial resolutions (up to $1m^2$) (Wilson et al. 2012). However, some other types of semi-grasslands in the UK may contain only a few species within the same area. The extent to which species are patch forming and the compositional complexity of the sward at smaller scales will largely determine the sensor choice.

1.10 Multi-temporal sampling and phenology

Temperate grasslands are seasonally dynamic systems, with phenological responses driven by temperate and precipitation (Shen et al. 2011), climate (Jentsch et al. 2009), land-use (Nguyen et al. 2020) and species composition (Wilsey et al. 2018). The study of vegetation phenology via satellite image time-series is called 'Land surface phenology' (Matongera et al. 2021). Satellite reflectance data can provide global and regional scale measures of vegetation development associated with chlorophyll build up after the dormant cold season in temperate climates. The most frequently used spectral metric for characterizing these processes is the Normalised Difference Vegetation Index (NDVI), (Pettorelli 2013a) a normalized ratio of red to infra-red bands, given by the equation:

$$NDVI = (NIR - Red)/(NIR + Red)$$

In temperate climates, as vegetation ‘green-up’ occurs in the spring the amount of photosynthetically active material in the plants start to develop, thus increasing absorption in the red and blue regions of the electromagnetic spectrum and decreasing absorption in the green and NIR. A peak NDVI value is eventually obtained later in the season, followed by a gradual drop due to plant flowering, seed setting and the senescence of leafy material. Alternative spectral measures of vegetation seasonal development also exist (Zeng et al. 2020). For example, the Green-Red Vegetation index, has been designed for use with sensors limited to capturing the visible part of the electromagnetic spectrum (Motohka et al. 2010). This monitoring of phenological phases, known as phenophases, has many potential uses, including the discrimination of vegetation community type (Ivanova et al. 2019); species identification (Isaacson et al. 2012, Donnelly et al. 2017); monitoring of management or disturbance events (Reinermann et al. 2022) and for assessing the impact of climate change (Badeck et al. 2004, Melaas et al. 2013).

As already discussed, (section 1.9), one of the major drawbacks in using satellite data to describe vegetation dynamics is that the pixel resolution is often coarse (Landsat thematic mapper imagery at 30m^2 is up to 100 times that of a tree canopy and 10,000 times that of the average grassland plant size). By inference, it is the phenological properties of whole plant communities that is being captured not those of individual plants or species (Helman 2018). The major advantage of using satellite data, however, is the ability to exploit both inter and intra annual time-series of optical proxies, on local, regional, and global scales, which allows medium to large scale evaluation of vegetation status and change (Petorelli 2013).

There is also the possibility to use handheld or aerially mounted sensors appropriate for observing species-specific phenological properties over time, which can be compared to the larger scale satellite data (Rossi et al. 2019). Comparison of coarse green-up from multi-spectral satellite images with in-situ plant-based recordings of leaf unfolding and first flowering have shown disparities between the two approaches. Fu et al. 2014, observed that from 1982-2000, both approaches observed an advance in spring phenology at an average rate of -0.45 days yr^{-1}, while for the period 2000 - 2011, the in-situ methods showed the trend slowing but still advancing, whereas the satellite data showed a reversal of the spring advancement trend. This disparity was accounted for by the effect of winter cooling on spring emerging species during this latter period. As satellite captured data is not able to differentiate between species, the signal of green up following the dormant period only reflects the activity of the earliest species but not the rest of the plant community, which may not be affected in the same way by temperature changes.

We can infer therefore, that a subset of species will have the greatest impact on the reflectance data. In most environments this is likely to be tree and shrub species due to their size and spatial coverage. In grasslands the family Poaceae (the grasses) is usually the dominant group in terms of cover. For example, in the prairies of North America, authors studied the migration of 'cheat-grass' (*Bromus tectorum*) an invasive grass from Europe, over five years in the state of Utah by use of NDVI time series and precipitation data (Clinton et al. 2010). The species causes ecological problems as it increases the frequency of the fire regime and replaces native diversity. Due to the life cycle of cheat grass, it can be detected using NDVI time series. The species responds with a very rapid growth period to rainfall and is evolved to take advantage of water availability quickly, thus outcompeting native grasses that do not have this ability. The authors found that the native ecosystem had a very different response to rainfall to that of the invaded system and that this was detectable even using Landsat time-series satellite data with a pixel size of 30m^2.

1.11 A mechanistic understanding of grassland reflectance through physical modelling

Our understanding of the drivers of vegetation reflectance has been represented in physically-based models, called Radiative Transfer Models (RTMS). These models describe the interaction of light with vegetation surfaces and can be used to quantify certain vegetation parameters. Radiative transfer is the physical phenomenon of energy transfer in the form of electromagnetic radiation. In general, the rad problem is understood as 'ill-posed', meaning that it has many possible solutions depending on the relationships between multiple co-varying vegetation properties. For this reason, models require parameterisation with known values for some variables, in order that others can be estimated. For an overview of frequently used RTMs in optical remote sensing see Jacquemoud and Ustin (2019), Verrelst et al. (2015) and Verrelst et al. (2019).

The most established RTM at the leaf level is PROSPECT, which has several incarnations of increasing complexity. PROSPECT is based on a plate model and describes the way light is reflected from or transmitted through the leaf across the electromagnetic spectrum between 400 and 2500nm, in the visible, Near Infra-Red (NIR) and Short-Wave Infra-Red (SWIR). The original PROSPECT model (Jacquemoud and Baret 1990), was trained on green leaves from a limited selection of plants, including commercial crop species (Maize and wheat) and common trees (Oak and maple species). The model was created to understand the extent to which leaf biochemical and structural properties could be successfully estimated using the reflectance and transmittance signatures. Later the model was extended to incorporate leaf senescent properties, such as the impact of cellulose and lignin using a larger data set of 50 species, including both woody and herbaceous samples (Jacquemoud et al. 1996). More recent models capture more information on non-photosynthetic pigments: PROSPECT 5 included information on carotenoids (Féret et al. 2008). PROSPECT-D (D for dynamic) on anthocyanin (Féret et al. 2017), and PROSPECT-PRO on the decomposition of leaf dry matter as nitrogen-based proteins and carbon-based constituents (Féret et al. 2021). These later model versions therefore enable study of leaves throughout their life cycle and seasonal development. Different versions of PROSPECT have also been combined with the canopy level Radiative Transfer Model SAIL (Scattering by Arbitrarily Inclined Leaves) (Verhoef 1984), to produce the hybrid model PROSAIL (Jacquemoud et al. 2009).

There are two main approaches to using these RTMs. Firstly, they can be employed in 'direct' mode, (Jacquemoud et al. 2000), whereby a set of parameters are used to created likely spectral reflectance or transmittance hyperspectral signatures. In 'inverse' mode, a spectral signature can be offered up, and likely outcomes of parameters produced (Jacquemoud 1993). Because prior knowledge is required of some model parameters in order to predict others, information can be collected as part of field campaigns or estimated apriori. For example, the leaf structural parameter N, a property that captures the internal leaf structure, such as the amount of spongy mesophyll, is commonly estimated as between the values 1 and 2, for live leaves (Jacquemoud and Baret, 1990). There also exist functional trait data bases for species (Kattge et al. 2019) and biomes (Asner et al. 2008), which can be drawn upon to inform the inversion process.

Sensitivity analysis is another way of looking at the impact of variables on spectral reflectance (Lee et al. 2016). There are two main approaches to sensitivity analysis of these models. The most frequently used approach is a 'factor at a time' where all parameters are held constant except for one (Ferretti et al. 2016). Secondly, a global sensitivity analysis (GSA) can be applied (Verrelst et al. 2016), where the percent global contribution to spectral variability per wavelength is calculated. These 'sensitivity analysis' approaches can give researchers interpretable and visual evidence for the physical drivers of reflectance at particular wavelengths within different instrumental conditions (Niu et al. 2021) and the certainty of their interpretations (Mousivand et al. 2014). For a GSA of the RTMs discussed here and their relationship to instruments see Figure 1.4.

Both PROSPECT and SAIL, and their amalgamation, PROSAIL, have been used extensively to help quantify the biochemical and structural properties of leaves and canopies in many field settings (Berger et al. 2018) and are one of the major means of retrieving bio-physical properties of vegetation (Verrelst et al. 2015). They have been used across different systems, for example in forests (Asner and

Martin 2008, Morcillo-Pallarés et al. 2019), wetlands (Feilhauer et al. 2018, Darvishzadeh et al. 2019), croplands (Boren and Boschetti, 2020, Liang et al. 2015) and grasslands (Atzberger et al. 2013, Darvishzadeh et al. 2008). Another application of these models is in development of vegetation indices (Féret et al. 2017, He et al. 2015, le Maire et al. 2008) which serve as univariate proxies for vegetation properties.

There are, however, some limitations to applying these models to field campaigns. PROSPECT is trained on reflectance and transmittance data from a radio spectrometer fitted with an integrating sphere, which measures hemispherical reflectance. However, most optical sensors in RS capture bi-directional reflectance. When estimating leaf traits from bi-directional reflectance, additional sources of variation must be considered and controlled for, such as Light Incident Angle and Illumination Zenith Angle and the specular properties of leaves. Jay et al. (2016) developed the PROCOSINE model, to account for these additional sources of variation, when using close range imaging sensors. These additional models require knowledge of additional parameters which can be problematic to control for in the field.

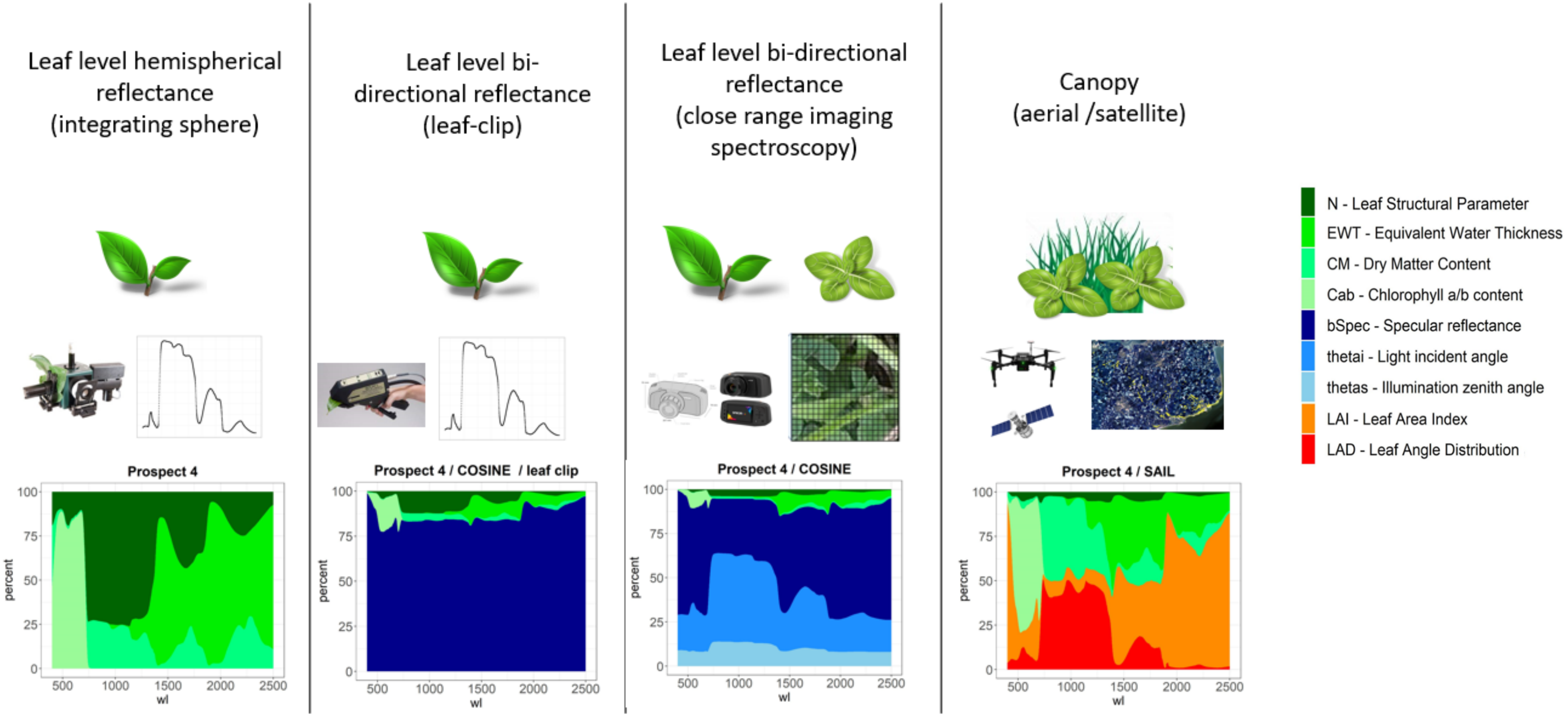

Figure 1.4: The principle leaf and canopy level trait drivers of reflectance from different RTMs and the relationship to sampling scale / instrumentation.

Herbaceous species pose additional challenges compared to woody species, which tend to have larger and more robust leaves, which are easier to measure. Vegetation traits within grassland systems are also extremely changeable in relation to the environment. For example, climate and rainfall (Bart et al. 2017), and between functional types (Ren et al. 2022). There is also evidence that the retrieval of plant functional traits from spectra and RTM inversion is affected by plant phenological stage (Schiefer et al. 2021) and may be more challenging to use within complex canopies (Lu et al. 2021).

Despite these constraints, RTM approaches have been used successfully at varying spatial scales to characterise plant communities and ecosystems (Abelleira Martínez et al. 2016). Globally they have been used to estimate the distribution of leaf (Croft et al. 2020) and canopy (Moreno-Martínez et al. 2018) traits. At the site level they have been used to map canopy traits such as Leaf area index and chlorophyll content (Ali et al. 2020). An extension of this research is to use RS to map broad functional types (Punelaker et al. 2016, Schweiger et al. 2017) and to predict trait variation in space (Ma et al. 2020, Van Cleemput et al. 2021).

The study of plant functional types and how they are related to ecosystem services is an active area of research (Wright et al. 2004). Formulating our understanding of vegetation within a plant functional framework lends itself well to the exploitation of remotely sensed reflectance data, due to the optical traits link. Optically measurable traits, such as Leaf Mass Area (LMA), the ratio of dry mass of a leaf to its surface area, has also been positively correlated with ecosystem functions such as peak biomass, above ground net primary production and soil water content (Rossi et al. 2020). Leaf Area Index (LAI), the one-sided area of leaves above an area on the ground surface, is an important optical trait in the PROSAIL model and is often treated as a proxy for biomass accumulation (Eon et al. 2019).

1.12 Spectral heterogeneity: a proxy for biodiversity?

An emerging and important approach to estimating biodiversity using remote sensing involves exploitation of an empirical relationship between spectral variation and the diversity of species in space, under the Spectral Variation Hypothesis (SVH) (Palmer et al. 2002, Rocchini et al. 2015). If valid, this concept would deliver a quantitative measure of species diversity from RS, which would enable relatively easy monitoring across biogeographical regions. There are two main ways in which researchers have approached this idea, which are largely based on the scale of the data acquisition, and therefore whether reflectance corresponds to leaf or canopy properties.

The first approach exploits our existing knowledge of land-cover mapping through RS (Ban et al. 2015). Forests and grasslands, for example, can be determined by differences in their NDVI profiles, which is likely to be associated with their differences in biomass. The idea that structural diversity in vegetation is a strong predictor of number of species present is based loosely on the species-area curve theory (Scheiner 2003), whereby species richness increases with area covered due to the likelihood of more habitats being incorporated in the area. This approach to the SVH is usually applied on the regional or global scale using data from satellite borne sensors (Rocchini 2007).

Secondly, at the leaf level, the SVH relies on the concept that each taxonomic unit has a 'spectral signature,' a unique set of reflectances that can be used to classify the taxa. Therefore, spectral variation acts as a direct proxy for the number of taxonomic units in space. This approach relies on the assumption that the chemical composition of leaves has a strong taxonomic drive. This has been proven in some systems (Wang et al. 2018a), however, there is also evidence of the convergent optical properties of photosynthetically active material (Ollinger 2011), the impact of taxonomic organisation (Rocchini et al. 2009) and the changing optical properties of leaves due to climate gradients (Asner et al. 2009). Consequently, this approach has been refined. Groups of emergent properties of leaves have been framed as 'optical types' (Ustin and Gamon, 2010) and the chemical study of leaves and their relation to biodiversity and environmental gradients has been termed 'spectranomics' (Asner and Martin, 2016). These lines of research have a wider application than merely mapping presence and absence of taxonomic units, but rather attempt to extend our understanding of ecosystem function and change through the 'trait' lens (Wright et al. 2016). For example, the diversity of functional traits, rather than habitat or species diversity, within a spatial unit has also been linked to spectral variation (Hauser et al. 2021, Rossi et al. 2020, Schweiger et al. 2018). However, there is also evidence that spectral variation can be driven by a subset of optical traits, such as LAI and Leaf inclination (Kattenborn et al. 2019).

There are very few studies that have tested the relationship between spectral variation and species or habitat diversity hypothesis over time. At the landscape scale, Schmidlein and Fassnacht (2017) used spectral variation captured using the MODIS satellite, with a pixel size of 500 x 500m^2. They found that regional richness measures could not be predicted across a German landscape. Inclusion of the time-series data (8-day revisit) offered by MODIS revealed seasonal fluctuations which further confounded the ability to predict species diversity. At the leaf or very high-resolution canopy scale, the SVH has not been tested over time. So, while the impact of vegetation changes (i.e.

phenology) on the spectral variation during a growing season is acknowledged, this additional source of uncertainty in estimations has not been evaluated.

1.13 Key questions being addressed in the thesis

Our ability to conserve the most important of our UK grasslands for biodiversity and the associated ecosystem services they provide is severely restrained by our lack of real time data on their changing taxonomic composition and condition. Under-appreciation of these systems is fuelled by our lack of understanding of their natural status and ecological importance, alongside agricultural systems that do not provide the correct type and intensity of management. Remote sensing holds much potential for enhancing our monitoring and understanding of these systems over time and space but monitoring systems must be carefully designed and tested before being implemented. This thesis is driven by the observation that much experimental work and ground-truthing data on plant communities is collected at one time of year and that the temporal stability of predictions is little explored.

Spectral heterogeneity provides an attractive approach to monitoring grasslands as it enables integration of multiple leaf and canopy properties into a simple empirical relationship. However, the instability of the spectral variation hypothesis over time is a major barrier to exploiting this method with confidence. Here I collect field and spectral data, over a growing season to test whether the timing of data collection affects our ability to predict diversity. I also explore the more fundamental question of whether plants found in grassland systems are optically different enough to enable their classification, and whether these differences are stable, or have a seasonal dimension. Although many analyses associated with classifying vegetation status or change have been carried out using satellite time-series the question of whether intra-habitat status can be detected has not been extensively explored. I use sentinel-2 optical time-series with repeat field condition assessments to assess the temporal stability of condition detection over two years.

1.14 Thesis structure

The thesis is presented as a series of papers, starting with a meta-analytical review paper, followed by two data papers that look at site level grassland biodiversity and taxonomic inventories, and a third data paper that examines semi-natural grassland condition at the landscape scale. The final chapter explores the implications of the thesis findings, outstanding challenges, and future projections for the field.

Chapter 2 is a meta-analytical review paper looking at existing research on the spectral variation hypothesis in relation to grassland diversity assessment. It contains a synthesis of results and examines possible moderating variables, which may explain the varying success of the theory. This chapter was published in the journal 'Remote Sensing' in January 2023.

Chapter 3 is a paper based on high resolution hyperspectral reflectance data collected over a growing season for two semi-natural grasslands. It tests the spectral variation hypothesis over time at the quadrat scale. It also examines the impact of phenological diversity on spectral variation and the interactions between phenological and taxonomic drivers of spectral variation. Chapter 3 was published in the journal 'Remote Sensing of Environment' in February 2022.

Chapter 4 examines the stability of leaf level hyperspectral reflectance over a growing season for 17 species characteristic of UK calcareous grasslands. It tests the ability of data driven classification models to select wavelengths of importance over the growing season, and to assess the advantages of the multi-temporal, as opposed to the uni-temporal, data approach to species discrimination. Chapter 4 was published in the journal 'Remote Sensing' in May 2022.

Chapter 5 tests the usefulness of the Sentinel-2 satellite mounted multi-spectral sensor for monitoring calcareous grassland condition at the landscape scale. Using Generalized Additive Models (GAMs), I examine intra-annual time-series of spectral indices, to differentiate between grasslands of varying condition status. This paper has been prepared in the style of the journal 'Ecological Indicators'.

Chapter 6 examines the collective implications of the results from the four research papers and possible future research directions in the field.

Chapter 2

Prediction of grassland biodiversity using measures of spectral variance: a meta-analytical review

This chapter comprises a review and meta-analysis of studies that have examined the use of spectral variation as a surrogate for plant or habitat diversity in grasslands. It uses three-level meta-analytical models to assess the strength of the relationship across studies and explores possible reasons why the relationship varies. The range of studies employ large pixel size spectral data from satellites down to very small detail imaging instruments, that capture leaf level reflectance. Some studies also explore the relationship over time.

This chapter has been published in the journal 'Remote Sensing' by MDPI, with the following reference:

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Author contribution statement

Rachael Thornley: conceptualisation, literature review, data collation, data analysis and writing. France Gerard: conceptualisation, review, and editing. Kevin White: conceptualisation. Anne Verhoef: conceptualisation, review, and editing.

Abstract

Over the last 20 years, there has been a surge of interest in the use of reflectance data collected using satellites and aerial vehicles to monitor vegetation diversity. One methodological option to monitor these systems involves developing empirical relationships between spectral heterogeneity in space (spectral variation) and plant or habitat diversity. This approach is commonly termed the 'Spectral Variation Hypothesis'. Although increasingly used, it is controversial and can be unreliable in some contexts. Here, we review the literature and apply three-level meta-analytical models to assess the test results of the hypothesis across studies using several moderating variables relating to the botanical and spectral sampling strategies and the types of sites evaluated. We focus on the literature relating to grasslands, which are less well studied compared to forests and are likely to require separate treatments due to their dynamic phenology and the taxonomic complexity of their canopies on a small scale. Across studies, the results suggest an overall positive relationship between spectral variation and species diversity (mean correlation coefficient = 0.36). However, high levels of both within-study and between-study heterogeneity were found. Whether data was collected at the leaf or canopy level had the most impact on the mean effect size, with leaf-level studies displaying a stronger relationship compared to canopy-level studies. We highlight the challenges facing the synthesis of these kinds of experiments, the lack of studies carried out in arid or tropical systems and the need for scalable, multitemporal assessments to resolve the controversy in this field.

2.1. Introduction

Grasslands are ecologically important systems, as they cover around 30–40% of the global terrestrial land mass (Gibson 2008), contain high levels of biodiversity (Veldman et al. 2015) and provide multiple ecosystem services (Bengtsson et al. 2019). However, much of our global grassland resource is undergoing, or is at risk of, degradation (Bardgett et al. 2021) due to changes in management intensity (Nakahama et al. 2016, Pipponen et al. 2022), climate (De Boeck et al. 2007, Ma et al. 2010) and eutrophication (Borer et al. 2014). To prevent further decline and ensure successful restoration, government agencies and research bodies require reliable, quantitative data on the changing status of the plant biodiversity within these systems, and remote sensing could be part of the solution (Nagendra 2001, Turner et al. 2003).

Although most remote sensing studies aimed at vegetation monitoring are focused on forests of late, grasslands have also received more attention (Ali et al. 2016, Reinermann et al. 2020, Wang et al. 2020). Herbaceous plants, which dominate grasslands, are often magnitudes smaller than their counterparts in woody vegetation, and this has been a major obstacle to applying remote sensing at the plant or leaf level. Some grasslands are dominated by a few species that can be mapped using satellite-mounted sensors (Irisarri et al. 2009,Muthoka et al. 2021); however, natural, or semi-natural grasslands are often characterized by a high community complexity within small areas (Wilson et al. 2021). In addition, grasslands are particularly dynamic over time due to variations in water availability (Zelikova et al. 2015) and other environmental factors. Despite these challenges, recent technological developments have made applications involving grasslands more feasible. There are now satellite missions providing small pixel sizes (10 m Sentinel-2), and high temporal resolutions (daily 250 m MODIS or every 5 days for Sentinel-2) (Cavender-Bares et al. 2017) and fast-developing sensors on Unmanned Aerial Vehicles are enabling observations at very high spatial and spectral resolutions (Gillan et al. 2020, Librán-Embid et al. 2020). Some researchers have also employed proximal field instrumentation such as tram-mounted sensors (Wang et al. 2018) to obtain extremely detailed spectral information.

One attractive approach to monitoring grassland diversity, due to its simple concept, is to utilize the 'Spectral Variation Hypothesis' (Palmer et al. 2002), which assumes that the spectral variation in space is correlated with the plant or habitat diversity. Plant diversity mapping using this method is based on the premise that individual species or plant communities have a distinct spectral reflectance signature, a product of optically detectable leaf and/or canopy traits (Asner and Martin 2016). At very small spatial scales, leaf-level optical properties drive the variance in reflectance, whereas, at larger scales, the canopy properties will be the main drivers. These relationships are well understood for single-species scenarios (Jacuemoud and Baret 1990, Jacquemoud et al. 2009) but are likely to be more complex in taxonomically diverse communities.

Although the Spectral Variation Hypothesis is widely recommended and examined, the theory is not without critics (Fassnacht et al. 2022). It can be unstable in space (see Schmidtlein and Fassnacht 2017, who used the approach across European landscapes) and temporally unstable interannually (Gholizadeh et al. 2020) and over growing seasons (Thornley et al. 2022a). Plant materials at the leaf level are plastic, reacting to the environment in diverse ways (Fritz et al. 2018, Wu et al. 2016). The extent of plasticity in optical traits is thought to be, in part, genetically based, meaning that the taxonomic component of communities is influential (Cavender-Bares et al. 2017) but not necessarily easy to predict across space and time. The approach to bio-diversity monitoring at the community type level could also be problematic when applied to grasslands. For example, at these scales, grassland plants may display convergent canopy-level traits due to weather parameters, such as increases in greenness and bio-mass due to increased precipitation (Cleland et al. 2006). In addition, the spectral variation of grassland fields is strongly influenced by management events such as mowing and grazing (Bastin et al. 2012, Giménez et al. 2017).

The motivations behind applying the Spectral Variation Hypothesis display some cohesion; however, the spatial scale, instrumentation and spectral resolution of the studies vary considerably. These experimental choices could explain some of the inconsistency in the results as follows. Our ability to map taxonomic units using reflectance data is thought to be dependent on small variations that can only be detected using hyperspectral resolution data (Andrew and Ustin 2008, Mansour et al. 2012). The Spectral Variation Hypothesis applied at the leaf level could therefore produce much weaker predictions when multi-spectral data are used. The spectral variation can be influenced by instrumentation. For example, in close range imaging spectroscopy situations, surface leaf reflectance can potentially have a large impact on spectral variance (Jay et al. 2016). The number of taxonomic units being examined may matter, as there is evidence that the spectral variation–species diversity relationship is saturated with more complex communities (Féret and Asner 2011). The timing of sampling campaigns is also critical, as plant traits change seasonally (Noda et al. 2020, Yang et al. 2016) and interannually (Noda et al. 2014), affecting the plant spectral reflectance (Hesketh and Sánchez-Azofeifa 2012). This is likely to have an impact on the temporal stability of the spectral variation–biodiversity relationship (Wang et al. 2022).

There have been several review papers published on the usefulness of remote sensing to assess biodiversity (Bush et al. 2017, Cavender-Bares et al. 2022, Lausch et al. 2016, Mairota et al. 2015, Pettorelli et al. 2014, Wachendorf et al. 2017, Wang and Gamon 2019), and some have specifically looked at the Spectral Variation Hypothesis (Rocchini et al. 2015, Fassnacht et al. 2022). However, these approaches are somewhat subjective and non-standardized. A better alternative is to use a quantitative synthesis, known as a meta-analysis. Here, it is possible to weigh differences between study outcomes using the sampling effort and to investigate the impact of proposed moderating variables (Gurevitch et al. 2018). The method has been previously used in ecology (Koricheva and Gurevitch 2014, Stewart 2009) and in optical remote sensing to evaluate the literature relating to, for example, plant pigment concentrations (Huang et al. 2015), functional traits (Van Cleemput et al. 2018), forestry variables (Chirici et al. 2016), crop variables (Weiss et al. 2019) and land cover classification (Khatami et al. 2016).

Here, we carry out a literature search and meta-analysis of studies that used optical remote sensing to estimate the biodiversity of grasslands under the Spectral Variation Hypothesis, with an emphasis on the effect of the spatial, temporal, and spectral resolutions of the remote sensing data used, alongside other features of the sampling campaigns.

2.2. Materials and Methods

2.2.1. Literature Search and Selection of Studies for Meta-Analysis

In April 2020 and May 2022, we carried out literature searches using Google Scholar and Scopus (Table S1) following the PRISMA (Preferred Reporting Items for Systematic re-views and Meta-analysis) methodology (Moher et al. 2009). We read paper abstracts to ascertain whether studies contained spectral data and dealt with plant biodiversity in grassland systems. We did not include studies that mapped specific taxonomic units or that aimed to differentiate between a small number of target species. Some of the searches produced a very large number of records. In these cases, after sifting through 100 pages of results, (of approximately 10 results per page), the search was abandoned. The initial searches produced 74 papers, with an extra 4 found through reference lists, giving a total of 78 papers. These were then examined in more detail, and duplicates were removed, giving 77 studies. These were included in the final data set if the authors:

(1) Explicitly tested whether plant species richness or diversity was correlated with a measure of spectral variance in space.

(2) Included a Pearson's Correlation Coefficient that resulted from a bivariate model or an r^2 value with an indication of the relationship direction.

(3) Did not deal with environments such as in savannahs or mixed planned country-side. This left 20 studies suitable for our quantitative synthesis. Figure S1 provides details of the selection in the PRISMA graphical format.

2.2.2. Extraction and Description of Likely Moderators

We extracted several moderating variables that are likely to affect the relationship between spectral variance and plant species diversity. These moderators related to (1) the spectral data, (2) the species data and (3) the sampling design.

2.2.2.1. Spectral Moderators

We identified five moderating variables relating to the spectral data. The ground sampling size of the instrument is essential to understand if the Spectral Variation Hypothesis was tested at the leaf level or at the community/habitat level. A continuous variable in meters was created called the 'pixel size'. In addition, a categorical variable called 'leaf–canopy' was generated that classified effect sizes according to whether the pixel size matched 'leaf'- or 'canopy'-scale measurements.

Next, we created a category called 'spectral region' to note the spectral region used. Here, we refer to the visible part of the spectrum as 400–699 nm, the NIR as 700–1299 nm and the SWIR as 1300–2519 nm. Since the variation within each of these spectral regions is broadly driven by differing optical leaf and canopy properties, we can use the results of this analysis to propose biochemical reasons for the link between spectral variation and species diversity. In addition, to understand if a better spectral resolution improves predictions, effect sizes were categorized as to whether they were calculated using hyper-spectral or multi-spectral data under the moderator ‘spectral resolution’.

Measures of spectral variation are calculated in different ways. Some authors select a simple dispersion around the mean reflectance value, such as the range, standard deviation or the coefficient of variation, whereas others take more complex approaches, such as the average spectral angle between species (Kruse et al. 1993), spectral entropy (Rocchini et al. 2017) or species spectral clustering measures (Feret and Asner 2012). To test whether there was an advantage in using these more complex measures, we created a variable called the ‘spectral diversity metric’, where measures were coded as either ‘simple’ or ‘complex’.

2.2.2.2. Species Moderators

We identified three moderating variables related to the species data. Species counts in space, also referred to as richness, is the basic measure in biodiversity assessments, but it does not capture the relative abundance of the taxa. The variable ‘species diversity’ was coded as either ‘richness’ or, where a metric also incorporated evenness or abundance, as ‘diversity’.

Additionally of interest is the number of species considered in the study. In grass-lands, the species richness levels can be very high per m^2. In previous works, it has been suggested that our ability to predict taxonomic units using spectral variance may be saturated as the number of species in a data set rises (Zhao et a. 2021, Thornley et al. 2022b). Therefore, effect sizes may be smaller when looking at communities where species richness is consistently high. To test this idea, the continuous moderator ‘richness level’ was created, using the minimum value of richness within an analysis, as a proxy for the taxonomic complexity of the analysis. We hypothesize that the mean effect will be negatively influenced by higher numbers of species.

The methods of assessing biodiversity are classified according to the scale of organization, known as alpha, beta, or gamma diversity (Whittaker et al. 2001). Alpha is the number of species within a unit area and can also include a measure of their relative abundance. Beta diversity captures community dissimilarities between patches or components of a landscape. Gamma diversity is an additive measure of both alpha and beta diversities and describes diversity at the landscape scale. We created the categorical moderator ‘level of diversity’ to capture these different scales.

2.2.2.3. Sampling Design

We identified four moderating variables related to sampling design. Firstly, we noted that the sampling effort difference between the spectral and the botanical data is often pronounced. For example, satellite sensors collect spectral data over large areas, whereas the accompanying field botanical data have a much sparser coverage and are extrapolated from small plots. In contrast, when aerial or handheld instruments are used, small plots are often sampled exhaustively for both spectral and botanical data. To understand if these differences in the sampling effort impact the effect size, we created the moderator ‘spatial matching’, which is the ratio of the area sampled botanically to the area sampled spectrally.

Secondly, the time of year that sampling occurs is likely to impact the relationship between spectral variance and species diversity. Leaf and canopy phenology drive changes in reflectance over a growing season, and therefore, the relationship between spectral variance and plant diversity is also expected to vary over time. Summer should be the most stable time of the year for sampling leaf spectra. To capture this, we created a variable called ‘sampling season’. We noted the first and the last month that spectral data were collected and categorized these months into seasons as follows: ‘summer’ (June–August) or ‘other’. We recognize the somewhat arbitrary nature of these sampling periods, as seasonality will not be uniform across our sites due to the latitude and continentality of sites.

Thirdly, we used the Köppen climate classification to classify sites into one of five main groups (tropical, arid, temperate, continental, and polar) according to their seasonal temperature patterns (Beck et al. 2018) in order to explore the impact of the ecological region on the reliability of the hypothesis. We called this variable ‘climate’.

Finally, the level of naturalness of systems may affect the extent to which the Spectral Variation Hypothesis works. More natural systems often have higher levels of complexity in terms of their species distribution in space. In experiments, diversity levels are manipulated through, for example, seeding or weeding. To test if this has an impact, the moderator ‘site type’ was coded with two levels: ‘natural’ and ‘experimental’.

2.2.3. Data Analysis

2.2.3.1. Extraction of Effect and Sample Sizes

To carry out a meta-analysis, we needed a standardized effect size for each result across all studies. Suitable effect sizes in studies that dealt with two continuous variables were generally based on Pearson's Correlation Coefficients or associated values of the co-efficient of determination (r^2), where additional information was available about the direction of the relationship. When results were only available as graphic displays, we extracted the estimates using the software 'Plot Digitizer' (Huwaldt 2022). The results based on Kendall's rank were converted to the Pearson's Correlation Coefficient (Walker 2003). We transformed all estimates to Fisher's Z (Hedges and Olkin 1985) to improve the fit to a normal distribution. Next, we weighted them for the meta-analysis using effect-level sample sizes based on the number of sampled botanical areas (e.g., plots or fields) used in the analysis. The sampling variances were calculated using large sample approximations and bias corrected correlation coefficients (Borenstein 2009). Model estimates based on Fisher's Z were converted back into the Pearson's Correlation Coefficient for interpretation purposes.

2.2.3.2. Three-Level Meta-Analytical Models

One of the challenges with synthesizing outcomes of remote sensing studies is that there are often multiple results reported within one study, leading to the challenge of modelling dependence of the effect sizes. Traditionally, this problem is handled by creating a mean effect size for each study (Cheung 2019). However, this discards useful information that can, for example, be used to assess the impact of moderators. A more recent approach has been to use a multi-level extension also known as a three-level model, which enables us to estimate the variance not attributable to sampling errors and to specify both the with-in-cluster and between-cluster variances (van der Noortgate 2013). Firstly, we specified models clustered by 'study', a common approach in meta-analyses. Secondly, we used 'site' as a clustering variable, as high levels of between-study variations could be driven by site specificity. In addition, to test if our likely moderating variables impact mean effect sizes, we evaluated their importance by carrying out a subgroup analysis within a mixed effects model framework. Due to the data set size, we first included these moderators individually, and then, if they were significant, we tested for interactions (Assink and Wibbelink 2016). We used the restricted maximum likelihood estimator (REML) to evaluate the significance of the main effect size for each model. For the moderator models, we estimated different effect sizes for each level of the categorical moderator. If the moderator was a continuous variable, we estimated the overall effect size and tested its significance.

When each study design is identical, all variances between study effect sizes should be attributable to the sampling error (i.e., sampling effort). Outside clinical trials, this is almost never true. Especially in ecological studies, we would expect there to be high levels of variance between study results due to the high levels of variation in natural systems. In meta-analyses, 'heterogeneity' is used to describe variances not attributable to sampling errors. Here, we report the significance level of Cochrane's Q for an overall test of 'heterogeneity' in the models, followed by I^2 (Higgins and Thompson 2002). The I^2 statistic is a relative value that indicates the percentage of total variance that is not attributable to a sampling error. It can be further decomposed into I^2 level-two and I^2 level-three variances, which are, respectively, the be-tween-cluster and within-cluster variances. We tested the significance of the variance decomposition by comparing the three-level model with the equivalent two-level model using a one-sided log-likelihood-ratio test. We also evaluated the changes in the I^2 value as different moderators were added to the basic model.

2.2.3.3. Sensitivity Analysis and Publication Bias

For each three-level model, we carried out a sensitivity analysis. Influential case di-agnostics were produced using a multivariate measure analogous to Cook's distance (Viechtbauer and Cheung 2010), which can be interpreted as the Mahalanobis distance between the entire set of predicted values, with the ith case included and excluded from the model fitting. These diagnostics were carried out at the study cluster level for each model. A robust cut off value for influential data does not exist, but generally, a Cook's distance > 4/n is used, where n is the number of clusters in the model. To test if outlier studies were having a strong effect on the results, outliers were removed and the models recalculated.

Publication bias arises when results from studies are more likely to be published if they fulfil existing expectations. In the case of testing the Spectral Variation Hypothesis, this would result in finding a strong positive correlation between species or habitat diversity and spectral variance and, within the meta-analytical framework, an overestimation of the mean effect size. There are limited methods available for estimating publication bias in data sets that display dependence (Nakagawa et al. 2021). One simple option is to visually inspect funnel plots where residual values from the meta-analysis are plotted against the standard error. Non-symmetrical plots indicate the presence of publication bias.

All data analyses were done using the metafor package (Vienchtbauer 2010) in R (R Core Team 2022).

2.3. Results

2.3.1. Overview of Studies

In terms of study location, there was a strong research bias towards sites in North America and Northwestern Europe. Three studies were carried out in Northern China (Figure 2.1A). There were no studies carried out in the Southern Hemisphere. All grasslands could be classed as temperate, continental, or alpine, with no examples of tropical or arid systems. There was a good mix of leaf- and canopy-level studies, captured using satellites, unmanned aerial vehicles and proximal instruments (Figure 2.1B). We found studies that looked at alpha and beta diversities but only one that investigated gamma diversity (Figure 2.1C). The effect size for gamma diversity was excluded from future analyses due to the small sample size. Three studies collected data at discrete time points and explicitly re-ported results on the temporal stability of the Spectral Variation Hypothesis. Two studies did this across a growing season and one over different years. Some authors treated field data collected across a few months as a single sampling point (Figure 2.1D).

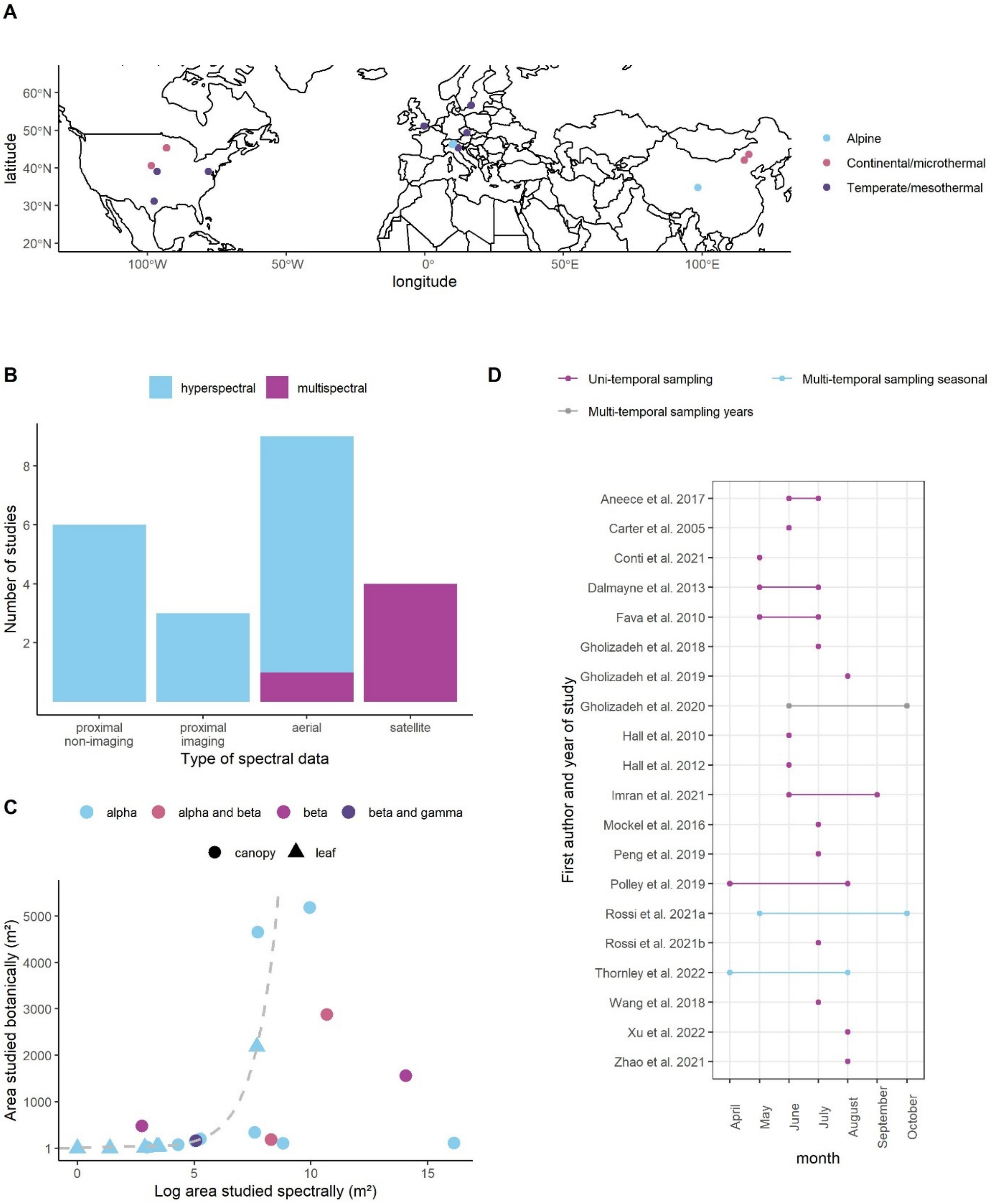


Figure 2.1. Literature search summary results. (A) The studies' geographical locations, alongside their climate zone classifications. (B) The sensor type used and spectral resolution. (C) The area sampled botanically and spectrally and whether the data was collected at the leaf or canopy scale (the grey dashed line represents equal sampling efforts for both variables). (D) The time of year the sampling took place and whether the author examined the data multi- or uni-temporally and if in multiple years.

Most studies focused on a particular aspect of the relationship between spectral variance and biodiversity: six tested different biodiversity metrics using the same data set, four looked at the relationship at spatial different scales (i.e., pixel sizes), three looked at the relationship over time, six calculated the spectral variation in different ways and five repeated the same experiment across different sites or fields. Table 2.1 lists the publications, alongside their thematic focus.

Table 2.1. An overview of the studies and their thematic focus. Sites that are shared across studies are uniquely numbered.

Paper Number	Paper	Botanical Diversity Metrics	Scale Diversity Measured	Temporal Stability	Spectral Diversity Metric	Grassland Types	Shared Experimental Location
1	Aneece et al. 2017	0	0	0	0	1	1
2	Carter et al. 2005	0	0	0	0	0	2
3	Conti et al. 2021	0	0	0	0	0	3
4	Dalmayne et al. 2013	0	0	0	0	0	4
5	Fava et al. 2010	0	0	0	0	0	5
6	Gholizadeh et al. 2018	0	1	0	1	1	6
7	Gholizadeh et al. 2019	1	1	0	0	1	7
8	Gholizadeh et al. 2020	0	0	1	1	0	7
9	Hall et al. 2010	0	0	0	0	0	4
10	Hall et al. 2012	1	0	0	0	0	4
11	Imran et al. 2021	1	1	0	0	1	8
12	Möckel et al. 2016	0	0	0	0	0	4
13	Peng et al. 2019	0	0	0	1	0	9
14	Polley et al. 2019	0	0	0	1	0	10
15	Rossi et al. 2021a	0	0	1	0	0	11
16	Rossi et al. 2021b	0	0	0	1	0	12
17	Thornley et al. 2022a	1	0	1	0	1	13
18	Wang et al. 2018	1	1	0	0	0	6
19	Xu et al. 2022	1	0	0	1	0	14
20	Zhao et al. 2021	0	0	0	0	0	15

2.3.2. Results of the Multi-Level Models

For the meta-analysis, we extracted 297 effect sizes from 20 studies over 15 experimental locations. A forest plot shows these effect sizes with their sampling variance by study category (Figure 2.2). The mean effect size (Pearson's Correlation Coefficient) calculated for the basic three-level meta-analysis models (no moderators) with study or site as the clustering variable, respectively, was 0.358 or 0.32 (confidence interval ±0.161 or 0.197), suggesting that, overall, there is a positive relationship between spectral variance and plant species diversity. We tested for the significance of the variance components by comparing the three-level model with the equivalent two-level model. Both three-level models, with level three heterogeneity constrained to zero, were a better fit for the data than their equivalent two-level models at $p = 6.897 \times 10^{-22}$ (study) and $p = 2.076 \times 10^{-20}$ (site) when using a likelihood-ratio test. Using the three-level approach, heterogeneity was de-composed into sampling between-cluster (level 2) and within-cluster (level 3) variances, each level being expressed as a percentage of the total model variance. The measure of heterogeneity (I^2) across all models was significant and substantial at about 80%, with about two-thirds of the heterogeneity occurring within studies. The results of the variance partitioning for the three-level models was very similar, whether study or site were de-fined as the clusters. Therefore, going forward, we report only the models clustered by study.

Most of the moderating variables were not found to be significant, and the inclusion of moderators did not change the proportion of variance attributable to level-two and - three variances in the models. The exceptions were moderator models that included the ‘leaf–canopy’ term, where leaf-level studies were predicted to have a higher effect size (0.49 ± 0.128) compared to canopy-level studies (0.31 ± 0.146) at $p = 0.0036$. The continuous moderator ‘richness level’ was also significant but with a very small effect size (0. 00161) at $p = 0.043$. Full model results, alongside their diagnostic criteria, are provided in Table 2.2. We also tested for interactions between ‘leaf–canopy’ and the other moderator variables. We found significant interaction terms of ‘leaf–canopy’ and ‘sampling season’, ‘site type’ and ‘richness level’. The results of these interaction models are in Table S2.

Cook’s distance values indicated which studies were influential on the outcome of the basic and moderator models (i.e., outliers; see Figure S2). The results of the reprocessed three-level models showed that the basic model without moderators was still significant without outliers but that the mean effect size was lower at 0.32 (±0.149) (Table S3). Outlier removal did not change the significance level of the moderating variables. The only exception was the addition of ‘site type’ as significant at $p = 0.0323$, with the category natural sites showing a stronger relationship compared to the experimental ones (0.5 (±0.191) and 0.24 (± 0.194), respectively). Funnel plots show no significant publication bias in any of the specified models (see Figure S3 for a basic model example).

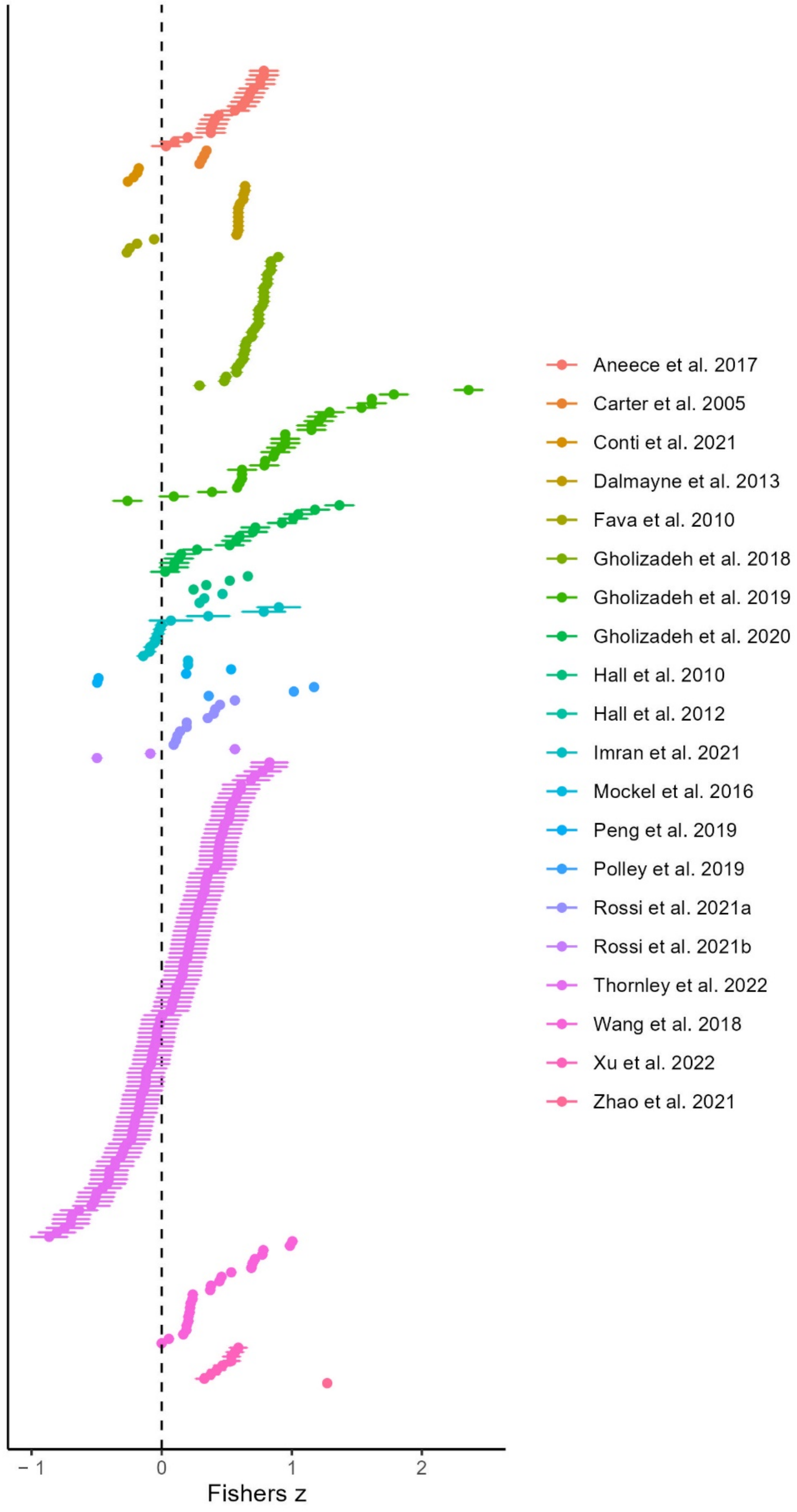


Figure 2.2. Forest plot showing the 297 effect sizes and their sampling variance ordered alphabetically by study. The dashed line represents the null hypothesis of no effect.

Table 2.2. Results of the three-level models with and without moderators. Significance levels of estimates are given as n.s. = $p > 0.05$, * = $p \leq 0.05$, ** = $p \leq 0.01$.

	Model Type	Cluster Variable	Moderators	Total Number of Effect Sizes (studies)	Number of Effect Sizes Per Group of Moderator	Pooled Correlation (Fisher's Z) with 95% CI	Pooled Correlation (r) with 95% CI	Significance Test of Pooled Correlation	Estimates for Moderators (if Significant) (r)	Significance Tests of Moderator Based Estimates	Random Effect Variance % (Sampling Error)	Random Effect Variance % ($\tau^2_{level\ 2}$)	Random Effect Variance % ($\tau^2_{level\ 3}$)	Multi-Level Variance % (I^2)
Basic	3 -level model	Study	-	297(20)	-	0.3741 (±0.162)	0.358 (±0.161)	8..3 x 10^{-6}	-	-	16.5	21.9	61.6	83.5
	3-level model	Site	-	297(20)	-	0.333 (±0.2)	0.32 (±0.197)	0.0012	-	-	14.6	22.2	63.1	85.4
Spectral data	3-level moderator model	Study	Pixel Size	297(20)	-	-	-	-	-	0.18 (n. s.)	17.88	22.31	59.81	82.12
	3-level moderator model	Study	Leaf or Canopy	297(20)	Leaf = 53; Canopy = 244	-	-	-	Leaf = 0.49 (±0.128);Canopy = 0.3111 (±0.146)	0.0036 (**)	16.01	18.76	65.22	83.99
	3-level moderator model	Study	Spectral Region	297(20)	Single = 153; Cross = 144	-	-	-	-	0.154 (n. s.)	17.13	22.76	60.12	82.87
	3-level moderator model	Study	Spectral Resolution	297(20)	Multi-spectral = 38; Hyperspectral = 259	-	-	-		0.2094 (n. s.)	16.8	22.29	60.9	83.2
	3-level moderator model	Study	Spectral Diversity Metric	297(20)	Complex = 97; Simple = 200	-	-	-	-	0.7448 (n. s.)	16.29	21.61	62.09	83.71

Species data	3-level moderator model	Study	Level of Diversity	296(20)	Alpha = 269; Beta = 27	-	-	-	-	0.24 (n. s.)	16.2	19.2	64.6	83.8
	3-level moderator model	Study	Species Diversity Metric	232(18)	Richness = 133; Diversity = 99	-	-	-	-	0.86 (n. s.)	13.9	23.8	62.2	86.1
	3-level moderator model	Study	Richness Level	247(15)	-	-	-	-	0.0161 ±- 0.0015	0.0433 (*)	15.82	13.95	70.2	84.2
Sampling Design	3-level moderator model	Study	Spatial Matching	297(20)	-	-	-	-	-	0.3199 (n. s.)	16.9	22.41	60.69	83.1
	3-level moderator model	Study	Climate	297(20)	Alpine = 26; Continental = 101; Temperate = 170	-	-	-	-	0.0878 (n. s.)	17.99	23.78	58.23	82.01
	3-level moderator model	Study	Sampling Season	297(20)	Summer= 252; Other = 45	-	-	-	-	0.8065 (n. s.)	16.4	21.89	61.71	83.6
	3-level moderator model	Study	Site Type	297(20)	Experimental = 175; Natural = 122	-	-	-	-	0.3122 (n. s.)	15.75	20.8	63.46	84.25

2.4. Discussion

2.4.1. The Spectral Variation Hypothesis across Studies and Moderator Impact

The positive pooled effect size across studies of +0.36 indicates that, overall, the Spectral Variation Hypothesis appears to hold in grassland systems. The sensitivity analysis showed there was a strong influence on this mean effect size by the findings of Zhao et al. 2021. This study contained the only leaf-level result where reflectance data was collected using a leaf clip as opposed to close range imaging spectroscopy instruments and contained a single correlation that was very high (0.85). This indicates that we should be cautious when scaling our inferences from the leaf clip to imaging devices, as the taxonomic component of reflectance is weaker with imaging devices due to additional variables such as the specular reflectance (Jay et al. 2016). However, even with the removal of this study, the mean effect size was still positive and significantly different from zero (+0.33 +/− 0.149) (see Table S3). The weak-to-moderate overall effect size could be due to a nonlinear relationship between spectral variation and plant species or habitat diversity. Amongst the studies examined, almost all the available results were produced when testing for a linear relationship (nonlinear relationships were only examined in one study (Anneece et al. 2017). Testing for these alternative relationships should be an avenue of future research.

We tested whether the magnitude of the effect sizes across studies depended on reflectance observations from within single spectral regions (the visible, NIR or SWIR) or across the spectrum. We proposed that certain spectral regions may be more important than others for assessing biodiversity. However, there was no evidence from the meta-analysis that this was the case, nor did models containing data sampled from across the spectrum have a stronger relationship with plant/habitat diversity. This finding is unfortunate for two reasons. Firstly, for practical applications, such as sensor design, we require a better understanding of which spectral bands matter more (Sun and Du 2019). Secondly, understanding which optical traits are driving the spectral variation–biodiversity relationship (Fassnacht et al. 2022), within which contexts, is important for ecological interpretation. The results from this meta-analysis support the idea that the grounds for detecting biodiversity within grasslands could be location-specific.

The only clearly significant moderating variable, at $p < 0.01$, was the ‘leaf–canopy’ variable. Leaf-level studies had a higher mean effect size (0.49) compared to the canopy-level studies (0.32), implying that biodiversity estimations using optical leaf traits as opposed to habitat/community heterogeneity are a distinct methodological approach. The moderator interaction term between the 'leaf–canopy' and 'sampling season' was also significant (see Table S2). There was no relationship between spectral variance and biodiversity for leaf-level studies outside the summer season, whereas, for canopy-level studies, the relationship held for non-summer sampling. This indicates that summer sampling is more critical for leaf-level than for canopy-level approaches and that the Spectral Variation Hypothesis, at the canopy scale, may be successfully used during the spring and autumn when non-mature or senescing vegetation is present. The results of the interaction model with 'leaf–canopy' and 'site type' as terms suggest that experimental sites, rather than natural grasslands, have larger effect sizes for leaf-level estimates compared to canopy-level and vice versa. At the canopy level, the effect of higher levels of species richness was very slightly positive compared to the leaf level, where there was no effect. This result does not support our hypothesis that, in data sets with high numbers of species, our ability to estimate diversity using the Spectral Variation Hypothesis decreases.

The low influence of outliers on the results of the moderator models further suggests that most of the methodological concerns associated with testing the hypothesis seem to be systematically unimportant across existing studies. The exception is perhaps the study by Thornley et al. 2022b when testing the moderating variable 'site type'. By removing this study, the difference between the two site types (natural or experimental) became significant (but only just at $p = 0.032$). This study stands out, as it is the only example where repeat sampling was carried out across a season at both a natural and an experimental site.

High levels of heterogeneity were observed across all the models. This may reflect what is known in meta-analyses as the 'apples and oranges' effect, where we are not strictly comparing like for like (Borenstein et al. 2009). High heterogeneity is, however, common in ecological meta-analyses (Senior et al. 2016), and values between 60 and 90% are usual. The high level of heterogeneity attributable to within-study variance, compared to between-study, indicates that the choice of data processing approaches within studies is responsible for more effect size variations than the study-level variables, such as site geographical location and instrumentation choice.

2.4.2. Limitation in the Scope of Studies

All studies included in the meta-analysis were carried out in the Northern Hemi-sphere. Evidence from the Southern Hemisphere and tropical and arid grasslands is notably absent. This reflects, in part, the lack of funding for experimental work in the developing world (Waldron et al. 2013). However, our exclusion of studies that dealt with partially wooded environments at the landscape scale, such as savannahs and chaparrals, impacted the scope. We predict that isolated trees in otherwise grass- and forb-dominated landscapes will probably increase the spectral diversity due to the inclusion of two very different land cover types. Other studies have shown good outcomes for the estimation of tree covers in these types of communities (Geesner et al. 2013, Zhang et al. 2019), and we may be able to utilize these estimates as co-variates alongside the Spectral Variation Hypothesis within these systems to separate out pixels that include trees and those that capture only grassland.

An observation from this meta-analysis is that, despite the phenological dynamism of grassland systems, there are only a few instances of multitemporal testing of the hypothesis. Explicit testing of temporal stability was only examined in three cases (Gholizadeh et al. 2020, Rossi et al. 2021a, Thornley et al. 2022a), with all studies reporting instability across time when using the same instrumentation and analytical approaches. Most other studies focused on a mid-summer assessment. The results from the interaction models suggest that this is a good choice, at least when dealing with spectral data captured at the leaf level.

There are likely to be some additional sources of study bias that we were not able to explore within this meta-analysis. For example, the quality of the spectral data between and within studies due to the variability in terrain variables. Rugged terrain creates shadows that affects reflectance (Sirguey 2009). This could be especially problematic when assessing the hypothesis across large-scale landscapes using satellite data. However, terrain effects can also be observed within high spatial resolution data sets, collected using un-manned aerial vehicle technology. In future analyses, more attention should be given to validate reflectance data that could be affected by the terrain.

Although we did not detect any significant publication bias in this meta-analysis using funnel plots, this result should be treated with caution, as methods for testing publication bias with dependent data sets are still under development (Nakagawa et al. 2021). While the non-publication of negative data is a well-known phenomenon amongst scientists (Fanelli 2011, Petty and Gross 2019), within this synthesis, we found that there was a range of both negative and positive results reported, which perhaps indicates that this phenomenon is not as prevalent in this research field as in others.

2.4.3. Spectral Variation as a Covariate in More Complex Models

The high level of heterogeneity in the models presented in this study imply that species diversity prediction using spectral variation is likely to require the consideration of additional covariates. Within the reviewed studies, more complex relationships were examined that incorporated biomass levels (Rossi et al. 2021b), vertical sward complexity (Conti et al. 2021) and the proportion of the canopy at a mature phenological stage (Thornley et al. 2022a). Spectral variance has also been found to be related to ecosystem productivity in grasslands (Sakowska et al. 2019), and spectral diversity, captured by satellites, has been shown to be principally influenced by the land cover type (Hauser et al. 2021). Combining reflectance data with structural characteristics, such as the tree height from LiDAR (Cho et al. 2012), has also proven promising in mapping species, suggesting that different types of remotely sensed variables can be combined to predict diversity.

4.4. Approaches to the Spectral Variation Hypothesis Outside This Meta-Analysis

While examining the literature on the Spectral Variation Hypothesis, we noted emerging approaches that expand on the traditional definition, which relates to the spectral variation in space. For example, some authors have looked at the spectral variance of a pixel or cluster of pixels over time (Fauvel et al. 2019, Lopes et al. 2017, Rapinel et al. 2021). This is based on the idea that plant species or community-specific responses to temperature, rainfall, day length and soil conditions can be exploited for diversity estimations. One step further is to combine temporal and spatial spectral variations into a composite measure (Rossi et al. 2021a). Spectral variance has also been used to estimate plant functional diversity (Frye et al. 2021, Schweiger et al. 2018). In addition, relationships have been found between phylogenetic and spectral distances among species (Meireles et al. 2020). It is evident that, as the field of biodiversity estimations from spectral data expands, these newer approaches will require scrutiny.

2.5. Conclusions

The results of this study indicate that there is some promise for the use of the Spectral Variation Hypothesis to estimate biodiversity in grasslands but that more work is needed before we can exploit the method with confidence. A diverse assemblage of approaches is in use by analysts, making this an exciting and active field of research. However, this also creates challenges when synthesizing results from studies. We encourage more work in extensive natural systems, especially in tropical and arid regions, and in the Southern Hemisphere. In addition, the repetition of experiments across phenological cycles and between years will also help increase our understanding of the stability of the hypothesis across time. Hyperspectral imaging sensors that capture data at very small scales and enable scaling up to the field level (while keeping all other site and analysis variables stable) are an important link in understanding the future possibilities

2.6 Supplementary Materials

Table S1

The search terms used in the literature search

Database name	Search terms	Unfiltered Results	pages shifted
Google Scholar	Remote + sensing + biodiversity	441,000	100
Google Scholar	Spectral + variation + grasslands	60, 800	100
Google Scholar	Spectral + heterogeneity + grasslands	30,500	100
Google Scholar	Grassland + remote sensing	240,000	100
Google Scholar	Plant + richness + remote + sensing	78,600	100
Scopus	Remote + sensing + biodiversity	4, 636	100
Scopus	Spectral + variation + grasslands	298	all
Scopus	Spectral + heterogeneity + grasslands	66	all
Scopus	Grassland + remote + sensing	55,137	100
Scopus	Plant + richness + remote + sensing	315	all

Figure S1

The PRISMA flow chart for standardised literature reviews and synthesis

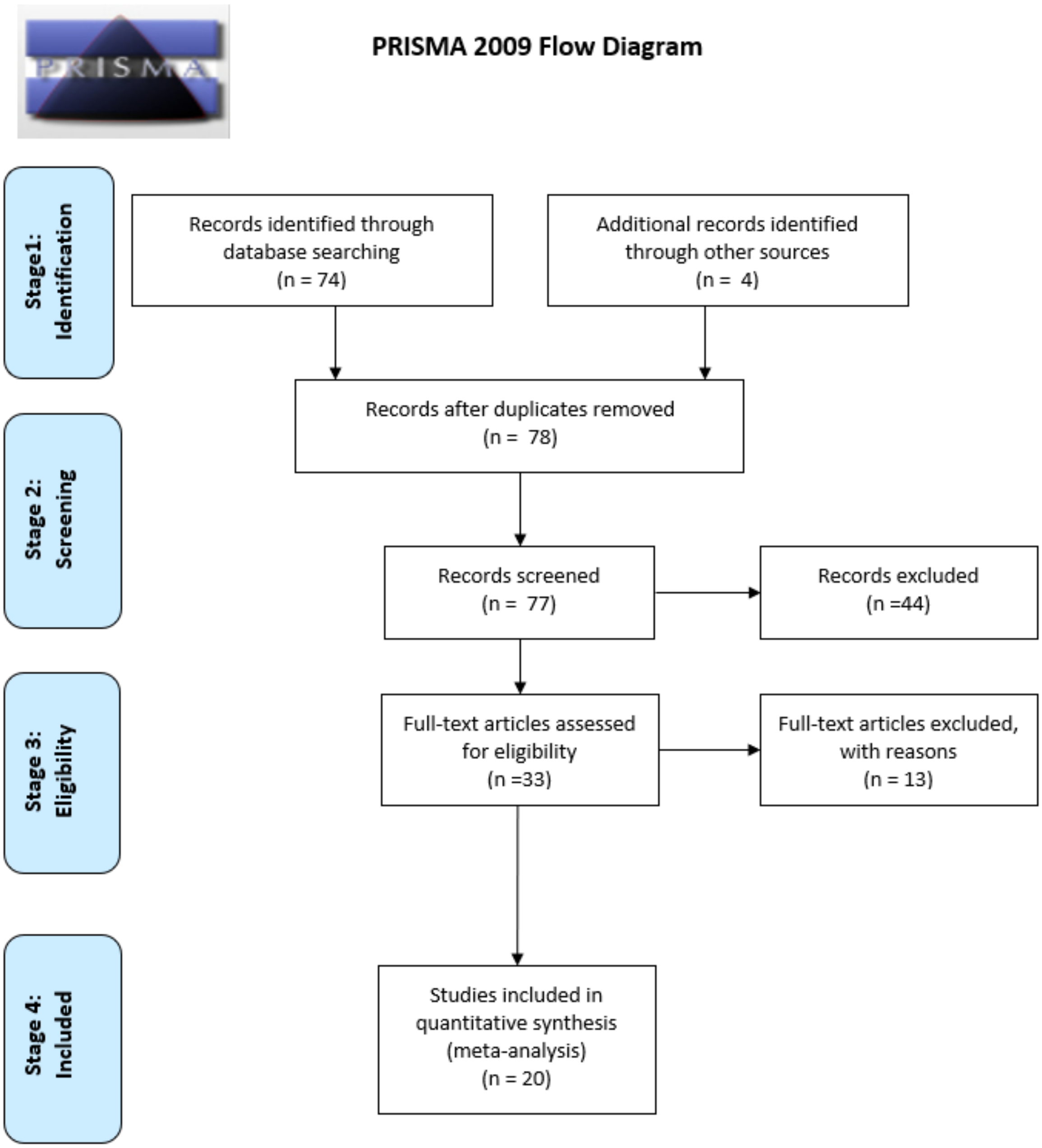


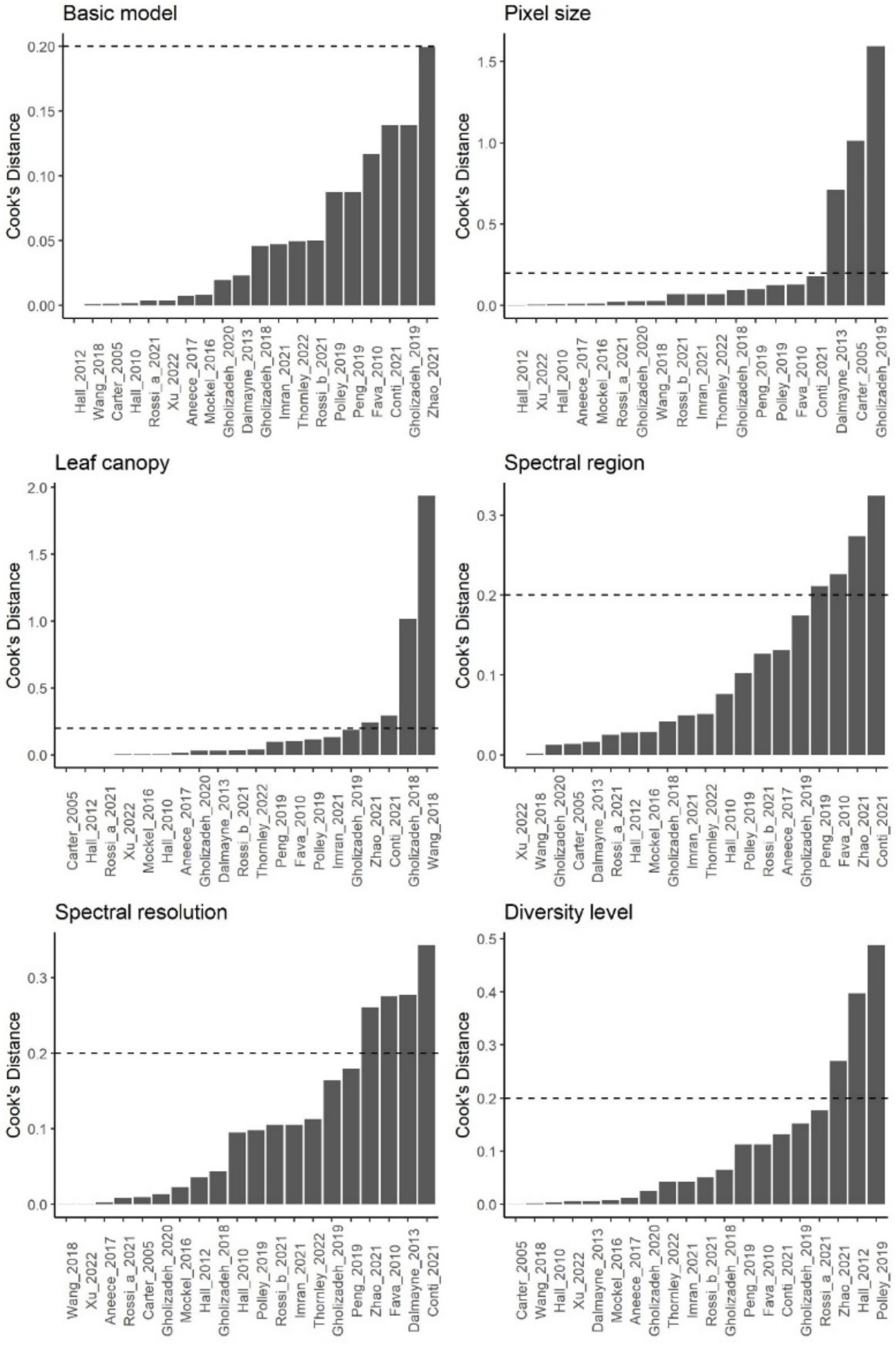
Table S2

The results of the three-level models with statistically significant interaction terms

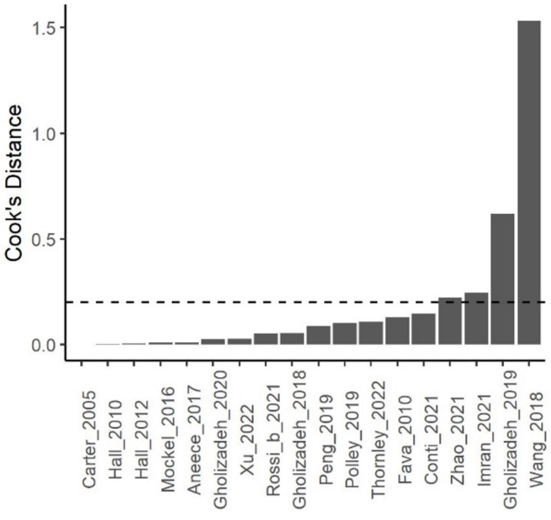
Cluster variable	**Moderators**	**Type of Moderator**	**Overall test of moderators (*p*)**	**Test of difference for model improvement with interaction term as opposed to additive terms**	**Estimates of interactions (*r*) with confidence interval**	**Significance of estimates (*p*)**
Study	1. Leaf canopy; 2. Sampling season	Categorical	0.0042(**)	0.03(*)	Canopy and Other = 0.3690 (0.1041)	0.0005(***)
					Leaf and Other = -0.2099 (0.3427)	0.5407 (n.s.)
					Canopy and Summer = 0.3552 (0.0816)	<0.0001 (****)
					Leaf and Summer = 0.5910 (0.0988)	<0.0001 (****)
Study	1. Leaf canopy; 2. Site Type	Categorical	0.0003(***)	0.0020(**)	Canopy and experimental = 0.2920 (0.0893)	0.0032 (**)
					Leaf and experimental = 0.5426 (0.1114)	<0.0001 (****)
					Canopy and natural = 0.4444 (0.1009)	<0.0001 (****)
					Leaf and natural = 0.0723 (0.2020)	0.7206 (n.s)
Study	1. Leaf canopy; 2. Richness level	Continuous	<0.0001(****)	0.0143(*)	Canopy and richness level = 0.022 (0.0069)	0.0015(**)
					Leaf and richness level = 0.0286 (0.0286)	0.2348(n.s)

Figure S2

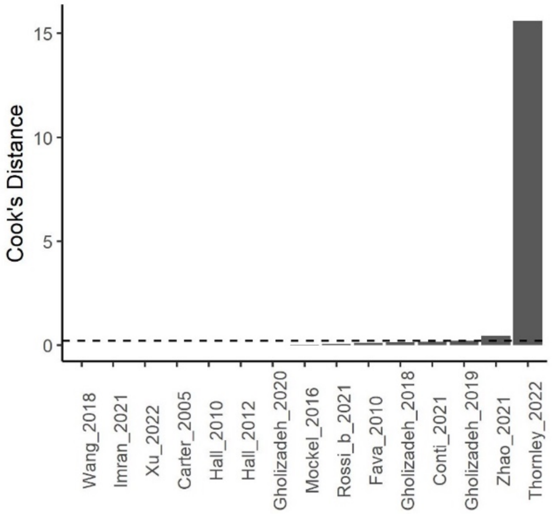
The results of the outlier analysis for the three-level models displaying the Cook's Distance Metric clustered at the study level. Study outliers are those studies with values above the dotted line representing 0.2.



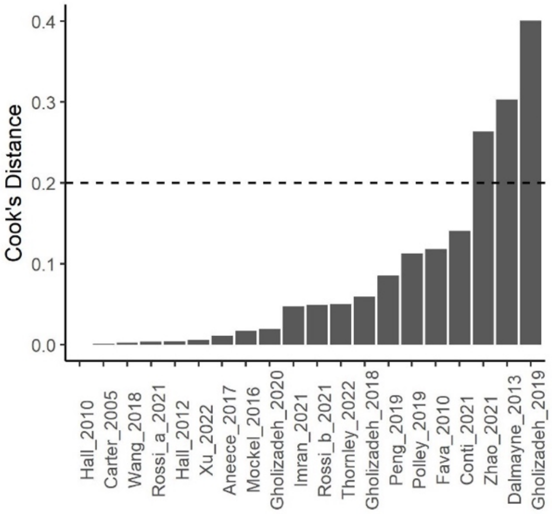
Species diversity metric



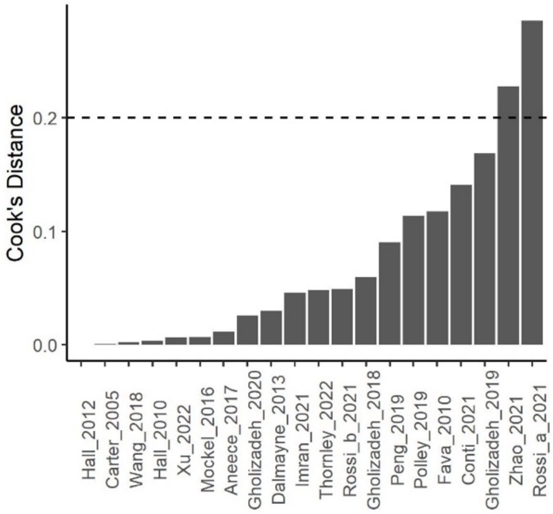
Richness level



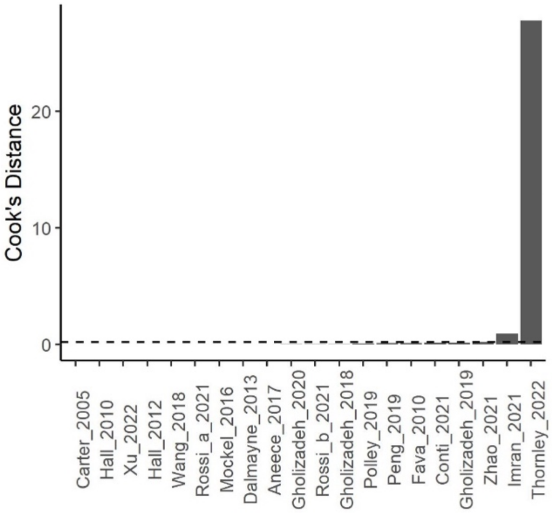
Scale matching



Sampling season



Site type



Climate

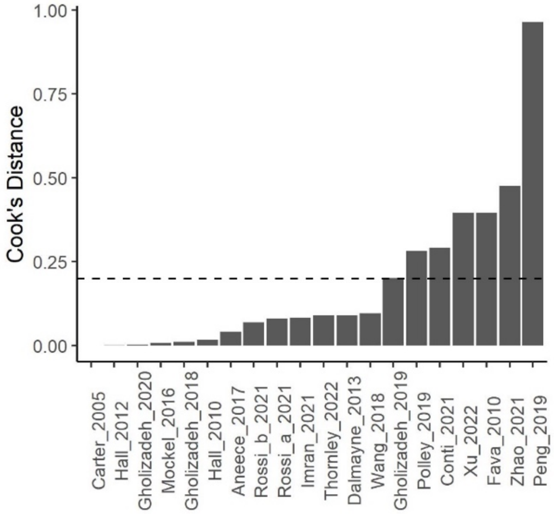


Table S3

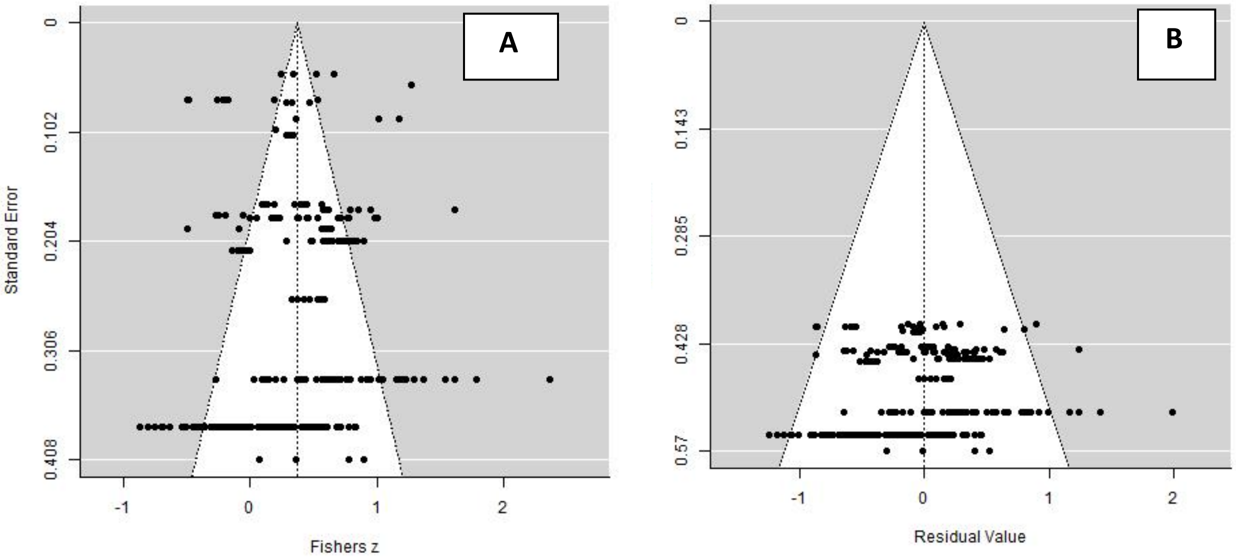
The results of the three-level model results after removal of the outliers at the study level.

Model category	**Model type**	**Cluster variable**	**Moderators**	**Outliers at study level**	**Pooled correlation (fisher's z) without outliers**	**Significance test of pooled correlation withour outliers**	**Estimates for moderators (if significant) without outliers**	**Significance tests of moderator based estimates without outliers**
Basic	Three-level model	Study	-	Zhao et al. 2021	0.325 (+/- 0.149)	1.45E-05	-	-
	Three-level model	Site	-	Zhao et al. 2021	0.275 (+/- 0.178)	0.002217545	-	-
Spectral data	Three-level moderator model	Study	Pixel Size	Dalmayne et al. 2013, Carter et al. 2005, Gholizadeh et al. 2019	-	-	-	0.5028
	Three-level moderator model	Study	Leaf or Canopy	Gholizadeh et al. 2019, Wang et al. 2018	-	-	-	0.1522
	Three-level moderator model	Study	Spectral Region	Peng et al. 2019, Fava et al. 2010, Conti et al. 2021, Zhao et al. 2021	-	-	-	0.0606
	Three-level moderator model	Study	Spectral Diversity Metric	Peng et al. 2019, Polley et al. 2019	-	-	-	0.8045
Species data	Three-level moderator model	Study	Level of Diversity	Zhao et al. 2021, Hall et al. 2012, Polley et al. 2019	-	-	-	0.5446
	Three-level moderator model	Study	Species Diversity Metric	Imran et al. 2021, Gholizadeh et al. 2019, Wang et al. 2018	-	-	-	0.86
	Three-level moderator model	Study	Richness Level	Thornley et al. 2022	-	-	-	0.2875
Sampling Design	Three-level moderator model	Study	Spatial Matching	Gholizadeh et al. 2019, Zhao et al. 2021, Dalmayne et al. 2013	-	-	-	0.3254
	Three-level moderator model	Study	Sampling Season	Zhao et al. 2021, Rossi et al. 2021a	-	-	-	0.1237

	Three-level moderator model	Study	Climate	Gholizadeh et al. 2019, Polley et al. 2019, Conti et al. 2021, Xu et al. 2022, Fava et al. 2010, Zhao et al. 2021, Peng et al. 2019	-	-	-	0.0636
	Three-level moderator model	Study	Site type	Thornley et al. 2022	-	-	Experimental = 0.2409 (+/- 0.194) Natural = 0.501 (+/- 0.191)	0.0323

Figure S3

Funnel plot for the basic three level model with study as cluster, showing A: the raw Fisher's *Z* plotted against the standard error; B: the model residuals plotted against the standard error



Chapter 3

Intra-annual taxonomic and phenological drivers of spectral variance in grasslands

This chapter comprises data collected during the first field season of the PhD (2019) on two semi-natural grasslands, a calcareous grassland in Surrey and an acid grassland in Berkshire. It tests the application of the spectral variation hypothesis over a growing season at the site level. Hyperspectral reflectance data was captured using a non-imaging spectroradiometer at very small canopy scales (10cm^2). The grassland communities were characterised both by their cross-seasonal changes in taxonomic composition and by their phenological development.

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Author contribution statement

Rachael Thornley: conceptualisation, spectral and botanical data collection, data analysis and writing. France Gerard: conceptualisation, review, and editing. Kevin White: conceptualisation, data collection, technical guidance on instruments, review, and editing. Anne Verhoef: conceptualisation, data collection, review, and editing.

Abstract

According to the Spectral Variation Hypothesis (SVH), spectral variance has the potential to predict taxonomic composition in grasslands over time. However, in previous studies the relationship has been found to be unstable. We hypothesise that the diversity of phenological stages is also a driver of spectral variance and could act to confound the species signal. To test this concept, intra-annual repeat spectral and botanical sampling was performed at the quadrat scale at two grassland sites, one displaying high species diversity and the other low species diversity. Six botanical metrics were used, three taxonomy based, and three phenology based. Using uni-temporal linear permutation models, we found that the SVH only held at the high diversity site and only for certain metrics and at particular time points. We also tested the seasonal influence of phenological stage dominance, alongside the taxonomic and phenological diversity metrics on spectral variance using linear mixed models. A term of percentage mature leaves, alongside an interaction term of percentage mature leaves and species diversity, explained 15-25% of the model variances, depending on the spectral region used. These results indicate that the dominant canopy phenology stage is a confounding variable when examining the spectral variance-species diversity relationship. We emphasise the challenges that exist in tracking species or phenology-based metrics in grasslands using spectral variance but encourage further research that contextualises spectral variance data within seasonal plant development alongside other canopy structural and leaf traits.

3.1 Introduction

3.1.1 Grassland monitoring

An important criterion when assessing field-level grassland condition is the complexity of the plant community, often summarised as the number of taxonomic units co-existing within the sward and their spatial distribution. These surveys are typically targeted at mid growing season when most plants are flowering, a time referred to as peak phenology (Stohlgren, 2006), with few data having been collected outside what is considered to be this 'optimal' window (Magurran, 2007). Plant communities can be dynamic in terms of the number of taxa present at a single time point during a growing season (Mellard et al., 2019; Wang et al., 2016), however, repeat intra-annual botanical surveys are very time-consuming and so little is understood about these community dynamics.

3.1.2 Spectral variation as a proxy for species diversity

An option to increase our understanding is to utilise remote sensing (Ali et al., 2016; Wachendorf et al., 2017) and in particular hyperspectral reflectance data (Fava et al., 2010; Mockel et al., 2016; Wang and Gamon, 2019). The Spectral Variation Hypothesis (SVH) proposes that the variance in spectral reflectance within a given area can be used as a proxy for plant taxonomic diversity. The concept of reflectance variance as an ecological surrogate was first described by Palmer et al. (2002). Rocchini et al. (2010) provide a review of the concept and the challenges to its implementation. Evidence to support the hypothesis has been gathered at the landscape scale (Hall et al., 2010) using broad-band satellite data products, down to the leaf-level with close-range imaging spectrometers (Wang et al., 2018). In some studies, however, the SVH been found to be unstable (Schmidtlein and Fassnacht, 2017; Torresani et al., 2019) and context dependent (Imran et al., 2021). Convergent optical properties of photosynthetically active material alongside the impact of environmental drivers, such as water availability, may prove obstacles to species differentiation (Asner et al., 2009; Ollinger, 2011). Furthermore, spectral distance between species may be affected more by functional variation rather than by the number of taxonomic units (Schweiger et al., 2018). This perspective is intrinsic to the 'optical type' theory (Ustin and Gamon, 2010), where, in the context of high spatial resolution reflectance data, 'diversity' can be framed as a product of leaf traits at the individual level (Leaf Mass Area, Nitrogen, Chlorophyll, Carotenoids, Lignin) rather than taxonomic variation (Ma et al., 2020).

3.1.3 Measures of spectral variance

One of the complications in assessing the SVH and prior findings in this field, is that measures of spectral variance are calculated in different ways. Sophisticated approaches have been employed to deal with the multi-variate data sets produced from hyperspectral data sensors, for example, the 'Spectral Angle Mapper' (Gholizadeh et al., 2018), k-means clustering (Rocchini et al., 2005) and Partial Least Squares regression (M¨ockel et al., 2016; Polley et al., 2019). It is arguable, however, that descriptive statistical approaches, such as taking dispersions around the mean value for a single wavelength or index value, are more useful at this stage to evaluate the hypothesis (Torresani et al., 2019; Wang et al., 2018) as they allow the strength of the relationship to be more effectively compared between study findings. The coefficient of variation (CoV) is an often-used metric when dealing with hyperspectral data (Aragon et al., 2011; Blanco-Sacristian et al., 2019; Lucas and Carter, 2008; Wang et al., 2018) as it normalises the dispersion against the mean reflectance for each wavelength, thus accounting for the differences in magnitude between spectral regions.

3.1.4 The performance of the SVH over time

Another obstacle is that the relationship between spectral variance and taxonomic diversity when examined over time has been shown to be inconsistent (Schmidtlein and Fassnacht (2017)). Inter-annual studies with similar sampling dates in temperate systems (Gholizadeh et al., 2020) suggest this inconsistency is not merely a product of 'time of year' but may be due to a complex relationship between reflectance and seasonally dynamic leaf and canopy traits (Feilhauer and Schmidtlein, 2011; Feilhauer et al., 2017). High spatial resolution hyperspectral data, to our knowledge, has been collected on multiple dates in grasslands for only a couple of studies (Feilhauer and Schmidtlein, 2011; Gholizadeh et al., 2020), both of which reported varying relationships over time between taxonomic diversity and spectral reflectance. These observations could be due to the dynamic nature of grassland canopies, in terms of their responses to rainfall and management (Li et al., 2013) and phenological variability (Ustin and Gamon, 2010).

3.1.5 The impact of phenological stage on the spectral variation

Hyperspectral reflectance data are usually collected at peak biomass or growth when assessing taxonomic diversity, and there is good theoretical basis for this decision. At these times, grassland canopies are generally less affected by dead plant tissue and exposed soil, which are significant additional sources of spectral variation (Asner, 1998). When these sources are minimised, leaf intracellular structure and chemical traits drive variation in leaf reflectance (Ollinger, 2011; Ustin et al., 2009) and it is this variation that has been shown to be strongly correlated with the species present (Asner and Martin, 2011, 2016). In addition, leaf traits and so reflectance, and derived vegetation indices, alter with leaf age (Chavana-Bryant et al., 2017) which, if not accounted for, could be confounded with, among others, a taxonomic signal. On the other hand, the effect of leaf age and plant life cycle stage on reflectance could be exploited when the aim is to map single species or functional types. For example, in temperate deciduous woodlands, species specific timing of leaf emergence and senescence, accompanied with species specific leaf colouring, have been instrumental in distinguishing between tree species (Fassnacht et al., 2016; Hill et al., 2010; Voss and Sugumaran, 2008). The spatial scale of data acquisition is highly significant when assessing the SVH (Gamon et al., 2019). At the leaf level, phenological stage affects reflectance through the process of leaf maturation (the development of palisade and spongy mesophyll and increase in chlorophyll cell number) (Noda et al., 2021), followed by senescence (reallocation of resources away from the leaf to over-wintering or reproductive structures). At the canopy scale, the quantity and developmental stage of leaves affect reflectance through increases in parameters such as leaf area index (LAI) and total canopy chlorophyll (Jacquemoud et al., 2009). Non-leaf plant architecture (buds, flowers, seeds) will also influence reflectance as these parts of the plant are generally not photosynthesising (Asner, 1998). The number of differing phenological stages present will therefore be an additional driver of spectral variation alongside taxonomic diversity. The extent to which individuals within plant communities exhibit phenological stages at simultaneous or staged phases is known as phenological synchrony or asynchrony (Rathcke and Lacey, 1985; Forrest and Miller-Rushing, 2010) and the number of co-occurring phenological stages can be understood as phenological diversity (Lasky et al., 2016; Ramos et al., 2014). These properties may, however, be difficult to estimate as they are likely to vary between years and within a growing season (Mazer et al., 2013; Tansey et al., 2017) due to individual-based responses to environmental conditions (Wolkovich et al., 2014).

3.1.6 Study aims

This study uses intra-annual repeat taxonomic and phenological observations alongside the variance of high-resolution spectral reflectance data collected at two grassland sites, with differing levels of species diversity, soil type and management regime, to test the following: 1) The temporal stability of the SVH across a growing season in relation to plant taxonomic metrics and to determine the best time of year for biodiversity surveys using this method. 2) The extent to which the phenological diversity of the canopy drives spectral variance. 3) The relative impact of phenological and taxonomic diversity and phenological stage dominance on spectral variance across the growing season.

3.2 Methods

3*.2.1. Grassland site description and sampling campaign overview*

Two sites in the southeast of England were used for the collection of remote sensing and botanical data. The first site is Dawcombe nature reserve, Betchworth, Surrey, UK (51.259, - 0.261). It is an example of medium quality chalk grassland situated on the scarp slope of the North Downs and is managed for biodiversity conservation. From here on, this site will be referred to as 'Dawcombe.' The second site is a long-term experimental grassland managed by Imperial College London and called 'Nash's Field' located at Silwood Park, Sunningdale, Berkshire, UK (51.413, - 0.645). It is composed of acid grassland plots that have undergone varying soil nutrient and grazing treatments (Crawley et al., 2005). The range of treatments have created a site with high levels of community variation within a small area. This site will be referred to as 'Silwood.' Both sites undergo annual late summer or winter biomass removal through mowing and grazing but are not managed during the spring and summer months when the sampling was undertaken. However, they are subject to some low-level grazing throughout the year by wild herbivores (deer and rabbits) and invertebrates. We selected the sites for their strong gradients of community composition. They are also grasslands where plant species undergo their reproductive cycles without significant interruption, unlike in many agricultural grasslands where high grazing pressures can occur throughout the year. Twenty 50 × 50 cm (0.25m2) quadrats sub-divided into twenty-five 10 × 10 cm subquadrats, using a botanical grid as a guide, were measured over the 2019 growing season. Ten of these were situated at Dawcombe and ten at Silwood (see Figure 3.1). Quadrats were used as they represent the most common approach to monitoring vegetation in grasslands. At Dawcombe, quadrat locations were randomly chosen along a slope gradient, intended to capture maximum variation in the plant community, and were simply labelled 1–10. At Silwood, the quadrats were chosen to represent a range of nutrient and grazing applications, to the same aim. They were labelled to reflect the experimental plot (L), whether they were subject to grazing (UF = unfenced) or not (F = fenced) and the nutrient treatment (N = nitrogen, K = potassium, P = phosphate, All = all nutrients, None = control). The quadrats were marked with pegs and geolocated to sub-cm accuracy by use of a differential GPS so the same areas could be revisited. The botanical sampling grid was painted matt black to avoid interference with the reflectance observations. Botanical observations were made at six time points at Silwood and ten at Dawcombe. Hyperspectral sampling events totalled five at Silwood and seven at Dawcombe. Details of botanical and spectral sampling are to follow.

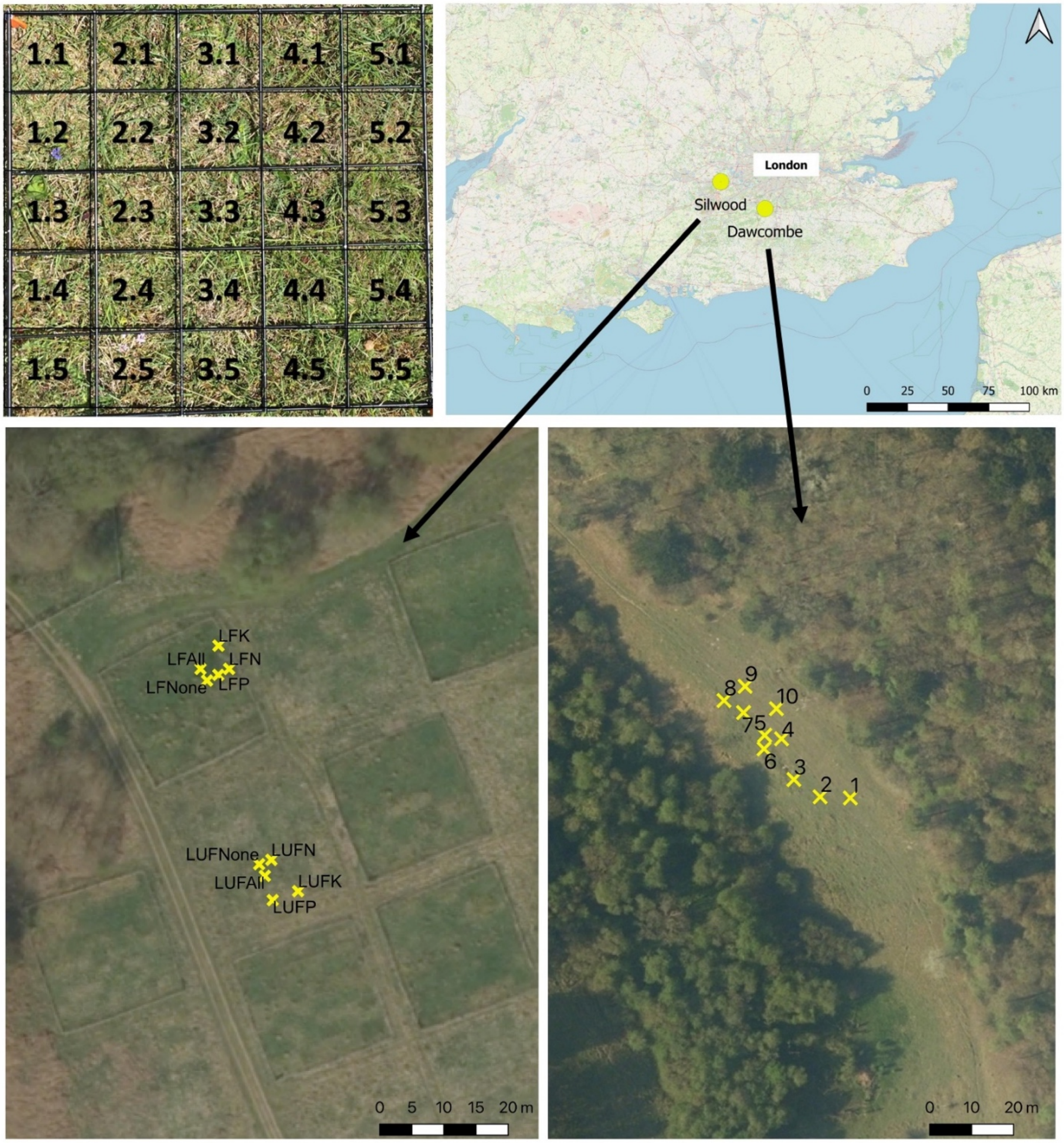


Figure 3.1 The sampling sites and the quadrat locations at Silwood and Dawcombe. Top left: the sampling grid used to acquire both spectral and botanical samples for each of the 20 quadrats at each time point.

3.2.2. Plant community metrics

We calculated two sets of metrics, which are listed with their respective equations in Table 3.1. The first set is based on *taxonomic* units and metrics were calculated per time point per quadrat. The second set is designed to evaluate the impact of plant phenological stage on spectral diversity. These metrics are based on *phenological* observations associated with the recorded taxa and were also calculated per time point per quadrat.

3.2.2.1 Taxonomic metrics

Plant taxonomic complexity can be described and summarised by using a range of metrics, each of which present a different aspect of, or approach to, diversity measurement (Magurran and McGill, 2011; Morris et al., 2014). The following three metrics were calculated (Table 3.1): 'species richness', which is a count of species occurrence and is the basic measure in biodiversity assessment; the 'Simpson evenness index', also known as relative abundance (Smith and Wilson, 1996) which describes the dominance distribution of the species present; and the 'Simpson diversity index', a composite measure which incorporates both richness and evenness. Visual estimations of percent cover per species, are often used in botanical assessments, but this measure is very subjective, especially in more complex species-rich quadrats. So, instead, the proportion of sub-quadrats in which the species occurred was used to calculate relative abundance and subsequently derive the Simpson's diversity and Simpson's evenness indices.

Because proximal remote sensing instruments are generally set to nadir viewing and so are limited to sensing the top part of canopies, only species within the quadrat that were observed when looking directly down were considered.

Table 3.1 Definitions of the taxonomic and phenological metrics used in this study.

Name of Metric	Description	Category	Equation
Species Richness (S)	The total number of species observed for each time point (*t*)	Taxonomic	S_t
Species Diversity (Simpson's diversity inverse or reciprocal) (D)	The inverse of the probability that two species drawn from the same sample will belong to the same species. Index ranges from 1 to infinity, where 1 represents a community with a single species and the higher the number the more complex the community.	Taxonomic	$D_t = \frac{1}{\sum_{i_t}^{S_t}(\frac{N_{i_t}}{N_t})^2}$ Where: *N* is the total species abundance*;* N_i is the abundance of species *i; t* is time point *t*
Species Evenness (Simpson's Evenness) (E)	Describes the distribution of individuals among classes and is therefore a measure of relative abundance. *E* is expressed as a proportion of the maximum value which *D* could assume if individuals in the community were completely evenly distributed (which equals *S*). The metric takes a value between 0 and 1 where 1 is complete evenness.	Taxonomic	$E_t = \frac{D_t}{S_t}$
Phenological Richness (P)	The number of phenological stages observed for each time point (*t*)	Phenology	P_t
Phenological Diversity (PhenD)	Index ranges from 1 to infinity, where 1 represents a community with a single phenological stage present (complete synchrony) and higher values indicate a greater number of phenological classes and a more complex distribution of classes among species.	Phenology	$PhenD_t = \frac{1}{\sum_{i_t}^{P_t}(\frac{M_{i_t}}{M_t})^2}$ Where *M* is the total number of phenology stages observed and M_i is the total number of phenology stages observed for species *i.*
Phenological Evenness (PhenE)	As definition for *E* *but for phenological stage*	Phenology	$PhenE_t = \frac{Phen\ D_t}{P_t}$

3.2.2.2 Phenology metrics

Firstly, for each taxonomic unit observed in in the quadrat, an observation of phenological stage was made according to the definitions in Table 3.2. Some species displayed multiple stages at a single time point. The number of observations in each phenological category were then summed and weighted to produce a measure of phenology stage dominance for each quadrat at each time point. Measures are available that describe the timing of plant phenology stages, such as frequency, regularity, amplitude, synchrony, and duration (Newstrom et al., 1994; Denny et al., 2014). However, to evaluate the impact of plant phenological stage on spectral variance we required metrics that capture the phenology stage diversity observed at any moment in time. As far as we are aware, these do not exist. We therefore adopted the above taxonomic metrics to produce the following phenological metrics: ‘phenological richness’, the number of different phenology stages present at a given time in a given quadrat’; phenological evenness’, a measure of the relative abundance of phenology stages present; and ‘phenological diversity’ which was designed to reflect the diversity of phenology stages present at any moment in time within a quadrat, as a product of the species richness and abundance. For full definitions of the metrics and the calculations used to produce them see Table 3.1.

Table 3.2 Descriptions of the phenology stages used to calculate the phenological diversity metrics.

Phenology stage code	Stage name	Stage description
SEN(1)	Senescent	Plant material in senescence (brown, lacking in chlorophyll) when quadrat was first examined in the spring (principally from last years' growth season).
YOU(2)	Young	Leaf material is thin/downy – displays colours (bright green) not in line with those expected from mature leaves.
MAT(3)	Mature	Leaf material is thickened / some cases waxy – displays colours in line with those expected from mature leaves
BUD(4)	Budding flowers	Plant has the beginnings of reproductive organs - flower buds for broadleaved herbs, or sheathed heads for grasses
FLO(5)	Flowering	Plant is in flower; flower heads emerged
SEE(6)	Seeding	Plant has seeds or seed encapsulating organs visible
SEN(7)	Senescent	The current growth season's plant material in senescence (brown or red/brown discoloured leaves).

3.2.3 Spectral data capture and calculation of spectral variance

The Coefficient of Variance (CoV) from hyperspectral reflectance observations was selected as the spectral variance metric. The next sections outline the in-situ instrument setup and hyperspectral data pre-processing steps taken to ensure a robust dataset for reliable derivation of reflectance CoV.

3.2.3.1 Hyperspectral field radiometry setup

Hyperspectral reflectance measurements (350 nm – 2500 nm) were collected for each sub-quadrat (25) of each quadrat (20). We used two SVC non-imaging spectrometers (SVC HR2024i spectroradiometers, Spectra Vista Corporation, USA) in a Dual Field Of View (DFOV) mode (Maclellan, 2017; Punalekar et al., 2018), to simultaneously record irradiance and reflected radiance. This approach is recommended when data is collected under fluctuating illumination conditions (which is often the case in the UK) and is expected to deliver more accurate observations, which are particularly important when, as in most vegetation studies, spectral distance between target classes is small. Before target sampling began, both spectrometers were mounted on tripods pointing at their respective Spectralon panels and reference readings were taken concurrently. The instrument measuring down-welling radiation was then set to timed-mode while the instrument measuring upwelling radiation was used on a boom held at nadir 70 cm above the grassland canopy, resulting in a sample spot size of 10 cm. Each grassland quadrat measured 50 × 50 cm and was subdivided into twenty-five 10 × 10 cm sub-quadrats using as a guide, the same matt black grid that was used in the botanical sampling. For each sub-quadrat one reading was taken. The target spots were intended to be non-overlapping but spatially correlated in order to emulate the effect of pixels from an imaging sensor. All measurements were taken between the hours of 10 am and 3 pm local time (BST). Twenty-five measurements were taken of each quadrat at each time point resulting in 250 measurements per sampling date for each site, totalling 3000 spectral samples.

3.2.3.2 Hyperspectral data pre-processing

Pre-processing of the spectrometry data involved calibration of each sub-quadrats' reflected radiance spectrum against its respective Spectralon white reference panel spectrum to produce reflectance. Parts of the spectrum affected by water absorption and scattering were removed (339–399 nm, 1900–2051 nm, 2450–2519 nm) and a Savitzky-Golay smoothing filter was applied. The spectrum was binned by 10 nm increments. Smoothing and binning was carried out with the package HSDAR (Lehnert et al., 2019) in R (R Core Team, 2022). Spectrometry data can suffer from erroneous measurements caused by slight changes in viewing angle and subject illumination (Wehrens, 2011). It is vital to ensure that the inclusion of these measurements is minimalised as we are dealing here with variance measures from a mean or a centroid value. A common practice is to carry out repeat measures of the same target and take an average. Due to the number of measurements required per day this process was not feasible. Instead, thorough data cleaning and pre-processing was carried out to identify the erroneous readings. Two principal sources of measurement error were considered; 1) time stamp mismatch between the two spectrometers (one measuring the quadrats, the other the white reference panel), especially in rapidly changing conditions and 2) changes in reflectance caused by variations in viewing and sun angle. To minimise these sources of error, we used 'Robust Principal Component

Analysis' (ROBPCA) (Hubert et al., 2005; Hubert, 2020) which was applied to the spectra grouped by time-point and quadrat (amounting to 120 data sets). Outliers are computed using 'projection pursuit' techniques and the Minimum Covariance Determinant (MCD) method (Hubert and Debruyne, 2010). The ROBPCA approach can be used to compute PC scores that are outlier resistant, but also to detect the outliers themselves. The level of data cleaning changed with the α parameter (0.5–0.9); lower values indicate more 'robust' outlier detection, with more samples being removed from the analysis. Data sets produced with five values of α (0.5, 0.6, 0.7, 0.8 and 0.9) were used to help assess the stability of the model fits for the uni-temporal data sets (Section 3.2.5.1). For the rest of the analysis, we used the ROBPCA corrected data with an α value of 0.8 resulting in a total sample size of 2561 spectra. For sample sizes, the sampling dates, and their corresponding day of year (DoY) see Table 3.3.

Table 3.3 Sample size and dates for the hyperspectral data set

Site	Date	Day of Year	Time point	Spectrometer sample size(n=)	
				All data	ROBPCA screen
Dawcombe	18th April 2019	108	1	250	216
	1st May 2019	121	2	250	215
	16th May 2019	136	3	250	209
	31st May 2019	151	4	N/A	N/A
	11th June 2019	162	5	N/A	N/A
	27th June 2019	178	6	N/A	N/A
	13th July 2019	194	7	250	215
	24th July 2019	205	8	250	217
	8th August 2019	220	9	250	211
	20th August 2019	232	10	250	213
Silwood	29th April 2019	119	1	250	210
	21st May 2019	141	2	250	217
	5th June 2019	156	3	250	218
	20th June 2019	171	4	N/A	N/A
	12th July 2019	193	5	250	210
	29th July 2019	210	6	250	210

3.2.3.3 Coefficient of variation

The coefficient of variation (CoV) was used as the spectral diversity metric and was calculated for each waveband *i* as follows:

$$CoV_i\ (\%) = \frac{\sigma_i}{\mu_i}\ x\ 100 \qquad (1)$$

where µi equals the mean reflectance of the 25 subplots and σi equals the standard deviation. Wang et al., 2018 used the mean of the band specific CoV values across spectral regions as a summary measure of hyperspectral variance and found strong positive correlations with taxonomic diversity metrics. Here we follow this method in order to compare findings. Firstly, the band specific measures of CoV were averaged across the full visible to short wave infra-red spectrum and then, secondly, across three spectral regions; the visible (400–699 nm), the near infra-red (700–1299 nm) and the short wave infra-red (1300–2519 nm). These averages are referred to as 'mean-CoV', 'vis-mean-CoV', 'NIR-mean-CoV' and 'SWIR-mean-CoV', respectively. Although the exact values of these regional cut-off points are somewhat arbitrary, spectral variation within these three chosen spectral regions (visible, NIR and SWIR) has been shown, through use of radiative transfer models and global sensitivity analysis, to be driven by different leaf or canopy traits (Li and Wang, 2011; Xiao et al., 2014). At the leaf level, use of the PROSPECT model (Jacquemoud and Baret, 1990) shows that global spectral variation is dominated by variation in chlorophyll concentration in the visible region (400 – 699 nm) and the leaf structural parameter (N) in the NIR (700–1299 nm). Although the influence of N is still relevant at certain spectral sub-regions beyond 1300 nm, equivalent water thickness (Cw) becomes the principal contributor to spectral variance throughout the SWIR region (1300– 2500 nm). Similarly, at the canopy scale, the PROSAIL model (Jacquemoud et al., 2009) shows that these spectral regions retain their discrete importance. Variation in reflectance in the visible region is driven by chlorophyll content and by water throughout the SWIR. In contrast to the leaf level, at this scale, spectral variation in the NIR is mainly driven by dry matter content (Cm) and leaf area index (LAI). We hypothesise that, during the growing season, different leaf and canopy traits will be dominant in driving spectral variance and summarising data by these regions will help with interpretation of results.

3.2.4 *Satellite NDVI to contextualise findings*

A time-series of satellite derived NDVI values obtained from the Sentinel-2 mission at 10 m spatial resolution was used to contextualise the findings of the field observations in terms of the main growing season periods: green-up, peak biomass and senescence (Figure 3.2). For each available time-point, cloud free MSI pixels corresponding with site quadrats locations were extracted and a site-specific mean NDVI (and standard error) was calculated. Seven pixels over 31 dates were used to construct the time-series for Dawcombe and five pixels over 19 dates for Silwood. The NDVI time-series were divided into three phenology stages, which we call “Pre-NDVI max” (representing ‘green-up’ of the site vegetation), “NDVI max” (the plateaux of maximum NDVI which we assume to coincide with the vegetation being at maximum growth stage) and “Post-NDVI max” (where vegetation begins to senescence). The period of peak growth (NDVI max) corresponded to 25 days either side of the highest NDVI value, although this value was more difficult to ascertain at Silwood, as the site exhibited cloudy conditions at this time of year. Pre-NDVI max covers the months of March, April, and May (DoY 50 to 150), NDVI max covers June and the first half of July (DoY 150 to 200) and Post-NDVI max covers late July, August, and September (DoY 200 to 300).

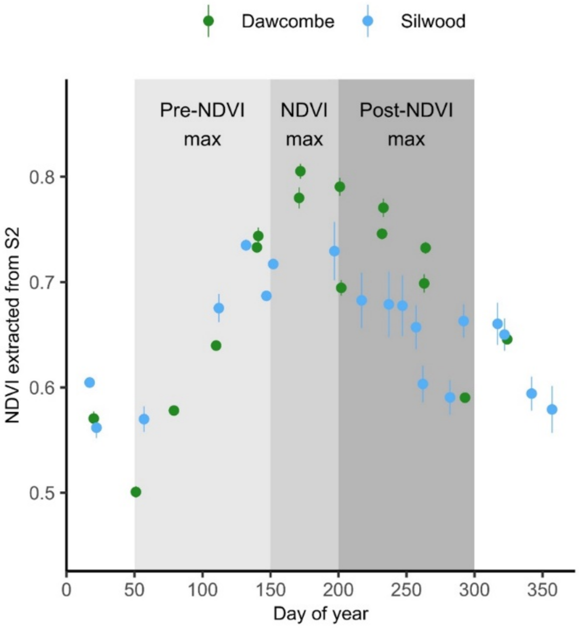


Figure 3.2 The three phenology stages (Pre-NDVI max, NDVI max and post-NDVI max) derived from Sentinel-2 MSI NDVI time-series for two grassland sites.

3.2.5 Statistical analysis of spectral variance and taxonomic and phenological metrics

The key aims of this study are to test the temporal stability of the SVH in relation to taxonomic metrics and to assess the extent to which phenological diversity drives spectral variance. In order to test these hypotheses two types of modelling were carried out. The first consisted of simple linear models which assessed the strength of the relationship between spectral variance and the three taxon, and three phenology based metrics at each sampling event at each site. The second utilised mixed models to evaluate the consistency of these same relationships over all sampling points and across both sites. We also used mixed modelling to investigate the third aim of the work which was to assess the relative impact of taxonomic and phenological diversity, alongside phenological stage dominance on spectral variance over all sampling points.

3.2.5.1 Simple linear models

Simple linear models were used to test the relationship between each narrow band value (the hyperspectral approach), as well as mean-CoV, vis-mean-CoV, NIR- mean-CoV and SWIR-mean-CoV, (the spectral regions approach) and the three species-based and three phenology-based diversity measures. For the spectral regions models, 288 uni-temporal model runs were carried out (Dawcombe: 7 time-points x 6 diversity metrics x 4 spectral regions =168 and Silwood: 5 time-points x 6 diversity metrics x 4 spectral regions = 120). Our data sets are small, when considered for each time point and site, so a permutation modelling approach was applied (LaFleur and Greevy, 2009), where *p* values for each linear model are assessed for stability using imputation, and the resulting adjusted r^2 values are reported.

3.2.5.2 Linear mixed models

One of the challenges associated with the data set collected is its structure, which includes temporal and spatial autocorrelation. Each quadrat was revisited several times so within-quadrat samples could be more similar to each other than to the data from other quadrats. It is also possible that samples taken at similar times of year will be more similar to each other. With this in mind, all data were modelled using a mixed model (Zuur et al., 2009), where the fixed effect is the taxonomic or phenological metric and the random effects, the quadrat and sampling time point (Pinheiro and Bates, 2000). The package *lme4* (Bates et al., 2015) in R was used for the mixed model analysis. The model random effects structure was determined following the procedure outlined in Barr et al. (2013). The model fitting was performed using restricted maximum likelihood (REML) and the most complex random structure that would converge, used sampling event (day of year) and quadrat as random effects, producing an intercept only model with two random terms. Site was added as a fixed effect, because it only has two levels (the recommended minimum number of levels in a random effect is five (Zuur et al., 2009)). Examination of model residuals displayed heteroscedasticity, so spectral variance was converted to the natural log. This brought the residuals into an acceptable distribution. Application to the model residuals of a first order auto-correlation function revealed no significant temporal autocorrelation (Mitchell et al., 2020). We also used mixed modelling to investigate the sources of spectral variance over time and used spectral variance as the response variable. Before modelling, all predictor variables are scaled from - 1 to +1 and centred to allow interaction effects to be suitably assessed. The maximal model, containing the same random effects structure as in the first modelling stage, was fitted by Maximum Likelihood (ML) with all six of the taxonomic and phenology-based community variables and the percentage canopy stages as predictors with interaction terms included. The most parsimonious model, assessed using Akaike Information Criterion and Bayesian Information Criterion, included the terms % Mature leaves (MAT(3)) and species diversity and a term of their interaction. For both stages of mixed modelling, reported coefficients, confidence intervals and *p*-values for fixed effects were obtained by fitting the models using Restricted Maximum Likelihood (REML) and by use of the Swatterwaite post-hoc test. Two pseudo r^2 were calculated to assist with the evaluation of the models: the marginal r^2, which is the fixed effects variance divided by the total variance (fixed + random + residual) and the conditional r^2, which is the fixed and random effects variance divided by the total variance. The marginal r^2 indicates the percentage of the total model variance explained by the fixed effects and the conditional r^2 indicates how much of the model variance is explained by the complete model (Nakagawa and Schielzeth, 2013). These values enable assessment of the relative impact of the spatial (quadrat) and temporal (sampling time) grouping variables and the fixed-effect predictor variables.

3.3 Results

3.3.1 Plant taxonomic and phenological diversity between sites and over time

The two sites were very distinct in terms of their species and phenology-based community composition (Figure 3.3a). Throughout the season, relatively speaking, species richness is low to medium at Silwood (1–10 species) and medium to high at Dawcombe (9–24 species). Dawcombe shows very high levels of quadrat evenness (0.4–0.6) in all quadrats at all times, meaning there is no single dominant species. Silwood displays a range of quadrat evenness from 1.0 (only one species present – so completely even) to levels comparable with Dawcombe for more uneven quadrats (0.4). In terms of phenological richness, Dawcombe shows higher values, partly reflecting the fact that the site has more species, so is more likely to have many phenological stages occurring at one time. Results for phenological evenness concur with species evenness, with Silwood having more phenologically homogenous swards compared to Dawcombe. All quadrats at Silwood have low phenological diversity, whereas at Dawcombe there is a large spread in the values of this metric with some quadrats displaying different species' specific phenological states simultaneously. In terms of seasonal patterns (Figure 3.3b), at Dawcombe phenological richness and diversity follow species richness and diversity, with a sharp build up at pre-NDVI max stage followed by a peak at around DoY 150, and then a decline into post-NDVI max. Both species and phenology diversity metrics at Silwood, the species poor site, peak slightly later than at Dawcombe, and less strongly, coinciding more with NDVI-max. These results suggest that if we want to capture the full extent of species and phenological diversity we should sample just before and during NDVI-max. We speculate that both spring and summer emerging species are occurring simultaneously at this time, thus maximising measures of both species and phenological diversity.

When comparing the community metrics for each site using pair-wise correlation (Figure 3.4), high intra-site positive correlation between species richness and species diversity can be observed (Pearson's correlation co-efficient of 0.83 at Dawcombe and 0.93 at Silwood). The strength of the pairwise correlations between the two types of community metrics (species and phenology based) is generally greater at Silwood than at Dawcombe. This result indicates that at the species poor site (Silwood), phenological traits of the community over the whole season are more closely aligned to species community traits and in contrast, at the more species rich site (Dawcombe), phenological and species traits are more divergent.

Phenological stage dominance was determined by use of the seven phenological stage definitions and weighted percentages of total canopy coverage per stage per quadrat were calculated (Figure 3.5). YOU(2) (young material) dominated the swards at both sites in early spring sampling (DoY 108 and 121 at Dawcombe and DoY 119 at Silwood). By DoY 156 at Silwood no new material was emerging, except in very small amounts in three quadrats (LUFN and LUFNone and LUFP). In contrast, at Dawcombe, young material was still emerging in all quadrats up to DoY 194 and 205, towards the end of the phenology period NDVI-max. YOU (2) material was absent in all quadrats during the last two sampling points (DoY 220 and 232). Peak MAT(3) was reached on DoY 151 at Dawcombe and occurred at the cusp of the two satellite derived phenology stages (pre-NDVI-max and NDVI-max). At Silwood, peak MAT(3) was recorded at DoY 171, well into the NDVI-max satellite period. The percentage of the sward in stages BUD(4), FLO(5) or SEE(6) (bud, flowering, or seed respectively) was very variable between quadrats at any one time.

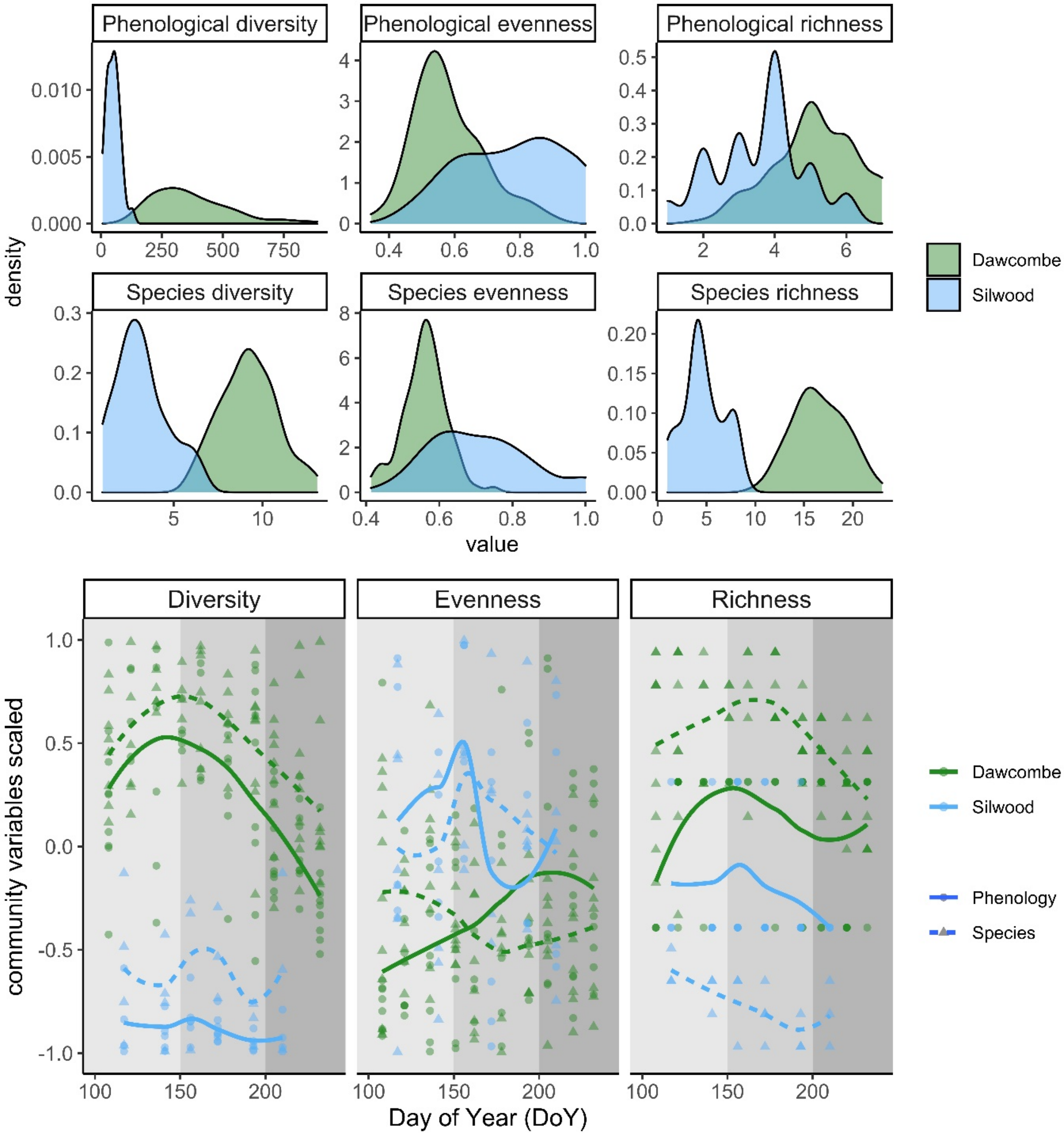
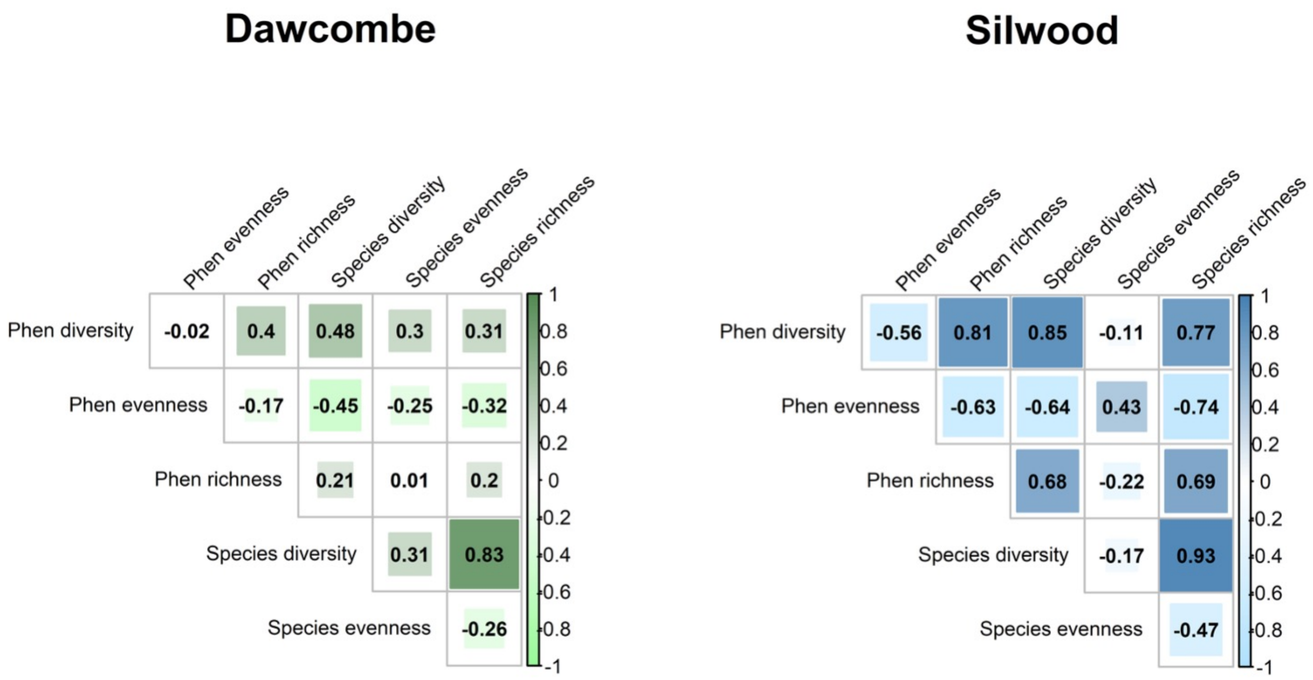


Figure 3.3 a (top): Density distributions of the community metrics across the two sites for all times. b: (bottom): Community metrics over time at the two sites. All metrics have been scaled so they can be displayed together, and the inter-site differences can be emphasised. A lowess smoother has been applied to emphasise any seasonal data trends.



Dawcombe

	Phen evenness	Phen richness	Species diversity	Species evenness	Species richness
Phen diversity	-0.02	0.4	0.48	0.3	0.31
Phen evenness		-0.17	-0.45	-0.25	-0.32
Phen richness			0.21	0.01	0.2
Species diversity				0.31	0.83
Species evenness					-0.26

Silwood

	Phen evenness	Phen richness	Species diversity	Species evenness	Species richness
Phen diversity	-0.56	0.81	0.85	-0.11	0.77
Phen evenness		-0.63	-0.64	0.43	-0.74
Phen richness			0.68	-0.22	0.69
Species diversity				-0.17	0.93
Species evenness					-0.47

Figure 3.4 Correlation heat map of the taxonomic (Species diversity, evenness, and richness) and phenology (Phenological diversity, evenness, and richness) metrics over all sampling times. Pearson's correlation coefficients are shown. Light colours indicate a negative correlation; dark colours a positive correlation.

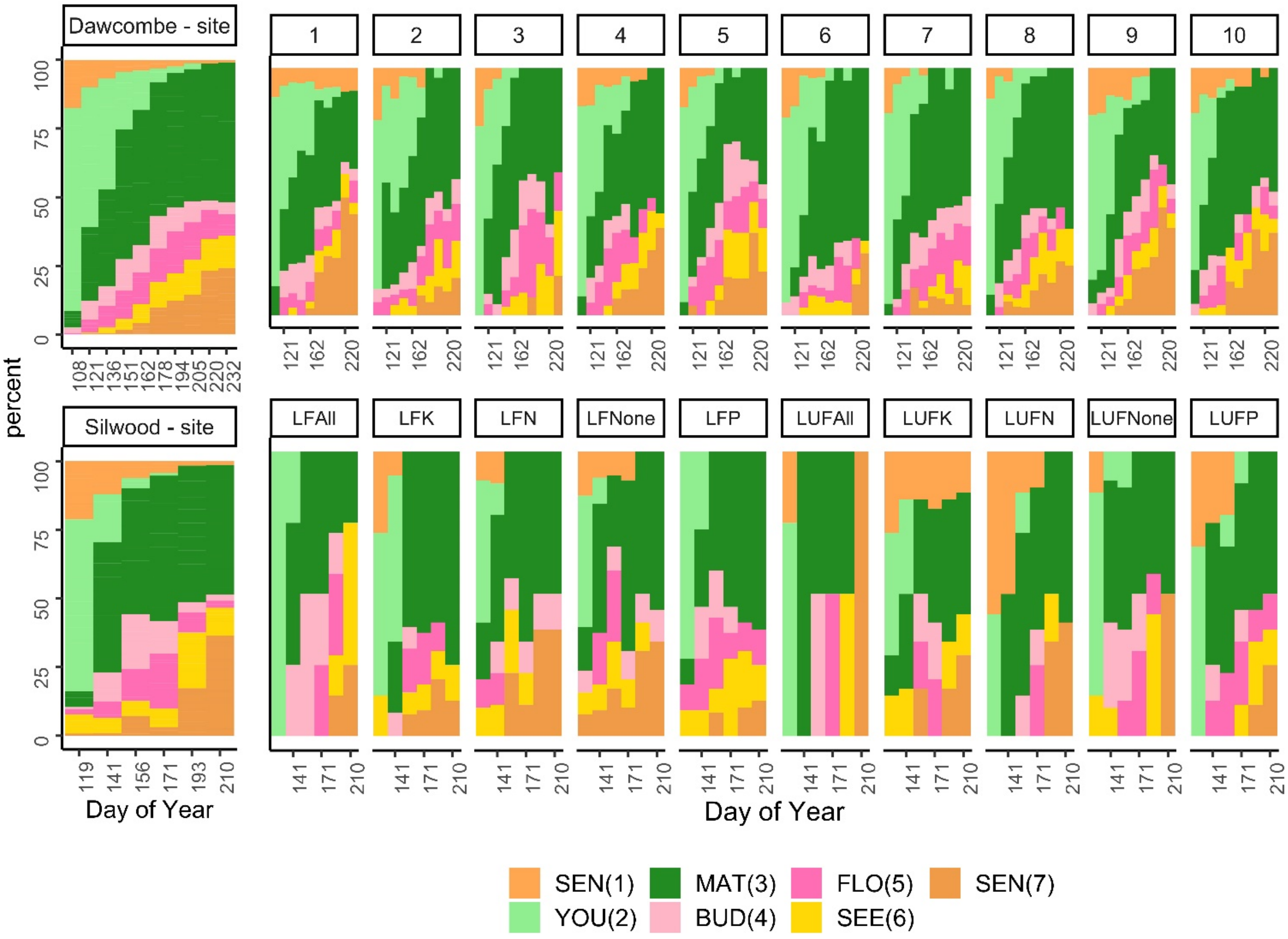


Figure 3.5 The percentage of the canopy dominated by each of the seven documented phenological stages at each time point over the growing season at the site and the quadrat level.

3.3.2 Spectral CoV over time

Mean reflectance values per quadrat, per sampling time are shown in Figures 3.6a and 3.6b alongside changes in the spectral variance for each wavelength. Mean reflectance for some quadrats (quadrat 5 at Dawcombe and quadrat LUFP at Silwood for example) remained very stable throughout the season whereas other quadrats displayed clear seasonal shifts (quadrat 4 at Dawcombe and quadrat LUFAll at Silwood). The largest magnitude in changes is observed in the NIR part of the spectrum. Seasonal patterns in CoV also changed dramatically in some quadrats but not in others. The temporal change in spectral variability were evaluated by the slope of a linear regression model CoV = f(DoY) for each quadrat (See Supplementary Material B, Table B1). Within quadrat rates of change were not very different between the spectral regions and the extent of change was principally a cross spectra phenomenon, therefore, only the mean-CoV is reported here. At Dawcombe quadrats 2, 3, 5 and 8 remained stable in time (model slope close to zero) whereas quadrats 1, 4, 6, 7, 9, and 10 increased over time (model slopes > +0.1, the fastest changing quadrat was quadrat 6 at +0.34). At Silwood, quadrats LFK and LUFK were stable, whereas all other quadrats at this site increased in spectral variability as the season progressed (with the maximum rate of change found at quadrat LFNone, model slope + 0.47). At the site level, mean-CoV followed the same overall trajectory at both sites, starting at a low level and increasing as the season progressed through pre-NDVI max and NDVI max, with the highest values occurring in late summer during post-NDVI max (Figures 3.6 and 3.7). The extent to which the CoV changed over time is expressed as the slope of a linear regression model (Dawcombe $r^2 = 0.18$, $p = 0.00021$ (2sf), slope = +0.08 and Silwood $r^2 = 0.20$, $p = 0.000069$, slope = +0.15). The rate of change was slightly higher at Silwood in all spectral regions compared to Dawcombe.

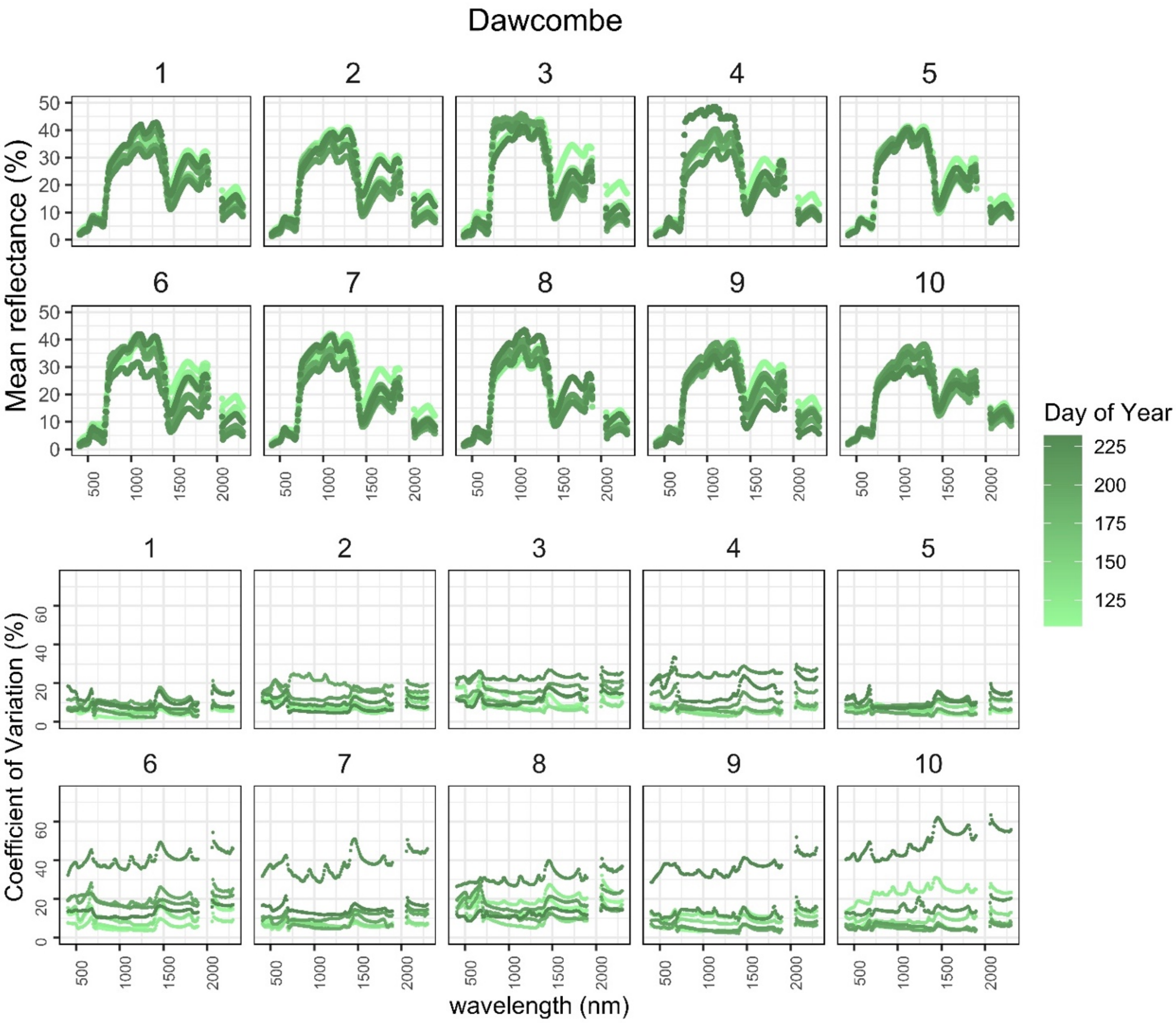
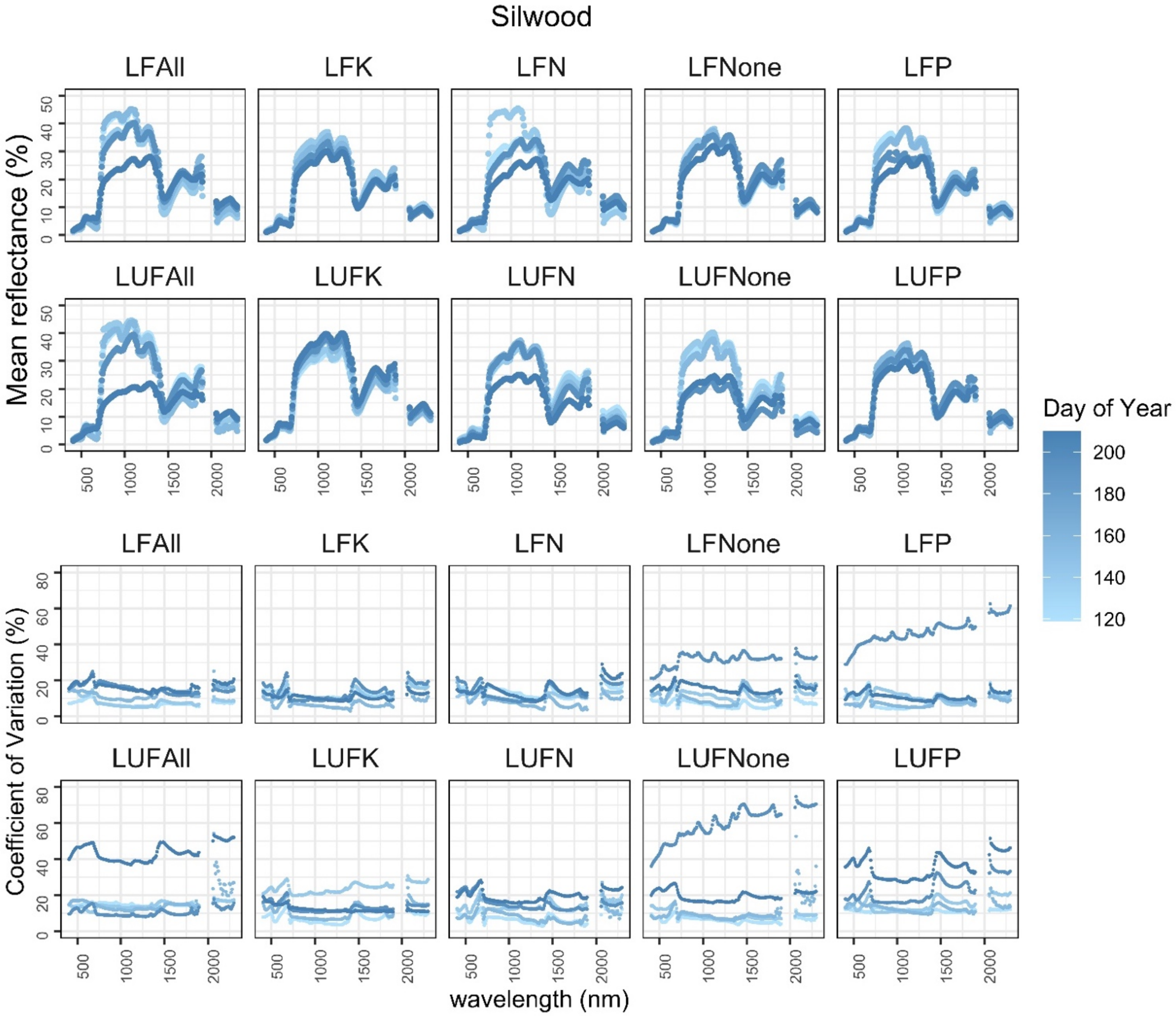


Figure 3.6 a: Mean spectral reflectance and Coefficient of Variation (CoV) per quadrat per wavelength over the sampling days from the quadrat-based spectrometry data for Dawcombe. b: Mean spectral reflectance and Coefficient of Variation (CoV) per quadrat per wavelength over the sampling days from the quadrat-based spectrometry data for Silwood.


Silwood
LFAll
LFK
LFN
LFNone
LFP
LUFAll
LUFK
LUFN
LUFNone
LUFP
Mean reflectance (%)
0
10
20
30
40
50
500
1000
1500
2000
Day of Year
200
180
160
140
120
Coefficient of Variation (%)
0
20
40
60
80
wavelength (nm)

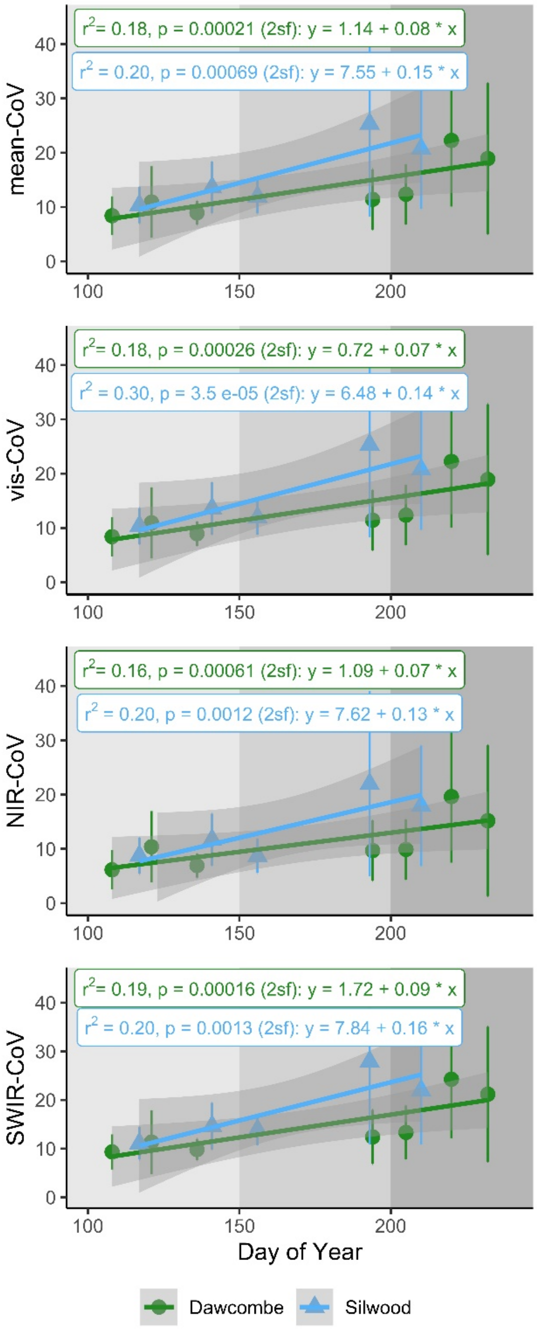


Figure 3.7 Mean-CoV and spectral-regions-CoV over time, with linear model results describing the temporal increase at the site level.

3.3.3 The relationship between spectral diversity (CoV) and taxonomic and phenological diversity using linear permutation models

3.3.3.1 The spectral regions approach

The strength of the relationship between spectral variance averaged across spectral regions and each of the six uni-temporal plant community metrics (species richness, species evenness, species diversity, phenological richness, phenological evenness and phenological diversity) was very variable across time (Figure 3.8), indicating that at the quadrat level spectral variance does not track changes in these metrics over a season. Values of adjusted r^2 for 209 out of 288 of the models were less than 0.1, meaning that at the majority of sampling points and for most community variables very little variation, if any, was explained by the metrics. Twenty-five out of 288 of the models were significant at $p < 0.05$. In eight of these models, mean-CoV was the predictor variable, in four, vis-mean-CoV, in seven, NIR-mean-CoV and in six, SWIR-mean- CoV. Three of the significant models predicted well values of phenological diversity, six phenological evenness, five phenological richness, six species diversity, one species evenness and four species richness (see Table 3.4). The sampling times when spectral variance best predicted taxonomic diversity (highest r^2 values and significant models) was at the end of pre-NDVI max (DoY 136) and post-NDVI-max (DoY 220 and 232) for Dawcombe and during NDVI-max (DoY 156) for Silwood. The stability of the model r^2 also depended on the level of data cleaning imposed by the alpha parameter in the ROBPCA (Supplementary Material, Section A Figure A1 and A2). At some time points, model r^2 steadily increased with more robust data cleaning. For example, at Dawcombe, Phenological diversity at DoY 194, during NDVI-max and Phenological evenness and diversity at DoY 136, during pre-NDVI-max, displayed this behaviour. Other model r^2 values remained constant, despite the level of data cleaning, for example for species evenness and species diversity at DoY 108. These results suggests that, at times, the quadrat level CoV can depend on a few reflectance outliers caused by, for example, bare soil, or a single plant dominating a sub quadrat or measurement errors such as un-intended off-nadir viewing. Our confidence in the SVH should increase with models that remain stable or improve in fit with data cleaning.

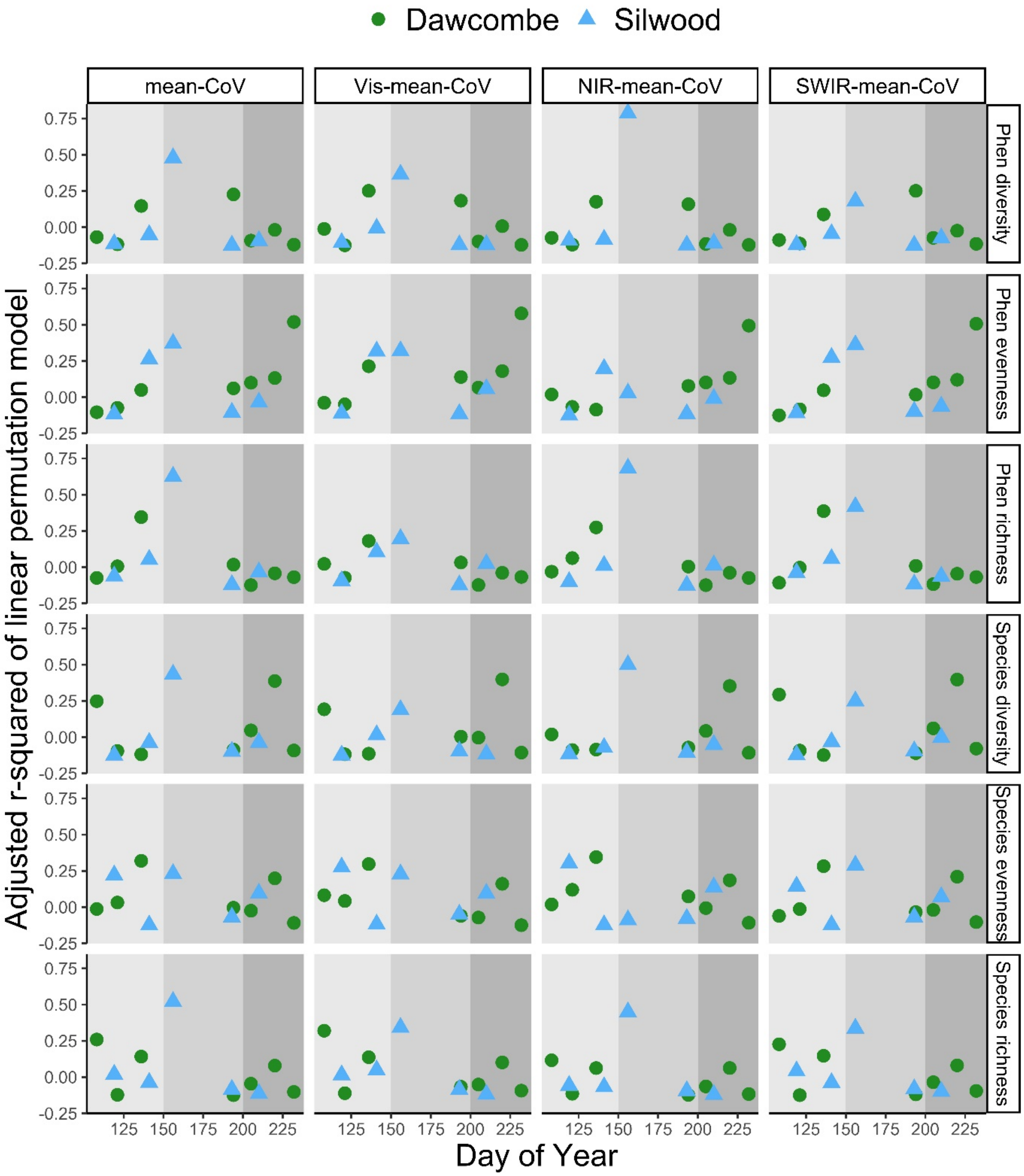


Figure 3.8 Adjusted r^2 of the simple uni-temporal linear permutation models (spectral variance = f(diversity metric) using the spectral regions approach.

Table 3.4 Results of the significant uni-temporal permutation models at $p < 0.05$. Results that do not support the SVH are highlighted in grey.

Site	Time Point	DoY	Satellite derived phenology stage	Community metric	Spectral variable	(Coefficient) Intercept	(Coefficient) Slope	adjusted r^2	p value
Dawcombe	**3**	136	Pre-NDVI-max	Species evenness	NIR-mean-CoV	8.652	**-13.775**	**0.345**	**0.043**
Dawcombe	**9**	220	Post-NDVI-max	Species diversity	mean-CoV	31.025	**7.716**	**0.387**	**0.032**
Dawcombe	**9**	220	Post-NDVI-max	Species diversity	NIR-mean-CoV	28.028	**6.770**	**0.353**	**0.041**
Dawcombe	**9**	220	Post-NDVI-max	Species diversity	SWIR-mean-CoV	33.774	**8.797**	**0.397**	**0.030**
Dawcombe	**9**	220	Post-NDVI-max	Species diversity	vis-mean-CoV	27.855	**6.006**	**0.398**	**0.030**
Dawcombe	**3**	136	Pre-NDVI-max	Phenological richness	mean-CoV	11.055	**2.129**	**0.345**	**0.043**
Dawcombe	**3**	136	Pre-NDVI-max	Phenological richness	SWIR-mean-CoV	12.307	**2.676**	**0.387**	**0.032**
Dawcombe	**10**	232	Post-NDVI-max	Phenological evenness	mean-CoV	27.107	**-129.600**	**0.519**	**0.011**
Dawcombe	**10**	232	Post-NDVI-max	Phenological evenness	NIR-mean-CoV	23.407	**-115.058**	**0.493**	**0.014**
Dawcombe	**10**	232	Post-NDVI-max	Phenological evenness	SWIR-mean-CoV	29.626	**-144.397**	**0.507**	**0.013**
Dawcombe	**10**	232	Post-NDVI-max	Phenological evenness	vis-mean-CoV	26.114	**-109.356**	**0.578**	**0.006**
Silwood	**3**	156	NDVI-max	Species richness	mean-CoV	13.237	**-0.803**	**0.521**	**0.011**
Silwood	**3**	156	NDVI-max	Species richness	NIR-mean-CoV	9.453	**-0.712**	**0.449**	**0.020**
Silwood	**3**	156	NDVI-max	Species richness	SWIR-mean-CoV	15.341	**-0.892**	**0.337**	**0.046**
Silwood	**3**	156	NDVI-max	Species richness	vis-mean-CoV	13.791	**-0.688**	**0.342**	**0.044**
Silwood	**3**	156	NDVI-max	Species diversity	mean-CoV	13.237	**-1.029**	**0.433**	**0.023**

Silwood	**3**	156	NDVI-max	Species diversity	NIR-mean-CoV	9.453	**-1.024**	**0.499**	**0.013**
Silwood	**3**	156	NDVI-max	Phenological richness	mean-CoV	13.237	**-1.528**	**0.626**	**0.004**
Silwood	**3**	156	NDVI-max	Phenological richness	NIR-mean-CoV	9.453	**-1.490**	**0.682**	**0.002**
Silwood	**3**	156	NDVI-max	Phenological richness	SWIR-mean-CoV	15.341	**-1.707**	**0.418**	**0.026**
Silwood	**3**	156	NDVI-max	Phenological evenness	mean-CoV	13.237	**9.911**	**0.371**	**0.036**
Silwood	**3**	156	NDVI-max	Phenological evenness	SWIR-means-CoV	15.341	**12.890**	**0.360**	**0.039**
Silwood	**3**	156	NDVI-max	Phenological diversity	mean-CoV	13.237	**-0.051**	**0.478**	**0.016**
Silwood	**3**	156	NDVI-max	Phenological diversity	NIR-mean-CoV	9.453	**-0.059**	**0.787**	**0.000**
Silwood	**3**	156	NDVI-max	Phenological diversity	vis-mean-CoV	13.791	**-0.046**	**0.365**	**0.038**

3.3.3.2 The hyperspectral approach

The linear permutation models were also applied to spectral variance at the hyperspectral level. The adjusted r^2 of these models is reported in Figure 3.9. At Dawcombe at DoY 220 and 232, the whole of the spectrum displayed strong correlations to the metrics, however, at DoY 136 only narrow regions of the spectrum were correlated. Examination of the model fits from the hyperspectral approach demonstrated that the spectral regions approach was largely effective at picking up the best sampling times and metrics of interest.

3.*3.4 Mixed models: relationships between variables over time*

All the spectral data, summarised as spectral regions (the spectral regions approach), was included in a series of mixed models, allowing for temporal and spatial pseudo-replication. In the first stage of mixed modelling, which tested the ability of the CoV of spectral variance to predict taxonomic or phenological metrics over all sampling times and both sites, none of the models contained significant terms (see Supplementary Material C, Table C1). A large amount of variance in these models was explained by the random terms. Values of the Intra-class Correlation Coefficient (ICC) (the ratio of the between group variance to the total variance) (Nakagawa et al., 2017) ranged from 0.32–0.43 (these are considered high values and validify the use of the mixed model approach). The random term, quadrat, had a much smaller impact on the model, with estimates of around 10% that of sampling-time. These results further support the results from the uni-temporal models, that the strength of the relationship between spectral variance and these metrics is heavily time dependent. During the second stage of mixed modelling, differing interaction effects of percent phenology stage dominance (SEN(1), YOU(2), MAT (3), BUD(4), FLO(5), SEE(6), SEN(7)) and taxon and phenology-based community metrics on spectral variance were tested. A significant effect of MAT(3) mature stage (slope = 0.19, p = 0.003) alongside a significant interaction effect of MAT(3) and species diversity (slope = 0.12, p = 0.014) was found for mean-CoV (Figure 3.10) with similar results for the other spectral regions (see Supplementary Material C, Table C2 for full model results). NIR was the spectral region with the highest marginal r^2, with around 25% of the variance explained by the fixed terms, and an effect size of 0.25 for the mature term and 0.15 for the interaction term mature and species diversity. The model using vis-mean-CoV as the response variable displayed the largest values of conditional r^2 with 43% variance explained, 16% of which was explained by the fixed terms.

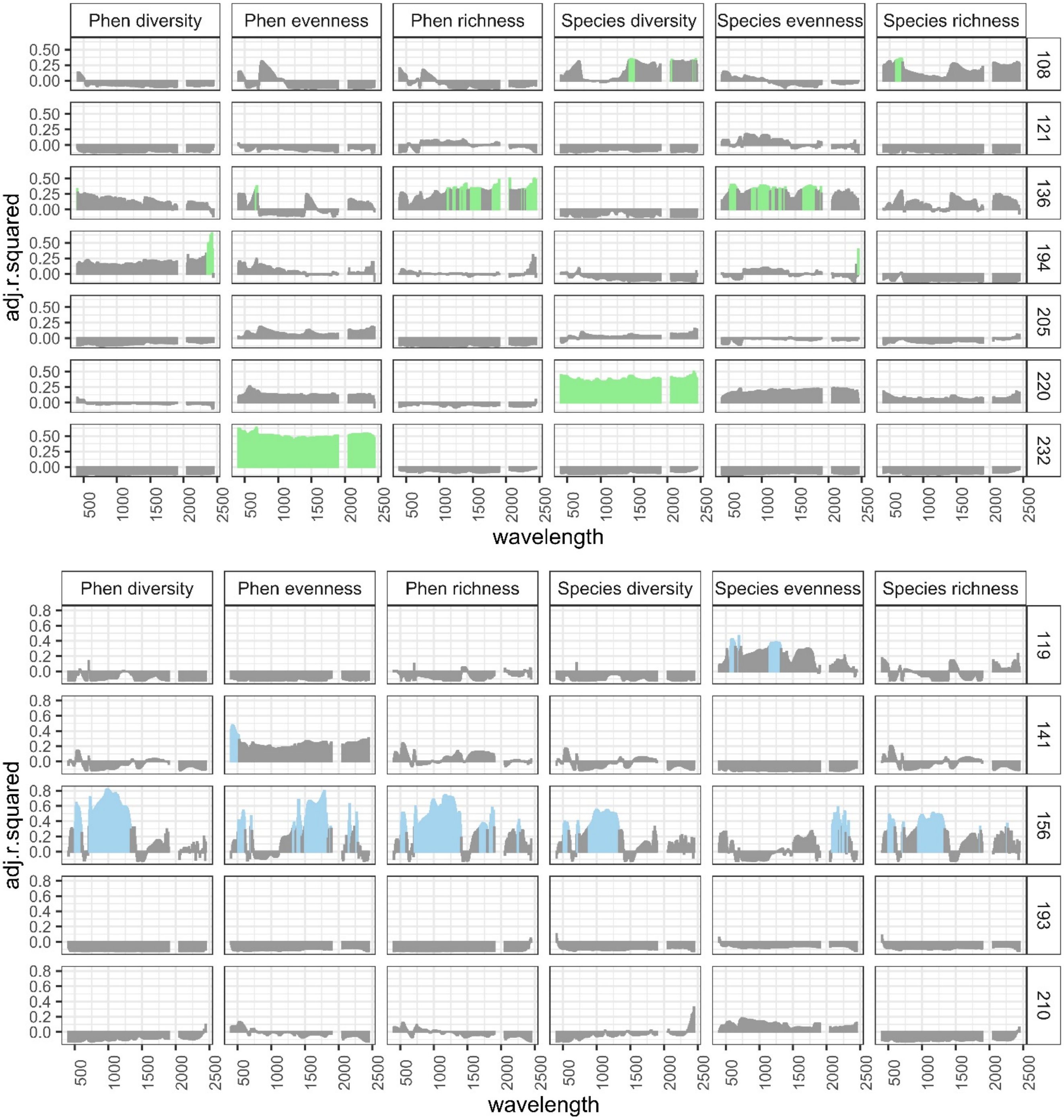


Figure 3.9 Adjusted r^2 of the simple uni-temporal linear permutation models (spectral variance = f(metric) using the hyperspectral approach. Significant wavelengths are shown in colour; non-significant in grey.

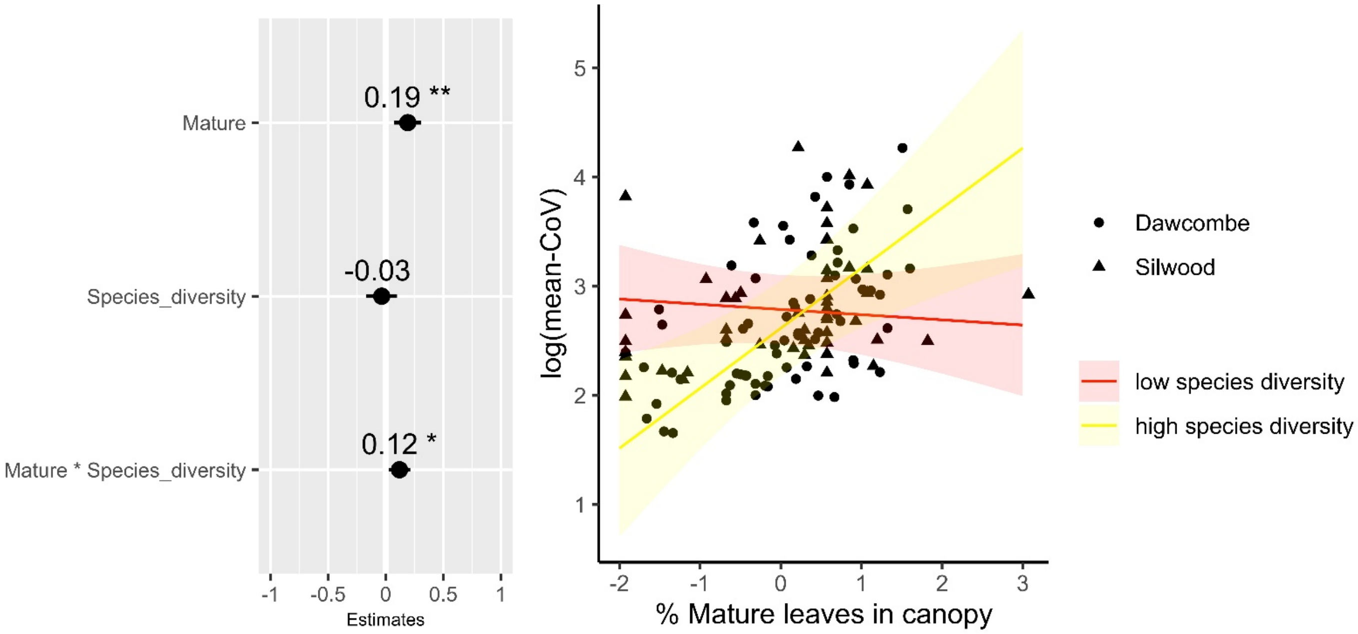


Figure 3.10 Left: Forest plot showing the standardised effect sizes of the fixed terms in the mixed model; percent mature leaves (MAT(3)) species diversity and their interaction term. Right: Significant interaction effect of MAT(3) and species diversity on mean spectral variance. Prediction lines with confidence intervals show values of species diversity at extremes of the data set (Dt = 1 and 13). MAT(3) is scaled with values ranging from - 2 = 0% and + 3 = 100%.

3.4 Discussion

3.4.1 Relationships between spectral variance and taxonomic and phenology metrics over time

The uni-temporal models at the site level were able to predict gradients of both taxonomic and phenology-based community metrics. However, the predictive ability of the models varied over time indicating that tracking these metrics across a growing season using spectral variation is problematic. The highest correlations between spectral and community metrics tended towards late pre-NDVI-max and early NDVI-max at both sites, suggesting that late spring (around DoY 150) is optimal for estimation of taxonomic and phenological traits in these grassland systems. These dates coincided with maximum species and phenological diversity at both sites. Late summer sampling (DoY 220 and 232 during post-NDVI-max) also proved productive at Dawcombe, although data was not collected on comparable dates for Silwood due to the site management regime. Using the mixed model approach, we found that none of the six metrics displayed a consistent relationship to spectral variance over time, further confirming that there is a temporal dependence in the relationship. However, at the low species diversity site, Silwood, the best models (DoY 156) consistently predicted a negative relationship between the metrics and spectral variance, for five out of six of the metrics (none of the models predicted well Species Evenness). At Dawcombe, regardless of the sampling time, for the best models, there was always a positive relationship between spectral variance and the metrics; a result that supports the SVH (note that species and phenological evenness should under the hypothesis display a negative relationship, as low measures of evenness represent more varied communities). We proposed that grassland community phenological dynamics could be responsible for some of the variation in spectral variance. We tested this hypothesis using our own phenological metrics and looked for interactions between these metrics and the species-based metrics in a mixed modelling approach. However, we found no significant interaction terms, implying that phenology-based spectral signals are not operating systematically across the growing season either to detract from species-based signals or to enhance them. When considering the overall trend in spectral variance between sites, despite Silwood and Dawcombe displaying low and high taxonomic and phenological diversity, respectively, the mean-CoV values at the site level were marginally higher at Silwood (Figure 3.7) clearly demonstrating that, in this instance, the site with the higher diversity did not have a higher spectral variance.

3.4.2 Taxonomic and phenological dynamics between sites

The trends in taxonomic and phenology metrics show how grasslands can display diverse temporal dynamics in terms of the seasonal development of their community composition which may have effects on our ability to monitor them using remote sensing techniques and the SVH. Judging from these results if we want to capture the full extent of taxonomic and phenological diversity we should sample just before and during NDVI-max. We speculate that both spring and summer emerging species are occurring simultaneously at this time, thus maximising measures of both species and phenological diversity. We observed that at Silwood the phenology and taxonomic metrics were more strongly correlated across the season than at Dawcombe (Figure 3.4). This suggests that at Silwood phenological diversity follows seasonal species turnover whereas at Dawcombe there is a more complex relationship. This complexity could be a direct result of the higher species diversity of the site or the type of species present. It could also reflect other phenomena such as assortative mating and the development of discrete sub-populations that over time become reproductively isolated (Elzinga et al., 2007).

3.*4.3 Mature leaves as drivers of spectral variance*

Across the growing season, at the site level an increase in spectral variance was observed, which was found to be independent of the taxonomic and phenological based metrics. Rather spectral variance was found to be partly driven by the occurrence of plant parts in MAT(3) phenology stage. There are different possible interpretations of this result. Firstly, mature leaves of plants could, in fact, be more spectrally variable than other leaf growth stages. Another perspective is that when plants are in their mature stages canopy structural attributes contribute to spectral variance through self-shading. This problem is difficult to eliminate in mixed 'pixel' situations, but if the pixel sizes were small enough (i.e., those obtained through drone acquisition), this problem could be reduced through removal of low NDVI 'shade' pixels, in a similar way to soil correction techniques (Gholizadeh et al., 2018). Additionally, as canopies develop over time, they could become more spectrally variable due to vertical complexity (Conti et al., 2021). The mixed model with the highest explained variance by the fixed terms (MAT(3) and species diversity) was in the NIR spectral region. This suggests that canopy traits such as LAI and leaf angle distribution could be influential. Irrespective of the ultimate driver of spectral variance associated with the presence of mature leaves, the observed underlying seasonal increase in spectral variance needs to be taken into account when sampling across dates for the purposes of taxonomic diversity evaluation as it will likely confound the desired signal. The observed interaction effect of species diversity and mature leaves implies that the usefulness of spectral variance as a surrogate for species diversity is dependent on the extent to which plant species are synchronous in terms of their display of mature leaves.

3.4.4 Issues of scale in estimations of species diversity

One of the major challenges in testing the SVH is that the strength of findings may rely on both the temporal *and* spatial scale of the observations. Here we investigated whether the species diversity of small grassland plots could be predicted using very high resolution (10 cm^2) simulated pixels. A similar study in grasslands showed significant correlations between spectral variance and species diversity at single points in time (Wang et al., 2018) and demonstrated that spectral variance calculated using the smallest pixels (1mm^2) had the strongest relationship to taxonomic-based metrics, with the relationship declining as pixel size increased and 10 cm^2 pixels being the largest size at which the relationship held. A possible explanation for this decline in the relationship with increased scale is that species diversity metrics per quadrat may not be well aligned to the spectral data. Consider the situation where one quadrat has many species, but they are small and evenly distributed throughout the quadrat. This situation is typical of the quadrats at Dawcombe in the species rich calcareous grassland. The spectral diversity of this quadrat at 10 × 10 cm pixel resolution could be very low, as each of the pixels are very similar. Compare this to a quadrat with only two species that are distributed in clumps and spectrally dissimilar. In contrast the spectral diversity of this quadrat could be high. To adequately assess community complexity using reflectance data our plant diversity metrics need to be robust in light of this type of dilemma with consideration given to the appropriate pixel size scaling to the community at hand. We tested the SVH using both the spectral regions approach and the hyperspectral approach. In this instance, the models fitted using the spectral variance of very narrow wavelengths did not perform better, or provide more insight, than using broad spectral bands (regions) in terms of the timing of sampling nor the taxonomic or phenological metrics. Wang et al., 2018 also showed that summary measures of variance taken across the spectrum were sufficient to predict species diversity. However, other previous studies have demonstrated that species discrimination is possible only by small differences in reflectance in narrow bands (Kokaly et al., 2003; Schmidt and Skidmore, 2003). These results suggest that high spectral resolution data may be less important for diversity studies than for detecting species classes.

3.4.5 Challenges and further study: other sources of spectral variance in grasslands

Diversity in temperate grasslands has been shown to be a product of structural lack of species dominance in the canopy and light ‘sharing’ (Borer et al., 2014; Pulungan et al., 2019). Diverse grasslands by consequence have sparser canopies, are lower in absolute biomass and are usually found in soils lower in nutrients (which determines the absence of nitrophiles, that tend to dominate the canopy) (Crawley et al., 2005; Silvertown et al., 2006). Grasslands that follow this definition may be detectable by virtue of their canopy structural parameters such as height and LAI (Stenzel et al., 2017). It is possible that the negative relationship between spectral variance and the diversity metrics at Silwood is linked to these variables. Self-shading or vertical complexity as a source of spectral variation in high biomass swards could be additional sources of variation at this small scale. At this site, high spectral variance was found in high fertilizer addition plots with single species (LUFAll at DoY 210) alongside a large seasonal growth in CoV (model slope 0.23–0.29 depending on spectral region, see supplementary material table B1). In this instance, we could say that high levels of intra-specific spectral variation are displayed as this change is not associated with changes in species composition. The principal challenge in interpreting the results of this study is that we don’t know the relative importance of leaf and canopy traits in driving spectral variance over time. A future option would be to monitor biomass variation both between sampling points and within a sampling unit. It is obviously impossible to monitor changes in biomass within a quadrat using destructive sampling techniques. However, biomass models using non-destructive measures of LAI and NDVI, in partnership with radiative transfer modelling, have been shown to provide reasonably accurate time-series of fluctuations (Punalekar et al., 2018). Some traits could therefore be simulated from spectral data. Future studies into the relationship between spectral variance and diversity metrics should attempt to incorporate at least some other leaf and canopy traits. Temporal variability in the relationship between floristic patterns and spectral response in grasslands have been demonstrated in other studies using multi-temporal hyperspectral sampling and the physical model, PROSAIL (Feilhauer et al., 2017). In this case, the driver of spectral variability was found to be local resource stressors (i.e., leaf dehydration) and had little to do with changes in the actual canopy composition. In other multi-temporal studies, seasonal burning of the sward was proposed to be responsible for the failure of spectral variance to predict species diversity in some years (Gholizadeh et al., 2020). Large scale disturbance events could be associated with a re-setting of phenological niche partitioning that drives phenological diversity causing the relationship between spectral variance and plant community diversity to break down. The observation that the amount of data cleaning changed the strength of the relationship between spectral variance and the taxonomic and phenology metrics also deserves further investigation. We may expect that in the early part of the growing season bare soil may be present in certain sub-quadrats. By recording total vegetation cover per plot it would be possible to infer if reflectance measurements were being affected by the present of bare ground. Later in the season, some plants with erect growth forms could cast shadow on other plants that display a more recumbent habit. Alongside erroneous data, these are the kinds of spectra that require filtering from the dataset. Ensuring the correct level of data cleaning and the most appropriate methods remain significant challenges.

3.5 Conclusion

Results of this study suggest that spatial variability in reflectance fails to hold across space and time as a predictor of species diversity in grasslands. It appears that at a single point in time stochastic combinations of species and/or phenological traits of canopies can drive spectral diversity. This may explain the instability of previous studies that examine similar questions. We observe that for these grasslands the canopy stage MAT(3) is positively correlated with canopy spectral variance over the season and that if this canopy stage is accounted for there may be an opportunity to predict well species diversity using these data. The full reasons for these observations remain unclear and we highlight the need for simultaneous collection of some leaf and canopy traits in future similar studies to help determine the cause. The fact that species and phenological properties of canopies were comparably estimated in the uni-temporal models suggests that spectral variance may be *at least as* suitable for looking at phenological properties as taxonomic ones. Establishing a link between spectral variance and phenological patterning of grassland communities would be an important addition to the study of plant phenology and conservation biology (Morellato et al., 2016) as well as furthering our understanding of the effects of climate change on species phenological partitioning. Under current knowledge, application of the SVH to within-site monitoring of taxonomic diversity should be approached with caution. More studies are required that incorporate multiple sampling dates, at differing spatial scales, to determine if the relationship is stable enough to be useful in ecological evaluations. However, verifying the results of this study by expanding the geographical extent of detailed multi-temporal studies will remain a significant challenge due to the time-consuming nature of repeat botanical and spectral sampling at a gradient of spatial resolutions.

3.6 Supplementary Materials

Supplementary Material A: The change in the sample sizes depending on the application of the ROBPCA

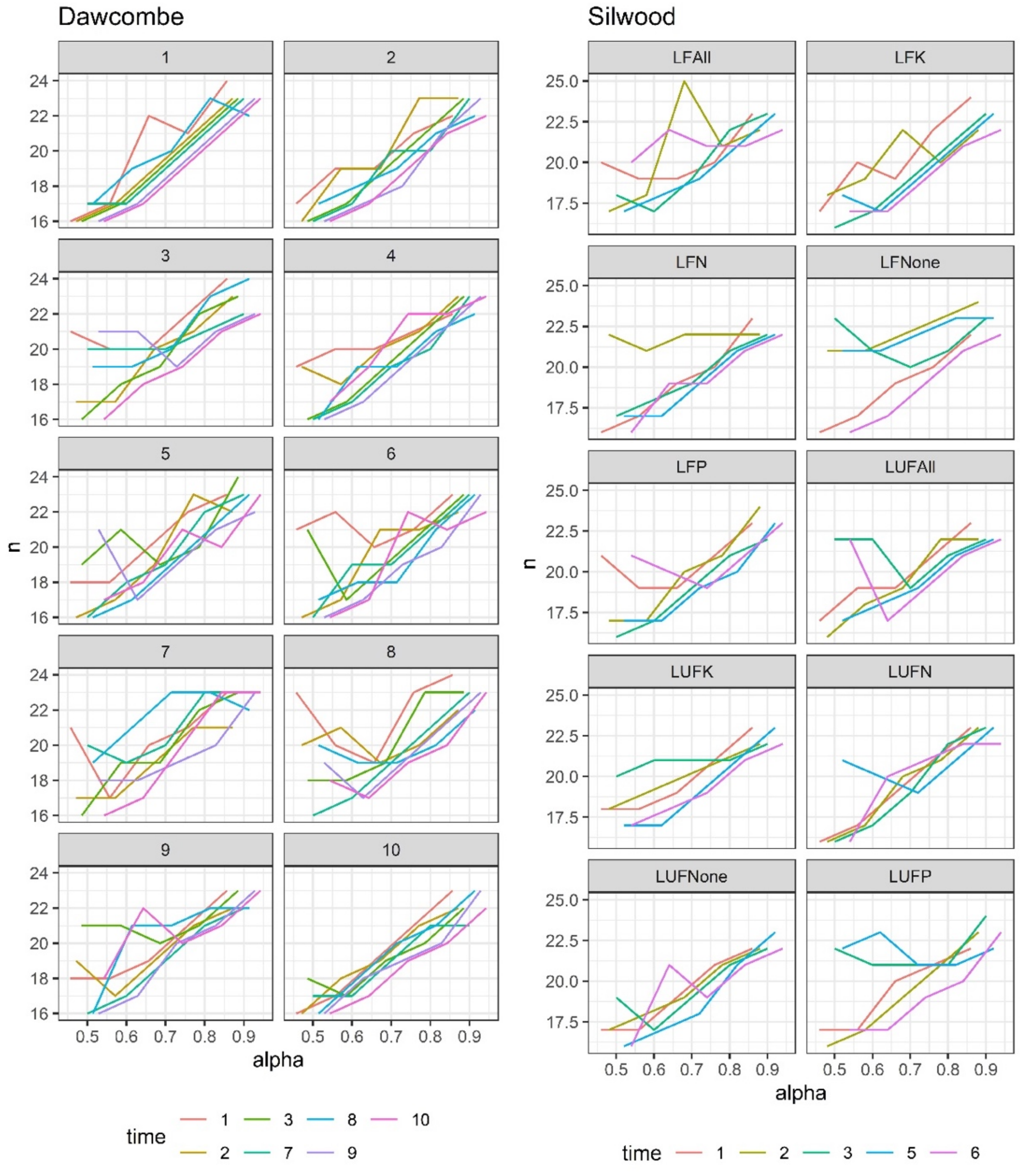
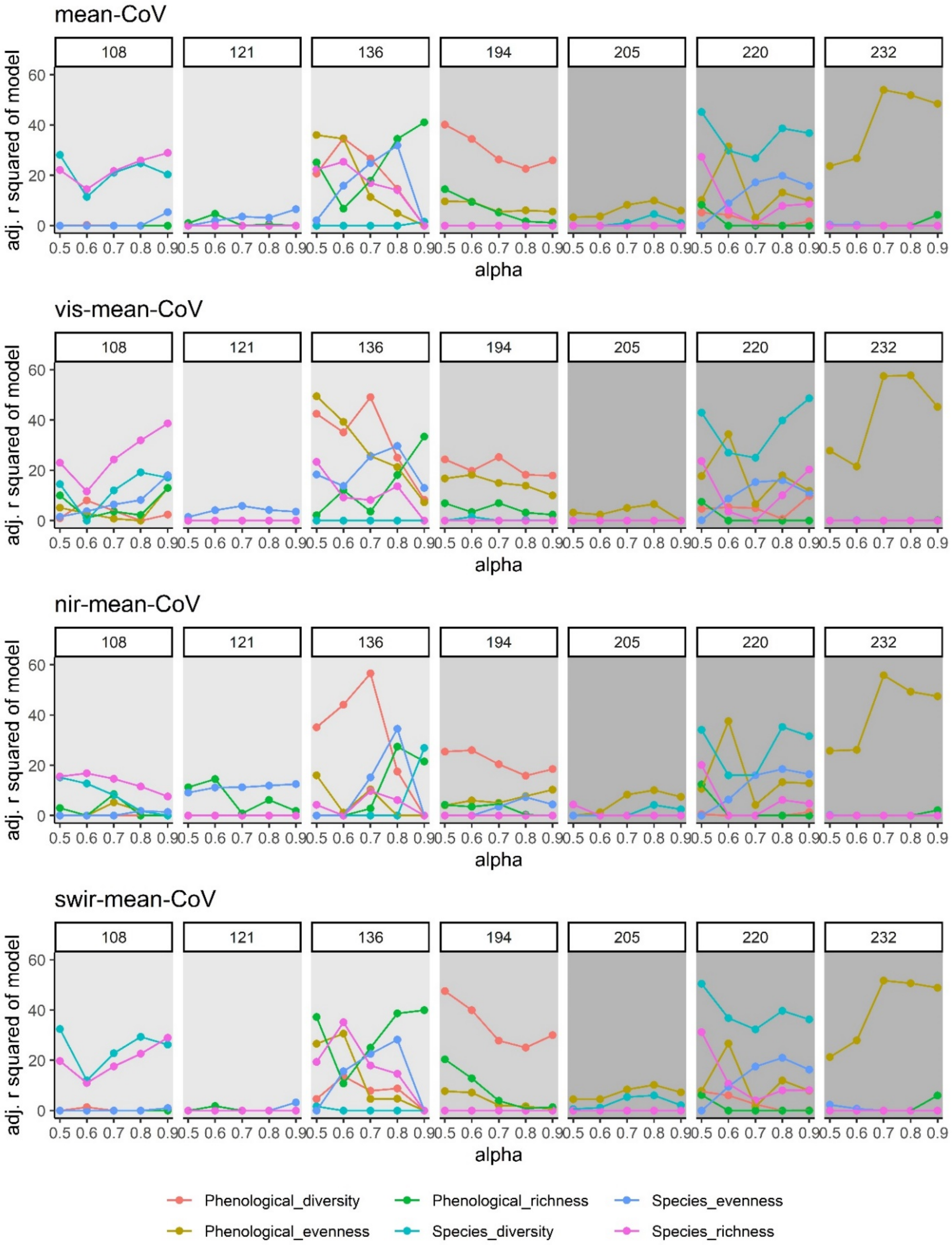


Figure A1: The application of Robust PCA to the raw data and the subsequent changes in the quadrat-based sample size dependent on values of the alpha parameter, which can range from 0.5 to 1.0. The lower the value the more 'robust' the PCA is at removing outliers.


mean-CoV
vis-mean-CoV
nir-mean-CoV
swir-mean-CoV
108
121
136
194
205
220
232
adj. r squared of model
0
20
40
60
0.5 0.6 0.7 0.8 0.9
alpha
Phenological_diversity
Phenological_evenness
Phenological_richness
Species_diversity
Species_evenness
Species_richness

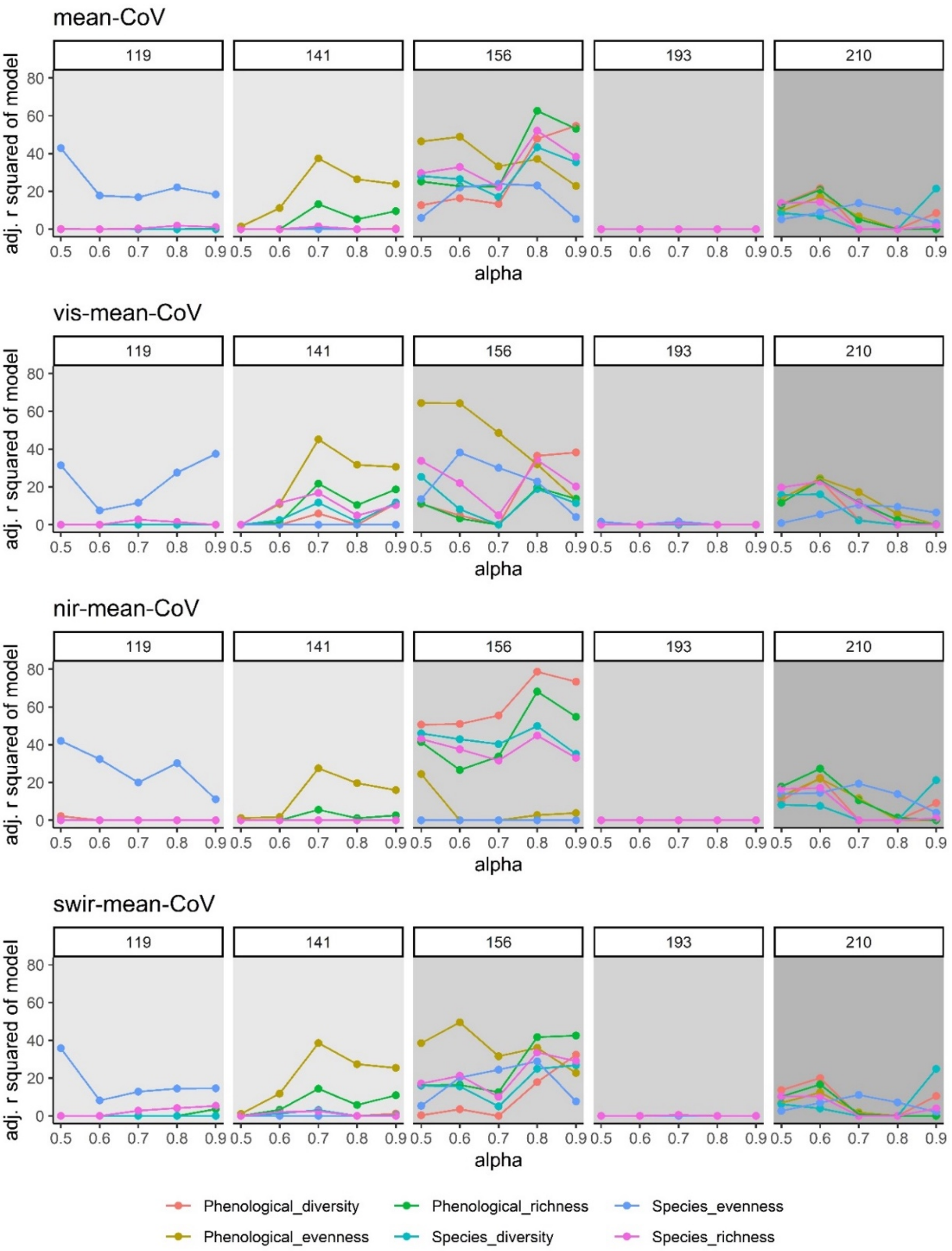


Figure A2: The change in variance explained (adjusted r^2) from the simple linear permutation models (CoV = F(taxonomic/phenology diversity metric) over time, dependent on the level of the alpha parameter in the ROBPCA data cleaning (Dawcombe top and Silwood below). The lower the value of alpha the more outliers are removed from the data set. Some model variances are stable despite the sample number and the robustness of the cleaning level, other model variances proved more volatile.

Supplementary Material B: Results of the quadrat based rate of change of the CoV over the growing season.

Table B1: Results of quadrat based linear models: CoV = f(doy)

Site	Quadrat	Model slope	std.error	statistic	p.value	adj_r	Spectral variable
Dawcombe	1	0.13	0.07	1.78	0.13	0.27	mean-CoV
Dawcombe	1	0.11	0.07	1.60	0.17	0.21	NIR-mean-CoV
Dawcombe	1	0.15	0.08	1.86	0.12	0.29	SWIR-mean-CoV
Dawcombe	1	0.12	0.07	1.78	0.13	0.27	vis-mean-CoV
Dawcombe	2	0.06	0.05	1.16	0.30	0.06	mean-CoV
Dawcombe	2	0.07	0.06	1.20	0.28	0.07	NIR-mean-CoV
Dawcombe	2	0.06	0.05	1.12	0.31	0.04	SWIR-mean-CoV
Dawcombe	2	0.06	0.05	1.12	0.31	0.04	vis-mean-CoV
Dawcombe	3	0.05	0.02	2.89	0.03	0.55	mean-CoV
Dawcombe	3	0.04	0.03	1.60	0.17	0.21	NIR-mean-CoV
Dawcombe	3	0.06	0.01	4.14	0.01	0.73	SWIR-mean-CoV
Dawcombe	3	0.04	0.02	1.86	0.12	0.29	vis-mean-CoV
Dawcombe	4	0.13	0.04	3.07	0.03	0.58	mean-CoV
Dawcombe	4	0.10	0.05	2.18	0.08	0.39	NIR-mean-CoV
Dawcombe	4	0.14	0.04	3.20	0.02	0.61	SWIR-mean-CoV
Dawcombe	4	0.14	0.04	4.01	0.01	0.72	vis-mean-CoV
Dawcombe	5	0.04	0.01	3.31	0.02	0.62	mean-CoV
Dawcombe	5	0.02	0.01	1.57	0.18	0.20	NIR-mean-CoV
Dawcombe	5	0.05	0.01	3.31	0.02	0.62	SWIR-mean-CoV
Dawcombe	5	0.04	0.01	2.87	0.03	0.55	vis-mean-CoV
Dawcombe	6	0.34	0.13	2.67	0.04	0.51	mean-CoV
Dawcombe	6	0.34	0.11	3.16	0.02	0.60	NIR-mean-CoV
Dawcombe	6	0.36	0.15	2.38	0.06	0.44	SWIR-mean-CoV
Dawcombe	6	0.26	0.09	3.07	0.03	0.58	vis-mean-CoV
Dawcombe	7	0.19	0.11	1.71	0.15	0.24	mean-CoV
Dawcombe	7	0.17	0.10	1.68	0.15	0.23	NIR-mean-CoV
Dawcombe	7	0.19	0.12	1.63	0.16	0.22	SWIR-mean-CoV
Dawcombe	7	0.19	0.09	2.04	0.10	0.35	vis-mean-CoV

Dawcombe	8	0.03	0.06	0.45	0.67	-0.15	mean-CoV
Dawcombe	8	0.04	0.06	0.75	0.49	-0.08	NIR-mean-CoV
Dawcombe	8	0.02	0.07	0.31	0.77	-0.18	SWIR-mean-CoV
Dawcombe	8	0.01	0.05	0.31	0.77	-0.18	vis-mean-CoV
Dawcombe	9	0.21	0.10	2.02	0.10	0.34	mean-CoV
Dawcombe	9	0.18	0.10	1.76	0.14	0.26	NIR-mean-CoV
Dawcombe	9	0.24	0.11	2.12	0.09	0.37	SWIR-mean-CoV
Dawcombe	9	0.19	0.09	2.16	0.08	0.38	vis-mean-CoV
Dawcombe	10	0.17	0.13	1.30	0.25	0.10	mean-CoV
Dawcombe	10	0.15	0.12	1.28	0.26	0.10	NIR-mean-CoV
Dawcombe	10	0.20	0.15	1.29	0.25	0.10	SWIR-mean-CoV
Dawcombe	10	0.15	0.11	1.38	0.23	0.13	vis-mean-CoV
Silwood	LFAll	0.12	0.03	4.17	0.03	0.80	mean-CoV
Silwood	LFAll	0.12	0.04	3.29	0.05	0.71	NIR-mean-CoV
Silwood	LFAll	0.11	0.04	3.14	0.05	0.69	SWIR-mean-CoV
Silwood	LFAll	0.13	0.02	6.10	0.01	0.90	vis-mean-CoV
Silwood	LFK	0.01	0.05	0.11	0.92	-0.33	mean-CoV
Silwood	LFK	0.03	0.02	1.12	0.35	0.06	NIR-mean-CoV
Silwood	LFK	-0.01	0.07	-0.11	0.92	-0.33	SWIR-mean-CoV
Silwood	LFK	0.01	0.06	0.18	0.87	-0.32	vis-mean-CoV
Silwood	LFN	0.14	0.13	1.01	0.39	0.00	mean-CoV
Silwood	LFN	0.13	0.14	0.96	0.41	-0.02	NIR-mean-CoV
Silwood	LFN	0.15	0.15	1.00	0.39	0.00	SWIR-mean-CoV
Silwood	LFN	0.08	0.08	1.05	0.37	0.03	vis-mean-CoV
Silwood	LFNone	0.47	0.12	3.88	0.03	0.78	mean-CoV
Silwood	LFNone	0.41	0.10	3.96	0.03	0.79	NIR-mean-CoV
Silwood	LFNone	0.54	0.15	3.69	0.03	0.76	SWIR-mean-CoV
Silwood	LFNone	0.38	0.11	3.47	0.04	0.73	vis-mean-CoV
Silwood	LFP	0.20	0.24	0.85	0.46	-0.07	mean-CoV
Silwood	LFP	0.22	0.22	0.98	0.40	-0.01	NIR-mean-CoV
Silwood	LFP	0.22	0.27	0.80	0.48	-0.10	SWIR-mean-CoV
Silwood	LFP	0.12	0.16	0.78	0.49	-0.11	vis-mean-CoV
Silwood	LUFAll	0.27	0.13	2.02	0.14	0.43	mean-CoV
Silwood	LUFAll	0.23	0.13	1.75	0.18	0.34	NIR-mean-CoV
Silwood	LUFAll	0.29	0.13	2.20	0.12	0.49	SWIR-mean-CoV

Silwood	LUFAll	0.27	0.15	1.85	0.16	0.38	vis-mean-CoV
Silwood	LUFK	-0.01	0.13	-0.10	0.93	-0.33	mean-CoV
Silwood	LUFK	0.00	0.13	-0.02	0.98	-0.33	NIR-mean-CoV
Silwood	LUFK	-0.03	0.14	-0.25	0.82	-0.31	SWIR-mean-CoV
Silwood	LUFK	0.04	0.12	0.31	0.78	-0.29	vis-mean-CoV
Silwood	LUFN	0.05	0.12	0.41	0.71	-0.26	mean-CoV
Silwood	LUFN	0.04	0.11	0.35	0.75	-0.28	NIR-mean-CoV
Silwood	LUFN	0.03	0.13	0.21	0.84	-0.31	SWIR-mean-CoV
Silwood	LUFN	0.13	0.08	1.67	0.19	0.31	vis-mean-CoV
Silwood	LUFNone	0.43	0.32	1.32	0.28	0.16	mean-CoV
Silwood	LUFNone	0.38	0.30	1.28	0.29	0.14	NIR-mean-CoV
Silwood	LUFNone	0.48	0.38	1.26	0.30	0.13	SWIR-mean-CoV
Silwood	LUFNone	0.33	0.18	1.79	0.17	0.36	vis-mean-CoV
Silwood	LUFP	0.26	0.10	2.60	0.08	0.59	mean-CoV
Silwood	LUFP	0.15	0.09	1.74	0.18	0.34	NIR-mean-CoV
Silwood	LUFP	0.33	0.11	2.99	0.06	0.67	SWIR-mean-CoV
Silwood	LUFP	0.27	0.11	2.40	0.10	0.54	vis-mean-CoV

Supplementary Material C: Results of the mixed models

Table C1: Results of the mixed models from stage 1

Model number	Spectral variable	Community metric	Estimate (Fixed term community metric)	Confidence interval	p-value	Estimate (Fixed term site)	Confidence interval	p-value	Estimate (random term quadrat)	Estimate (random term sampling event)	Marginal R2	Conditional R2
1	mean-cov	Phenological Diversity	-0.10	-0.25 – 0.06	0.22	0.02	-0.44 – 0.49	0.917	0.01	0.10	0.037	0.362
2	mean-cov	Phenological Evenness	-0.01	-0.12 – 0.10	0.849	0.19	-0.26 – 0.64	0.405	0.01	0.11	0.024	0.401
3	mean-cov	Phenological Richness	-0.05	-0.16 – 0.06	0.366	0.13	-0.30 – 0.56	0.559	0.01	0.11	0.03	0.399
4	mean-cov	Species Diversity	0.06	-0.14 – 0.26	0.567	0.28	-0.28 – 0.84	0.325	0.01	0.11	0.025	0.406
5	mean-cov	Species Evenness	-0.01	-0.12 – 0.10	0.884	0.19	-0.26 – 0.63	0.411	0.01	0.11	0.024	0.4
6	mean-cov	Species Richness	0.10	-0.12 – 0.33	0.373	0.37	-0.23 – 0.97	0.230	0.01	0.11	0.028	0.405
7	vis-mean-CoV	Phenological Diversity	-0.11	-0.25 – 0.03	0.119	0.03	-0.40 – 0.45	0.901	0.01	0.08	0.058	0.397
8	vis-mean-CoV	Phenological Evenness	-0.01	-0.11 – 0.09	0.863	0.21	-0.21 – 0.63	0.320	0.02	0.1	0.036	0.449
9	vis-mean-CoV	Phenological Richness	-0.05	-0.15 – 0.05	0.298	0.15	-0.25 – 0.55	0.461	0.01	0.09	0.045	0.441
10	vis-mean-CoV	Species Diversity	0.04	-0.15 – 0.23	0.689	0.27	-0.25 – 0.79	0.313	0.02	0.1	0.037	0.453
11	vis-mean-CoV	Species Evenness	-0.01	-0.11 – 0.09	0.888	0.21	-0.20 – 0.62	0.322	0.02	0.09	0.036	0.448
12	vis-mean-CoV	Species Richness	0.10	-0.12 – 0.33	0.373	0.37	-0.23 – 0.97	0.230	0.01	0.11	0.028	0.405

13	NIR-mean-CoV	Phenological Diversity	-0.11	-0.28 – 0.06	0.193	-0.01	-0.52 – 0.49	0.959	0	0.12	0.043	NA
14	NIR-mean-CoV	Phenological Evenness	-0.03	-0.15 – 0.09	0.634	0.19	-0.29 – 0.68	0.437	0.01	0.13	0.017	0.384
15	NIR-mean-CoV	Phenological Richness	-0.07	-0.19 – 0.05	0.237	0.09	-0.38 – 0.56	0.7	0	0.13	0.026	0.381
16	NIR-mean-CoV	Species Diversity	0.06	-0.15 – 0.27	0.588	0.26	-0.34 – 0.85	0.392	0.01	0.14	0.018	0.387
17	NIR-mean-CoV	Species Evenness	-0.02	-0.13 – 0.10	0.795	0.18	-0.30 – 0.65	0.473	0.01	0.13	0.016	0.379
18	NIR-mean-CoV	Species Richness	0.10	-0.12 – 0.33	0.373	0.37	-0.23 – 0.97	0.230	0.01	0.11	0.028	0.405
19	SWIR-mean-CoV	Phenological Diversity	-0.10	-0.26 – 0.07	0.244	0.03	-0.44 – 0.49	0.909	0.01	0.09	0.035	0.339
20	SWIR-mean-CoV	Phenological Evenness	0	-0.12 – 0.11	0.958	0.18	-0.26 – 0.62	0.427	0.01	0.10	0.023	0.375
21	SWIR-mean-CoV	Phenological Richness	-0.04	-0.16 – 0.07	0.462	0.14	-0.30 – 0.57	0.536	0.01	0.10	0.027	0.375
22	SWIR-mean-CoV	Species Diversity	0.06	-0.14 – 0.27	0.554	0.29	-0.27 – 0.85	0.317	0.01	0.11	0.025	0.380
23	SWIR-mean-CoV	Species Evenness	0	-0.12 – 0.11	0.938	0.18	-0.26 – 0.62	0.420	0.01	0.10	0.023	0.375
24	SWIR-mean-CoV	Species Richness	0.10	-0.12 – 0.33	0.373	0.37	-0.23 – 0.97	0.230	0.01	0.11	0.028	0.405

Table C2: Results of the mixed models from stage 2 (NA = model would not converge)

Model number	Spectral variable	Predictors	Effect type	Estimates	Confidence interval	p-value	Marginal R2	Conditional R2
1	mean-CoV	%Mature	Fixed	0.19	0.06 – 0.31	0.003	0.153	0.38
1	mean-CoV	Species Diversity	Fixed	-0.03	-0.77 – 0.63	0.612		
1	mean-CoV	%Mature * Species Diversity	Fixed	0.12	-0.16 - 0.21	0.014		
1	mean-CoV	Site	Fixed	0.21	-0.27 – 0.69	0.392		
1	mean-CoV	Quadrat	Random	0	NA	NA		
1	mean-CoV	Sampling time point	Random	0.07	NA	NA		
2	vis-mean-CoV	%Mature	Fixed	0.15	0.05 – 0.26	0.005	0.155	0.427
2	vis-mean-CoV	Species Diversity	Fixed	0.01	-0.17 – 0.18	0.923		
2	vis-mean-CoV	%Mature * Species Diversity	Fixed	0.11	0.03 – 0.20	0.010		
2	vis-mean-CoV	Site	Fixed	0.21	-0.25 – 0.67	0.380		
2	vis-mean-CoV	Quadrat	Random	0.01	NA	NA		
2	vis-mean-CoV	Sampling time point	Random	0.07	NA	NA		
3	NIR-mean-CoV	%Mature	Fixed	0.23	0.11 – 0.36	<0.001	0.247	NA
3	NIR-mean-CoV	Species Diversity	Fixed	0	-0.19 – 0.19	0.998		
3	NIR-mean-CoV	%Mature * Species Diversity	Fixed	0.15	0.05 – 0.25	0.002		
3	NIR-mean-CoV	Site	Fixed	0.14	-0.34 – 0.62	0.564		
3	NIR-mean-CoV	Quadrat	Random	0	NA	NA		
3	NIR-mean-CoV	Sampling time point	Random	0.06	NA	NA		
4	SWIR-mean-CoV	%Mature	Fixed	0.2	0.08 – 0.31	0.001	0.194	NA
4	SWIR-mean-CoV	Species Diversity	Fixed	0.02	-0.16 – 0.21	0.805		
4	SWIR-mean-CoV	%Mature * Species Diversity	Fixed	0.11	0.01 – 0.20	0.025		
4	SWIR-mean-CoV	Site	Fixed	0.21	-0.24 – 0.65	0.366		
4	SWIR-mean-CoV	Quadrat	Random	0	NA	NA		
4	SWIR-mean-CoV	Sampling time point	Random	0.04	NA	NA		

Chapter 4

The Feasibility of Leaf Reflectance-Based Taxonomic Inventories and Diversity Assessments of Species-Rich Grasslands: A Cross-Seasonal Evaluation Using Waveband Selection

This chapter presents data collected during the 2021 field season on a single calcareous grassland in Kent. It comprises of a very dense time-series of leaf level hyperspectral reflectance data for 17 herbaceous plant species and examines the possibility over time of distinguishing between these species optically.

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Author contribution statement

Rachael Thornley: conceptualisation, funding acquisition, data collection, methodology, software, data analysis, investigation, writing. France Gerard: Conceptualisation, supervision, review and editing. Kevin White: Conceptualisation, supervision, review and editing. Anne Verhoef: Conceptualisation, supervision, funding acquisition, review and editing

Abstract

Hyperspectral leaf-level reflectance data may enable the creation of taxonomic inventories and diversity assessments of grasslands, but little is known about the stability of species-specific spectral classes and discrimination models over the course of a growing season. Here, we present a cross-seasonal dataset of seventeen species that are common to a temperate, dry, and nutrient-poor calcareous grassland, which spans thirteen sampling dates, a week apart, during the spring and summer months. By using a classification model that incorporated waveband selection (a sparse partial least squares discriminant analysis), most species could be classified, irrespective of the sampling date. However, between 42 and

95% of the available spectral information was required to obtain these results, depending on the date and model run. Feature selection was consistent across time for 70 out of 720 wavebands and reflectance around 1410 nm, representing water features, contributed the most to the discrimination. Model transferability was higher between neighbouring sampling dates and improved after the “green-up” period. Some species were consistently easy to classify, irrespective of time point, when using up to six latent variables, which represented about 99% of the total spectral variance, whereas other species required many latent variables, which represented very small spectral differences. We concluded that it did seem possible to create reliable taxonomic inventories for combinations of certain grassland species, irrespective of sampling date, and that the reason for this could lie in their distinctive morphological and/or biochemical leaf traits. Model transferability, however, was limited across dates and cross-seasonal sampling that captures leaf development would probably be necessary to create a predictive framework for the taxonomic monitoring of grasslands. In addition, most variance in the leaf reflectance within this system was driven by a subset of species and this finding implies challenges for the application of spectral variance in the estimation of biodiversity.

4. 1 Introduction

The conservation and management of species-rich semi-natural grasslands require temporally and spatially detailed information on community composition (Critchley et al. 2004, de bello et al. 2010, Lark 2020). However, these data are very difficult and expensive to collect using traditional field-based surveys. It is now possible to create very high-resolution hyperspectral maps of grasslands due to advances in airborne remote sensing, with pixel sizes that are comparable to leaf sizes. Analyses of species-specific leaf and canopy spectra in herbaceous habitats have demonstrated that there is the potential for mapping taxonomic units (Mansour et al. 2012, Marcinkowska-Ochtyra et al. 2018, Pfitzner et al. 2021), phylogenetic groups (Meireles et al. 2020) and plant functional types (Irisarri et al. 2009, Punalekar et al. 2016). However, large variances in intra-specific leaf reflectance have been reported (Petibon et al. 2021, Wang et al. 2018), corroborating concerns about whether hyperspectral data can be used to reliably discriminate between taxonomic units (Price 1994). There has also been mounting evidence that the biophysical drivers of spectral reflectance vary significantly over time as they are influenced by the phenological stage of the plant (Yang et al. 2016) and/or leaf age (Chavana-Bryant et al. 2017). In addition, variation in leaf traits across environmental gradients, such as soil water availability (Guo et al. 2017) climate (Serbin et al. 2014) and soil fertility (Asner et al. 2011) have been found. These results suggest that when using spectral data to predict species classes, both the temporal dimensions of the sampling campaign and the environmental context of the plant community need to be considered. As a consequence, the ability of spectral reflectance at specific wavelengths to predict species may be unstable and the relative positions of species within spectral space could vary over the course of a growing season. It seems likely that the temporal and spatial configurations of field campaigns will affect our ability to monitor species in varied and complex ways (Hesketh and Sánchez-Azofeifa 2012). Certainly, the use of models that are built using data that capture evolving leaf states could improve our understanding of the spectral spaces that taxonomic classes occupy (Dudley et al. 2015) and allow the determination of optimal temporal windows within leaf phenology for taxonomic assessments.

There is also an important link between the spectral separability of taxonomic units and the spectral variation hypothesis (SVH), which proposes a positive correlation between spectral variance and the number of taxonomic units or functional classes that are present within an area at the leaf or plant scale. Variations in leaf-level spectral reflectance have been successfully correlated with the number of species that are present (Wang et al. 2018) and functional diversity (Schweiger et al. 2018). In forests ecosystems, where more research has been conducted, direct linkages have been found between spectral diversity and the diversity of the biochemical properties of leaves within taxonomically complex stands (Carlson et al. 2007). However, Féret and Asner 2011, demonstrated that the ability of spectral variation to predict species diversity and taxonomic classes becomes saturated with a higher number of species. Recent studies on grasslands have also demonstrated the scale (Wang et al. 2018) and temporal dependence of the SVH (Gholizadeh et al. 2020, Thornley et al. 2022). Different grassland types have displayed positive and negative relationships with spectral variance (Imran et al. 2021), independent of space and time. Thus far, there has been a limited understanding of these results. It is probable that spectral variation is unevenly influenced by differing leaf and canopy properties, depending on the spatial scale of the data acquisition and the trait space that is occupied by the community in question.

Hyperspectral data have a particular structure and contain many highly correlated bands. These types of data have been described as having "the curse of dimensionality" and several approaches have been used to deal with this challenge within the context of species differentiation, namely decision trees (Maschler et al. 2018), support vector machines (Dalponte et al. 2014, Lopatin et al. 2017) partial least squares discriminant analysis (Peerbhay et al. 2013) and neural networks (Zhong et al. 2020). Most methods used for class determination involve projection to latent variables and/or data splitting. Some processing chains also include an assessment of the importance of the variables, which is followed by variable selection (Vaiphasa et al. 2007). As the number of species classification studies has increased, it has become possible to determine whether any consistencies in waveband selection can be observed (Fassnacht et al. 2016). Although feature selection has been analysed in terms of spatial scale (leaf or canopy) and plant group (woody or herbaceous) (Hennessy et al. 2020), to date, to our knowledge, the temporal dependence of waveband selection has not been assessed. In this study, we collected the leaf-level hyperspectral reflectance spectra of a complex community of herbaceous species, which is characteristic of UK calcareous grasslands, throughout a growing season. Our principal aims were to:

(1) Determine whether the species within the community could be separated using classification models and to what extent the classification of these species changed over time;

(2) Explore the temporal stability of band selection during classification and test the transferability of classification models across sampling dates;

(3) Test whether the species that were more easily classified displayed particular leaf traits or were more phylogenetically distant from other species within the community;

(4) Examine the importance of the biochemical traits of a leaf in classification over time.

4.2. Materials and Methods

4.2.1 Experimental system

A species-rich ancient grassland with a calcareous rendzina soil type, which is called “Wrotham Water” and is situated in the North Downs in Kent, southeast England (51° 19’ 15” N, 00° 20’ 04” E), was selected as the study site. Plants within this system are either specialists that have adapted to low nutrient and water regimes or more plastic species that undergo dwarfism. To characterise the site, we used the Ecological Flora of the British Isles database (Fitter and Peat 1994), which contains the ecological traits of species, to acquire Ellenberg’s indicator values. These values can be interpreted as follows: species light demand from low to high (1–9); moisture demand from low to high (1–12); soil pH from very acid to very alkaline (1–9); and nitrogen demand from the least to excessive (1–9). These values provide evidence of the environmental niche within which these species are typically found. We also used the CRS (competitor/ruderal/stress-tolerator) functional strategy framework that was developed by Grime (2006). Thirteen out of the seventeen species in this study have been provided with autecological accounts (Grime et al. 2006). We used these accounts to understand the extent to which the species were obligate stress tolerators or more plastic species that had adapted to this environment. To situate our sampling dates within a temporal context in terms of precipitation and seasonal vegetation development, we used the Enhanced Vegetation Index (EVI), surface soil moisture values (both of which were derived from Copernicus Sentinel data) and regional precipitation data. All three time series were created for the period day of year 90 to 260. A site-based EVI time series was obtained from Sentinel-2 to describe the green-up trajectory. The EVI was derived of 60 pixels at a 10-m resolution over 10 cloudless dates. A time series of surface soil moisture derived from Sentinel-1 Synthetic Aperture Radar data at a 1-km pixel resolution was also created. The temporal resolution of the product was between 2 and 5 days and resulted in 99 measurements. Daily regional precipitation records were also sourced from the UK Met Office Hadley Centre observations database (Alexander and Jones 2001).

4.2.2 Leaf spectra acquisition and pre-processing

Seventeen species that are typical of the habitat were selected from the grassland (Figure 4.1A). Starting in the spring, on day of year 119 (29 April 2021), bi-directional leaf level reflectance spectra were collected using a spectrometer that was fitted with a fibre optic cable and leaf clip over the visible, NIR and SWIR regions of the spectra (SVC HR2024i spectroradiometers, Spectra Vista Corporation, Ploughkeepsie, New York State, USA). Data were collected approximately every seven days over three months of the growing season until day of year 204 (23 July 2021). The intention was to capture the period of leaf thickening and maturation but avoid the period of the year in which leaves begin to senesce. In total, 13 dates were sampled, which represented a multi-temporal spectral signature for each species. On each sampling date, a single leaf from five separate plants that were situated along transects was cut for each of the 17 species. Leaves that were trampled, insect damaged or otherwise unhealthy were avoided, as were shaded plants. Within a few minutes of the leaves being collected, three leaf clip readings were taken for each sample and the average of these readings was used in the analysis. The spectra were examined after capture and filtered for erroneous measurements (Schweiger 2020). Reference readings were taken regularly throughout the sampling campaigns using a Spectralon white panel. In three instances, less than five acceptable mean spectra were available (*Inula conyza* n = 2 and *Fragaria vesca* n = 4 on DoY 174 and *Brachypodium sylvaticum* n = 4 on DoY 126). We included these data in the analysis but the results for these dates and species must be treated with caution. The sampling campaign resulted in 1100 averaged leaf spectra.

The spectra were pre-processed through the removal of sensor overlap using SVC HR-1024i PC data acquisition software. They were then smoothed using a Savitzky–Golay filter. Different filter lengths were applied to the spectra and the optimal smoothing was obtained using a filter length of 55. The spectra were trimmed to 340–2500 nm and resampled to a 3-nm resolution (720 wavebands). The nominal bandwidth of the spectrometer was ≤1.5 nm in the region of 350–1000 nm, ≤3.8 nm in the region of 1000–1890 nm and ≤2.5 nm in the region of 1890–2500 nm. 3 nm was chosen so as to exploit the maximum spectral information without overly replicating information in neighbouring bands. All pre-processing was carried out using the HSDAR package in R (Lehnert et al. 2019). Example spectra at each stage of pre-processing are provided in the Supplementary Materials, Figure S1.

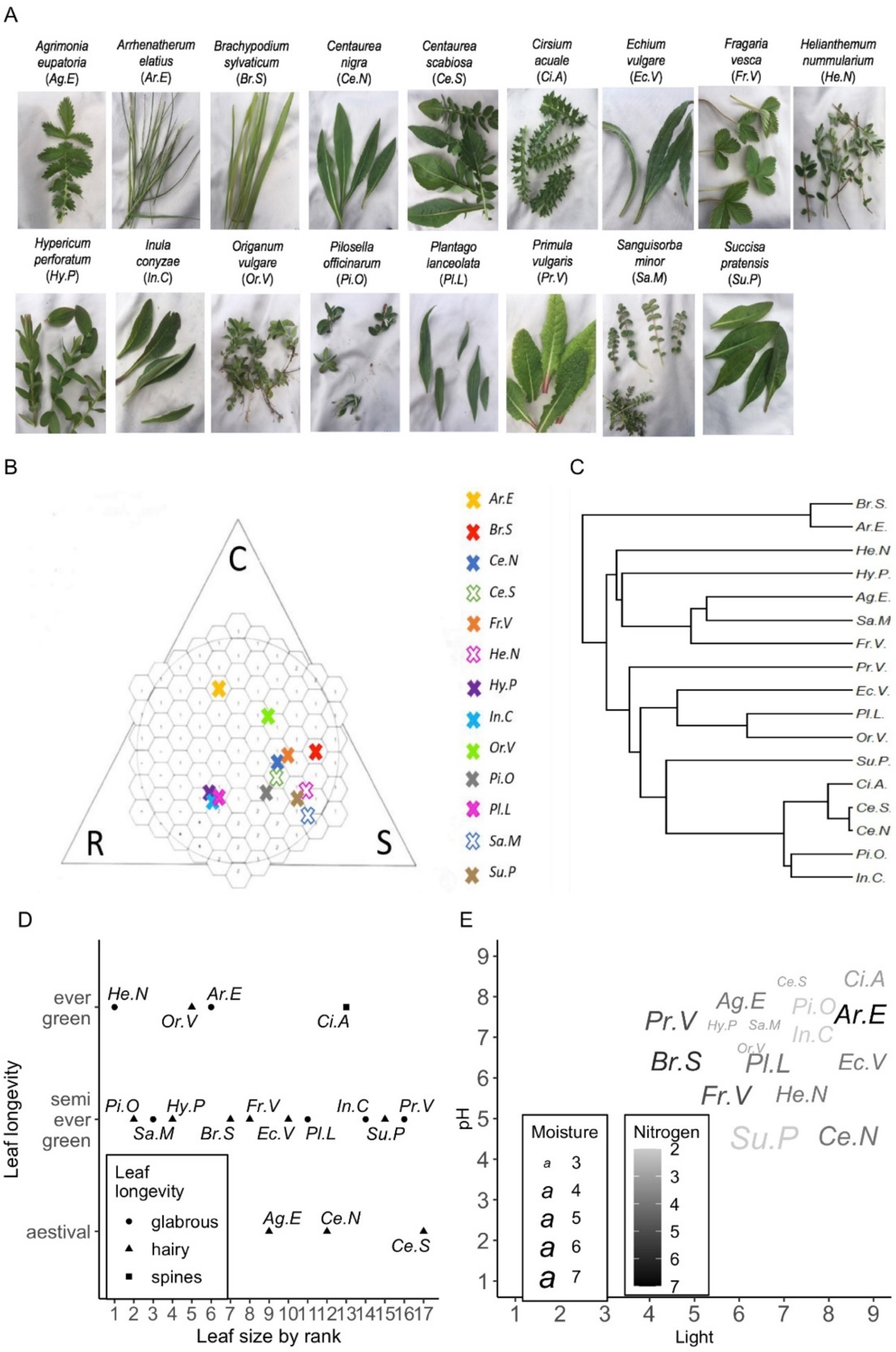


Figure 4.1 (A) The 17 grassland species that were involved in this study; (B) a plot of 13 of those species within the Grime strategy space, where data were available; (C) the phylogenetic relationship between species; (D) the morphological and phenological characteristics of the leaves; (E) Ellenberg's indicator values for light, moisture, pH, and nitrogen.

4.2.3 Spectral dissimilarity within and between species

The spectral distances between pairs of mean spectra were measured using two different algorithms: the Spectral Angle Mapper (Kruse et al. 1993) and the Euclidean distance. We wanted to ascertain whether the distance between pairs of intra-specific spectra was generally smaller than the distance between pairs of spectra from our target species and the other species (inter-specific distance) at certain times of the year. The two chosen distance metrics represent slightly different things: SAM measures the differences in angles for a pair of spectra and, therefore, minimises the effects of illumination and albedo; the Euclidean distance is calculated as the square root of the sum of the squared differences between two vectors. The distribution of the intra-specific distances was compared to the distribution of the interspecific distances for each species at each time point (see Supplementary Materials S1 and S2 for the distributions). A two-sided Kolmogorov–Smirnov test (Marsaglia et al. 2003) was performed on the two distributions and the statistic D was reported to ascertain whether the two distributions were likely to be made up of samples from the same population. Lower levels of D indicated that the distributions were the same and higher values indicated that the distributions were likely to be different. The p values for the test were also calculated.

4.2.4 Sparse PLS-DA for the class determination of species

To establish how easily species could be separated from each other, we used a sparse partial least squares discriminant analysis (sPLS-DA), which is a supervised version of the classic partial least squares regression. In the sPLS-DA approach, a sparsity assumption is made that only a limited number of variables (wavebands within this context) are necessary for the classification of samples (Ruis-Perez et al. 2020). Non-sparse PLS models tend towards the creation of independent latent variables (also known as components), which each contain very small amounts of information from multiple original variables. The sparse approach ensures that variables that make very small contributions to the model are excluded from the analysis, which is in line with other so-called “lasso” approaches (Mehmood et al. 2020). In the context of leaf-level hyperspectral reflectance, variability in optical leaf traits has a cross-spectral effect (Feret et al. 2008); however, reflectance at neighbouring wavelength values is highly correlated, which makes much hyperspectral data redundant. The minimum waveband selection from the sparse approach had several advantages within this context. Firstly, it enabled a wavelength selection comparison across the sampling dates, which was vital for the aims of this study. Secondly, it has been demonstrated more generally that the ratio of samples to variables affects the performance of PLS-DA models (Saccenti and Timmerman 2016). Hence, by reducing the number of wavebands, we minimised the magnitude of this ratio and increased the likelihood of producing more reliable results. Thirdly, hyperspectral imaging devices that are capable of very high spatial resolution often require prior band selection. This is because of the time that is needed to capture many simultaneous bands. Therefore, results from the sparse approach are more useful for transferability to imaging systems.

A sPLS-DA was performed for each of the thirteen sampling dates in the dataset for each of the seventeen species classes. The classes were dummy coded and linear combinations of the Y classes and X variables (the spectral data matrix) were created to maximise the co-variance. Each model was tuned, whereby both the number of latent variables (components) and the number of wavebands that were required for classification were minimised. To tune the model, three criteria were required: (1) the optimal distance metric for the assignment of new samples into classes during the cross-validation process (a choice of maximum distance, Mahalanobis distance or centroids distance); (2) the number of components; and (3) the number of wavebands to be used in each component (more generally, the minimum number of X variables that were necessary to explain the variance in the Y classes). The optimal number of components was selected by observing the stabilisation of the error after the introduction of an increasing number of latent variables. The waveband selection was based on the stability and frequency of the wavebands that were selected during model permutations. The distance metric was selected by the optimisation of the model error that was achieved by the use of the three metrics. One of the main limitations of PLS models is that they are prone to overfitting (Lee et al. 2018). Therefore, this model optimisation was achieved by M-fold cross-validation and an evaluation of the RMSE of the model. The number of folds was selected as the number of classes plus two (17 + 2 = 19) and 50 runs were performed within each model. When the specified number of folds was too large, the number of folds was reduced until cross-validation became possible. The whole process was repeated 10 times (over 10 model runs) for each sampling point. The sPLS-DA, model tuning and performance assessment were executed using the mixOmics package (Rohart et al. 2017) in R (R Core Team 2022). Detailed instructions on the procedure for the above approach can be found in Lê Cao et al. 2011.

4.2.5 Assessment of waveband selection and model stability

To assess the stability of the wavelength selection at each time point, the frequency with which each waveband was selected in the 10 model runs was determined. Wavebands that were consistently selected, both between runs and between times, could be said to have cross-seasonal importance for discrimination. Other wavebands that were consistently selected within a sampling point for all model runs but were not always selected for all sampling dates could be said to have temporally dependent importance. To assess the extent to which models that were trained using data from a single time point were over-fitted, we used the model that was trained using one time point to predict species from the data that were collected on the other sampling dates. By examining the mean model error of the 10 model runs, we could determine whether the wavelength selections were temporally dependent. When models performed better on neighbouring data than on data that were further away in time, we could say that the relative position of the species within spectral space was evolving with leaf age and phenology.

4.2.6 Grounds for the "ease" of species separation

We defined a "well-classified" species as a species for which a classification error rate of less than 0.1 (10%) was obtained. Each species was assigned a value at each time point, which was based on the number of latent variables that were required to achieve this classification accuracy (see Supplementary Materials Figure S4). We equated this value to the "ease" of the classification of a species within our framework. In some cases, it was not possible to classify species to this level of accuracy, so those classes were dummy coded with a value of 25 so that they could be included in the analysis. The mean and standard error of these values across the time points were also calculated.

To assess the possible causes of the "ease" of the classification of a species, we tested several hypotheses:

(1) Species that were taxonomically or phylogenetically more distinctive were easier to classify;

(2) Species with smaller, and therefore harder to measure, leaves were harder to classify (due to increased noise within the leaf clip dataset);

(3) The leaf longevity that is typical of this species affected the ease of species classification;

(4) The leaf surface defence mechanisms affected the ease of species classification;

(5) The amount of bi-directional leaf reflectance affected the ease of species classification;

(6) The spectral distance between pairs of species-specific spectra compared to interspecific spectral distances (as denoted by the Kolmogorov–Smirnov statistic D) was a good predictor of the ease of species classification.

To test Hypothesis 1, a phylogeny for the 17 species was generated using the phylomaker software in R (Jin et al. 2019). From this phylogeny, a relative measure of phylogenetic distance was created for each species within the community. To test Hypothesis 2, the relative leaf sizes of the species were judged according to observations in the field and ranked from smallest (1) to largest (17). It has been shown that leaf surface properties can be contributing factors to reflectance (Sims and Gamon 2002). To test Hypotheses 3 and 4, we used the Ecological Flora of the British Isles database (Fitter and Peat 1994) to access species traits on leaf longevity (whether leaves were evergreen, semi-evergreen, or spring emerging (aestival)) and leaf surface properties that are related to defence (whether the leaves are glabrous, hairy, or covered in spikes). Sims and Gamon 2002 observed that reflectance at 445 nm is almost entirely driven by leaf surface properties. Here, reflectance at 445 nm was used as a proxy for leaf specular reflectance and these values were used to test Hypothesis 5. Hypothesis 6 was tested using the data that were mentioned in Section 2.3. For all hypotheses, a linear regression model was used to test the proposed relationship and when the dependent variable was categorical, Tukey post hoc tests were used to determine the differences between the groups.

4.2.7 Use of the PRO-COSINE radiative transfer model to understand the biochemical basis of shifting waveband Importance

As the PROSPECT model (Jacquemoud and Baret 1990) was developed for use with hemispherical reflectance data that were measured with an integrating sphere, it may not be appropriate for understanding wavelength selection in bi-directional reflectance data that were collected using a leaf clip. PRO-COSINE offers an approach for unifying the PROSPECT-4 model with data that were collected using a leaf clip to enable a mechanistic understanding of the results (Jay et al. 2016). The principal additional factor that needed to be accounted for was the specular reflection of the leaves through the *bspec* parameter. The *bspec* ranges in value from -0.2 to 0.6 (unitless) and increases in value with increased specular reflectance, which influences reflectance in strong absorption regions (around 400 nm and at

1930 nm and 2500 nm). Studies so far have shown that specular reflectance can be explained to some extent by the species (Li et al. 2018, Wan et al. 2021). It has also been demonstrated that the impact of specular leaf properties on reflectance is relatively small compared to the variance within and between individuals of the same species (Petibon et al. 2021). Values ranging from 0 to 0.10 were used as the parameters for the *bspec* input of the model. N was constrained to the range of 1–2, following the method of Jacquemoud and Baret 1990, which are the values that are suitable for healthy leaves that are not in senescence. The additional model inputs of chlorophyll content (Cab), leaf mass area (LMA) and equivalent water thickness (EWT) were not parameterised.

We wanted to understand the biochemical relevance of the wavelength selections across time. Traditionally, leaf chemical assays have been used to determine variance partitioning in conjunction with radiative transfer models (Feret and Asner 2011). However, this approach is time and effort prohibitive and has only been attempted for woody species and never over time. Here, we used an alternative method: we performed a global sensitivity analysis (GSA) of PRO-COSINE using the Saltelli method and the ARTMO toolbox V1.14 in MATLAB (Verrelst et al. 2015). The total sensitivity effects (the first-order effect plus interactions with other input variables) were calculated for each of the model input variables for each spectral band. We then used the waveband selection of each of the sPLS-DA models, which were trained using data from each time point, to extract from the results of the GSA, thus representing the probability of relative trait importance for each of the first six components per sampling point.

4.3 Results

4.3.1 Ecological context of the plant community and timing of sampling campaigns

The species-specific CSR strategies revealed a community comprising of mainly stress tolerating specialists. A few species were more competitive (*Arrhenatherum elatius* and *Origanum vulgare*) or more ruderal in their preferences (*Plantago lanceolata*, *Inula conyzae* and *Hypericum perforatum*). In terms of the four Ellenberg's indicators, the species were all light demanding and suited to either neutral or high pH soils. Their preferences for water and nitrogen were more variable (Figure 4.1).

The start of the sampling season (DoY 119) was preceded by very low rainfall in the region and low surface soil moisture (Figure 2A, B). Later in the season, the peaks and troughs in surface soil moisture were driven by precipitation events throughout the sampling period and there was evidence of the repeated wetting and drying of the soil. A likely consequence of the very dry conditions in the spring was the slowing of the green-up. The first five sampling dates (DoY 119, 126, 132, 140 and 147) appeared to be during the green-up period of the grassland prior to the period of peak biomass (Figure 4.2C). Unfortunately, due to frequent cloud cover during 2021, the Sentinel-2 time series was sparse; so, the end of the green-up period was speculative but appeared to occur around DoY 160. The remaining eight sampling events took place during peak biomass.

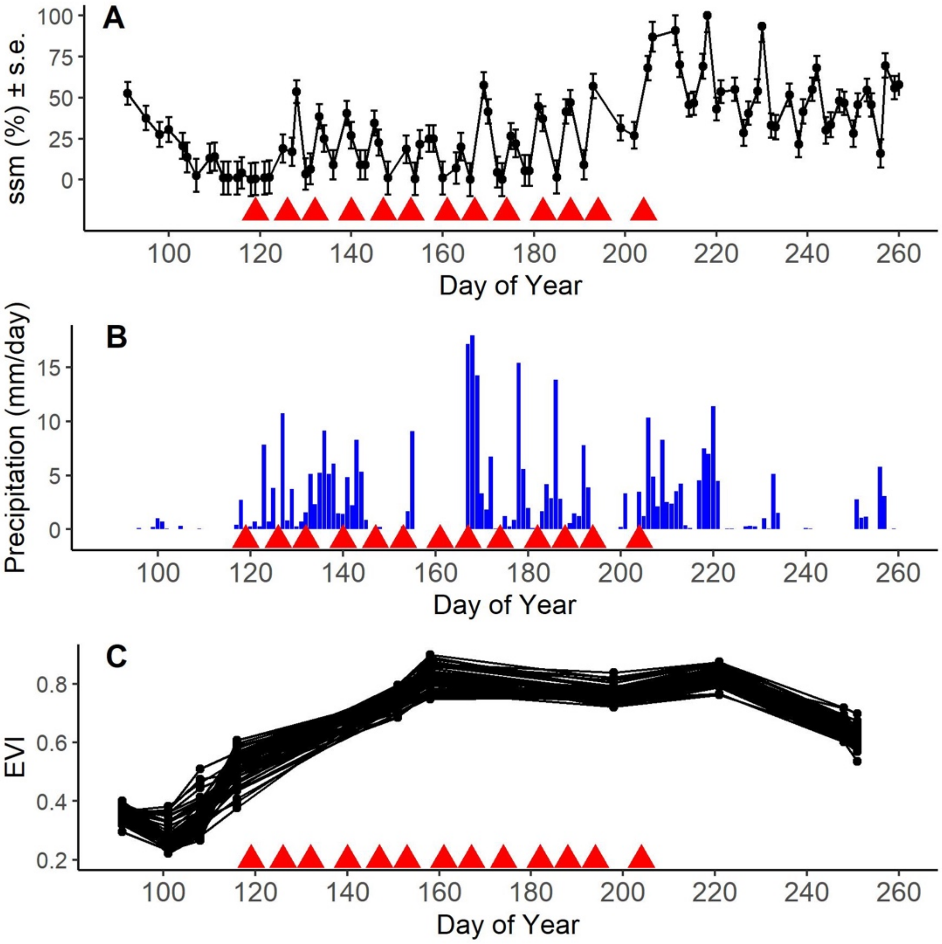


Figure 4.2 (A) Satellite-derived time series of surface soil moisture (Sentinel-1) at a 1-km resolution; (B) regional daily precipitation averages; (C) the site-based green-up trajectory using EVI (Sentinel-2) at a 10-m resolution. The 13 field sampling dates are shown as red triangles.

4.3.2 Spectral distance over time

The lowest cumulative Euclidean distance and SAM value between pairs of spectra across all species occurred on day of year 174 (13 July 2021) and the highest occurred on day of year 204 (2 June 2021). There was a moderate to strong correlation between the pairwise spectral distances, whether calculated using SAM or the Euclidean distance (Spearman's rank correlation = 0.7142857; *p* value = 0.008143).

The mean intra-specific distances for each species and time point were smaller than the mean inter-specific distances for both distance metrics (bar *Sanguisorba minor* at DoY 153, 194 and 204). This indicated that the leaf samples that shared the same species were generally more spectrally similar (see Supplementary Materials Figures S2 and S3 for the distributions and means of the distances). The Kolmogorov–Smirnov test statistic (*D*) was used to determine whether the distribution of the intra specific distances was significantly different from that of the inter-specific distances for each species at each time point. The values of *D* and their associated p values are presented in Figure 4.3. The values of *D* for five of the species (*Primula vulgaris*, *Inula conyza*, *Fragaria vesca*, *Cirsium arvense* and *Agrimonia eupatoria*) were always significant, regardless of the sampling point or distance metric. The values of *D* that were calculated using SAM were more stable in two of the species (*Brachypodium sylvaticum* and *Cirsium arvense*) than those that were calculated using the Euclidean distance. However, overall, there appeared to be no advantage to using either metric in terms of species separability from the single sampling point perspective. In contrast, the value of D was equivalent or larger for SAM than the Euclidean distance across all sampling dates for all species except *Primula vulgaris* and *Inula conyza.* So, cross-seasonally, SAM may be a more useful metric to use for species discrimination problems.

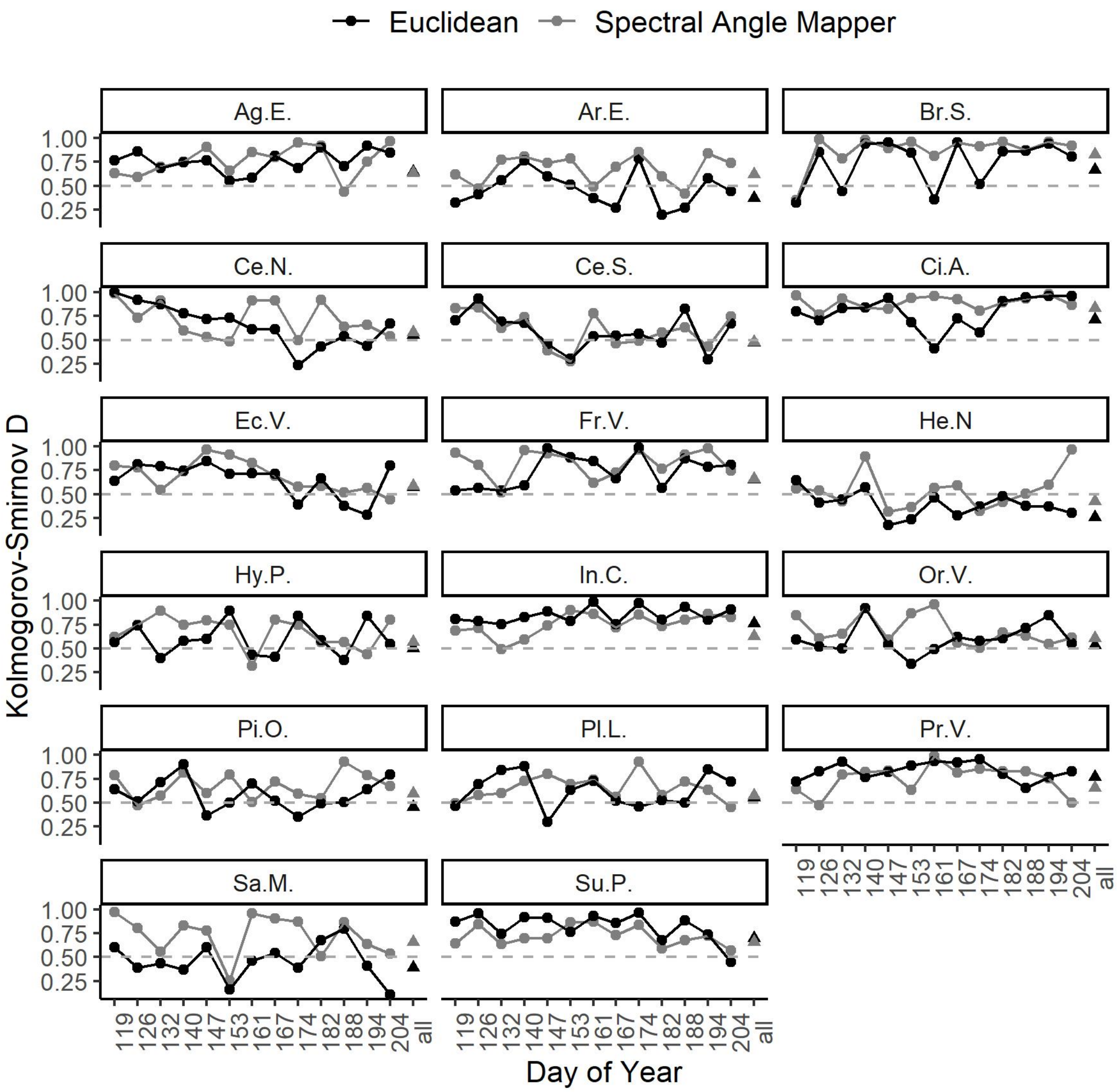
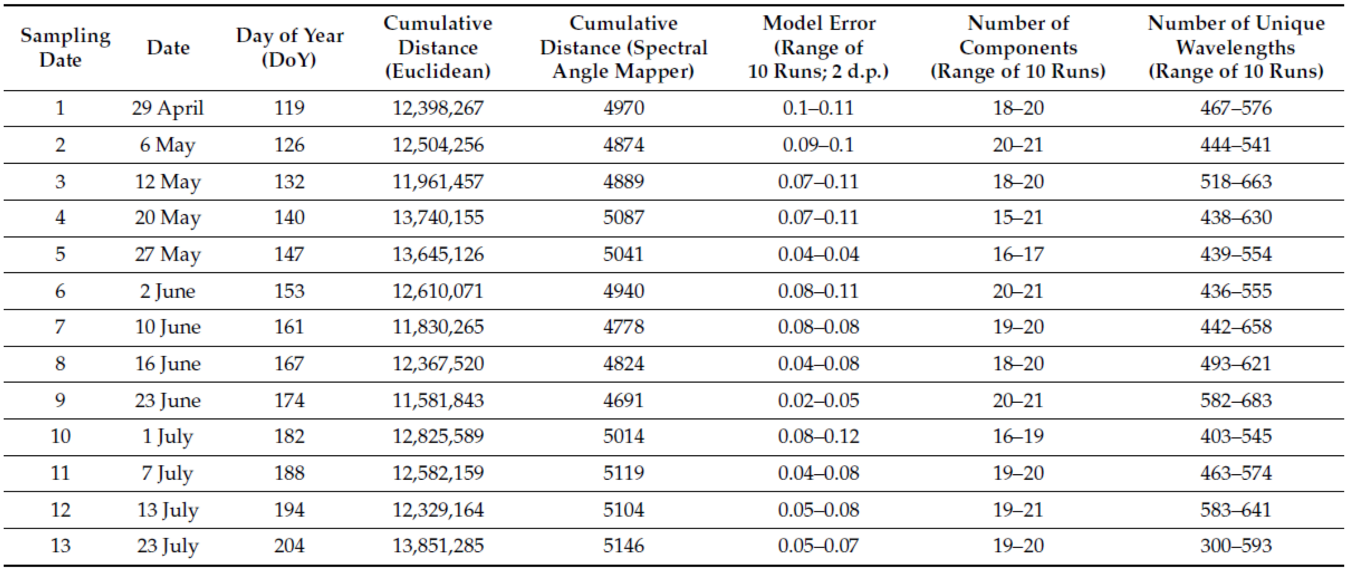


Figure 4.3 The value of *D* (the Kolmogorov–Smirnov statistic): a test of whether the distributions of the intra-specific and inter-specific distances were different from each other at each time point and across all sampling dates for each species class. The results are shown for both the Spectral Angle Mapper and the Euclidean distance. The values of *D* ranged from 1–0, with higher values representing distributions that were more distinct. A p value = 0.01 for the test is shown by a dashed line. Values above the line denote significantly different distributions.

4.3.3 Performance of PLS-DA over time: waveband and model stability

The sPLS-DA models at each time point performed well, with overall model errors ranging from 0.02 on DoY 174 (23 June) to 0.12 on DoY 182 (1 July) (Table 4.1). The number of independent components that were required to obtain these low errors was quite high, ranging from 15 components on DoY 140 (20 May) to 21 components on several of the other dates. The number of wavebands that were used to obtain this level of classification ranged from 300 to 683, with 42–95% of available bands being exploited. In other words, even when using the sparse approach, a large proportion of the spectra was required to classify the 17 species for some time points and model runs.

Table 4.1 A summary of the results of the cumulative spectral distances and sPLS-DA models for each sampling date.



Sampling Date	Date	Day of Year (DoY)	Cumulative Distance (Euclidean)	Cumulative Distance (Spectral Angle Mapper)	Model Error (Range of 10 Runs; 2 d.p.)	Number of Components (Range of 10 Runs)	Number of Unique Wavelengths (Range of 10 Runs)
1	29 April	119	12,398,267	4970	0.1–0.11	18–20	467–576
2	6 May	126	12,504,256	4874	0.09–0.1	20–21	444–541
3	12 May	132	11,961,457	4889	0.07–0.11	18–20	518–663
4	20 May	140	13,740,155	5087	0.07–0.11	15–21	438–630
5	27 May	147	13,645,126	5041	0.04–0.04	16–17	439–554
6	2 June	153	12,610,071	4940	0.08–0.11	20–21	436–555
7	10 June	161	11,830,265	4778	0.08–0.08	19–20	442–658
8	16 June	167	12,367,520	4824	0.04–0.08	18–20	493–621
9	23 June	174	11,581,843	4691	0.02–0.05	20–21	582–683
10	1 July	182	12,825,589	5014	0.08–0.12	16–19	403–545
11	7 July	188	12,582,159	5119	0.04–0.08	19–20	463–574
12	13 July	194	12,329,164	5104	0.05–0.08	19–21	583–641
13	23 July	204	13,851,285	5146	0.05–0.07	19–20	300–593

Within each time point, the variable selection across the 10 model runs was consistent for some wavebands but not for others (Figure 4.4). There are also multiple different solutions for the model at any one time in terms of waveband selection.

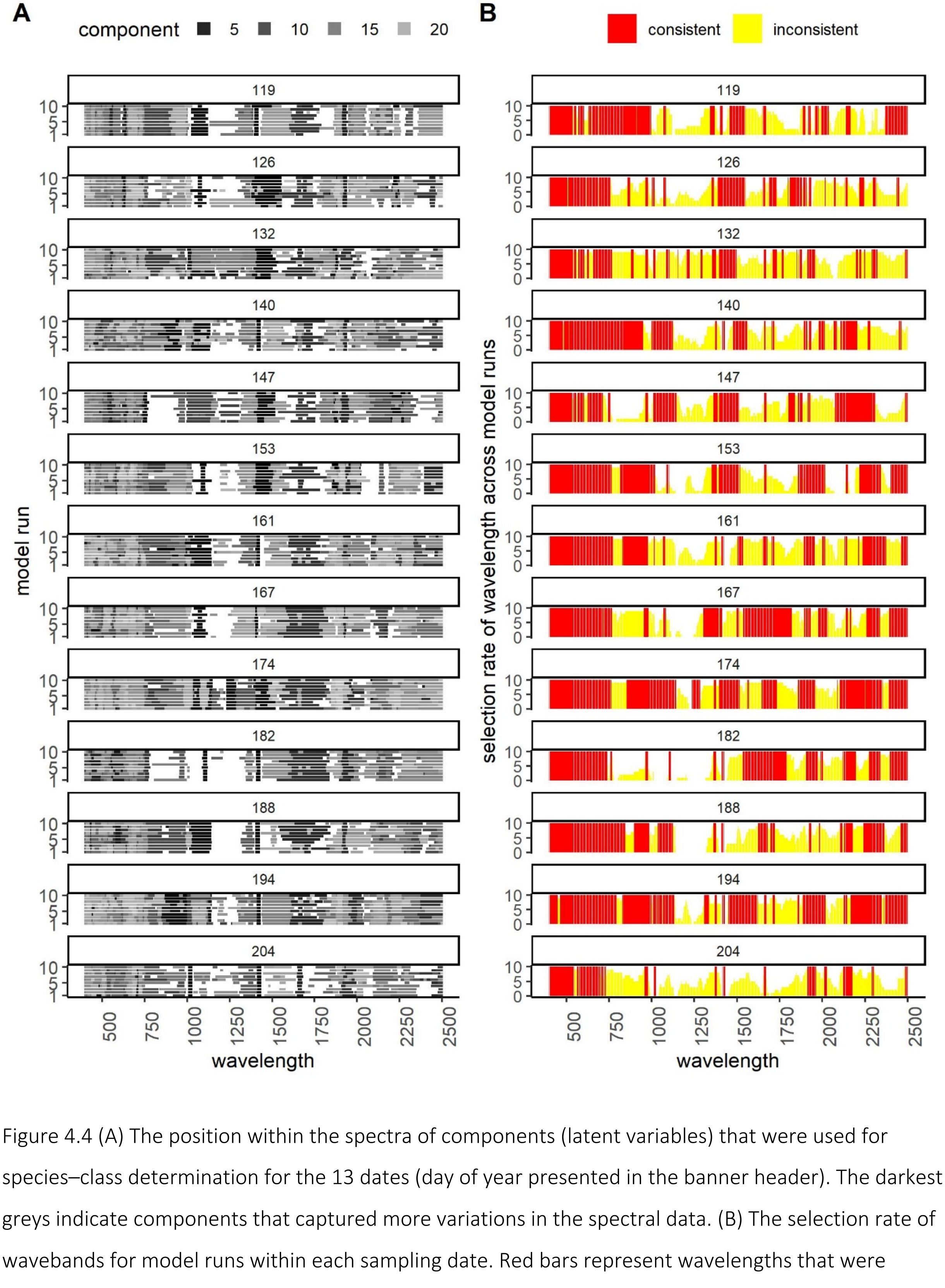


Figure 4.4 (A) The position within the spectra of components (latent variables) that were used for species–class determination for the 13 dates (day of year presented in the banner header). The darkest greys indicate components that captured more variations in the spectral data. (B) The selection rate of wavebands for model runs within each sampling date. Red bars represent wavelengths that were

consistently selected in 10/10 runs; yellow bars are those that were only selected for some of the model runs.

The wavebands that were consistently selected in all 10 models runs within a time point are shown in Figure 4.5. The number of times that these same wavebands were selected out of the 13 sampling dates is also shown. In total, 70 wavebands were selected in all model runs and time points (i.e., in 13 x 10 = 130 models) and 65 of these were in the visible part of the spectrum. The overlaid example spectrum in Figure 4.5 reveals the consistent general importance of wavelengths in both the visible and red-edge regions. Other important features can be seen at 1000 nm, the minimum points of reflectance in the SWIR at 1400 nm, 1950 nm and 2500 nm, the peak of 1800 nm in the SWIR and the slopes on either side of the peak at 2200 nm. The conformity of selection in the rough locations of important spectral features can also be observed. In contrast, there was a large variability in the exact location of band selection between sampling dates. Figures 4.4 and 4.5 show the need to exploit much of the spectra to classify the taxonomic units.

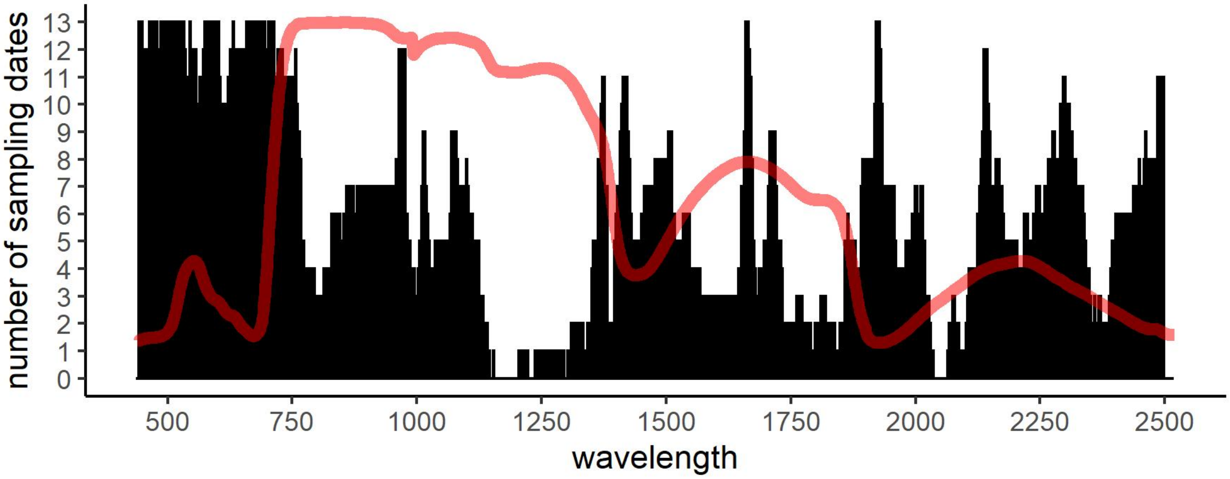
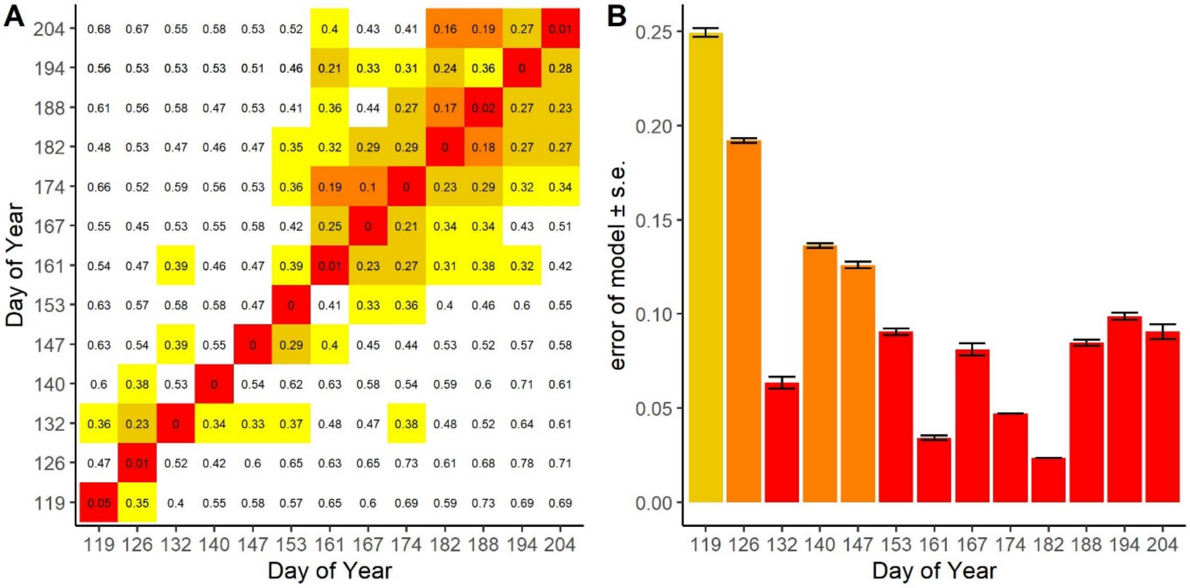


Figure 4.5 The number of times within each of the 13 sampling dates that wavebands were consistently selected in all model runs. A reference leaf spectrum (red line) is superimposed on the plot for contextualisation.

To assess model transferability across time, we tested the ability of the models that were trained using data from each sampling date to predict species using data from each of the other sampling dates (Figure 4.6A). We also used the model that was trained using all of the data to predict the species for each individual date (Figure 4.6B). In both cases, there was an observable increase in temporal dependence in the models after DoY 153. This stabilisation correlated with the end of “green-up” (see Figure 4.2C). When using the model that was trained using the cross-temporal data, the error rates were noticeably lower in the second half of the sampling campaign, which further indicated the stabilisation of waveband selection for species classification later in the growing season.



	119	126	132	140	147	153	161	167	174	182	188	194	204
204	0.68	0.67	0.55	0.58	0.53	0.52	0.4	0.43	0.41	0.16	0.19	0.27	0.01
194	0.56	0.53	0.53	0.53	0.51	0.46	0.21	0.33	0.31	0.24	0.36	0	0.28
188	0.61	0.56	0.58	0.47	0.53	0.41	0.36	0.44	0.27	0.17	0.02	0.27	0.23
182	0.48	0.53	0.47	0.46	0.47	0.35	0.32	0.29	0.29	0	0.18	0.27	0.27
174	0.66	0.52	0.59	0.56	0.53	0.36	0.19	0.1	0	0.23	0.29	0.32	0.34
167	0.55	0.45	0.53	0.55	0.58	0.42	0.25	0	0.21	0.34	0.34	0.43	0.51
161	0.54	0.47	0.39	0.46	0.47	0.39	0.01	0.23	0.27	0.31	0.38	0.32	0.42
153	0.63	0.57	0.58	0.58	0.47	0	0.41	0.33	0.36	0.4	0.46	0.6	0.55
147	0.63	0.54	0.39	0.55	0	0.29	0.4	0.45	0.44	0.53	0.52	0.57	0.58
140	0.6	0.38	0.53	0	0.54	0.62	0.63	0.58	0.54	0.59	0.6	0.71	0.61
132	0.36	0.23	0	0.34	0.33	0.37	0.48	0.47	0.38	0.48	0.52	0.64	0.61
126	0.47	0.01	0.52	0.42	0.6	0.65	0.63	0.65	0.73	0.61	0.68	0.78	0.71
119	0.05	0.35	0.4	0.55	0.58	0.57	0.65	0.6	0.69	0.59	0.73	0.69	0.69

Figure 4.6 (A) Confusion matrix of the mean errors of the 10 model runs that were trained using data from single sampling dates and tested using data that were collected on the other sampling dates. The temporal dependence of the data was higher after DoY 153. (B) The error of the model that was trained using data from all sampling dates and tested using data from single sampling dates. The error bars show the standard error of the mean model error after 10 runs.

4.3.4 *Ease of species separability*

We noted that 99% of the spectral variance in the single date models was explained by only six independent components (see Figure 4.7A, the “scree plot” of the models). This was the case in all model runs and at all time points. The species classification error was examined for each species across time.

With the recommended number of components in the model, all species achieved a satisfactory error rate (<0.1) for at least seven of the sampling dates. Three of the species (*Cirsium acaule*, *Fragaria vesca* and *Sanguisorba minor*) were well classified at all time points (Figure 4.7C). A very high error rate was found for *Inula conyza* on DoY 174. This was due to the low number of samples (n = 2) that was obtained for this species on this date. The class-based error rate of the 99% spectral variance and the six components was very stable across model runs within time points but overall, it was very temporally dependent (Figure 4.7B). Using this reduced number of components, almost all species (apart from *Centaurea scabiosa*) were well classified at certain times, but none of the species were consistently well classified, irrespective of the time point. The classification error was high for most species, which suggested that very small differences in spectral reflectance were responsible for most of the class differentiation of species within this community throughout the season. We used the number of components that were required to achieve a classification error of less than 0.1 as an indicator of the "ease of classification" for each species. The mean value of the standard errors across all time points and models runs (n = 130) per species is presented in Figure 4.8A. *Fragraia vesca* and *Cirsium acaule* were clearly the easiest to classify according to our criteria. The other species all showed large standard errors around the mean, which implied that the ease of classification was more temporally dependent. The same evaluation was carried out for the sPLS-DA model that was trained using the cross-seasonal data (Figure 4.8B). These results provided a clearer picture, with six species requiring under 10 components to be well-classified, five species requiring between 10 and 20 components and the remaining six species being impossible to classify to the desired level of error. When the species were ranked from the easiest to hardest to classify, the means of the results from the single time points and the model that was trained using the cross-seasonal data were well correlated (Spearman's rank correlation = 0.8). In the further analyses, the classification "ease" metric from each of the single time point models was used.

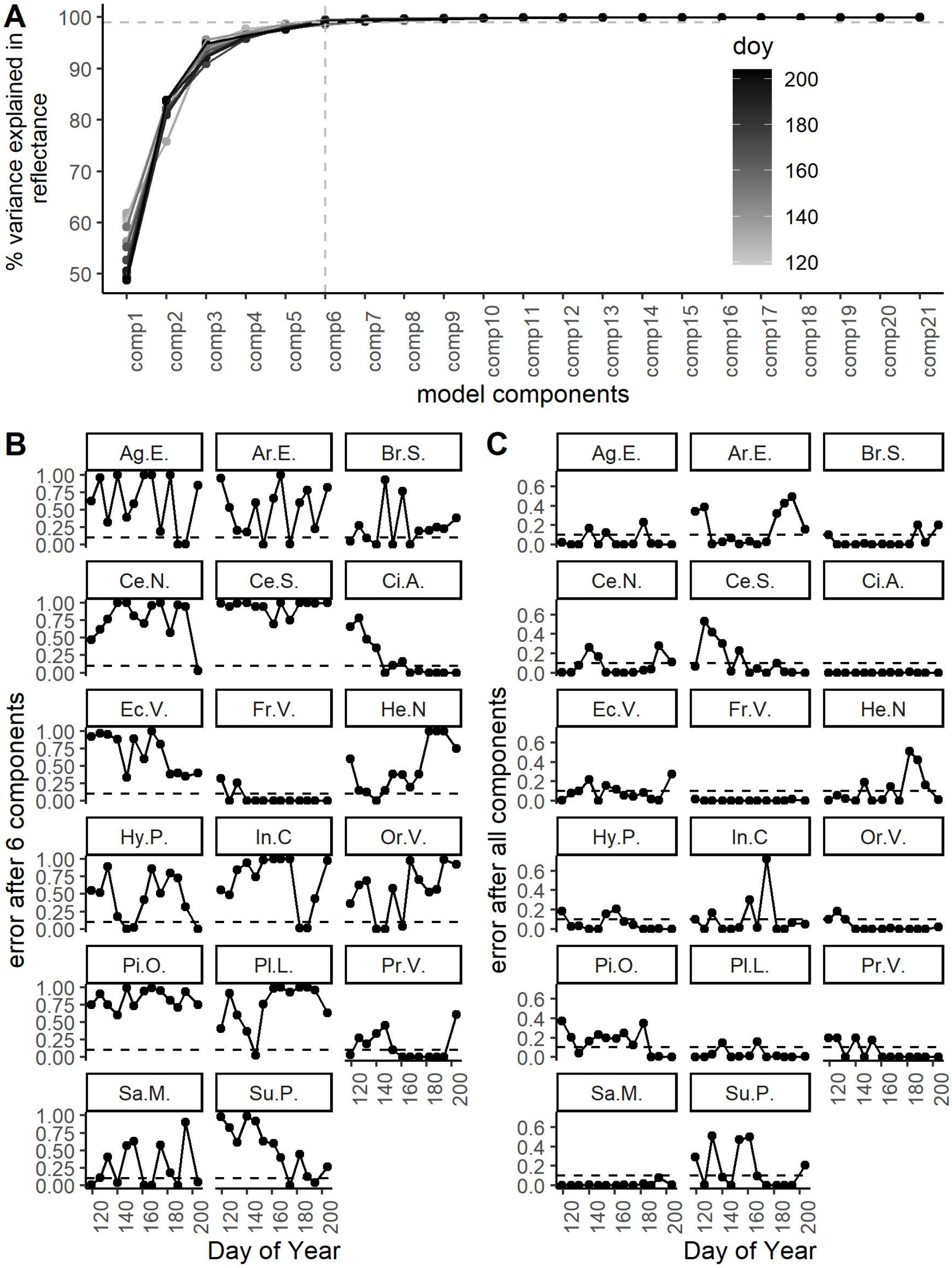


Figure 4.7 (A) The "scree plot" of the models at each time point, i.e., the variance in the X variable as explained by the model latent variables/components. The grey reference line represents the 99% variance in the X variable that was captured by six components, irrespective of sampling time; (B) species classification error over time with six components; (**C**) species classification error with the chosen number of components (i.e., the final model for each time point). Mean error is shown for each time point over the 10 model runs (the S.E. of the model runs was very small and is not shown).

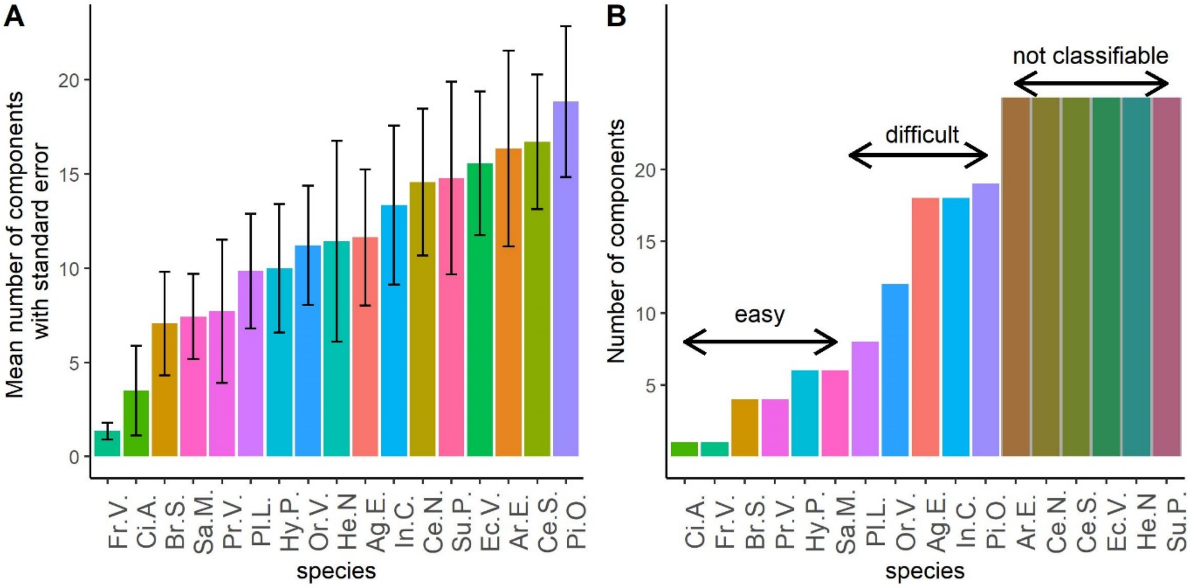


Figure 4.8 The "ease" of classification, defined as the number of components (latent variables) produced from the sPLS-DA models that were required to classify a species to a <10% error rate. Species are ranked from easiest to hardest to classify (left to right); (A) the mean and SE of the models across sampling dates; (B) the results from the model that was trained using the cross-seasonal dataset. Shaded bars show the species that were not classifiable to the required error rate.

4.3.5 Phylogenetic and Morphological Drivers of Species Separability

We used linear models to test whether the ease of classification was related to the phylogenetic and morphological aspects of the community (see Figure 4.1C–E). Firstly, we tested whether smaller phylogenetic distances between pairs of species made them more difficult to separate. We found that phylogenetic distance was very weakly correlated with the ease of classification within this community ($r^2 = 0.05$, slope = 0.03, p = 0.00287), with species that had smaller evolutionary distances being slightly harder to classify. We proposed that species with smaller leaves would be harder to measure using the leaf clip and that the measurements of these leaves would be subject to increased noise. However, we found no effects of leaf size on the ease of classification. We found bi-directional leaf reflectance at 445 nm to be very weakly correlated with the ease of separation; however, this finding was driven by two species (*Helianthenum nummularium* $r^2 = 0.36$, slope = -119, $p < 0.001$ and *Sanguisorba minor* $r^2 = 0.168$, slope = -98, $p < 0.001$). The more specular the reflectance, the easier these two species were to classify. We performed an ANOVA and a paired Tukey test to test whether leaf longevity or leaf surface mechanisms had any effects on classification ease. We found that aestival (spring emerging) leaves were harder to classify than evergreen and semi-evergreen leaves (ANOVA: F = 4.445, $p < 0.05$); the post hoc Tukey test showed that aestival leaves differed significantly from the other two groups at $p = 0.03$ and $p = 0.01$. We also found that species with spines were easier to classify than those with glabrous or hairy leaves (ANOVA: F = 8.552, $p < 0.0001$); post hoc Tukey test showed that spines differed significantly from the other two groups at $p < 0.0001$ and $p < 0.001$. However, this latter result should be treated with caution as only one species in the community had spines (*Cirsium acaule*). By using the GSA of PRO-COSINE and the waveband selections from the sPLS-DA models, we were able to understand which leaf traits were likely to be the principal drivers of spectral variations within the plant community (Figure 4.9A). The consistent results for Component 1 (Figure 4.9B) highlighted the importance of the SWIR water feature in explaining the variances between species. Regardless of the sampling date, the wavebands that accounted for the largest amount of independent variation (between 49–61%) were situated in the region of Cw maximum, around 1410 nm. The second most variable region (21–35%) was represented by wavelength selections in the NIR at all time points, except for one (DoY 132). This is the region where the structural parameter of the leaf, N, is most strongly expressed. Component 3 represented variations in the visible region and hence, the region of chlorophyll expression. In the second half of the sampling season (DoY 161, 174, 182, 188 and 204), specular reflectance (*bspec*) also became an important trait for certain sampling dates. Components 4–6 captured variations in Cm that only represented between 1 and 5% of the total spectral variance.

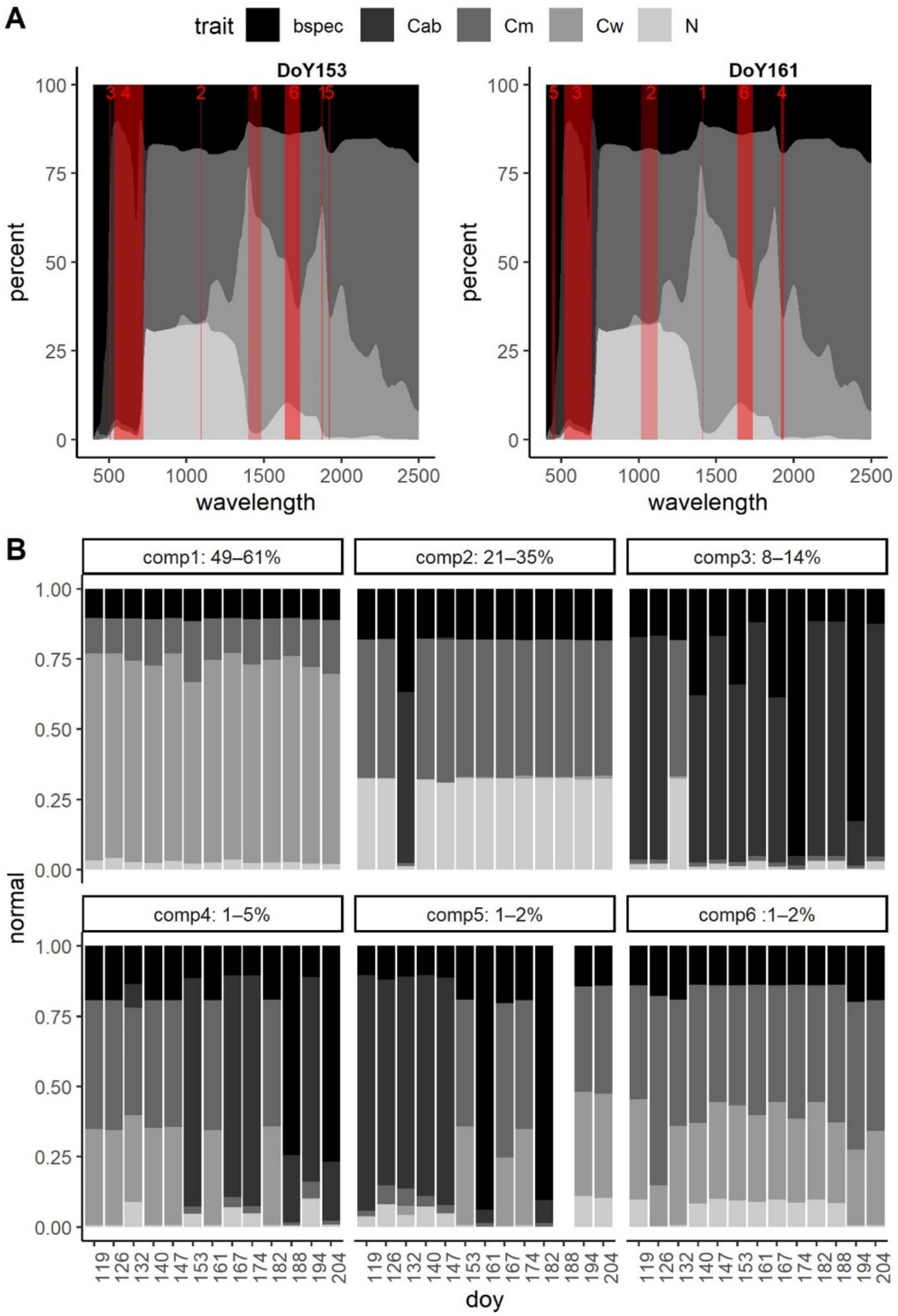


Figure 4.9 (A) The global sensitivity analysis of the radiative transfer model PRO-COSINE for leaf clip data with overlaid waveband selection for the first six components for two example time points (DoY 153 and 161); (B) the probability of the importance of traits for each of the six components over time using the wavelength selection from the best performing sPLS-DA models for each sampling date. The range of variance between model runs for each model component is presented in the panel header.

4.4 Discussion

Using the sparse PLS-DA approach with leaf clip data, species were classifiable to a very good error rate of 0.1% in most cases across the season. This result was obtained using a small sample size (n = 5) per species per time point and it was possible to collect these samples for 17 species within a single sampling day. However, the models that were produced were complex and required between 15 and 21 components, depending on the sampling date and model run. These results suggest that species classification within complex communities will not be an easy task. In addition, 99% of the spectral variance for any one of the sampling dates was explained by only six model components. All model runs and sampling dates were very consistent in this respect. After the application of six components, most species displayed an unsatisfactory error rate for any single sampling date. This meant that a large amount of the discriminatory ability of reflectance data for the species was based on extremely small differences between spectra, which probably resulted from the complex co-varying relationships between the leaf optical traits.

The results from across the growing season showed that some species were consistently easy to classify using a small number of components. Another group was possible to identify but required more components, most of which represented a very small amount of the total spectral variance. The final group of species was impossible to classify to the desired error rate of 10% across time, but at certain time points, the species were well classified. For sampling campaigns in which data are collected during a single day, there is the possibility that species discrimination results from sampling errors and instrumental noise when it is based on very small differences in leaf spectra. It has been shown through simulation studies that when there are more than twice as many classes as samples, the PLS-DA readily finds a hyperplane that is stochastic in nature (Ruiz-Perez et al. 2020). We showed that for species that are easy to classify, the model that was produced from cross-seasonal sampling merely confirmed the results of the models that were produced from single time points; however, for species that are more difficult to classify, it could provide confidence when discriminating between noise and biological signals. We may be able to understand the reasons for the variations in classification error over time in some cases. For example, we saw that for two of the species examined here (*Helianthenum nummularium* and *Sanguisorba minor*), variations in specular reflectance over the course of a growing season strongly affected the ease of classification.

The result that 99% of the spectral variance classified only six species to less than a 10% error rate across time suggested that the ability of the SVH to hold at the leaf level in single date sampling campaigns depended on the extent to which the community was composed of species that were "easy" to detect. SVH, as an unsupervised form of biodiversity assessment, assumes that cross-spectral variance in reflectance can account for the diversity of taxonomic units. However, from the results that are presented here, we could not infer that spectral variance was necessarily correlated with species numbers or their abundances.

The global variance decomposition that resulted from the radiative transfer modelling, alongside the waveband selections that were required for each model run, revealed that leaf EWT was the most important and consistent driver of spectral variance that was related to species classification, followed by N, Cab, *bspec* and LMA (although the relative importance of these traits was more temporally dependent). The importance of the wavebands that related to EWT did not vary with sampling date nor seasonal soil moisture content, as simulated by Sentinel-1. Grime's CSR strategy and Ellenberg's indicator values for the species that were examined here revealed a plant community that was dominated by stress tolerators and adapted to high pH soils. However, the moisture and nitrogen demand of these species was more variable. Similar sampling campaigns that involve the collection of leaf-level water content alongside leaf-level reflectance may help us to better understand why this feature is so important and whether this is limited to this type of stress tolerator system. The transferability of the models during the sampling period (day of year 161 to 204) could also coincide with the trait stabilisation of the leaves and, in turn, the stabilisation of the spectral representation of traits (waveband selection). Yang et al. 2016 found that in tree leaves, LMA and chlorophyll a/b content increase with green-up and then remain steady until leaf fall.

The detection of leaf traits using reflectance data is optimal when using a leaf clip and integrating sphere (Hovi et al 2018) which provides both reflectance and transmittance. In contrast, bi-directional reflectance data that are obtained using a leaf clip result in the over-estimation of cross spectral reflectance due to surface reflectance of the leaves. The application of the results of this study (and others that take a similar approach) in close range imaging spectroscopy requires a consideration of additional sources of variation that relate to anisotropy (light incident angle and illumination zenith angle). These variables can also be modelled using the COSINE radiative transfer model (Jay et al. 2016) but would be additional sources of uncertainty in species determination. Reflectance variance in grasslands at the very high-resolution canopy scale has already been attributed to non-taxonomic properties, such as the vertical complexity of the sward (Conti et al. 2021) the presence of mature leaves (Thornley et al. 2022) and pixels containing soil (Gholizadeh et al. 2018). In this work, we avoided sampling plants that were growing in shaded environments, but there is also evidence that chlorophyll levels vary between leaves that are in the sun and those that are in the shade (Murchie and Horton 1997). These additional sources of variation are likely to further increase the difficulty of species discrimination using close range imaging spectroscopy.

Feature selection and classification model specifications over time could also be affected by methodological choices in the analysis. Here, we applied the sPLS-DA approach to data that were pre-processed using a Savitzky–Golay filter. When utilising close range imaging spectroscopy, spectra are likely to contain more noise than when using a leaf clip. Therefore, the type and optimal amount of spectral smoothing need to be examined in more detail and within differing instrumental contexts. Here, spectra were resampled to a 3-nm bandwidth; however, when optimising classification, the bandwidth choice within differing spectral regions could vary. Finally, sPLS-DA is only one modelling approach for classification and feature selection. In order to develop more robust species discrimination models over time, it is likely that more advanced methods would also need to be tested and compared (Douzas et al. 2019, Banerjee and Raval 2021).

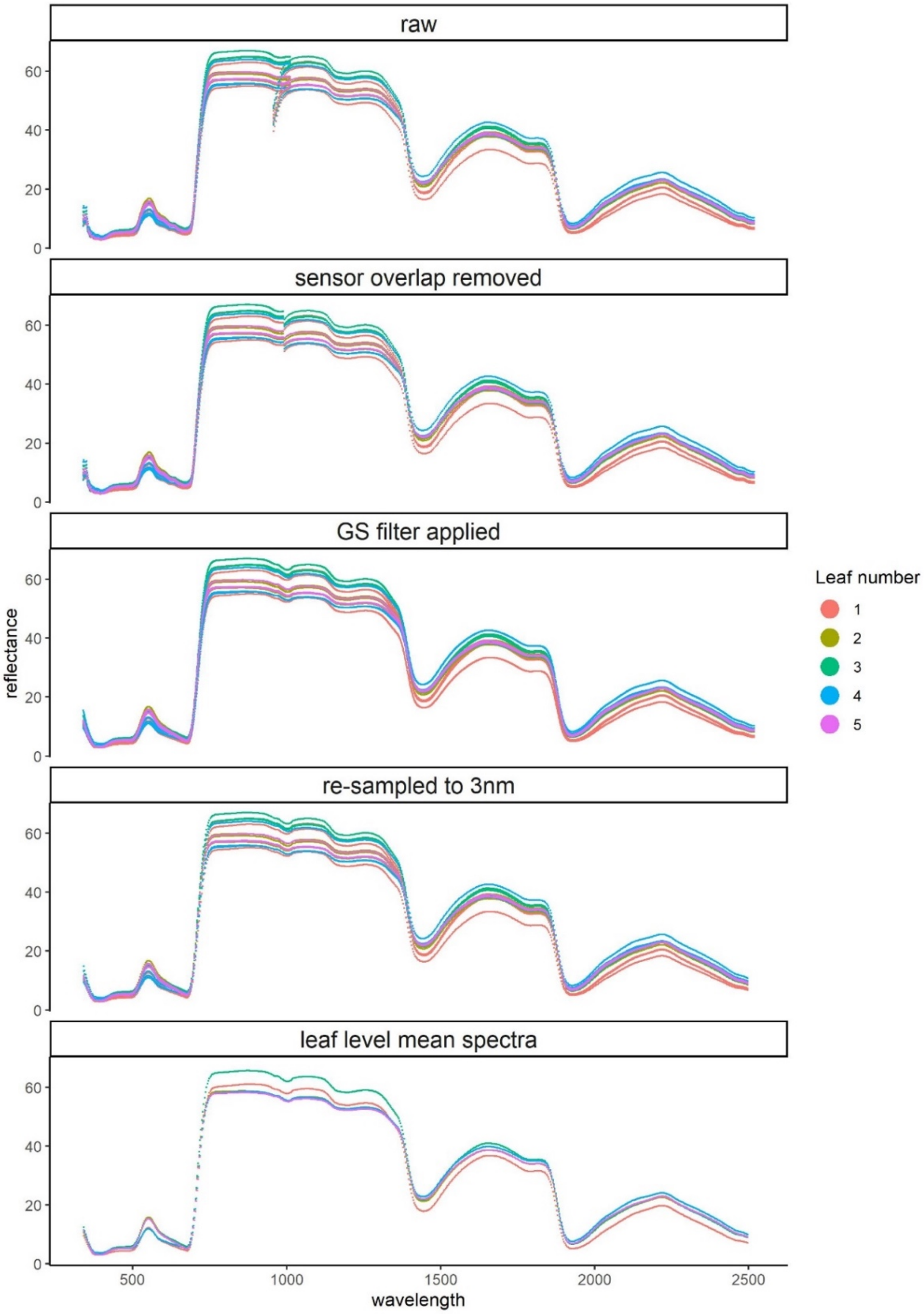
4.5 Conclusion

To date, species discrimination tasks using hyperspectral data have generally been focused on woody species. Despite their conservation status and importance, herbaceous species are less studied and when they are, observations are mostly confined to the dominant species rather than attempting to capture the full botanical composition of the sward. Plant trait studies have shown that the spectral determination of the leaf properties of herbaceous species may be more difficult to obtain than that of the leaf properties of woody species (Roelofsen et al. 2014); therefore, we should practice caution when applying results from studies that are performed in forests to grasslands and we should instead conduct similar work on grassland communities.

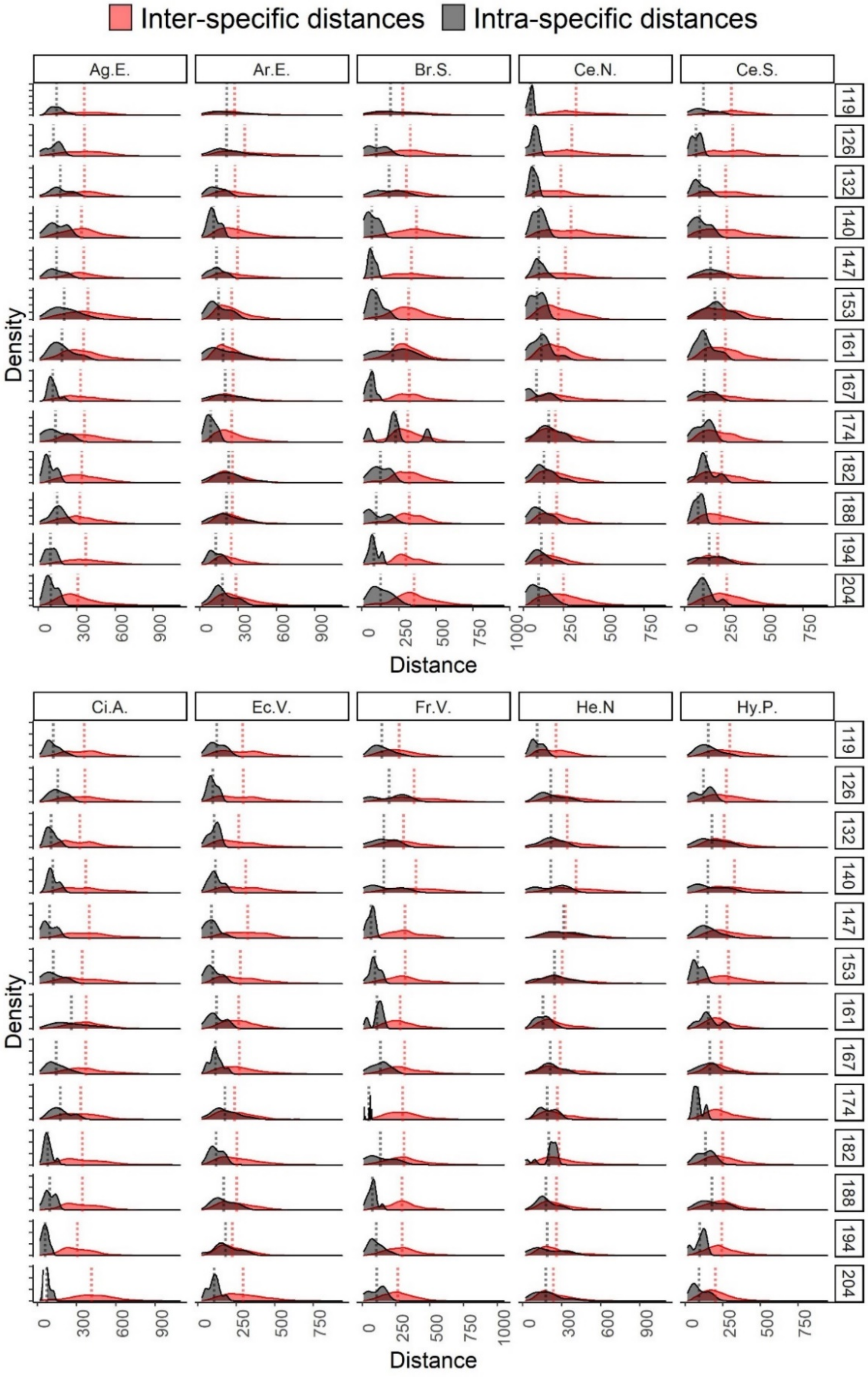
In this study, we found that some species within a community framework were easier to discriminate across the season than others. This pointed to a relative distinction in their leaf reflectance properties. Other species that were more difficult to discriminate required complex waveband combinations, which fluctuated across time. Cross-seasonal sampling, even with small sample sizes, could help to verify which species are driving measures of spectral diversity. Studies that explore species-specific chemical and structural leaf properties and relate these to leaf spectral signatures (Falcioni et al. 2020) are needed to help us to explain with more certainty why some species are easier to distinguish than others and to create a predictive framework for species monitoring and diversity assessment using leaf reflectance. We recommend further studies that explore functional trait frameworks when making predictions of species classes and exploit GSMs and RTMs, alongside biochemical assays, to estimate the importance of traits across different scales and instruments.

4.6 Supplementary Materials

Supplementary Materials 1: Spectra at each pre-processing stage prior to inclusion in the sPLS-DA models. Example spectra are for the species *Agrimonia eupatoria* for sampling date July 01.



Supplementary Materials 2: Differences in distributions using Euclidean Distance between pairs of spectra within species (grey) and between species (red). Mean distances are shown with vertical dotted lines.



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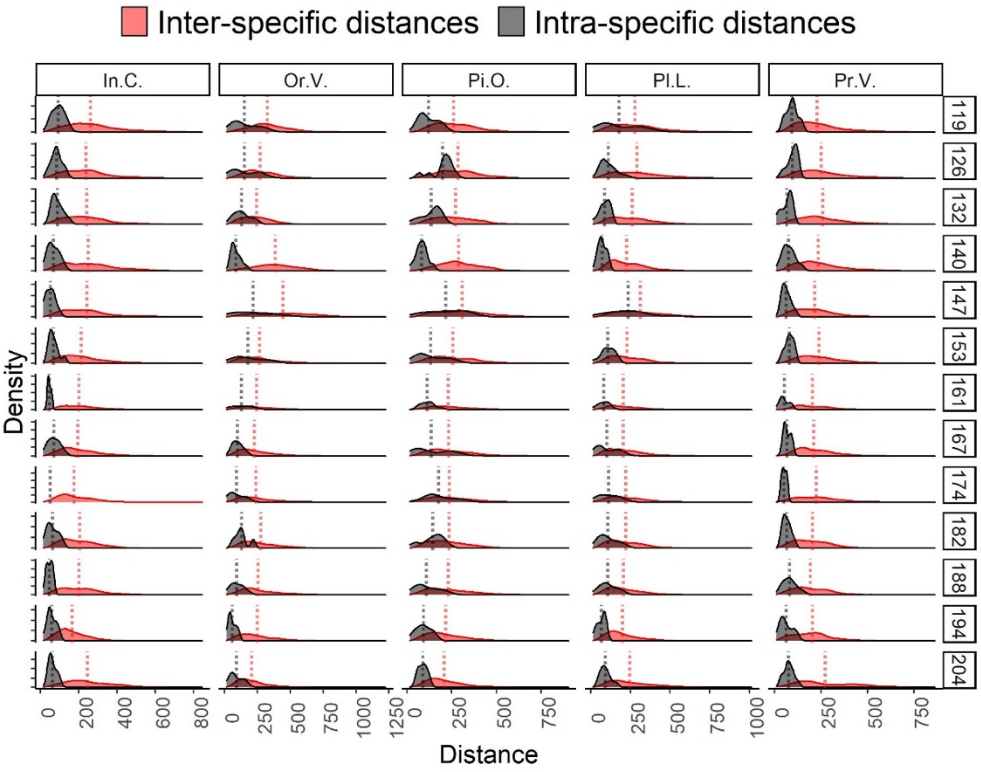
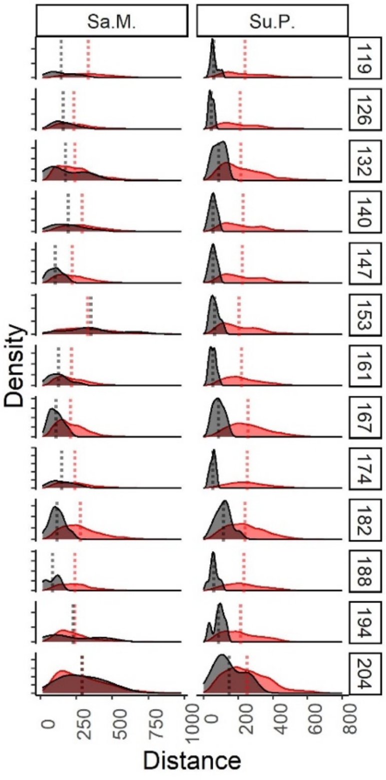
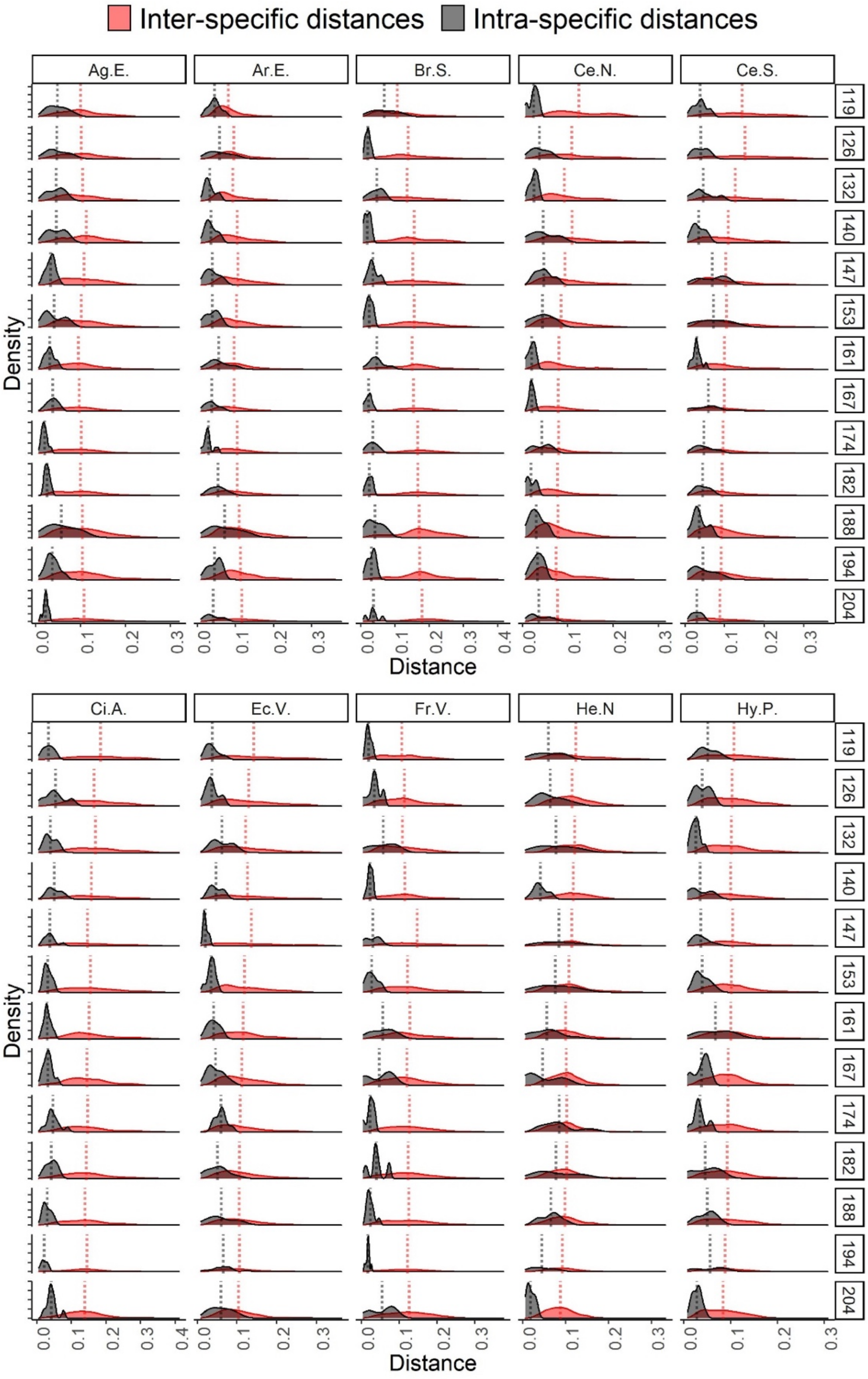


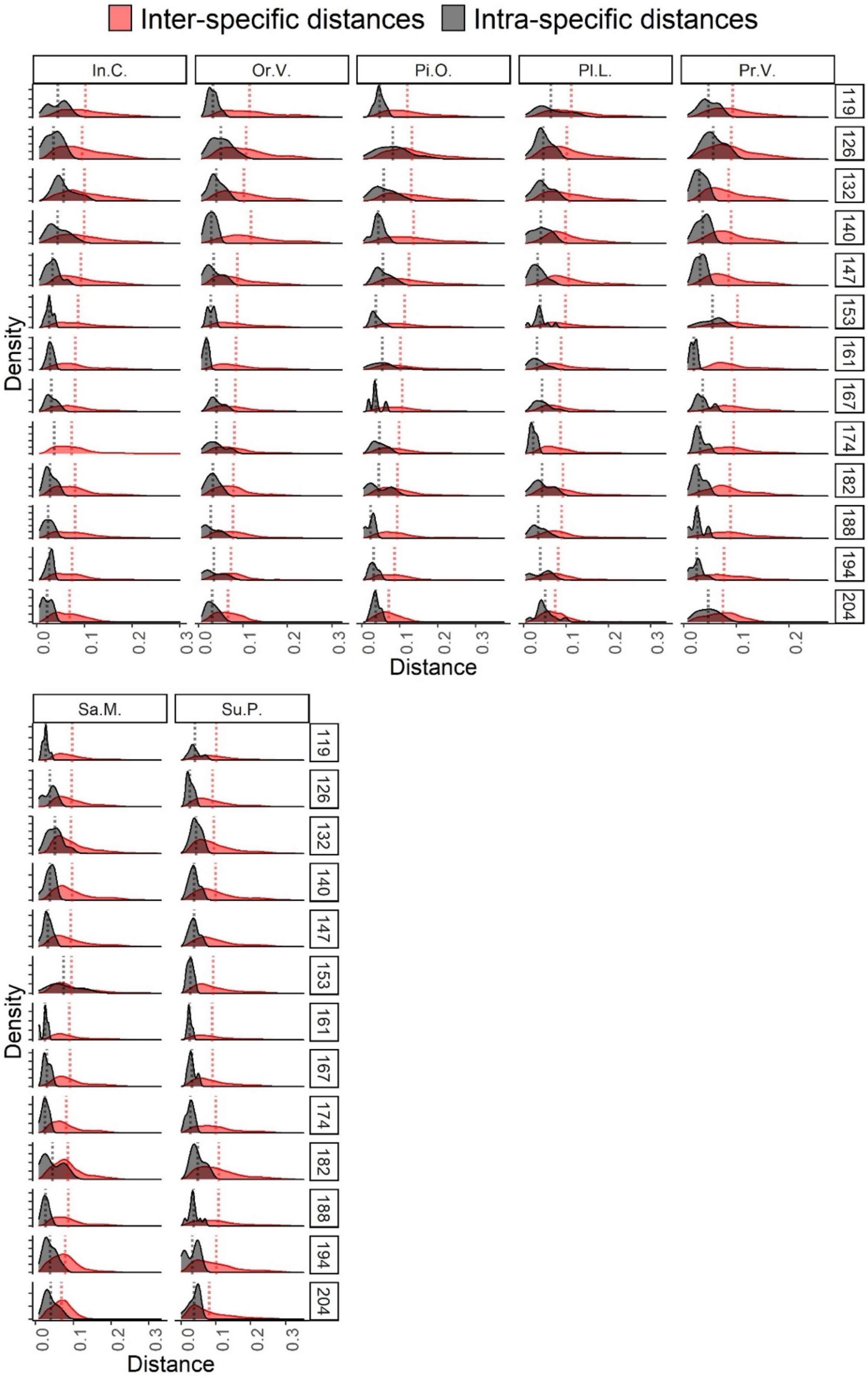
Inter-specific distances
Intra-specific distances
In.C.
Or.V.
Pi.O.
Pl.L.
Pr.V.
119
126
132
140
147
153
161
167
174
182
188
194
204
Density
0
200
400
600
800
0
250
500
750
1000
1250
0
250
500
750
0
250
500
750
1000
0
250
500
750
Distance



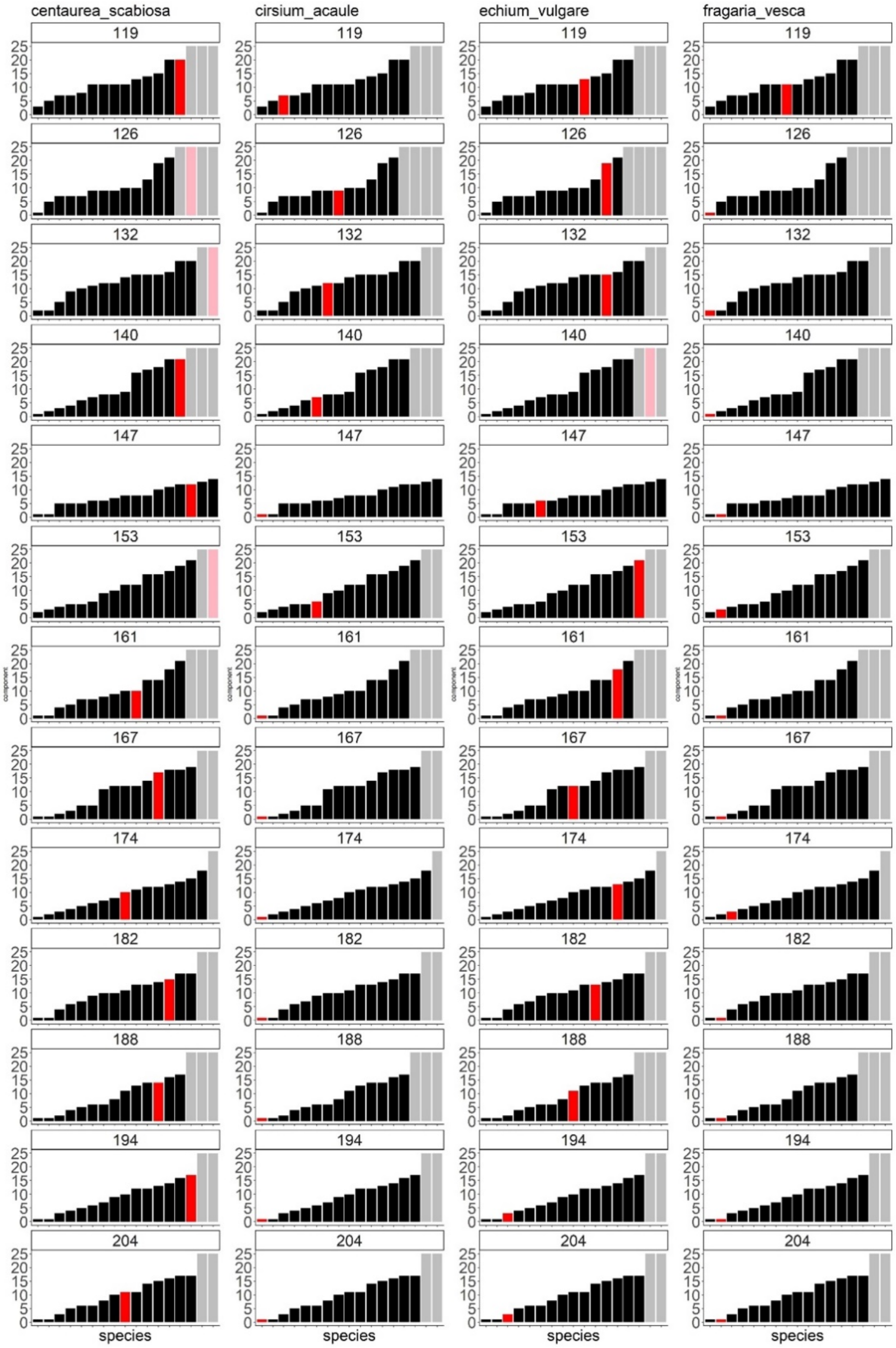
Sa.M.
Su.P.
119
126
132
140
147
153
161
167
174
182
188
194
204
Density
0
250
500
750
1000
0
200
400
600
800
Distance

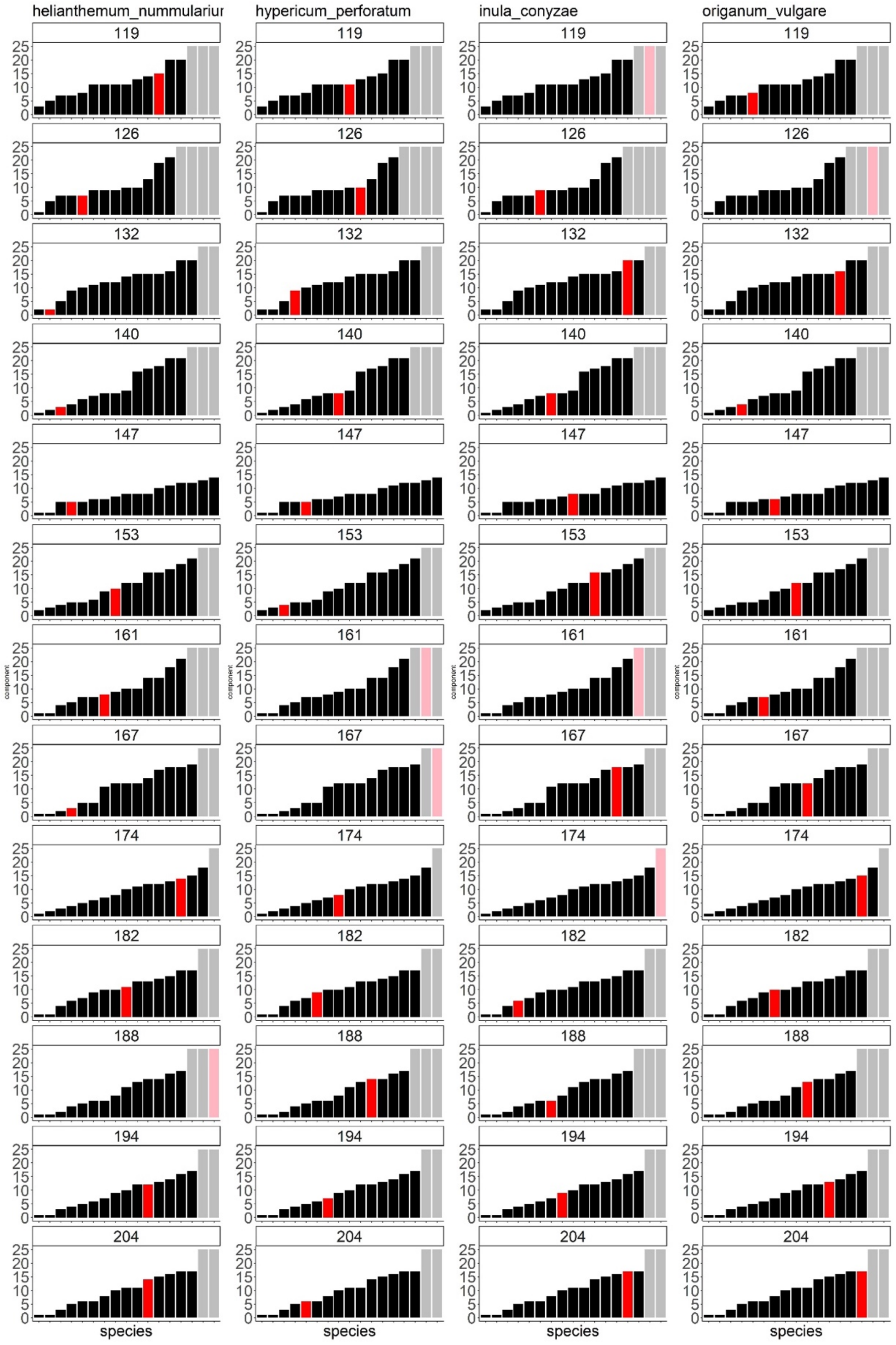
Supplementary Materials 3: Differences in distributions using SAM between pairs of spectra within species (grey) and between species (red). Mean distances are shown with vertical dotted lines.




Inter-specific distances
Intra-specific distances
In.C.
Or.V.
Pi.O.
Pl.L.
Pr.V.
Sa.M.
Su.P.
119
126
132
140
147
153
161
167
174
182
188
194
204
Density
Distance
0.0
0.1
0.2
0.3

Supplementary Materials 4: Ease of classification based on the number of components required for a classification error of less than 10% for each species for each time point (given as day of year on panel). For emphasis, classifiable focal species are shown in red and non-classifiable focal species are in pink. The grey and black bars show non-focal species.

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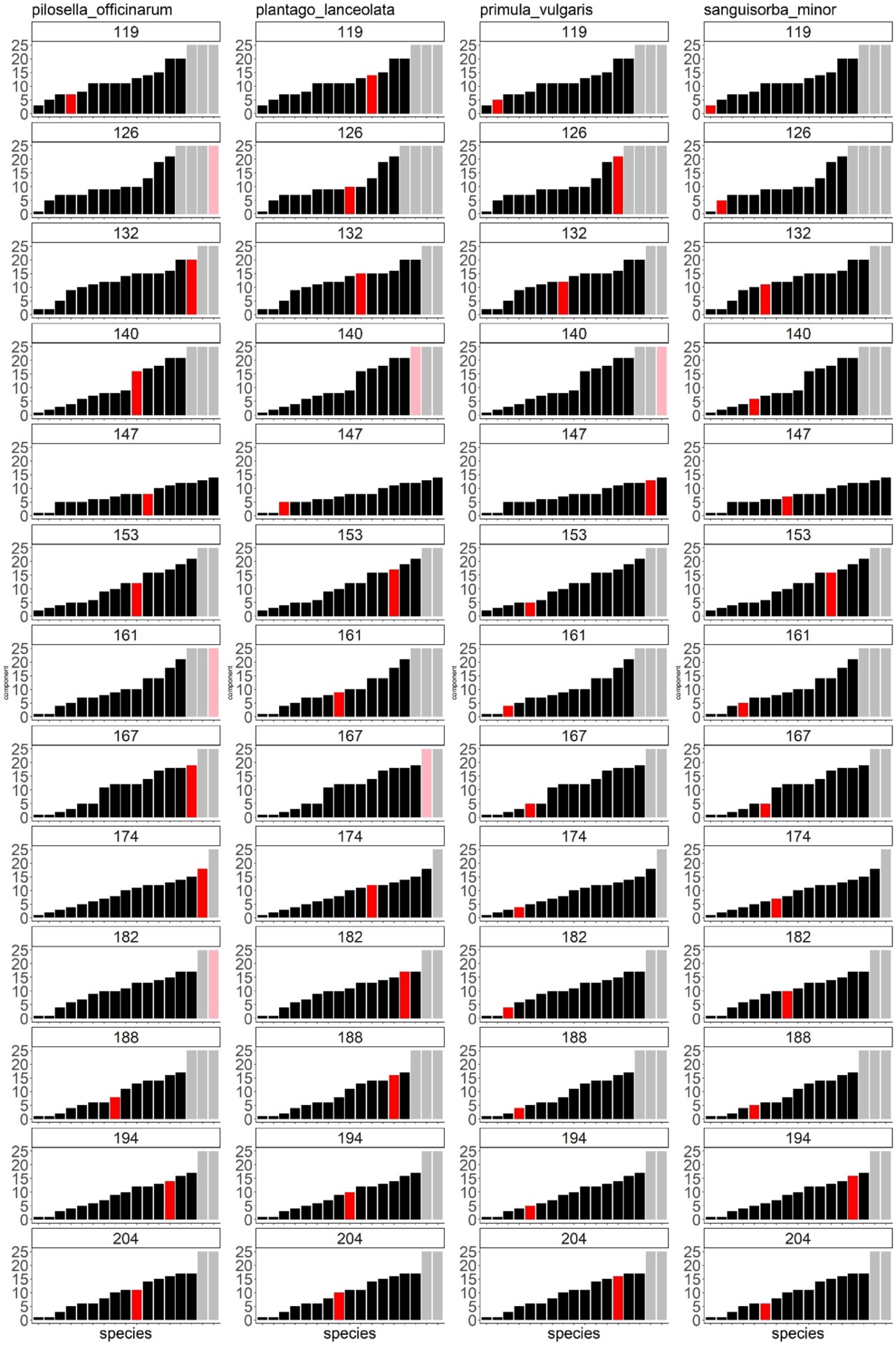
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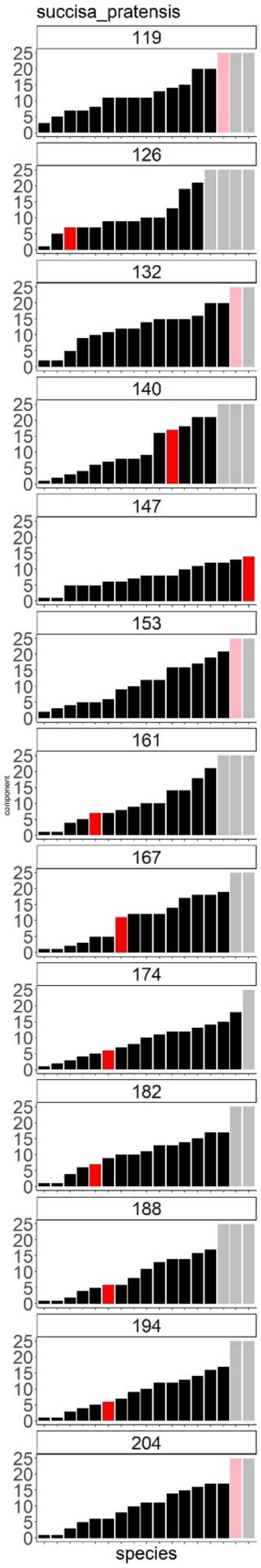
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Chapter 5

Sentinel-2 optical time-series for condition assessment of conservation grasslands

This chapter comprises botanical condition assessments collected in partnership with the 'Old Chalk New Downs' conservation project during the 2020 and 2021 field seasons, across the Kent North Downs, UK. It uses satellite optical time-series data, sourced from the Sentinel-2 MSI sensor to predict different condition criteria within lowland calcareous grasslands at a landscape scale. Thirty-seven vegetation indices were tested for their ability to detect the different indicators of grassland condition and the time-series were analysed using Generalised Additive Models.

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Author contribution statement

Rachael Thornley: conceptualisation, botanical data collection, spetcral data collation, data analysis and writing. France Gerard: conceptualisation, review, and editing. Kevin White: conceptualisation. Anne Verhoef: conceptualisation, review, and editing.

Abstract

Grasslands of conservation value are under threat from conversion and inappropriate management and are in urgent need of monitoring to ensure their longevity. Field collected indicators of vegetation habitat condition, such as sward height, woody vegetation cover, the percentage cover of herbaceous species and the presence of positive indicator species comprise the traditional approach to monitoring in these systems. However, the time-consuming nature of these surveys means they are rarely carried out at the landscape scale. Proxies of vegetation condition could be developed using intra-annual optical satellite time-series which capture how vegetation changes in respect to variation in weather. Here we test the capacity of 37 vegetation indices, derived from the European Space Agency sensor, Sentinel-2, to distinguish between surveyed fields that were classified as favourable or unfavourable for several condition criteria, over two survey years. We used semi-parametric, data-driven Generalised Additive

Models (GAMs), to ascertain whether the time-series of reflectance data for the two levels of condition outcome were distinguishable. We found that vegetation indices have the potential to provide useful information on different aspects of vegetation condition across years, but that the performance of the indices depended on intra-annual weather variations. The best times of year for estimating condition were in the dormant period of the year (mid-winter). We also discussed our results with reference to global sensitivity analyses of the radiative transfer model PROSAIL. It seems likely that differences in chlorophyll and Leaf Area Index are driving much of the optical difference between favourable and unfavourable grassland fields. We conclude that although intra-annual time series of vegetation indices have great potential for predicting differences in condition within conservation grassland systems, there is an urgent need for multi-annual collection of field data to make these predictors more reliable across time.

Keywords: grasslands, condition assessment, Sentinel-2, optical time-series, radiative transfer models

5.1 Introduction

5.1.1 Decline in grassland condition

Globally, the character of natural and semi-natural grassland ecosystems is changing due to land abandonment (Lasanta et al. 2017), conversion (Cvitanović et al. 2017), nitrogen deposition (Stevens et al. 2010), eutrophication (Smart et al. 2003), and climate change (Barnett and Facey 2016). These drivers are accelerating the trend away from complex plant communities to simplified ones (Hülber et al. 2017, Barbosa de Silva et al. 2016), dominated by a few invasive species (Buckland et al. 2001). Grasslands are maintained by complex and locally restrained interactions between climate, soil fertility and biomass removal (Bond 2019). Within a European context, withdrawal of mowing and grazing regimes in semi-natural systems, quickly leads to the advancement of successional processes, with encroachment of woody species and eventual conversion to secondary woodland (Cao et al. 2019).

Species rich semi-natural grasslands in the UK are often found within complex mosaics of intensively managed farmland and woodland. Due to their ecological importance, over recent decades payment under agri-environment schemes has been awarded to landowners for provision of seasonal grazing or cutting regimes. However, there is concern over the effectiveness of these mitigations (Kleijn et al. 2006, Kleijn et al. 2011). There is evidence that tohave the desired impact, agri-environment schemes need to be targeted and require careful planning (Arponen et al. 2013, Batâry et al. 2015). Another frequent criticism of farm payments is that they were made for implementing measures rather than achieving results (Swartz et al. 2008). However, the challenge with a results-based scheme, is that it requires stratified monitoring, to detect management events and to allow recommendation of subsequent desired improvements in habitat condition.

5.1.2 Current conservation monitoring frameworks

To protect landscapes of conservation importance quantifiable aims are required and realistic means of achieving these aims (Lindermayer et al. 2007). One of the problems is the lack of data on system condition and a lack of implementation of appropriate management regimes. Site level rapid vegetation condition assessments are the basis of ecological change monitoring (Bibbons and Freudenberger 2006). They capture system specific variables of vegetation state which can be used as proxies for decline or restoration. In grassland systems, these variables are often linked to successional processes. For example, lack of biomass removal leads to dense grass dominated swards, scrub encroachment and eventually development of secondary woodland. Losses of herbaceous plant species specially adapted to the edaphic conditions of the grasslands usually follow this trajectory, leading to declines in biodiversity across trophic levels (Webb et al. 2010). However, these kinds of condition assessments are rarely collected across landscapes, nor with high enough temporal frequency to detect decline early enough and to drive incentives towards more positive management practices. One of the reasons for the data deficit, is a lack of funding for these endeavours which are expensive to achieve through traditional field methods

5.1.3 Satellite remote sensing time-series and vegetation phenology as indicators of system condition

Some of the indicators of decline used by ecologists in grasslands systems could be retrieved through use of satellite remote sensing data products, thus reducing the financial burden of field surveys. One approach is to use satellite image time-series (SITS) to detect proxies of vegetation phenology, known as Land Surface Phenology (Caparros-Santiago et al. 2021). Reflection data collected in broad bands, and the vegetation indices derived from them, can be exploited to indicate vegetation greenness and development. The most famous of such indices is the Normalised Difference Vegetation Index (NDVI) (Tucker 1979, Petorelli 20013). However, many alternative metrics also exist such as the Enhanced vegetation index (EVI) (Huete et al. 2002), the Green index (Motohka et al. 2010), and the Normalised Difference Phenology Index (NDPI) (Wang et al. 2017, Xu et al. 2021), which have been developed for specific sensors or contexts.

Use of SITS has enabled estimation of global changes in climate (Garonna et al. 2016), species detection (Aragones et al. 2019, Landmann et al. 2020), and functional type mapping (Wang et al. 2013). Phenological proxies derived from the time-series are known as phenometrics, and include measures such as, onset of greenness, green-up midpoint, maturity, peak greenness, senescence, green-down midpoint, and dormancy (Gray et al. 2019). Phenological trends and the derived proxies, vary with the sensor employed (Younes et al. 2021), in part due to differing pixel sizes. Therefore the choice of sensor is an important consideration depending on the required spatial detail of the target vegetation. The MODIS (Moderate Resolution Imaging Spectrometer) (Ganguly et al. 2010) sensor captures daily reflectance data at coarse scales (250m), useful for global studies, while the Landsat Thematic Mapper (Young et al. 2017) with a pixel size of 30m is useful for more regional analysis. The more recently launched ESA Sentinel-2 offers a higher resolution alterative of 10m (Gómez-Giráldez et al. 2020), providing opportunities to capture the phenology of complex mosaics of land cover types. Revisit times of Landsat and Sentinel sensors depend on latitude (Li and Roy (2017)) and the availability of good quality data is also limited by cloud cover (Mercury et al. 2012). This makes the construction of phenological time series difficult in some parts of the globe and within some years.

5.1.4 Use of satellite image time-series for habitat discrimination and relationship to trait detection

Because grassland canopies react strongly to changes in precipitation, and so display temporal dynamism in their phenology, the use of SITS to study them could be especially apt. Existing studies have looked at grassland habitat extent and land cover change over time (Ge et al. 2018), grassland 'degradation' due to over grazing (Gong et al. 2015), and the frequency of management events, such as mowing and grazing intensity (Dara et al. 2020, Franke et al. 2012, Griffiths et al. 2020, Reinermann et al. 2022, Stumpf et al. 2020). Temporal change in greenness indices have also been exploited to discriminate between habitats (Rapinel et al. 2019, Rapinel et a. 2022, Schuster et al. 2015, Tarantino et al. 2021) and characterise ecologically important sites (Maynard and Karl 2017, Lopes et al. 2017), while phenological differences between functional groups, for example C3 and C4 grasses, have been used to map species invasions (Shoko and Mutanga 2017). The ability to use SITS for very detailed habitat specific change detection has also been suggested as an extension of these tested. Bradter et al. 2020, looked at the possibility of classifying grass-dominated sub-habitat types as defined by the UK National Vegetation Classification scheme, at different levels of vegetation distinctiveness. They also examined the influence of spectral resolution, acquisition time and the vegetation classification system on accuracy of classification. They found that particular vegetation indices were important for discrimination at different times of the year.

Much retrieval of plant traits in grasslands has been achieved using hyperspectral reflectance, carried out at the leaf or very high canopy spatial resolution (Zhao et al. 2021), but some work has also been carried out with vegetation indices. Ji and Peters (2007) found that the sensitivity of remotely sensed vegetation indices to vegetation traits depended on the value of the index and that these sensitivities are not constant across the whole index range. For example, NDVI at higher values has been shown to saturate in high biomass environments. As a response to this, indices such as EVI were developed to overcome these issues (Huete et al. 2002). Recently there has been interest in re-framing satellite image time-series to go beyond merely monitoring climate change and assessing vegetation seasonality to exploiting the seasonal time-series as ecological proxy (Lawley et al. 2016, Dronova et al. 2022).

Commonly, methods that employ remotely sensed products for habitat condition assessment tend towards national or global scope encompassing multiple habitat types or classes. However, land parcels of conservation importance in a European context are largely formed by human management and are defined by specific biophysical parameters which vary between systems. Therefore, it would be favourable to develop a system specific suite of remote sensing variables for monitoring habitat condition (Gerard et al. 2015). This approach requires determination of appropriate optical variables that can indicate the specific habitat qualities and the drivers of deterioration and improvement.

5.1.5 Aims and objectives

Here we present the use of a habitat specific 'state and transition model' in conjunction with a suite of intra-annual time-series of remotely sensed optical indices to predict grassland condition based on data collected using field-based rapid condition assessments, collected over two years. We also use a Radiative Transfer Modelling framework to understand the relationship between the wavebands used in optical indices and the canopy level trait drivers of optical variance. Although the analysis is applied to a particular grassland type, the approach and methods could be applied to any system of conservation interest. Our main aims are to test:

1) Whether optical time-series can be used for detection of grassland condition in this system.
2) Which indices are the most effective at picking up the condition elements and at what time of year.
3) Whether the results of the analyses are stable across the two sampling years.

5.2 Materials and Methods

5.2.1 The study system

Here we apply our method to a specific UK habitat, 'lowland calcareous grassland' (JNCC 2011) of the Kent North Downs (KND) Area of Outstanding Natural Beauty (AONB) in the south-east of the UK. Thirty-seven fields were chosen as part of a conservation management project, covering approximately 1km^2 of chalk grassland habitat of varying condition status. The KND consists of a fragmented patchwork of land parcels, interspersed with woodlands, croplands, re-seeded pastures, and valuable semi-natural grassland fields. Lowland calcareous grasslands in the UK correspond to more wide-spread European communities belonging to the class Bromion (otherwise known as Meso-bromian) (Rodwell et al. 2007). These habitats are consistently found on well-drained rendzina soils above chalk or limestone substrate and are significant in terms of their high species richness and sward diversity. Grasslands of this type have very specific soil properties of high pH (7.7–7.9) with low nutrient levels generally, but particularly low phosphorus (Critchley et al. 2002). Calcareous grassland covers 1,929ha of the southern UK county of Kent, comprising 0.5% of the county's surface area, and 5% of the total UK calcareous grassland resource. Most of this habitat occurs along the North Downs, a narrow chalk ridge, which at its most eastern edge meets the sea at the famous white cliffs of Dover (Kent Habitat Survey 2012). According to the UK National Vegetation Communities Classification (NVC) the calcareous grasslands in Kent range from short, grazed species rich swards (CG2 *Festuca-Avenula*) graduating into the longer, ranker, less intensively grazed communities CG3 (*Bromus erectus*), CG4 and CG5 (*Brachypodium pinnatum*) (Rodwell 2008). Locations of fields surveyed in this study are given in Figure 5.1.

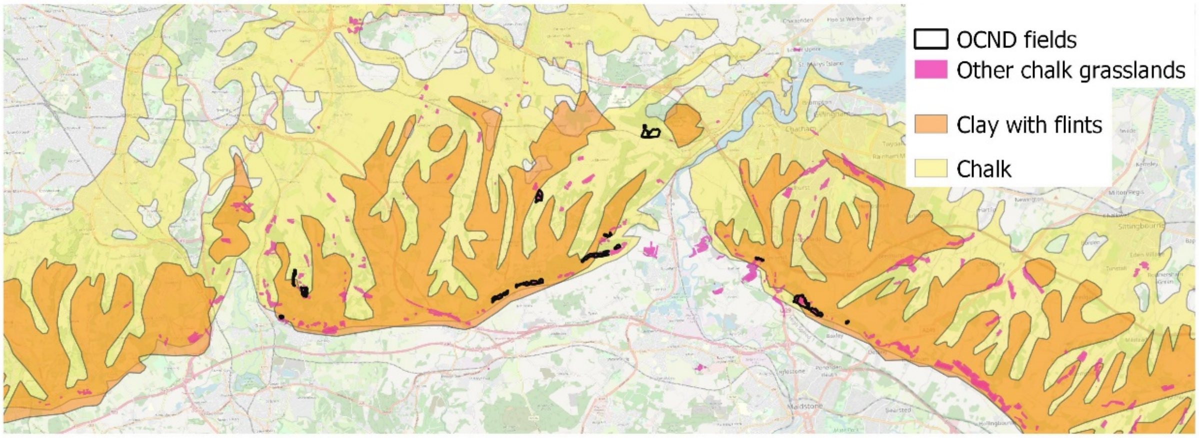

Figure 5.1 Map of the Kent North Downs showing the location of the fields used in this study.

5.2.2 A state and transition model to describe changes in habitat condition

Conceptualising vegetation states, and the drivers of habitat change that cause transitions across states, is essential so we can develop spectral indicators of grassland condition. One option is to use a 'state and transition model.' This is a conceptual approach which identifies the target communities, the community phases at risk of degradation, transitions to 'alternative states,' as well as habitat thought to be un-restorable. Pathways of decline and restoration are also indicated.

In the case of lowland calcareous grasslands, maintenance depends on removal of a significant amount of annual biomass through grazing or cutting, otherwise species poor grasslands develop. Failure to remove annual biomass leads to dominance of grass species and eventually woody species become established. Scrub encroachment has been observed globally as a threat to grasslands (Cao et al. 2019). In these systems mosaics of grassland and scrubland transition phases can add to the diversity of both the floristic and faunal component of these systems so should not be discouraged. However, these scrub/grassland interfaces are difficult to maintain and generally lead to damage to the grassland component (Ratajczak et al. 2012). Currently natural grazing levels are low, principally due to the decline since the 1950s of wild rabbit populations as a result of their biological control through myxomatosis (Lees et al. 2008). Domestic livestock numbers in the area have also been greatly reduced due to the movement towards industrialised cereal production and the remaining grassland systems are unattractive to food producers because of their low productivity compared to fertilized and re-seeded pastures

(Sienkiewicz-Paderewska et al. 2021). The maintenance of these systems relies heavily on subsidised conservation grazing regimes, through charities and stewardship payments.

5.2.3 Field condition assessments

Criteria for field site condition assessments have been established over the last few decades. Rapid grassland condition assessments (Robertson 2000) involve observations of sward structure, the presence of invasive species (including woody species) and the decline in herbs compared to grass species. Chronosequences of grasslands of known or suspected age have been used to develop lists of 'ancient grassland indicators' (Wagner et al. 2019) and these taxonomic indicators of grassland state are also regularly included in condition criteria for habitats of conservation concern. In this study, established rapid condition assessments were carried out according to the condition criteria outlined in Table 5.1. Twenty surveys were carried out in the summer of 2020 and 35 surveys in the summer of 2021. Altogether 37 separate fields were surveyed. Nineteen fields only received one assessment, 2 in 2020 and 17 in 2021, whereas 18 fields were surveyed twice (once in 2020 and once in 2021).

Table 5.1 Overview of condition criteria and how they were assessed for the grassland rapid field condition assessments.

Summary Name of condition criteria	**Biophysical Indicator of Condition**	**Criteria for assignment of favourable condition**	
Sward	Lack of seasonal biomass removal through grazing or mowing or adversely over-grazing	Average height of sward across the field Target: 2-15cm	
Species (positive)	Prescence of specialist species associated with habitat	Presence of positive indicators species Target: At least two indicator species are frequent, and three occasional.	
		Anthyllis vulneraria *Arenaria serpyllifolia* *Asperula cynanchica* *Blackstonia perfoliata* *Bromopsis erecta* *Campanula glomerata* *Campanula rotundifolia* *Carex flacca* *Carlina vulgaris* *Centaurea scabiosa* *Cirsium acaule* *Clinopodium vulgare* *Euphrasia spp* *Galium verum* *Gentiana spp* *Helianthemum nummularium*	*Hippocrepis comosa* *Hypericum hirsutum* *Leontodon hispidus* *Linum catharticum* *Ononis repens* *Onobrychis viciifolia* *Ophioglossum vulgatum* *Origanum vulgare* *Pilosella officinarum* *Pimpinella saxifraga* *Plantago media* *Polygala austriaca* *Polygala calcarea* *Polygala vulgaris* *Primula veris* *Rhinanthus minor* *Sanguisorba minor* *Scabiosa columbaria* *Thymus spp* *Viola hirta* *Orchidaceae (any species)*
Species (negative)	Gain of non-specialist species not associated with habitat	Presence of negative indicator species Target: Frequency no more than occasional.	
		Cirsium arvense *Cirsium vulgare* *Rumex crispus* *Rumex obtusifolius* *Senecio jacobaea* *Urtica dioica*	
Herbs	Community shift away from herb rich swards towards grass dominated	Percentage of grasses and broad-leaved herbs Target: Cover of wildflowers and sedges make up more than 30% of the vegetation.	
Scrub	Dominance of woody species	Percentage cover of trees and shrubs Target: Less than 5% cover (can be site adjusted dependent on invert/reptile requirements)	
Overall	-	All condition criteria have passed	

5.2.4 Sentinel-2 satellite image time-series (SITS)

The European Space Agency satellite mission Copernicus Sentinel-2 offers open source, multi-spectral reflectance data through two polar orbiting satellites with a revisit time of approximately five days at mid-latitudes. Data is collected within twelve bands, ten of which are useful for vegetation monitoring (see Supplementary materials 1). The high spatial resolution of the visible/NIR bands (10m) and the NIR/SWIR (20m) makes possible detection of vegetation change within small field sizes, such as those found in complex agricultural mosaics.

We used the package Sen2r (Rangetti et al. 2020) in the R programming environment (R Core Team 2022) to search for ESA Sentinel-2 products processed to level 2A (bottom of atmosphere reflectance) for tiles overlapping the study site (30UYB and 30UYC). Images were only selected with less than 75% total cloud cover and for the time-period starting 1st January 2020 to 1st January 2022 coinciding with the years field condition assessments were carried out.

Cloud cover is a major obstacle to obtaining satellite time-series. The UK has a humid temperate oceanic climate (Koppen climate classification system) (Beck et al. 2018), with average total sunshine at about 30-40% of the maximum possible hours. To further filter the data for cloud contamination, we used the Scene Classification Layer from the Level 2-A products, and selected pixels that contained only vegetation (class 4 of the SCL).

Using the Index Database (Henrich et al. 2009) (https://www.indexdatabase.de/db/si-single.php?rsindex_id=209&sensor_id=96), we searched for spectral indices recommended for the Sentinel-2A mission and vegetation monitoring, resulting in 182 indices. From these we selected 37 unique indices that had been used in previous studies to monitor grasslands or other herbaceous structures. Indices were included that had been used for estimation of biomass, cellulose, leaf area index, starch, or water content. Full details of indices are given in Supplementary Materials 2. Site polygons of grassland parcels where geolocated during the field campaigns using a differential GPS and indices were calculated for each pixel located within the site polygons.

5.2.5 Global sensitivity analysis of PROSAIL radiative transfer model

To link the condition assessment criteria with the optical time-series of indices, a global sensitivity analysis (GSA) was performed (Ferretti et al. 2016) of the radiative transfer model, PROSAIL (Jacquemoud et al. 2009), a combination of the leaf level model PROSPECT-4 (Feret et al. 2008) and the canopy level model (SAIL) (Verhoef 1984). PROSAIL is a mechanistic model that enables an understanding of the drivers of reflectance across the electromagnetic spectrum dependent on the values of a selection of canopy and leaf traits. For a list of the model variables and their parameters see Table 5.2. By performing a GSA we can compartmentalise the variation space of the optical model into the relative influence of the canopy parameters, for each of the optical indices in our data set. Therefore, the method offers a way of understanding the biophysical basis for the ability of the indices to pick up states of vegetation condition.

Table 5.2 The variables of the PROSAIL Radiative Transfer Model and their range of values used in the global sensitivity analysis.

Model	Symbol	Quantity	Unit	Range
PROSPECT-4	N	Leaf structure parameter	-	1-4
	C_{ab}	Chlorophyll a+b content	ug cm^{-2}	0-100
	C_w	Equivalent Water Thickness	cm	0.0001-0.08
	C_m	Dry Matter Content	g cm^{-2}	0.0001-0.05
SAIL	LAI	Leaf Area Index	-	0-15
	LAD	Leaf Angle Distribution	-	0-90
	S_L	Hotspot parameter	-	0-100
	SKYL	Ratio of diffuse total incident radiation	-	0-1
	sza	Solar Zenith Angle	degrees	0-90
	vza	Viewing Zenith Angle	degrees	0-90
	raa	Relative Azimuth Angle	degrees	0-180

5.2.6 Generalised Additive Models (GAMs)

Curve fitting to satellite image time series is a substantial field of research (Bradley et al. 2008). The approaches largely dependent on the length of the time-series and the frequency of the acquisition.

Traditional time-series and change detection techniques require long time-series, where trend and seasonality can be extracted (Verbesselt et al. 2010). To exploit these methods for small changes in condition, we would need to have accompanying field data over similarly long periods. Here field data sets are available for two years only, so these approaches are not suitable. An alternative approach is to analyse intra-annual time-series separately for each year, and then to compare the results between years for an assessment of stability. A Generalised Additive Model (GAM) is a semi-parametric, data driven statistical approach to dealing with non-linear, complex data structures (Zuur et al. 2009). The advantage of using GAMS over machine learning approaches, is that they require less data, and they are easier to interpret, thus avoiding the 'black box' criticism (Zhu et al. 2017). However, GAMs, like machine learning models, are very flexible to the data structure so are prone to over-fitting and must be carefully employed. GAMs are frequently applied to time-series analysis in general (Curtis and Simpson 2014, Simpson 2018) as well as to satellite image time-series (Lee et al. 2020, Zhang et al. 2018).

GAMs use a smoothing function, which changes throughout the data range, and the complexity of the model can be understood as the extent to which the curve changes shape (Wood 2017a). Here we fitted GAMs with the mgcv package (Wood 2017b) inR (R Core Team 2022) using a type of smooth term called a penalised regression spline. It is possible to determine the amount of smoothing manually or the optimal amount can be estimated using cross validation and likelihood-based methods. Here the smoothing parameter estimation problem was solved by using the minimised generalised cross validation criteria (GCV) (Wood et al. 2016). A predetermined degree of smoothing is also specified by the number of 'knots'. Here we applied values 4, 6, 8 and 10. As GAMs can suffer from under-smoothing (over-fitting on the data), deciding on smoothing extent relies on knowledge of the system under study, as well as visual observation of the smooths for different levels of knots. Here we presumed that lower numbers of knots, and hence a simple smooth, would reflect the seasonal trend of green-up in the spring, biomass build up during summer and then senescence during autumn and winter months. Likewise, the more complex smooths with higher numbers of knots would additionally capture the impact of weather events (wet and dry periods) within the simpler seasonal trend. However, they could potentially also be affected by any noise that had remained in the data set after the pre-processing caused by cloud or haze.

Here we use GAMS to predict two outcomes (i.e. 'favourable' and 'unfavourable') for the grassland condition criteria which describe the vegetation state of the fields. To achieve this, we created different smooths for the different levels of the categorical term. This approach is called a 'factor smooth interaction'. Statistical differences between the two smooths, representing the two levels of the factor can be assessed and the magnitude of difference at any section of the time-series can also be obtained. GAMs were run for each year (2020 and 2021), with the two outcomes, 'favourable' and 'unfavourable', as categorical smooths for each of the condition criteria (Table 5.1) for each of the 37 vegetation indices. Dependency in the spatial data was accounted for by inclusion of the categorical variable 'field' as a random effect. Model assumptions were assessed using residual plots, which showed that there were no violations of independence. P- values of smooths were assessed using Restricted Maximum Likelihood (REML). To assess the performance of the indices, we also examined the 'fit' of the model. Within a linear modelling framework, the coefficient of determination (r^2) is used for this purpose. However, this is not appropriate for the GAM models, instead the 'deviance explained', which is a measure of the likelihood that the model fits the data, is reported, with higher values indicating a better fit. When we ran the models, using cross-validation, whereby the amount of smoothing was estimated based on the minimisation of prediction error, the resulting GCV score was also reported.

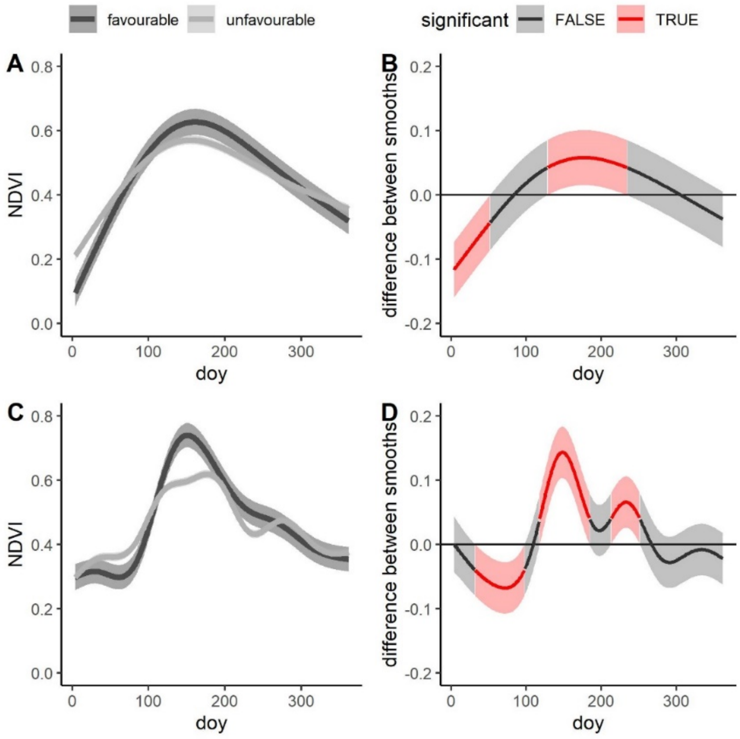

Figure 5.2 An example of how the Generalised Additive Model was used to predict the favourable and unfavourable outcomes for the field condition criteria, using the time-series of EVI for the year 2020.

Firstly, a factorial smooth was fitted, over different parameterisations of the smooth (here A, is the smooth fitted at 4 knots, and C at 10 knots). Secondly, the difference between the factorial smooths was estimated and the regions of significant difference between smooths used for further analysis (C at 4 knots and D at 10 knots).

During the course of this analysis, we aim to use GAMs in the following ways:

1) To test if factorial smooths of vegetation indices can differentiate between the fields in favourable / unfavourable condition, for the five field condition criteria (Table 5.1).
2) To test how methodological changes in the parameterisation of the GAMs affects our ability to differentiate between the field conditions.
3) To assess which indices are most effective in differentiating between the different aspects of condition.
4) To assess what time of year is most important for different aspects of condition.
5) To assess how transferable the observations are across the two years

To do this, we extracted the regions of the time-series where the factorial smooths were statistically different from each other (see Figure 5.2). We then assessed the magnitude of the maximum difference, with more suitable indices having larger differences. We performed the assessments on GAMs that had been parameterised over four different smoothing levels (knots set at 4, 6, 8, 10) as well as the smoothing level selected by cross-validation (cv) and examined whether the ability of the index time-series to predict the condition criteria remained stable over the different smoothing levels. We used the day of year on which the maximum difference value occurred to gain an insight into the best times of the year for discrimination between condition criteria outcomes.

We expect that some of the smooths for different indices will be correlated. Normally correlation of smooths is assessed within a model framework via a process called concurvity (Pedersen et al. 2019). However, due to large number of co-variates (indices) within the study that was not computationally possible. Instead, the predicted values of the difference smooths were compared using distance correlation (Székely et al. 2007). Correlation coefficients such as Pearson's Correlation Coefficient assess linear correlation between variables but are not suitable for non-linear relationships. Distance correlation is not the correlation between the distances themselves, but the correlation between the scalar products which the 'double-centred' matrices are composed of (Edelmann et al. 2021).

5.3 Results

5.3.1 Field based condition assessments and the state and transition model

Although most of the fields were favourable for the condition criterion positive indicator species present, the majority of the fields were found to be unfavourable overall. This was due to the strict criteria, that to be favourable in this category required all of the other condition criteria (species, herbs, scrub, and sward) to be favourable. The field criterion 'Negative Indicator Species' was omitted from the analysis as none of the listed species were found in the fields. Therefore, results will be shown for the condition criteria 'Positive Indicator Species', 'Scrub cover', 'Sward height' and 'Percentage herbs', and for the overall condition. The sample sizes of the data used in the analysis in terms of both the number of fields and the number of Sentinel-2 pixels for each category are shown in Figure 5.3.

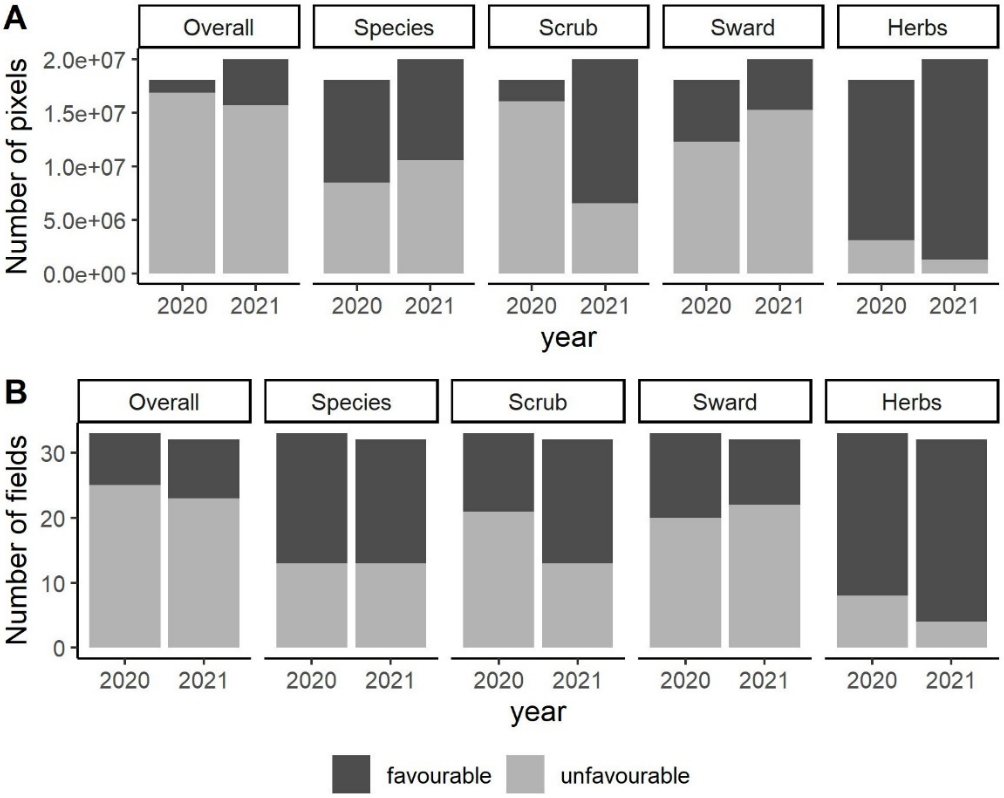

Figure 5.3 The sample sizes of the data set by year, condition criteria and condition outcome, expressed as, A, the number of pixels, B, the number of fields.

For the eighteen fields that were sampled twice (in 2020 and 2021), overall condition changed in only 3 out of 18 fields, with two fields changing from unfavourable to favourable, and only one field changing from favourable to unfavourable. The outcome for positive indicators species changed in 5 out of 18 fields, herbaceous plant cover changed in 4 out of 18 fields, scrub cover changed in 8 out of 18 fields and sward height in 8 out of 18 fields (Figure 5.4).

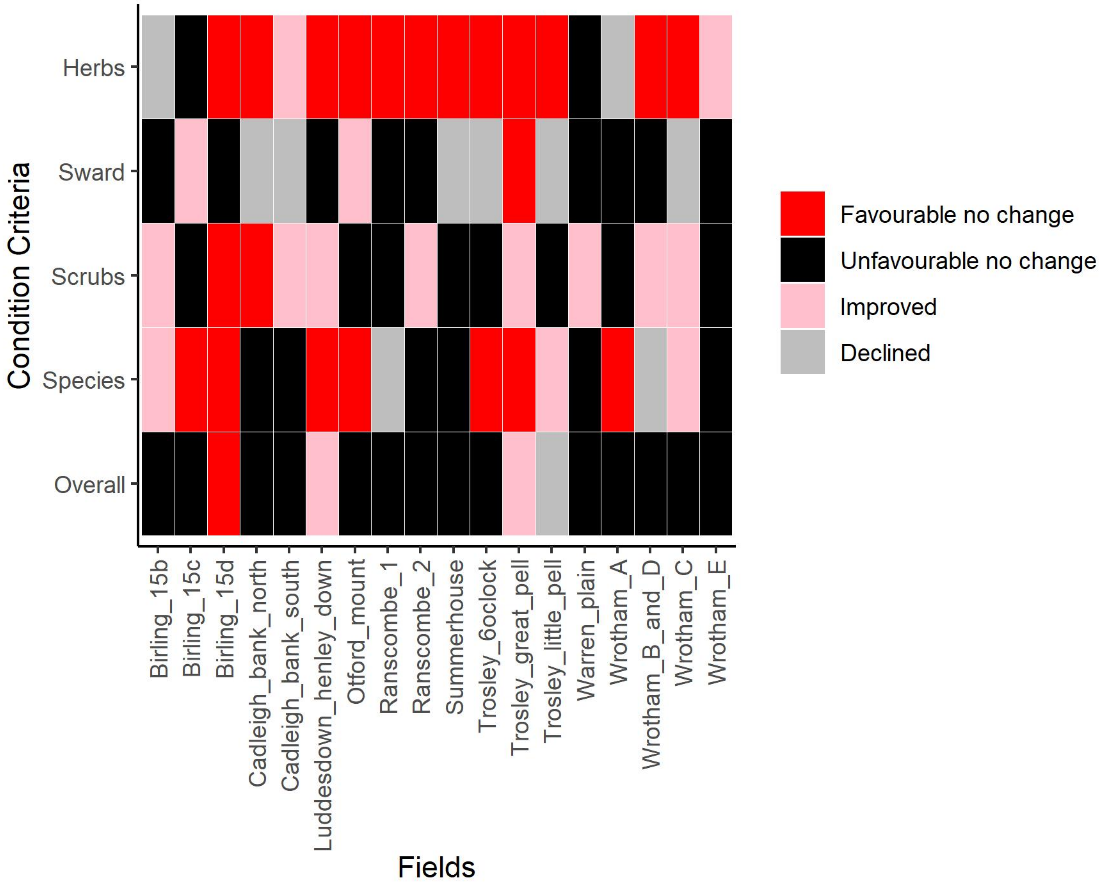

Figure 5.4 The change in condition between the two years for fields that were surveyed twice

We related the changes in condition, as outlined in the condition assessment (Table 5.1), to a state and transition model (Figure 5.5). The drivers of decline in this system relate to biomass build up, caused by lack of grazing. In the first instance, this biomass build-up leads to long grass dominated swards. Eventually, succession to woodland begins, with scattered small scrub, leading to development of larger patches. Finally, the grassland component of the habitat is lost. Equally, re-introduction of appropriate cutting and grazing regimes, alongside scrub control can reverse these processes, providing that the taxonomic communities have not been lost.

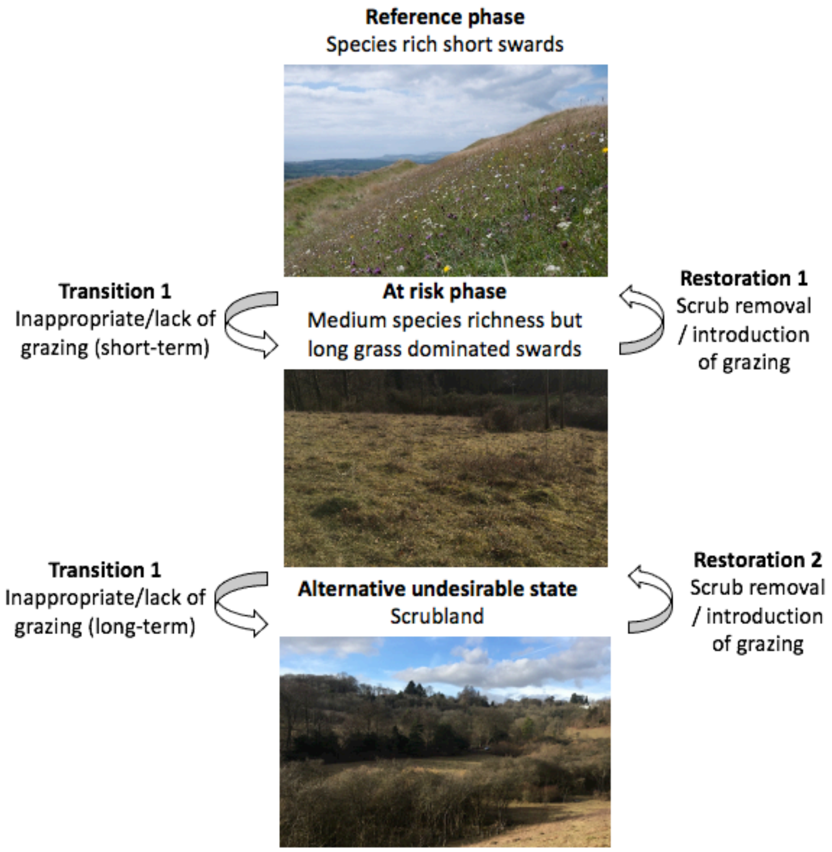

Figure 5.5 A state and transition model for the lowland calcareous grasslands of the Kent North Downs, detailing the management drivers of the different states.

5.3.2 The ability of the vegetation indices to predict grassland condition

The GAM results showed that factorial smooths of the vegetation indices were overall statistically different from each other at ($p < 0.001$) irrespective of the number of knots applied to the model or the condition criteria examined. The random term of ‘field’ which was used to account for spatial autocorrelation in the analysis was also consistently significant at $p < 0.001$. The deviance explained by the models depended mostly on the index, but also somewhat on the year. The highest values found in the cross-validated models were for the indices SR735-710, SR672-550, MSI and GI in 2021, where the deviance explained by the model ranged from 50-65%. Results of the GAMs can be found in

Supplementary Materials 3. The cross-validated smooths returned an effective degrees of freedom (EDF) value of around 9 (rounded to whole number), irrespective of year or index. This is equivalent to setting the knots manually to 10. Therefore, we will report on the four manual choices of knots (4, 6, 8, 10), keeping mind that the model with 10 knots should be considered to have a better data fit than the models with a lower number of knots. From the example in Figure 5.2, we can see that the knots set at 4, captured the typical vegetation green up trajectory, for the index EVI, and that when the knots were set at 10, there was evidence of a late summer dip in the magnitude of the index, which was expressed more strongly in the unfavourable sites. Other similar variation was observed in the time-series for the other vegetation indices (not shown).

The use of SITS in prediction of whether fields were in favourable or unfavourable condition was generally successful for both years, with several indices proving useful for discrimination between the condition outcomes of all the condition criteria. The most important indices, as assessed by the magnitude of the maximum difference between factorial smooths, varied between the different levels of smooth parameterisation (knots 4-10) and for the different condition criteria and between years (Figures 5.6 A and B). The starkest difference was between the smooths parameterised at 4 knots and between the other levels of parameterisation (6, 8, 10 knots). Generally, the indices performed better in 2021 compared to 2020, especially when a higher number of knots was applied to the models. The highest absolute difference smooth from the 2021 data (1.27, for the estimation of positive indicator species) was almost twice the magnitude of the highest difference smooth in 2020 (0.613, for the estimation of overall condition). Both highest values were predicted using the time-series for the index CI red-edge. During 2021, it was possible to predict differences in all five of the condition criteria by using one of a number of indices(GI, CVI, SR700, SR672-550, SR735-710, CI green) and this was largely independent of the number of knots applied to the model. In contrast, during 2020, a few indices could be used to predict all elements of condition (SR700, SR735-710) but these were very dependent on the number of knots.

To assess the difference in results between years, we calculated the cumulative difference between significant regions of smooths for all the indices (Table 5.3). For the overall condition, the smooth differences were comparable between years. The results of 2020 were better for estimating sward height, but 2021 was the better year for estimation of positive indicator species, scrub cover and percentage herb cover. In both years and across condition criteria, the greater differences between smooths were found when the GAM was set to a higher number of knots, thus capturing seasonal changes in weather conditions. This indicates that responses to rainfall and drought within a growing season, are important in distinguishing states of condition in these habitats.

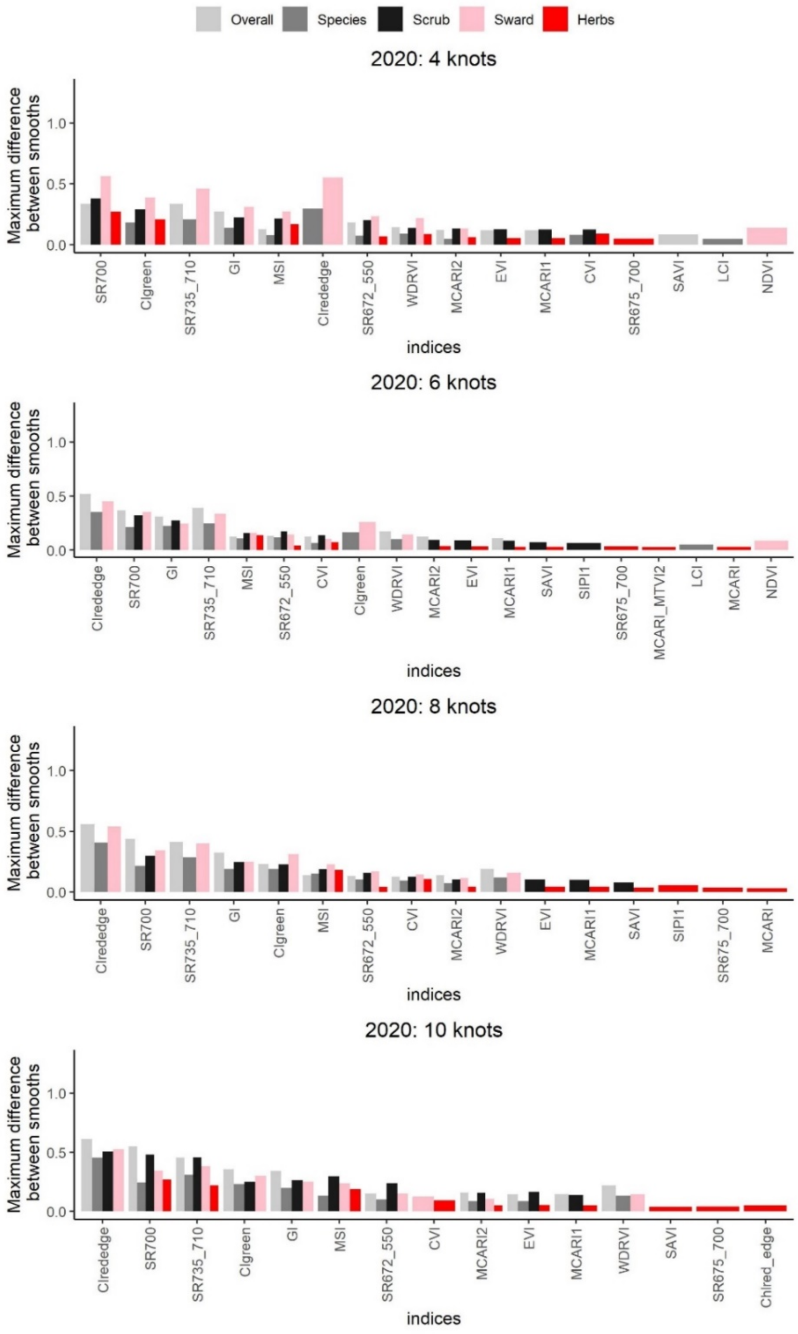

Figure 5.6 A The magnitude of the highest difference between smooths obtained from the predictions of the two-level factorial GAMs for the 10 best performing indices. Results are shown for the cross validated smooths for 2020.

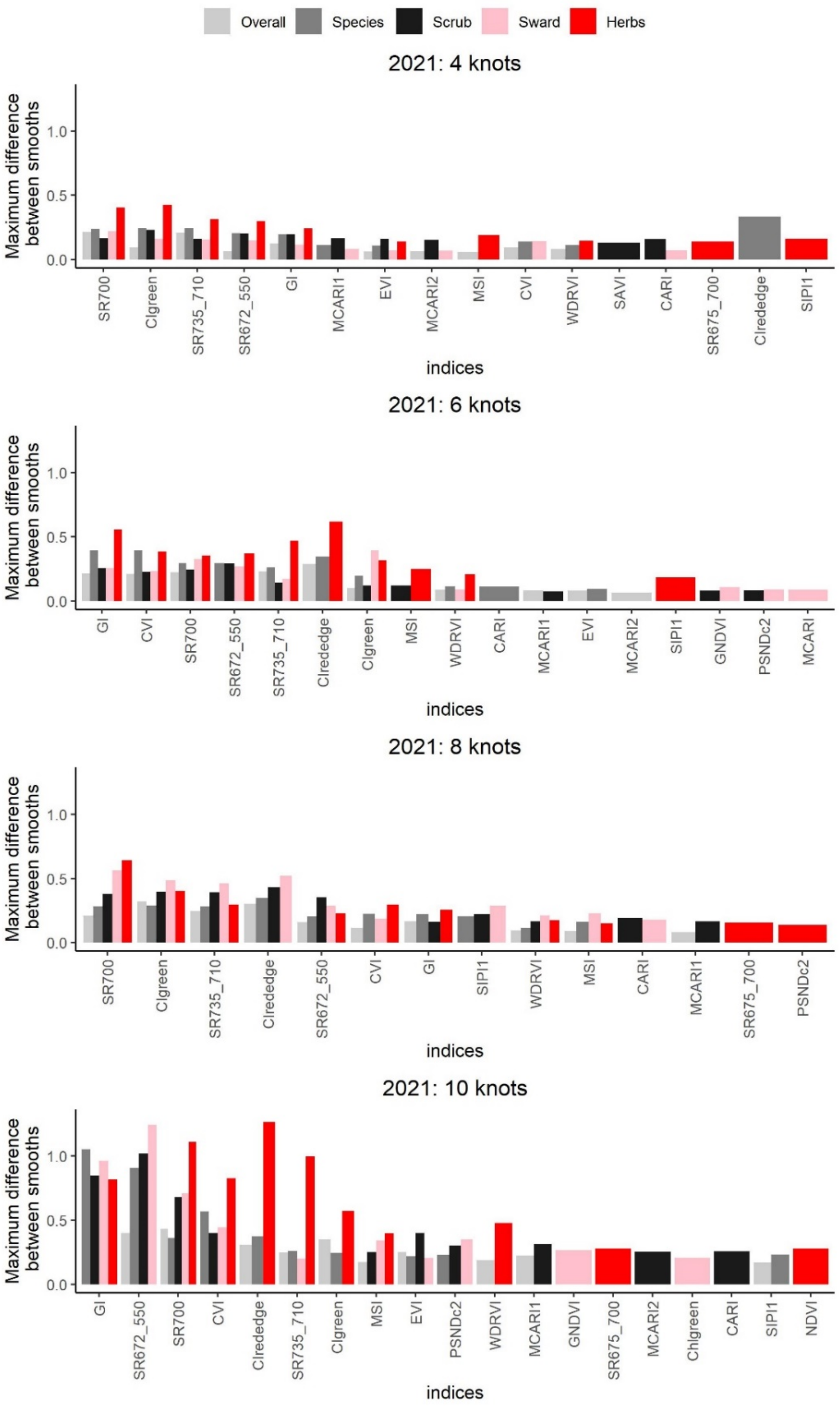

Figure 5.6 B The magnitude of the highest difference between smooths obtained from the predictions of the two-level factorial GAMs for the 10 best performing indices. Results are shown for the cross validated smooths for 2021.

Table 5.3 The cumulative difference between significantly different regions of smooths across all indices in estimation of the five condition criteria (as favourable / unfavourable) for each of the GAM parameterisation levels and across years. The years where the performance of the GAMs are distinctly different from each other are distinctly shaded.

Condition Criteria	knots	2020	2021
Overall	4	245	202
	6	296	281
	8	319	294
	10	338	333
Species	4	162	456
	6	242	485
	8	269	508
	10	246	568
Scrub	4	188	245
	6	166	213
	8	189	280
	10	204	341
Sward	4	509	178
	6	598	222
	8	631	308
	10	621	319
Herbs	4	31	477
	6	41	494
	8	38	445
	10	41	553

To assess the most important times of year for the assessment of the condition criteria, we counted the number of times in each month that the highest difference between smooths occurred (Figure 5.7). In 2020, for overall condition, late spring (May and June) were the optimal months, but late summer (August) was also promising, as well as mid-winter (January). For the condition criteria, positive indicator species, both summer (July) and mid-winter (December), were the times of year when the smooths were most different. For scrub cover and sward height, the highest differences between the smooths, were found in the winter months only (January and December). In contrast to the results for 2020, during 2021, the maximum difference between smooths for all the condition criteria was during January, when vegetation was largely dormant.

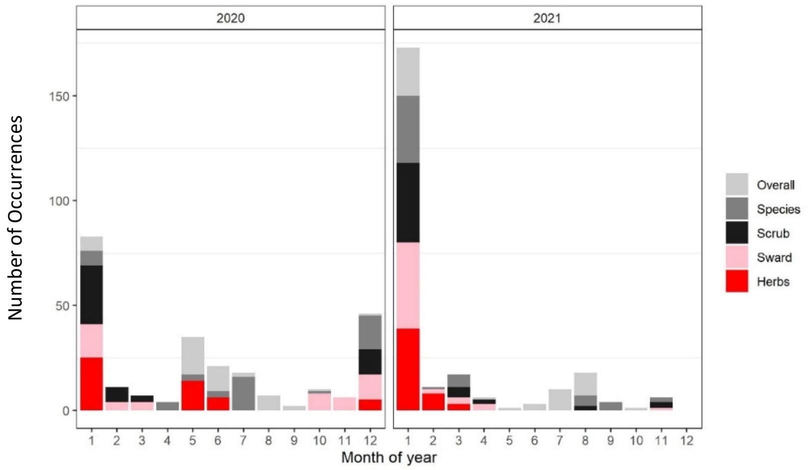

Figure 5.7 The most important times of the years for estimating the five condition criteria as assessed by the count of occurrences of the maximum difference between smooths.

5.3.3 The use of the distance correlation and the RTM PROSAIL to understand drivers of condition

We used a global sensitivity analysis of the RTM PROSAIL to understand the physical drivers of the variance in the values of the vegetation indices. For clarity, we confine our reporting of results of the GSA to only the ten best indices for estimating the different condition criteria, using the results of the GAMs with 10 knots (equivalent to the results found with cross-validation). The best indices out of the 37 tested were: the Chlorophyll Index Green (CI green), the Chlorophyll Index Red-edge (CI red-edge), the Chlorophyll Vegetation Index (CVI), the Enhanced Vegetation Index (EVI), the Greenness Index (GI), the Modified Chlorophyll Absorption in Reflectance Index 2 (MCARI2), the Moisture Sensitive Index (MSI), the Simple Ratio 672-550 (SR672-550), the Simple Ratio 700 (SR700), and the Simple Ratio 735-710 (SR735-710).

We found, using distance correlation, that the difference smooths for the two factorial levels of condition, favourable and unfavourable, were strongly correlated with each other for most of the best performing indices (Figure 5.8 A and B), but the extent to which this was true, did vary dependent on the condition criteria considered. For example, for the condition criteria sward, scrub, and overall condition, CVI and CI green were correlated with each other but not strongly with the other indices. The indices were generally much less correlated with each other when used to estimate the condition criteria herbs or species.

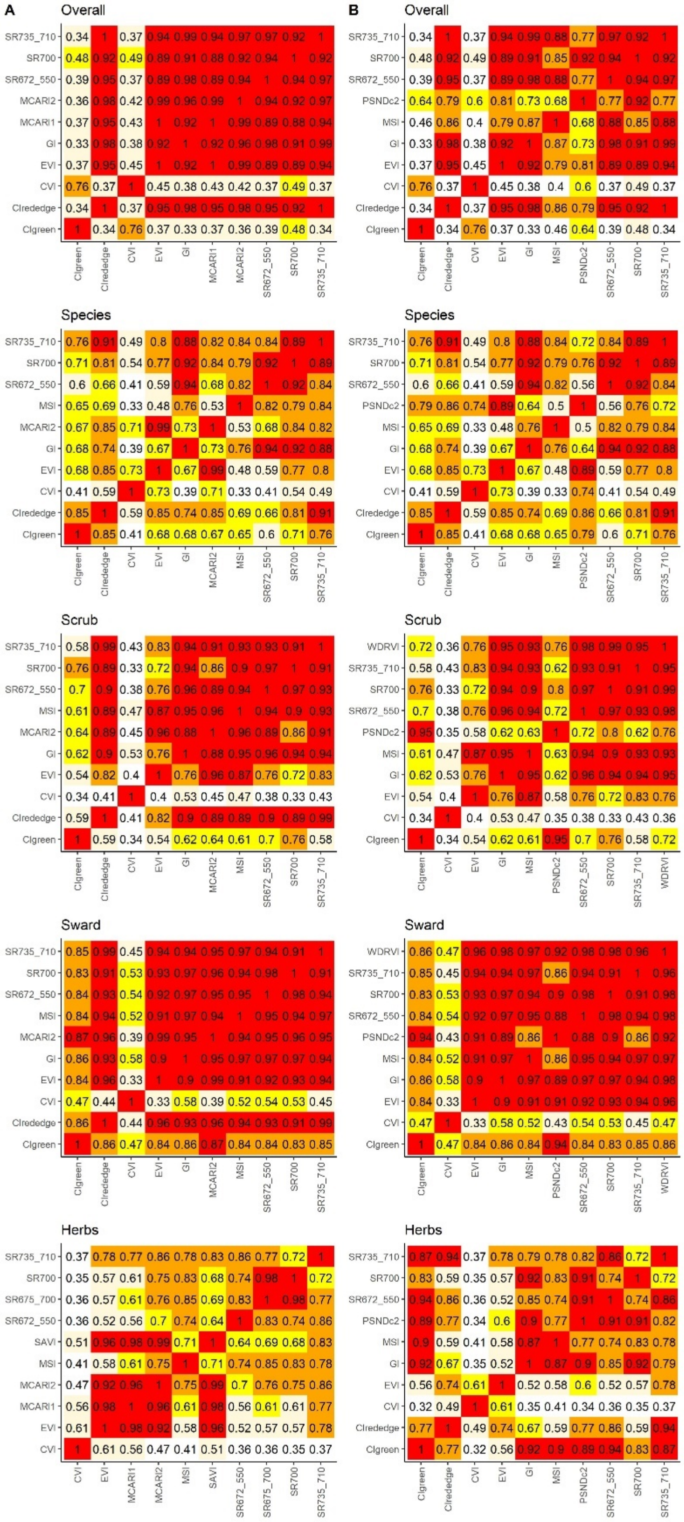

Figure 5.8 Distance correlation heatmaps of the difference smooths using the cross validated level of smoothing for each of the condition criteria for A: 2020 and B: 2021.

By combining the measures of distance correlation with the RTM results, we can start to understand the biophysical drivers of changes in the values of the indices (Figure 5.9). We found that many of the best indices for all condition criteria were likely proxies of chlorophyll content (Cab), with the six best performing indices (PSNDc2, WDRVI, SR735-710, CI red-edge, CI green and CVI) being driven by very similar combinations of traits (mostly Cab, but also Cm, LAI and to some degree N). The indices SR 675-700, SAVI, EVI, MCARI1 and MCARI2, are driven by a number of traits, with the most dominant influence being Cm (Dry matter content). SR700 is mostly influenced by Leaf Angle Distribution, whereas MSI is jointly influenced to an equal extent by Leaf Area Index (LAI) and Water content (Cw).

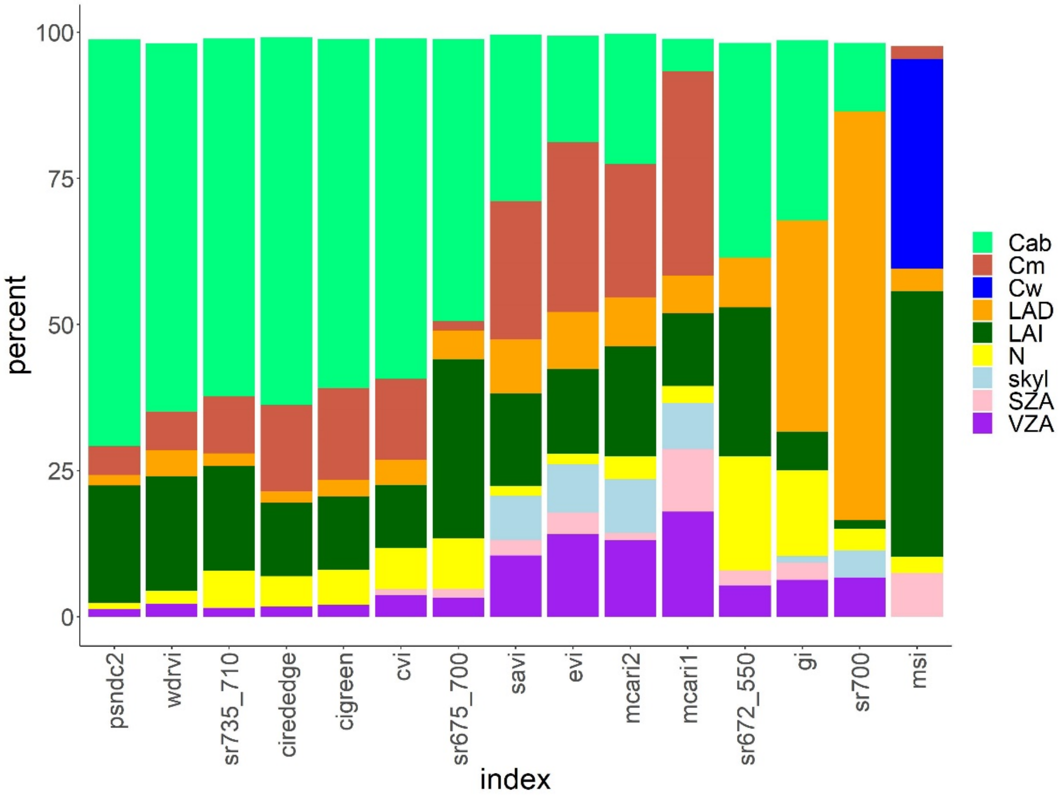

Figure 5.9 A Global Sensitivity Analysis of the RTM PROSAIL for the best performing indices for the cross-validated GAMs. For clarity, the optical traits are shown where percent variance contribution is > 1% of the total global variance.

5.4 Discussion

In this study, we tested the ability of intra-annual time-series to predict components of vegetation condition. We found that particular indices at particular time-points were useful, but that these observations were only partially transferable between the two years. We also found that many of the indices that were used successfully in predicting different aspects of condition are correlated to each other. This could be for different reasons. Either they are sensitive to the same combination of vegetation traits (see Figure 5.9), or different canopy traits are reacting to green-up and senescence along similar temporal trajectories throughout the time-series. For example, we would expect Leaf Area Index (LAI) and Chlorophyll content of vegetation (Cab) to both rise rapidly during spring and to gradually decline over autumn. In the past, indices have been tested for their ability to vary with measured vegetation properties. For example, the greenness index (GI) is presumed to be a biophysical proxy for both chlorophyll and water content (see Supplementary Materials 2). However, the GSA of the index showed that this index is driven by a combination of Chlorophyll, Leaf Angle Distribution, and the N parameter in equal measure, but not water content (Cw). The only index that showed potential as being a proxy for water content was the MSI, but we should also be cautious in our application of this index for water content estimation as it is also strongly influenced by changes in LAI.

NDVI is by far the most commonly used index in land surface phenology (Caparros-Santiago et al. 2021) and has been used as a proxy to assess many aspects of vegetation condition, from biodiversity (Oindo et al. 2002) to ecosystem health (Lloret et al. 2007). In this study, we found that NDVI did not prove very useful for estimating condition in lowland calcareous grassland systems. Another very commonly used metric that encapsulates 'green-up' is the EVI (Huete et al. 2002). In contrast, this index did prove useful for estimating condition, although not to the same extent as less well known and used indices such as SR700, CI red-edge and CI green.

The advantage of using a smoother approach, is that the full temporal trajectory of the spectral data can be exploited, across the entire growing season. However, the smoother can be over-trained on the data, making the application of results across years problematic. There are several aspects of time-series data that will affect the produced optical variables for the year in question. Firstly, the amount of data available per annum due to the number of cloud free days. In this study the number of pixels available per year was comparable between years, but this may not always be the case. A major issue is large gaps in the time-series due to clouds which have to be interpolated from neighbouring dates, thus creating a high level of uncertainty in the time-series. Cloudy days are more likely to occur during the winter months. Interestingly we found that mid-winter is one of the most important times of the year for discrimination between favourable and unfavourable fields. The application of these methods may therefore be limited to years with enough cloudless days during the dormant season. It may be possible, in future studies, to use a limited number of scenes per annum, if we can extrapolateinformation gained from studies such as this one to ascertain which times of year are most important. Schmidt et al. (2014) looked at the minimum number of data acquisitions from Rapid Eye satellite (6.5m spatial resolution) required over a four-year period for land cover classification. Only a three-scene composite was needed to obtain maximum accuracy with the most important seasons for differentiation of semi-natural grasslands being dates in early summer, midspring, late summer, and midsummer.

The seasonal dependence of detailed ecological vegetation mapping using remote sensing has been shown in several contexts. For example, within salt marshes (Belluco et al. 2006, Sanchez-Hernandez et al. 2007), mountainous grasslands (Dirnböck et al. 2003) and floodplain grasslands (Rapinel et al. 2019), and dry grasslands, wet heaths, and floodplain meadows (Feilhauer et al. 2013). In nutrient poor grasslands, the importance of litter and canopy height over a season varies. Litter cover is normally highest during the winter months and reduces with new vegetation growth in the spring and throughout the summer (Feilhauer et al. 2013). Broadly it could be said to be inversely proportionate to canopy height (fresh biomass) across a season and therefore Chlorophyll content. This could partly explain why the smooths were most different from each other during the dormant time of the year, when chlorophyll content is minimised, the differences in biomass can be detected more easily.

The results from Generalised Additive Models are very likely to be sensitive to outliers when whole scenes within a satellite image is faulty or cloudy and this has failed to be picked up during the data pre-processing stage. A recent study on the estimation of biodiversity using both spectral response and variation from the Sentinel-2 mission, showed the importance of different atmospheric correction methods on the temporal consistency of the reflectance data (Chraibi et al. 2022), and hence any inferences that may be made. Here we analysed data extracted from the Sentinel archive, which had been processed using the atmospheric correction method, Sen2Cor, followed by pixel classification derived from the SLC layer of the Bottom of Atmosphere product. In the Chraibi et al. (2022) study, the Sen2Cor correction method was found to be one of the more variable and hence unreliable of the correction methods. Within the workflow of our study, the shape of a GAM is likely to be influenced by pre-processing of satellite products. Analysis of the sensitivity of results from data driven models, such as GAMs, to pre-processing techniques would be an important future avenue of research.

Unlike many other cases of the application of smoothers in remotely sensed time-series, we also modelled spatial autocorrelation to account for the repeat measures on the same fields, by addition of the categorical variable 'fields' as a random effect in the GAMs. This did not severely affect the shape of the GAM, but it did affect the confidence intervals on the smooth parameter and the regions of the time-series where the factorial smooths were statistically different. When we included the random effect within the models, we found that most regions of the smooths were not statistically different from each other. We recommend that remote sensing researchers incorporate such terms within semi-parametric models so as to increase confidence in their inferences.

One of the challenges in generalising beyond the scope of this study to enable automated detection of grassland condition at the field scale, is the lack of transferability of the results across the two years. Some knowledge of weather variables for each year could assist with this. For example, Maynard and Karl (2017) linked long term Landsat optical time-series variability in weather patterns, to determine ecological site classification. They encapsulated variation in weather by use of a 'Standardised precipitation index'. There are also additional challenges associated with applying observations made under one sensor to another. Phenological trends and derived pheno-metrics have been shown to vary with the sensor employed (Younes et al. 2021) so the results here may only be valid in the context of the Sentienl-2 mission. This is not a problem per se, as equivalent sensors are planned to be operational for at least another 10 years.

In order to apply our methods to different grassland systems the inclusion of additional co-variates may be necessary. An important component of the classification and condition monitoring of distinct ecological sites is the tight coupling between potential vegetation composition and climo-edaphic properties. By introducing both soil, elevation and topographical variables to similar models, alongside parallel data on other grassland types, we would be able to develop a system for all conservation grade grasslands within a region.

5.5 Conclusion

The challenges in linking the knowledge of ecologists and conservation practitioners, with remote sensing specialists is something that has already been highlighted (Walsh et al. 2014). One of the important challenges identified is how to best combine existing knowledge systems already in use by conservation managers with the technical frameworks of remote sensing specialists, in an accessible way. Here the use of state and transition models, alongside the field condition assessments help to link field level observations, familiar to practitioners, to satellite vegetation index time-series, and the optical drivers of condition.

We show that intra-annual GAMs, used with factorial difference smooths, could be a suitable alternative to the more frequently used pheno-phase metrics such as start of spring and peak biomass, for estimation of vegetation condition. Many vegetation indices, tested here, are seldom used to create SITS for discrimination between vegetation classes, yet they performed far better for these purposes than the more frequently employed indices, such as NDVI and EVI.

Although there is great potential to establish reliable optical proxies of the properties of the changing state of grasslands, we need to expand field observations across several years, to build up knowledge of how the usefulness of proxies vary between years, largely due to weather conditions. This would be a worthwhile endeavour because if we can predict condition criteria within conservation grade grasslands through remote sensing methods, there would be vast improvements in how we administer agri-environment schemes and protect these systems for the future.

5.6 Supplementary Materials

Supplementary Materials 1

Bands of the Sentinel-2 satellite sensor

Band number	Band description	Central wavelength (nm)	Bandwidth (nm)	Spatial resolution(m)
Band 1	Coastal aerosol	442.7	21	60
Band 2	Blue	492.4	66	10
Band 3	Green	559.8	36	10
Band 4	Red	664.6	31	10
Band 5	Vegetation red edge	704.1	15	20
Band 6	Vegetation red edge	740.5	15	20
Band 7	Vegetation red edge	782.8	20	20
Band 8	NIR	832.8	106	10
Band 8A	Narrow NIR	864.7	21	20
Band 9	Water vapour	945.1	20	60
Band 10	SWIR – Cirrus	1373.5	31	60
Band 11	SWIR	1613.7	91	20
Band 12	SWIR	2202.4	175	20

Supplementary Materials 2

Indices used in this study with their formulas and their presumed biophysical proxies

Index Number	Name of index (short)	Name of index (long)	Formula using S2 bands	Effective resolution of indices (m)	Biophysical proxy	Example of use (reference)
1	CARI	Chlorophyll Absorption Ratio Index	3*((band_5-band_4)-0.2*(band_5-band_3)*((band_5)/(band_4)))	20	Chlorophyll	Moon et al. 1993
2	Chlgreen	Chlorophyll Green	power(((band_7)/(band_3)),(-1))	20	Chlorophyll	Gitelson et al. 2006
3	Chlred-edge	Chlorophyll Red-Edge	power(((band_7)/(band_5)),(-1))	20	Chlorophyll	Gitelson et al. 2006
4	CIgreen	Chlorophyll Index Green	(band_8)/(band_3)-1	10	Chlorophyll / LAI	Gitelson et al. 2003
5	CIrededge	Chlorophyll IndexRedEdge	(band_8)/(band_5)-1	10	Chlorophyll / LAI	Gitelson et al. 2003
6	CVI	Chlorophyll vegetation index	band_8*(band_4)/(power(band_3,2))	10	Chlorophyll	Vincini et al. 2008
7	D800-550	Difference 800/550	band_8-band_3	10	Chlorophyll	Bushmann and Nagel 1993
8	D800-680	Difference 800/680	band_8-band_4	10m	Chlorophyll/ PAR	Jordan 1969
9	Datt1	Datt1	(band_8-band_5)/(band_8-band_4)	20	Chlorophyll	Datt 1998
10	EVI	Enhanced Vegetation Index	2.5 * (NIR - RED) / (NIR + 6.0 * RED - 7.5 * BLUE+ 1.0)	10	Vegetation Biomass / LAI	Huete et al. 2022
11	GI	Greenness Index	(band_3)/(band_4)	10	Chlorophyll / Water	le Maire et al. 2004
12	GLI	Green leaf index	(2*band_3-band_4-band_2)/(2*band_3+band_4+band_2)	10	Chlorophyll	Gobron et al. 2000
13	GNDVI	Green NDVI	(band_8-band_3)/(band_8+band_3)	10	Chlorophyll	Bushmann and Nagel 1993
14	LCI	Leaf Chlorophyll Index	(band_8-band_5)/(band_8+band_4)	10	Chlorophyll	Datt 1998
15	MCARI	Modified Chlorophyll Absorption in Reflectance Index	((band_5-band_4)-0.2*(band_5-band_3))*((band_5)/(band_4))	20	Chlorophyll	Daughtry et al. 2000
16	MCARI-MTVI2	Modified Chlorophyll Absorption in Reflectance Index	(((band_5-band_4)-0.2*(band_5-band_3))*((band_5)/(band_4)))/((1.5*(1.2*(band_8-band_3)-2.5*(band_4-band_3))/(sqrt(power((2*band_8+1),2)-	20	Chlorophyll	Eitel et al. 2007
17	MCARI1	Modified Chlorophyll Absorption in Reflectance Index 1	1.2*(2.5*(band_8-band_4)-1.3*(band_8-band_3))	10	Chlorophyll	Haboudane et al. 2004

18	MCARI2	Modified Chlorophyll Absorption in Reflectance Index 2	(1.5*(2.5*(band_8-band_4)-1.3*(band_8-band_3))/(sqrt(power((2*band_8+1),2)-(6*band_8-5*sqrt(band_4))-0.5)))	20	Chlorophyll	Haboudane et al. 2004
19	MSI	simple ratio 1600/820 moisture stress index	(band_11)/(band_8)	20	Water	Hunt and Rock 1989
20	NDVI	Normalized Difference NIR/Red	(band_8-band_5)/(band_8+band_5)	10	Vegetation Biomass / cellulose / starch / water	Gamon et al. 1995
21	PSNDc2	Pigment specific normalised difference C2	(band_8-band_2)/(band_8+band_2)	10	Chlorophyll	Blackburn 1998
22	SAVI	Soil Adjusted Vegetation Index	(band_8-band_4)/(band_8+band_4+par_a)*(1+par_a)	10	Vegetation Biomass	Bausch 1993
23	SB1580	Single band 1580	band_11	20	Starch	Curran 1989
24	SB2100	Single Band 2100	band_12	20	Cellulose / nitrogen / protein / starch	Curran 1989
25	SB460	Single Band 460	band _2	10	Chlorophyll	Blackburn 1998
26	SB550	Single Band 550	band_3	10	Chlorophyll	le Maire et al. 2004
27	SB660	Single Band 660	band_4	10	Chlorophyll	Curran 1989
28	SB703	Single Band 703	band_5	20	Chlorophyll	le Maire et al. 2004
29	SIPI1	Structure Intensive Pigment Index 1	(band_8-band_1)/(band_8-band_4)	10	Chlorophyll	Blackburn 1998
30	SIPI3	Structure Intensive Pigment Index 3	(band_8-band_2)/(band_8-band_4)	10	Chlorophyll	Blackburn 1998
31	SR550-800	Simple Ratio 550/800	(band_3)/(band_8)	10	Chlorophyll	le Maire et al. 2004
32	SR672-550	Simple Ratio 672/550 Datt5	(band_4)/(band_3)	10	Chlorophyll	Datt 1998
33	SR675-700	Simple Ratio 675/700	(band_4)/(band_5)	20	Chlorophyll	Chappelle et al. 1992
34	SR700	Simple Ratio 700	(1)/(band_5)	20	Chlorophyll	Gitelson et al. 1999
35	SR735-710	Simple Ratio 735/710	(band_6)/(band_5)	20	Chlorophyll	Gitelson et al. 1999

36	TCIdx	Triangular chlorophyll index	1.2*(band_5-band_3)-1.5*(band_4-band_3)*sqrt((band_5)/(band_4))	20	Chlorophyll	Hunt et al. 2011
37	WDRVI	Wide Dynamic Range Vegetation Index	(0.1*band_8-band_4)/(0.1*band_8+band_4)	10	Vegetation Biomass / LAI	Gitelson 2004

Supplementary Materials 3

The results of the Generalised Additive Models with Cross-Validation

2020							
Condition Criteria	Index	edf (favourable)	edf (unfavourable)	sig. of smooths (favourable)	sig. of smooths (favourable)	sig. random term (field)	Deviance explained
Overall	SR735_710	9	9	***	***	***	53.1
Species	SR735_710	9	9	***	***	***	52.4
Scrub	SR735_710	9	9	***	***	***	52.2
Sward	SR735_710	9	9	***	***	***	53.6
Herbs	SR735_710	9	9	***	***	***	51.8
Overall	SR700	9	9	***	***	***	31.6
Species	SR700	9	9	***	***	***	31.2
Scrub	SR700	9	9	***	***	***	31
Sward	SR700	9	9	***	***	***	33.6
Herbs	SR700	9	9	***	***	***	30.9
Overall	SR672_550	9	9	***	***	***	54.9
Species	SR672_550	9	9	***	***	***	54.3
Scrub	SR672_550	9	9	***	***	***	54.5
Sward	SR672_550	9	9	***	***	***	56.5
Herbs	SR672_550	9	9	***	***	***	53.9
Overall	EVI	9	9	***	***	***	48.5
Species	EVI	9	9	***	***	***	48.3
Scrub	EVI	9	9	***	***	***	47.8
Sward	EVI	9	9	***	***	***	48.7
Herbs	EVI	9	9	***	***	***	47.9
Overall	CVI	9	9	***	***	***	13.4
Species	CVI	9	9	***	***	***	13.3
Scrub	CVI	9	9	***	***	***	13.1
Sward	CVI	9	9	***	***	***	13.8
Herbs	CVI	9	9	***	***	***	13.2
Overall	MSI	9	9	***	***	***	48.3
Species	MSI	9	9	***	***	***	48.5
Scrub	MSI	9	9	***	***	***	48.3
Sward	MSI	9	9	***	***	***	51.4
Herbs	MSI	9	9	***	***	***	48.4
Overall	GI	9	9	***	***	***	48.9
Species	GI	9	9	***	***	***	48.2
Scrub	GI	9	9	***	***	***	47.9
Sward	GI	9	9	***	***	***	49.8

Herbs	GI	9	9	***	***	***	47.7
Overall	CIgreen	9	9	***	***	***	18.6
Species	CIgreen	9	9	***	***	***	19.6
Scrub	CIgreen	9	9	***	***	***	18.6
Sward	CIgreen	9	9	***	***	***	19.6
Herbs	CIgreen	9	9	***	***	***	18.6
Overall	CIred-edge	9	9	***	***	***	42.6
Species	CIred-edge	9	9	***	***	***	42.4
Scrub	CIred-edge	9	9	***	***	***	42
Sward	CIred-edge	9	9	***	***	***	43
Herbs	CIred-edge	9	9	***	***	***	41.7

		2021					
Condition Criteria	Index	edf (favourable)	edf (unfavourable)	sig. of smooths (favourable)	sig. of smooths (favourable)	sig. random term (field)	Deviance explained
Overall	SR735_710	9	9	***	***	***	62.6
Species	SR735_710	9	9	***	***	***	64.6
Scrub	SR735_710	9	9	***	***	***	62
Sward	SR735_710	9	9	***	***	***	62.4
Herbs	SR735_710	9	9	***	***	***	62.4
Overall	SR700	9	9	***	***	***	40.2
Species	SR700	9	9	***	***	***	41.8
Scrub	SR700	9	9	***	***	***	40
Sward	SR700	9	9	***	***	***	39.9
Herbs	SR700	9	9	***	***	***	39.7
Overall	SR672_550	9	9	***	***	***	61.1
Species	SR672_550	9	9	***	***	***	63.6
Scrub	SR672_550	9	9	***	***	***	61.6
Sward	SR672_550	9	9	***	***	***	61.5
Herbs	SR672_550	9	9	***	***	***	61
Overall	EVI	9	9	***	***	***	47.9
Species	EVI	9	9	***	***	***	49.5
Scrub	EVI	9	9	***	***	***	47.9
Sward	EVI	9	9	***	***	***	48
Herbs	EVI	9	9	***	***	***	47.6
Overall	CVI	9	9	***	***	***	25.6
Species	CVI	9	9	***	***	***	26.5
Scrub	CVI	9	9	***	***	***	25.9
Sward	CVI	9	9	***	***	***	25.8
Herbs	CVI	9	9	***	***	***	25.6
Overall	MSI	9	9	***	***	***	61.7
Species	MSI	9	9	***	***	***	63.8
Scrub	MSI	9	9	***	***	***	62.2
Sward	MSI	9	9	***	***	***	62.2
Herbs	MSI	9	9	***	***	***	61.8
Overall	GI	9	9	***	***	***	60
Species	GI	9	9	***	***	***	61.8
Scrub	GI	9	9	***	***	***	60
Sward	GI	9	9	***	***	***	59.7
Herbs	GI	9	9	***	***	***	59.6
Overall	CIgreen	9	9	***	***	***	14.1
Species	CIgreen	9	9	***	***	***	14.7
Scrub	CIgreen	9	9	***	***	***	14

Sward	CIgreen	9	9	***	***	***	14
Herbs	CIgreen	9	9	***	***	***	14.2
Overall	CIred-edge	9	9	***	***	***	51
Species	CIred-edge	9	9	***	***	***	52.8
Scrub	CIred-edge	9	9	***	***	***	50.5
Sward	CIred-edge	9	9	***	***	***	50.8
Herbs	CIred-edge	9	9	***	***	***	50.9

Chapter 6
Conclusions and Future Directions

6.1 Overview of findings relating to multi-temporal testing of hypotheses

In this thesis I exploited reflectance of grasslands at both the leaf and canopy levels, in the hope of developing useful proxies of biodiversity in these systems. The specific aim was to understand the extent to which observations obtained at one time point were transferable to other sampling times and situations. I was motivated by a concern that although many positive outcomes in this field of remote sensing of biodiversity are reported in the literature, most studies do not contain repeat measurements. Despite researchers often alluding to the impact of phenological development on their observations, few carry out longitudinal studies and so this aspect is rarely studied. My experiments with multi-temporal testing of hypotheses, both within years (chapters 3 and 4), and between years (chapter 5), overall support the speculation that many findings in this field are temporally unstable. These instabilities show that depending on the time of data acquisition very different inferences are likely to be drawn from experimental results. This is a worrying observation and shows the desperate need for long-term experiments where data is captured across time if we are to use these methods reliably.

Due to nature of this PhD studentship, some of the data sets collected were quite small. For example, in chapter 3 only twenty botanical plots were sampled over the growing season. Likewise, in chapter 4, only five leaf samples of 17 species (85 reflectance measures), were collected per sampling day. It is therefore possible that sampling error had an impact on the fluctuations of the findings over time. These sample sizes were the upper limit of what could be practically collected due to instrumentation and weather restrictions. In contrast, in chapter 5, by collaborating with a partner organisation, we were able to generate 55 vegetation condition surveys, carried out in 37 unique field locations, over two survey years, thus expanding the scope of observations to a land scape scale and increasing the reliability of any inferences.

We know that the relationship between vegetation phenology and optical reflectance can be exploited to provide information on biodiversity, as this has been widely demonstrated at the spatial scales of data collected by satellite borne sensors. However, data gained from the multi-temporal sampling approach, at high spatial resolutions, (such as found in chapters 3 and 4 of this thesis) have not yet been analysed. For example, the instability of species-specific responses over time could be made use of in the future for biodiversity assessment. In chapter 4, I was able to test the models trained on data collected at one time point on the data collected from the other time points. I showed that models performed best on data collected at neighbouring time-points. This indicates that at least in some of the species, we were able to detect leaf development via reflectance data, and that such changes in reflectance are associated to some extent with taxonomic units.

I suggested in chapter 3 of the thesis that phenological synchrony or asynchrony of plant communities could be responsible for optical variation, and that older, more natural grasslands could be optically more complex due to the temporal partitioning of their phenological stages. This theory was not supported by data collected from two fields across a growing season. One possible conclusion is that the spatial scale of the experiment was sub-optimal for the detection of phenological diversity using optical diversity. Reflectance data was collected at approximately 10cm^2 (very high-resolution canopy scale), with the idea that the experiment could be scaled up to sensors mounted on Unmanned Aerial Vehicles (UAVs). In this thesis, we did not explicitly test the effect of scale on our observations as we were not able to obtain very high spatial resolution imaging spectrometry data which lends itself to being resampled to larger pixel sizes (Wang et al. 2018a), but this is certainly a desirable future avenue of investigation.

In chapter 5, I examined how calcareous grassland condition over two years could be monitored using time-series of spectral indices. I found that between years, differing time-points within phenological cycles would need to be exploited to distinguish between the condition criteria. This analysis was limited by the fact that field condition data was only available on the fields for two years, and that within these systems we cannot assume that fields remain stable in terms of their biophysical parameters. Very long optical time-series are now available through fusion of different satellite sensors (Houborg and McCabe 2018). However, we lack the long-term field data to exploit these spectral time-series to provide detail on changing habitats at the field level. This is something that requires urgent communication by the remote sensing community to policy makers.

6.2 Detection of biodiversity using spectral variation

The exploitation of spectral variance (SVH) in estimation of biodiversity is very much an on-going research area. At the time of writing-up this thesis, several more publications were produced on the subject (Chraibi et al. 2022, Kamoske et al. 2022, Schweiger and Laliberté 2022, Wang et al. 2022). From a cursory examination of this very recent literature, it appears that research in this field is still forest focused. The results of chapter 2 of this thesis, a meta-analysis of the detection of biodiversity via the spectral variation hypothesis, shows that there is considerable promise in applying remote sensing approaches, normally carried out in forests, to grassland systems. Although grasslands are likely to remain a more challenging land cover type to monitor, those interested in evaluating more spatially complex canopies, such as those found in herbaceous systems, should take encouragement from these findings.

The meta-analysis results also showed that the ability to extract useful optical proxies of biodiversity varied greatly within studies. This variation was a product of differences in temporal sampling, instrumentation, the exact optical and vegetation metrics used, and the type of data processing employed. These results generate many methodological questions, which are difficult to disentangle, and it is apparent that more thorough testing of the SVH will require stricter formulisation of data campaigns. In view of this, a few authors have outlined specific methodologies for developing spectral diversity metrics. For example, Féret and de Boissieu 2020, introduced an open-source software package in the R environment, called biodivMapR, which produces alpha, and beta spectral diversity metrics based on data reduction (Principal Component Analysis) and spectral clustering of pixels. Similarly, Rocchini et al. 2021, produced a package in R called rasterdiv, which facilitates the production of landscape and ecosystem heterogeneity metrics. This package allows for different types of pairwise spectral distances between pixels, also known as spectral entropy measures, to be calculated on satellite images (rasters). Sun et al. (2021) developed an alternative method which they named 'RSPD' (Remote Sensing of Plant Diversity) which combines spectral response and spectral variation, derived from broad spectral bands. They found that the technique outperformed the classical application of the CoV (Coefficient of Variation, as used in chapter 3 of this thesis), when applied to estimation of biodiversity.

6.3 Spectral detection of species

The results of chapter 4 indicate that the production of taxonomic inventories in grasslands using hyperspectral data captured at the leaf level is challenging. When Féret and Asner 2011, used leaf level spectral reflectance to classify taxonomic units or predict taxonomic diversity, they found this failed with higher numbers of species per unit area. Here I found that some species within a grassland community were optically distinct over a growing season, whereas others could not be distinguished cross-temporally from other species without unacceptable levels of classification error. This finding corroborates the idea that in many systems, taxonomic inventories (detecting all species present) will prove very difficult with only reflectance data. Interestingly, all the species were distinguishable from the others at least during one of the 17 sampling campaigns. The basis for this finding is not clear. One possible reason is sampling error, as only five leaves from each species were sampled per time point. Another reason could be instrumental error. Improvements in very high spatial resolution imaging spectroscopy in the future will hopefully enable scaling up of species-specific sample sizes, to explore these hypotheses further.

I concluded that one of the reasons why some species are more optically detectable than others, is likely to be related to their leaf traits. I found some evidence that leaf surface properties will be an important contribution to species detection, alongside other traits such as water content and chlorophyll. To verify this hypothesis, further sampling of leaves with varying surface reflectance properties, alongside collection on their leaf biochemical traits would be necessary. For within community assessment of biodiversity, with a limited number of species, such experiments like the one implemented in chapter 4 could be repeated, with this additional information.

An alternative option is to search for optically detectable indicator species within particular contexts, is an alternative avenue of research, which may be more feasible in the short to medium term. This is already happening to a certain extent with the targeting of invasive species monitoring in herbaceous vegetation (Papp et al. 2021, Gholizadeh et al. 2022). However, our ability to generalise using this approach is limited, and except where indicator species hold validity across wide geographic areas, this line of research may be financially untenable.

In single species environments, the sources of spectral variation also need to be deciphered, for example, canopy level sources of spectral variation, such as self-shadowing and leaf angle changes due to variation in growth habitat, need to be separated out from leaf sources of variation. Methods that enable detection of these canopy properties, and filtering of the data, accordingly, are required. Already simple approaches for shadow detection have been experimented with (Lopatin et al. 2017), and these could be built upon. There have also been attempts to address some of the known complexities of vegetation structure by modelling the 3D structure of plant communities (Shen et al. 2020).

To test more thoroughly the hypothesis that taxonomic units are in some way optically distinct, requires a much broader understanding of species-specific spectral responses and optical traits along appropriate phylogenetic and functional gradients. It would be physically impossible to sample all species exhaustively, so selective sampling across phylogenetic gradients will be necessary using phylogenetic sampling protocols (Plazzi et al. 2010). Intra-specific genetic diversity is an essential component of biodiversity (Ramel 1998) and could be partly responsible for species being difficult to classify optically. The concept of intra-specific spectral variance has already begun to be explored in more detail and could prove an important direction in the field of vegetation remote sensing (Petibon et al. 2021). If we can demonstrate a link between spectral variance and genetic diversity, we would be able to use remote sensing to go beyond mapping single species' presence or abundance and instead assess species population vulnerability to external drivers such as climate change and unfavourable land management.

6.4 Towards the use of drones in spectral data collection

The methods of data collection carried out within chapters 3 and 4 (spectrometers in dual mode and spectrometers fitted with leaf clips, respectively), are not scalable. In order to obtain similar data at the field level to practically carry out biodiversity assessments, we would need to employ alternative sensors, such as those mounted on UAVs. Recently, a review study from Müllerová et al. 2021, examined from a theoretical standpoint some of the advantages of using UAVs to characterise vegetation complexity, including phenology and diversity. They emphasised the importance of the choice of sensor, pixel size and knowledge of the phenology. In addition, there are also likely to be additional sources of uncertainty associated with collecting data with aerial sensors that could obscure the species-specific signal. For example, angular properties of the UAV mounted sensor and the canopy can strongly affect hyperspectral measurements from 2D images (Aasen and Bolten 2018). Therefore, sampling campaigns that combine field spectroscopy methods and those with sensors mounted on UAVs will be necessary to understand the increased uncertainty introduced by scaling up our observations, and in turn how we may reduce the impact of these uncertainties. This is an especially important, and to date missing, research strand in this field. Much of our confidence in the concept of species-specific spectral reflectance is grounded in leaf spectroscopy data. It may well be possible that these observations are not scalable in any workable way to field level instrumentation.

In the specific case of grassland swards, the complexity of the canopy and small size of plants makes leaf level spectral observations particularly difficult to achieve. This is compounded in the post-production of UAV images where algorithms are used to stitch together the individually captured images into larger maps. Currently, errors in the stitching process require the maps to be re-sampled to larger pixels sizes, roughly comparable with the spectrometer data collected in chapter 3 of this thesis (10cm^2). Therefore, the detailed procedures required to identify individual leaves are not yet commercially available. This is the likely reason why leaf level imaging spectroscopy in grasslands has only really been approached using tram mounted spectrometers or using single images from handheld imaging sensors.

Under current legislation and technological availability, UAV sampling campaigns are very time-consuming, expensive and require extensive pilot training and experience. Large companies are hoping to automate the use of pilot-less drones for several commercial applications, such as parcel delivery (Di Puglia Pugliese et al. 2020). Vegetation monitoring could profit from these innovations to develop automated image capture campaigns, which if scaled up, could be cost effective. However, it is likely, at least in the short to medium term, that within any of these innovation fields highly trained field ecologists will be required to ‘ground-truth’ the optical data.

In chapters 2 and 3 of this thesis, I focused on the analysis of very high spectral resolution (hyperspectral) data for observing species. However, some interesting alternative approaches using lower spectral grade sensors have also been proposed using UAVs. Kattenborn et al. 2019 used a consumer grade, Red Green Blue, sensor for segmentation of plant communities in different successional stages. This approach exploits spatial patterns, such as morphology and structural components of canopies rather than detailed spectral differences. A type of machine learning algorithms, Convolutional Neural Nets, employed in computer vision, were used to detect ‘texture’ differences. The future possibilities of extending this technology for image segmentation actual are also promising. Already mobile phone applications offer, to varying degrees of success, automated species detection based on leaf morphology. RGB data captured by drones has also been used to providethe ground-truthing data for analyses using satellite data. For example, Ge et al. 2018 used UAVs to estimate the fraction grassland cover for use with a MODIS phenological time-series.

In phenology monitoring, reflectance data collected by UAVs could also be exploited. Images captured from UAVs can produce dense time-series of optical data, as flights can be planned around cloud free days, but this approach is very costly, and the ground area covered much smaller than that offered by satellite sensors. One major advantage in using UAVs is the ability to accurately correlate spatially biological data with the reflectance signal and to attribute cause and effect to species' densities and growth stages. For example, drones have been used to study heterogeneity in greenness (i.e. phenology), that is impossible to capture with satellites. Klosterman et al. (2018) showed that a time series of the Green Chromatic Co-ordinate (GCC) derived from RGB images collected by a UAV over a temperate North America forest can delineate habitat types. By comparing the GCC values of specific tree species at a 10 x 10m resolution, they were able to show that species distribution was the principal driver of ecosystem level greenness measures and that at some times of the year more than others phenological time-series from MODIS and Landsat mask the diversity of species phenological stages, with MODIS data capturing the mode rather than the mean values of greenness in both spring and autumn stages.

6.5 Leaf and canopy traits in biodiversity monitoring

Currently, we need to improve our understanding of the optical traits that are driving reflectance and variation in reflectance diversity in conservation grade grassland systems and why, in certain contexts, it may be possible to exploit them as a proxy for plant diversity. These traits are probably related to species specific phenologies but could also be confounded by changes in reflectance over time due to seasonal changes in canopy and leaf level structural traits.

Species within specialist grassland communities (chapter 4), are more likely to display convergent leaf traits, as they are adapted to narrow environmental conditions. This could be viewed as a kind of 'functional redundancy' within these sorts of systems. A contrasting situation could be that in disturbed or 'novel ecosystems', where we may be more likely to find plants with different survival strategies. This could explain why so many authors have had success with detecting invasive non-native species using reflectance. Different functional types, which occupy niches within systems may also display distinct leaf traits which enable detection (Punalekar et al. 2016).

In order to advance this line of research, it is vital to improve our understanding of the way in which optically detectable traits vary within taxonomic groupings. It would be desirable to link functional trait variation and taxonomic variation within different types of environmental gradients. A global synthesis of plants traits from over 26,000 species from 1 million plots (Bruedlheide et al. 2018) showed that at local scales within plot trait variation does not vary systematically with the macro-environment, instead local scale factors such as disturbance, soil conditions, niche partitioning and biotic interactions are more important. This study looked at many plant traits, not all of which are likely to be detected optically. However, it suggests ideas of environmental gradients which could be tested.

Radiative Transfer Models (RTMs) are developed on 'average' leaf spectra across species. This means that they are useful for looking at how leaf and canopy optical traits covary to influence reflectance, on average. Therefore, without collecting a data set on species specific traits the models can only be used to guide inferences about the probable causes of spectral variation. This is how I have employed the models in this thesis. Future work could involve parameterisation of RTMS for specific habitat types or even taxonomic units. They could also be employed to further our understanding of the reasons why spectral variation is a proxy for species diversity. Already phylogenetic drivers of optical trait variation have been established. For example, the value of N in the PROSPECT and PROSAIL models differs dependent on whether leaves are sampled from monocots or dicots. However, because N is a calibration parameter in the model it is not easy to relate this phenomenon to measurable field traits, such as the mesophyll structure of leaves.

6.6 Alternative approaches to linking vegetation quality and reflectance

In capturing the link between spectral data and biodiversity, alternative methods of vegetation assessment should also be considered. An alternative approach to traditional field mapping of plant communities are floristic plant gradients (Schmidtlein and Sassin 2004, Schmidtlein et al. 2007). These methods exploit a vegetation continuum based on dominant species to link different communities using ordination axes scores (McIntosh 1963). As they do not try to detect all taxonomic units, they could provide a much more useful conceptual space for monitoring using spectral reflectance. The idea of vegetation transitions is well documented in habitat condition monitoring and has also been explored using these techniques (Neumann et al. 2015). Another perspective in the classification of vegetation is that instead of thinking in terms of higher taxonomic groupings (i.e species), more encompassing metrics can be used, such as phylogenetic species variability (PSV) and phylogenetic species evenness (PSE) (Wang et al. 2018a, Schweiger et al. 2018). These metrics capture the phylogenetic distances between observed taxa in communities, and their use is driven by the idea that the differences in spectral reflectance between species are related to their phylogenetic relationships. This has been shown to work well within certain taxonomic groupings such as the genus Quercus (the Oaks) (Cavender-Bares et al. 2016) and more broadly across the seed plants (Meireles et al. 2020).

6.7 Optical variance as a co-variate in more complex models

Land surface models (LSMs) are used by environmental researchers to simulate the exchange of water, energy, and carbon at the land surface. They are vital tools in climate research. A current area of desired improvement in these models is the provision of reliable proxies of land surface heterogeneity within grid cells (Fisher and Koven 2020). The use of optical variance could potentially be a useful avenue in this context. Because LSMs operate at the regional to global scale, the data on heterogeneity would be required at a relatively coarse resolution (the smallest resolution of global models would be at the 1km-scale), while at the same time trying to capture the type of land cover variation which causes significant error in our estimation of water and energy fluxes. The requirement for development of such heterogeneity metrics via spectral variation would be a distinct application (compared to say the local estimation of biodiversity) and may require different scale and spectral parameters to achieve its objectives.

Remote sensing data could also be utilised in the improvement of species distribution models (He et al. 2015, Randin et al. 2020). One important addition to this field of ecology is in the ability of remote sensing data to model micro-climate (Zellweger et al. 2019). Integration of measures of spectral variation into other types of ecological experiments may also be appropriate. For example, there are many cases where structural heterogeneity is presumed to be a driver of biodiversity, and so conservation management is aimed at maintaining this heterogeneity (Borer et al. 2014, Diacon-Bolli et al. 2012). If we can demonstrate that spectral heterogeneity is a good proxy for structural heterogeneity in vegetation, we can then go on to use such proxies to test whether heterogeneity in canopy structure really does promotes biodiversity. We could also couple spectral heterogeneity measures with other ecological models, for example, individual growth models, such as GRASSMIND (Taubert et al. 2020), to test how individual plant species contribute to canopy and community level characteristics.

6.8 Concluding remarks

As we look to the future of remote sensing, we see the possibility of 'having it all', with automated space borne sensors that record hyperspectral reflectance, alongside high resolution spatial and temporal coverage. The improvement of UAV technologies and their accompanying sensors will also extend the types of reliable relationships we can build between reflectance data and grassland systems. These applications look set to extend our understanding of grassland biodiversity and conservation condition, beyond what is currently possible. The speed in which these observations will become operational in day-to-day monitoring of grassland for biodiversity policy and spending, depends on the extent to which government agencies and NGOs will invest in larger and more standardised field campaigns. Traditional field observations will be a large component in the development of these operational systems and will be expensive to implement. However, if we do seek to protect the biodiversity associated with semi-natural grasslands, remote sensing looks to be the only viable option for vegetation monitoring at scale.

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Appendices

The published versions of chapters 2, 3 and 4 of this thesis

remote sensing

MDPI

Review

Prediction of Grassland Biodiversity Using Measures of Spectral Variance: A Meta-Analytical Review

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Abstract: Over the last 20 years, there has been a surge of interest in the use of reflectance data collected using satellites and aerial vehicles to monitor vegetation diversity. One methodological option to monitor these systems involves developing empirical relationships between spectral heterogeneity in space (spectral variation) and plant or habitat diversity. This approach is commonly termed the 'Spectral Variation Hypothesis'. Although increasingly used, it is controversial and can be unreliable in some contexts. Here, we review the literature and apply three-level meta-analytical models to assess the test results of the hypothesis across studies using several moderating variables relating to the botanical and spectral sampling strategies and the types of sites evaluated. We focus on the literature relating to grasslands, which are less well studied compared to forests and are likely to require separate treatments due to their dynamic phenology and the taxonomic complexity of their canopies on a small scale. Across studies, the results suggest an overall positive relationship between spectral variation and species diversity (mean correlation coefficient = 0.36). However, high levels of both within-study and between-study heterogeneity were found. Whether data was collected at the leaf or canopy level had the most impact on the mean effect size, with leaf-level studies displaying a stronger relationship compared to canopy-level studies. We highlight the challenges facing the synthesis of these kinds of experiments, the lack of studies carried out in arid or tropical systems and the need for scalable, multitemporal assessments to resolve the controversy in this field.

Keywords: Spectral Variation Hypothesis; grasslands; biodiversity; remote sensing; meta-analysis

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1. Introduction

Grasslands are ecologically important systems, as they cover around 30–40% of the global terrestrial land mass [1], contain high levels of biodiversity [2] and provide multiple ecosystem services [3]. However, much of our global grassland resource is undergoing, or is at risk of, degradation [4] due to changes in management intensity [5,6], climate [7,8] and eutrophication [9]. To prevent further decline and ensure successful restoration, government agencies and research bodies require reliable, quantitative data on the changing status of the plant biodiversity within these systems, and remote sensing could be part of the solution [10,11].

Although most remote sensing studies aimed at vegetation monitoring are focused on forests of late, grasslands have also received more attention [12–14]. Herbaceous plants, which dominate grasslands, are often magnitudes smaller than their counterparts in woody vegetation, and this has been a major obstacle to applying remote sensing at the plant or leaf level. Some grasslands are dominated by a few species that can be mapped using satellite-mounted sensors [15,16]; however, natural or semi-natural grasslands are often characterized by a high community complexity within small areas [17]. In addition, grasslands are particularly dynamic over time due to variations in water availability [18] and other environmental factors. Despite these challenges, recent technological developments have made applications involving grasslands more feasible. There are now satellite missions providing small pixel sizes (10 m Sentinel-2), and high temporal resolutions (daily 250 m MODIS or every 5 days for Sentinel-2) [19] and fast-developing sensors on Unmanned Aerial Vehicles are enabling observations at very high spatial and spectral resolutions [20,21]. Some researchers have also employed proximal field instrumentation such as tram-mounted sensors [22] to obtain extremely detailed spectral information.

One attractive approach to monitoring grassland diversity, due to its simple concept, is to utilize the 'Spectral Variation Hypothesis' [23], which assumes that the spectral variation in space is correlated with the plant or habitat diversity. Plant diversity mapping using this method is based on the premise that individual species or plant communities have a distinct spectral reflectance signature, a product of optically detectable leaf and/or canopy traits [24]. At very small spatial scales, leaf-level optical properties drive the variance in reflectance, whereas, at larger scales, the canopy properties will be the main drivers. These relationships are well understood for single-species scenarios [25,26] but are likely to be more complex in taxonomically diverse communities.

Although the Spectral Variation Hypothesis is widely recommended and examined, the theory is not without critics [27]. It can be unstable in space (see [28], who used the approach across European landscapes) and temporally unstable interannually [29] and over growing seasons [30]. Plant materials at the leaf level are plastic, reacting to the environment in diverse ways [31,32]. The extent of plasticity in optical traits is thought to be, in part, genetically based, meaning that the taxonomic component of communities is influential [19] but not necessarily easy to predict across space and time. The approach to biodiversity monitoring at the community type level could also be problematic when applied to grasslands. For example, at these scales, grassland plants may display convergent canopy-level traits due to weather parameters, such as increases in greenness and biomass due to increased precipitation [33]. In addition, the spectral variation of grassland fields is strongly influenced by management events such as mowing and grazing [34,35].

The motivations behind applying the Spectral Variation Hypothesis display some cohesion; however, the spatial scale, instrumentation and spectral resolution of the studies vary considerably. These experimental choices could explain some of the inconsistency in the results as follows. Our ability to map taxonomic units using reflectance data is thought to be dependent on small variations that can only be detected using hyperspectral resolution data [36,37]. The Spectral Variation Hypothesis applied at the leaf level could therefore produce much weaker predictions when multi-spectral data are used. The spectral variation can be influenced by instrumentation. For example, in close range imaging spectroscopy situations, surface leaf reflectance can potentially have a large impact on spectral variance [38]. The number of taxonomic units being examined may matter, as there is evidence that the spectral variation–species diversity relationship is saturated with more complex communities [39]. The timing of sampling campaigns is also critical, as plant traits change seasonally [40,41] and interannually [42], affecting the plant spectral reflectance [43]. This is likely to have an impact on the temporal stability of the spectral variation–biodiversity relationship [44].

There have been several review papers published on the usefulness of remote sensing to assess biodiversity [45–51], and some have specifically looked at the Spectral Variation Hypothesis [27,52]. However, these approaches are somewhat subjective and non-standardized. A better alternative is to use a quantitative synthesis, known as a meta-analysis. Here, it is possible to weigh differences between study outcomes using the sampling effort and to investigate the impact of proposed moderating variables [53]. The method has been previously used in ecology [54,55] and in optical remote sensing to evaluate the literature relating to, for example, plant pigment concentrations [56], functional traits [57], forestry variables [58], crop variables [59] and land cover classification [60].

Here, we carry out a literature search and meta-analysis of studies that used optical remote sensing to estimate the biodiversity of grasslands under the Spectral Variation Hypothesis, with an emphasis on the effect of the spatial, temporal and spectral resolutions of the remote sensing data used, alongside other features of the sampling campaigns.

2. Materials and Methods

2.1. Literature Search and Selection of Studies for Meta-Analysis

In April 2020 and May 2022, we carried out literature searches using Google Scholar and Scopus (Table S1) following the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-analysis) methodology [61]. We read paper abstracts to ascertain whether studies contained spectral data and dealt with plant biodiversity in grassland systems. We did not include studies that mapped specific taxonomic units or that aimed to differentiate between a small number of target species. Some of the searches produced a very large number of records. In these cases, after sifting through 100 pages of results, (of approximately 10 results per page), the search was abandoned. The initial searches produced 74 papers, with an extra 4 found through reference lists, giving a total of 78 papers. These were then examined in more detail, and duplicates were removed, giving 77 studies. These were included in the final data set if the authors:

1. Explicitly tested whether plant species richness or diversity was correlated with a measure of spectral variance in space.
2. Included a Pearson's Correlation Coefficient that resulted from a bivariate model or an r^2 value with an indication of the relationship direction.
3. Did not deal with environments such as in savannahs or mixed planned countryside.

This left 20 studies suitable for our quantitative synthesis. Figure S1 provides details of the selection in the PRISMA graphical format.

2.2. Extraction and Description of Likely Moderators

We extracted several moderating variables that are likely to affect the relationship between spectral variance and plant species diversity. These moderators related to (1) the spectral data, (2) the species data and (3) the sampling design.

2.2.1. Spectral Moderators

We identified five moderating variables relating to the spectral data. The ground sampling size of the instrument is essential to understand if the Spectral Variation Hypothesis was tested at the leaf level or at the community/habitat level. A continuous variable in meters was created called the 'pixel size'. In addition, a categorical variable called 'leaf–canopy' was generated that classified effect sizes according to whether the pixel size matched 'leaf'- or 'canopy'-scale measurements.

Next, we created a category called 'spectral region' to note the spectral region used. Here, we refer to the visible part of the spectrum as 400–699 nm, the NIR as 700–1299 nm and the SWIR as 1300–2519 nm. Since the variation within each of these spectral regions is broadly driven by differing optical leaf and canopy properties, we can use the results of this analysis to propose biochemical reasons for the link between spectral variation and species diversity. In addition, to understand if a better spectral resolution improves predictions, effect sizes were categorized as to whether they were calculated using hyperspectral or multi-spectral data under the moderator 'spectral resolution'.

Measures of spectral variation are calculated in different ways. Some authors select a simple dispersion around the mean reflectance value, such as the range, standard deviation or the coefficient of variation, whereas others take more complex approaches, such as the average spectral angle between species [62], spectral entropy [63] or species spectral clustering measures [64]. To test whether there was an advantage in using these more complex measures, we created a variable called the 'spectral diversity metric', where measures were coded as either 'simple' or 'complex'.

2.2.2. Species Moderators

We identified three moderating variables related to the species data. Species counts in space, also referred to as richness, is the basic measure in biodiversity assessments, but it does not capture the relative abundance of the taxa. The variable 'species diversity' was coded as either 'richness' or, where a metric also incorporated evenness or abundance, as 'diversity'.

Additionally of interest is the number of species considered in the study. In grasslands, the species richness levels can be very high per m^2. In previous works, it has been suggested that our ability to predict taxonomic units using spectral variance may be saturated as the number of species in a data set rises [65,66]. Therefore, effect sizes may be smaller when looking at communities where species richness is consistently high. To test this idea, the continuous moderator 'richness level' was created, using the minimum value of richness within an analysis, as a proxy for the taxonomic complexity of the analysis. We hypothesize that the mean effect will be negatively influenced by higher numbers of species.

The methods of assessing biodiversity are classified according to the scale of organization, known as alpha, beta or gamma diversity [67]. Alpha is the number of species within a unit area and can also include a measure of their relative abundance. Beta diversity captures community dissimilarities between patches or components of a landscape. Gamma diversity is an additive measure of both alpha and beta diversities and describes diversity at the landscape scale. We created the categorical moderator 'level of diversity' to capture these different scales.

2.2.3. Sampling Design

We identified four moderating variables related to sampling design. Firstly, we noted that the sampling effort difference between the spectral and the botanical data is often pronounced. For example, satellite sensors collect spectral data over large areas, whereas the accompanying field botanical data have a much sparser coverage and are extrapolated from small plots. In contrast, when aerial or handheld instruments are used, small plots are often sampled exhaustively for both spectral and botanical data. To understand if these differences in the sampling effort impact the effect size, we created the moderator 'spatial matching', which is the ratio of the area sampled botanically to the area sampled spectrally.

Secondly, the time of year that sampling occurs is likely to impact the relationship between spectral variance and species diversity. Leaf and canopy phenology drive changes in reflectance over a growing season, and therefore, the relationship between spectral variance and plant diversity is also expected to vary over time. Summer should be the most stable time of the year for sampling leaf spectra. To capture this, we created a variable called 'sampling season'. We noted the first and the last month that spectral data were collected and categorized these months into seasons as follows: 'summer' (June–August) or 'other'. We recognize the somewhat arbitrary nature of these sampling periods, as seasonality will not be uniform across our sites due to the latitude and continentality of sites.

Thirdly, we used the Köppen climate classification to classify sites into one of five main groups (tropical, arid, temperate, continental and polar) according to their seasonal temperature patterns [68] in order to explore the impact of the ecological region on the reliability of the hypothesis. We called this variable 'climate'.

Finally, the level of naturalness of systems may affect the extent to which the Spectral Variation Hypothesis works. More natural systems often have higher levels of complexity in terms of their species distribution in space. In experiments, diversity levels are manipulated through, for example, seeding or weeding. To test if this has an impact, the moderator 'site type' was coded with two levels: 'natural' and 'experimental'.

2.3. Data Analysis

2.3.1. Extraction of Effect and Sample Sizes

To carry out a meta-analysis, we needed a standardized effect size for each result across all studies. Suitable effect sizes in studies that dealt with two continuous variables were generally based on Pearson's Correlation Coefficients or associated values of the co-efficient of determination (r^2), where additional information was available about the direction of the relationship. When results were only available as graphic displays, we extracted the estimates using the software 'Plot Digitizer' [69]. The results based on Kendall's rank were converted to the Pearson's Correlation Coefficient [70]. We transformed all estimates to Fisher's Z [71] to improve the fit to a normal distribution. Next, we weighted them for the meta-analysis using effect-level sample sizes based on the number of sampled botanical areas (e.g., plots or fields) used in the analysis. The sampling variances were calculated using large sample approximations and bias corrected correlation coefficients [72]. Model estimates based on Fisher's Z were converted back into the Pearson's Correlation Coefficient for interpretation purposes.

2.3.2. Three-Level Meta-Analytical Models

One of the challenges with synthesizing outcomes of remote sensing studies is that there are often multiple results reported within one study, leading to the challenge of modelling dependence of the effect sizes. Traditionally, this problem is handled by creating a mean effect size for each study [73]. However, this discards useful information that can, for example, be used to assess the impact of moderators. A more recent approach has been to use a multi-level extension also known as a three-level model, which enables us to estimate the variance not attributable to sampling errors and to specify both the within-cluster and between-cluster variances [74]. Firstly, we specified models clustered by 'study', a common approach in meta-analyses. Secondly, we used 'site' as a clustering variable, as high levels of between-study variations could be driven by site specificity. In addition, to test if our likely moderating variables impact mean effect sizes, we evaluated their importance by carrying out a subgroup analysis within a mixed effects model framework. Due to the data set size, we first included these moderators individually, and then, if they were significant, we tested for interactions [75]. We used the restricted maximum likelihood estimator (REML) to evaluate the significance of the main effect size for each model. For the moderator models, we estimated different effect sizes for each level of the categorical moderator. If the moderator was a continuous variable, we estimated the overall effect size and tested its significance.

When each study design is identical, all variances between study effect sizes should be attributable to the sampling error (i.e., sampling effort). Outside clinical trials, this is almost never true. Especially in ecological studies, we would expect there to be high levels of variance between study results due to the high levels of variation in natural systems. In meta-analyses, 'heterogeneity' is used to describe variances not attributable to sampling errors. Here, we report the significance level of Cochrane's Q for an overall test of 'heterogeneity' in the models, followed by I^2 [76]. The I^2 statistic is a relative value that indicates the percentage of total variance that is not attributable to a sampling error. It can be further decomposed into I^2 level-two and I^2 level-three variances, which are, respectively, the between-cluster and within-cluster variances. We tested the significance of the variance decomposition by comparing the three-level model with the equivalent two-level model using a one-sided log-likelihood-ratio test. We also evaluated the changes in the I^2 value as different moderators were added to the basic model.

2.3.3. Sensitivity Analysis and Publication Bias

For each three-level model, we carried out a sensitivity analysis. Influential case diagnostics were produced using a multivariate measure analogous to Cook's distance [77], which can be interpreted as the Mahalanobis distance between the entire set of predicted values, with the *i*th case included and excluded from the model fitting. These diagnostics were carried out at the study cluster level for each model. A robust cut off value for influential data does not exist, but generally, a Cook's distance $> 4/n$ is used, where n is the number of clusters in the model. To test if outlier studies were having a strong effect on the results, outliers were removed and the models recalculated.

Publication bias arises when results from studies are more likely to be published if they fulfil existing expectations. In the case of testing the Spectral Variation Hypothesis, this would result in finding a strong positive correlation between species or habitat diversity and spectral variance and, within the meta-analytical framework, an overestimation of the mean effect size. There are limited methods available for estimating publication bias in data sets that display dependence [78]. One simple option is to visually inspect funnel plots where residual values from the meta-analysis are plotted against the standard error. Non-symmetrical plots indicate the presence of publication bias.

All data analyses were done using the metafor package [79] in R [80].

3. Results

3.1. Overview of Studies

In terms of study location, there was a strong research bias towards sites in North America and Northwestern Europe. Three studies were carried out in Northern China (Figure 1A). There were no studies carried out in the Southern Hemisphere. All grasslands could be classed as temperate, continental or alpine, with no examples of tropical or arid systems. There was a good mix of leaf- and canopy-level studies, captured using satellites, unmanned aerial vehicles and proximal instruments (Figure 1B). We found studies that looked at alpha and beta diversities but only one that investigated gamma diversity (Figure 1C). The effect size for gamma diversity was excluded from future analyses due to the small sample size. Three studies collected data at discrete time points and explicitly reported results on the temporal stability of the Spectral Variation Hypothesis. Two studies did this across a growing season and one over different years. Some authors treated field data collected across a few months as a single sampling point (Figure 1D).

Most studies focused on a particular aspect of the relationship between spectral variance and biodiversity: six tested different biodiversity metrics using the same data set, four looked at the relationship at spatial different scales (i.e., pixel sizes), three looked at the relationship over time, six calculated the spectral variation in different ways and five repeated the same experiment across different sites or fields. Table 1 lists the publications, alongside their thematic focus.

3.2. Results of the Multi-Level Models

For the meta-analysis, we extracted 297 effect sizes from 20 studies over 15 experimental locations. A forest plot shows these effect sizes with their sampling variance by study category (Figure 2). The mean effect size (Pearson's Correlation Coefficient) calculated for the basic three-level meta-analysis models (no moderators) with study or site as the clustering variable, respectively, was 0.358 or 0.32 (confidence interval ±0.161 or 0.197), suggesting that, overall, there is a positive relationship between spectral variance and plant species diversity. We tested for the significance of the variance components by comparing the three-level model with the equivalent two-level model. Both three-level models, with level three heterogeneity constrained to zero, were a better fit for the data than their equivalent two-level models at $p = 6.897 \times 10^{-22}$ (study) and $p = 2.076 \times 10^{-20}$ (site) when using a likelihood-ratio test. Using the three-level approach, heterogeneity was decomposed into sampling between-cluster (level 2) and within-cluster (level 3) variances, each level being expressed as a percentage of the total model variance. The measure of heterogeneity (I^2) across all models was significant and substantial at about 80%, with about two-thirds of the heterogeneity occurring within studies. The results of the variance partitioning for the three-level models was very similar, whether study or site were defined as the clusters. Therefore, going forward, we report only the models clustered by study.

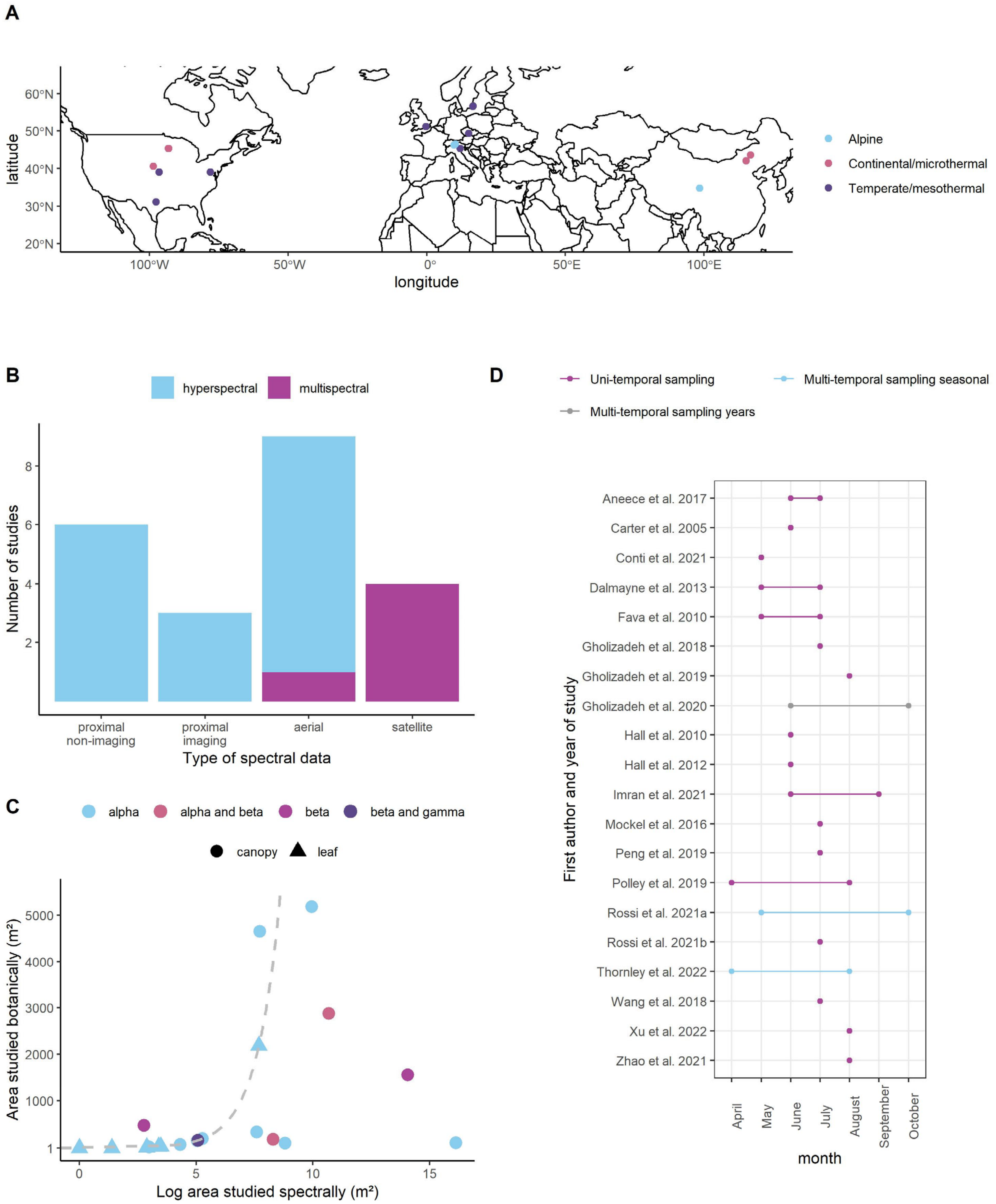

Figure 1. Literature search summary results. (**A**) The studies' geographical locations, alongside their climate zone classifications. (**B**) The sensor type used and spectral resolution. (**C**) The area sampled botanically and spectrally and whether the data was collected at the leaf or canopy scale (the grey dashed line represents equal sampling efforts for both variables). (**D**) The time of year the sampling took place and whether the author examined the data multi- or uni-temporally and if in multiple years.

Table 1. An overview of the studies and their thematic focus. Sites that are shared across studies are uniquely numbered.

Paper Number	Paper	Botanical Diversity Metrics	Scale Diversity Measured	Temporal Stability	Spectral Diversity Metric	Grassland Types	Shared Experimental Location
1	Aneece et al. 2017 [81]	0	0	0	0	1	1
2	Carter et al. 2005 [82]	0	0	0	0	0	2
3	Conti et al. 2021 [83]	0	0	0	0	0	3
4	Dalmayne et al. 2013 [84]	0	0	0	0	0	4
5	Fava et al. 2010 [85]	0	0	0	0	0	5
6	Gholizadeh et al. 2018 [86]	0	1	0	1	1	6
7	Gholizadeh et al. 2019 [87]	1	1	0	0	1	7
8	Gholizadeh et al. 2020 [29]	0	0	1	1	0	7
9	Hall et al. 2010 [88]	0	0	0	0	0	4
10	Hall et al. 2012 [89]	1	0	0	0	0	4
11	Imran et al. 2021 [90]	1	1	0	0	1	8
12	Möckel et al. 2016 [91]	0	0	0	0	0	4
13	Peng et al. 2019 [92]	0	0	0	1	0	9
14	Polley et al. 2019 [93]	0	0	0	1	0	10
15	Rossi et al. 2021a [94]	0	0	1	0	0	11
16	Rossi et al. 2021b [95]	0	0	0	1	0	12
17	Thornley et al. 2022a [31]	1	0	1	0	1	13
18	Wang et al. 2018 [23]	1	1	0	0	0	6
19	Xu et al. 2022 [96]	1	0	0	1	0	14
20	Zhao et al. 2021 [66]	0	0	0	0	0	15

Most of the moderating variables were not found to be significant, and the inclusion of moderators did not change the proportion of variance attributable to level-two and -three variances in the models. The exceptions were moderator models that included the 'leaf–canopy' term, where leaf-level studies were predicted to have a higher effect size (0.49 ± 0.128) compared to canopy-level studies (0.31 ± 0.146) at $p = 0.0036$. The continuous moderator 'richness level' was also significant but with a very small effect size (0. 00161) at $p = 0.043$. Full model results, alongside their diagnostic criteria, are provided in Table 2. We also tested for interactions between 'leaf–canopy' and the other moderator variables. We found significant interaction terms of 'leaf–canopy' and 'sampling season', 'site type' and 'richness level'. The results of these interaction models are in Table S2.

Cook's distance values indicated which studies were influential on the outcome of the basic and moderator models (i.e., outliers; see Figure S2). The results of the reprocessed three-level models showed that the basic model without moderators was still significant without outliers but that the mean effect size was lower at 0.32 (±0.149) (Table S3). Outlier removal did not change the significance level of the moderating variables. The only exception was the addition of 'site type' as significant at $p = 0.0323$, with the category natural sites showing a stronger relationship compared to the experimental ones (0.5 (±0.191) and 0.24 (± 0.194), respectively). Funnel plots show no significant publication bias in any of the specified models (see Figure S3 for a basic model example).

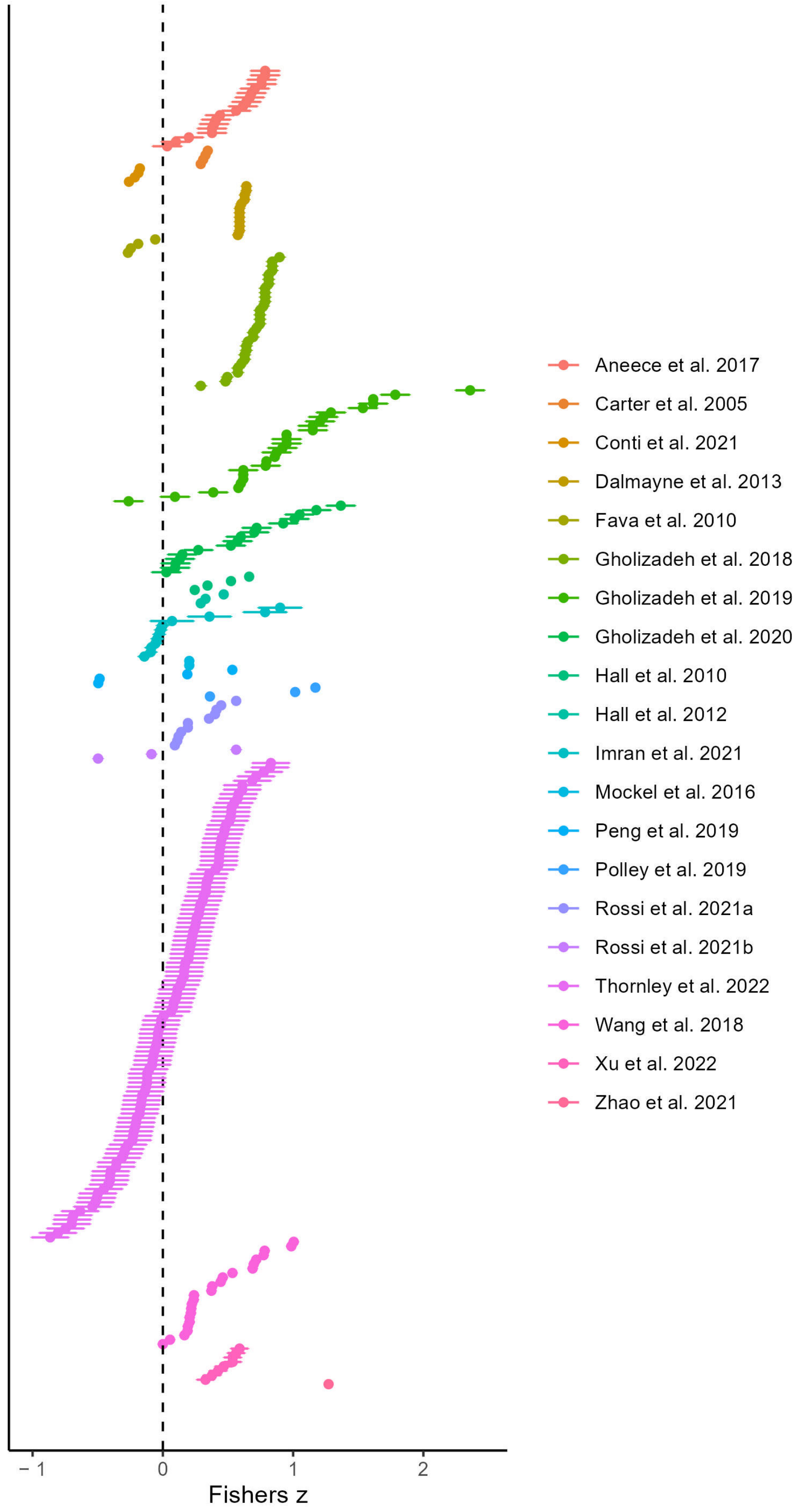

Figure 2. Forest plot showing the 297 effect sizes and their sampling variance ordered alphabetically by study. The dashed line represents the null hypothesis of no effect.

Table 2. Results of the three-level models with and without moderators. Significance levels of estimates are given as n.s. = $p > 0.05$, * = $p \leq 0.05$, ** = $p \leq 0.01$.

	Model Type	Cluster Variable	Moderators	Total Number of Effect Sizes (studies)	Number of Effect Sizes Per Group of Moderator	Pooled Correlation (Fisher's Z) with 95% CI	Pooled Correlation (r) with 95% CI	Significance Test of Pooled Correlation	Estimates for Moderators (if Significant) (r)	Significance Tests of Moderator Based Estimates	Random Effect Variance % (Sampling Error)	Random Effect Variance % (τ^2 level 2)	Random Effect Variance % (τ^2 level 3)	Multi-Level Variance % (I^2)
Basic	3 -level model	Study	-	297(20)	-	0.3741 (±0.162)	0.358 (±0.161)	8.3×10^{-6}	-	-	16.5	21.9	61.6	83.5
	3-level model	Site	-	297(20)	-	0.333 (±0.2)	0.32 (±0.197)	0.0012	-	-	14.6	22.2	63.1	85.4
Spectral data	3-level moderator model	Study	Pixel Size	297(20)	-	-	-	-	-	0.18 (n. s.)	17.88	22.31	59.81	82.12
	3-level moderator model	Study	Leaf or Canopy	297(20)	Leaf = 53; Canopy = 244	-	-	-	Leaf = 0.49 (±0.128); Canopy = 0.3111 (±0.146)	0.0036 (**)	16.01	18.76	65.22	83.99
	3-level moderator model	Study	Spectral Region	297(20)	Single = 153; Cross = 144	-	-	-	-	0.154 (n. s.)	17.13	22.76	60.12	82.87
	3-level moderator model	Study	Spectral Resolution	297(20)	Multi-spectral = 38; Hyperspectral = 259	-	-	-		0.2094 (n. s.)	16.8	22.29	60.9	83.2
	3-level moderator model	Study	Spectral Diversity Metric	297(20)	Complex = 97; Simple = 200	-	-	-	-	0.7448 (n. s.)	16.29	21.61	62.09	83.71
Species data	3-level moderator model	Study	Level of Diversity	296(20)	Alpha = 269; Beta = 27	-	-	-	-	0.24 (n. s.)	16.2	19.2	64.6	83.8
	3-level moderator model	Study	Species Diversity Metric	232(18)	Richness = 133; Diversity = 99	-	-	-	-	0.86 (n. s.)	13.9	23.8	62.2	86.1
	3-level moderator model	Study	Richness Level	247(15)	-	-	-	-	0.0161 ± 0.0015	0.0433 (*)	15.82	13.95	70.2	84.2
Sampling Design	3-level moderator model	Study	Spatial Matching	297(20)	-	-	-	-	-	0.3199 (n. s.)	16.9	22.41	60.69	83.1
	3-level moderator model	Study	Climate	297(20)	Alpine = 26; Continental = 101; Temperate = 170	-	-	-	-	0.0878 (n. s.)	17.99	23.78	58.23	82.01
	3-level moderator model	Study	Sampling Season	297(20)	Summer= 252; Other = 45	-	-	-	-	0.8065 (n. s.)	16.4	21.89	61.71	83.6
	3-level moderator model	Study	Site Type	297(20)	Experimental = 175; Natural = 122	-	-	-	-	0.3122 (n. s.)	15.75	20.8	63.46	84.25

4. Discussion

4.1. The Spectral Variation Hypothesis across Studies and Moderator Impact

The positive pooled effect size across studies of +0.36 indicates that, overall, the Spectral Variation Hypothesis appears to hold in grassland systems. The sensitivity analysis showed there was a strong influence on this mean effect size by the findings of Zhao et al. 2021 [66]. This study contained the only leaf-level result where reflectance data was collected using a leaf clip as opposed to close range imaging spectroscopy instruments and contained a single correlation that was very high (0.85). This indicates that we should be cautious when scaling our inferences from the leaf clip to imaging devices, as the taxonomic component of reflectance is weaker with imaging devices due to additional variables such as the specular reflectance [38]. However, even with the removal of this study, the mean effect size was still positive and significantly different from zero (+0.33 +/−0.149) (see Table S3). The weak-to-moderate overall effect size could be due to a nonlinear relationship between spectral variation and plant species or habitat diversity. Amongst the studies examined, almost all the available results were produced when testing for a linear relationship (nonlinear relationships were only examined in one study [81]). Testing for these alternative relationships should be an avenue of future research.

We tested whether the magnitude of the effect sizes across studies depended on reflectance observations from within single spectral regions (the visible, NIR or SWIR) or across the spectrum. We proposed that certain spectral regions may be more important than others for assessing biodiversity. However, there was no evidence from the meta-analysis that this was the case, nor did models containing data sampled from across the spectrum have a stronger relationship with plant/habitat diversity. This finding is unfortunate for two reasons. Firstly, for practical applications, such as sensor design, we require a better understanding of which spectral bands matter more [97]. Secondly, understanding which optical traits are driving the spectral variation–biodiversity relationship [27], within which contexts, is important for ecological interpretation. The results from this meta-analysis support the idea that the grounds for detecting biodiversity within grasslands could be location-specific.

The only clearly significant moderating variable, at $p < 0.01$, was the 'leaf–canopy' variable. Leaf-level studies had a higher mean effect size (0.49) compared to the canopy-level studies (0.32), implying that biodiversity estimations using optical leaf traits as opposed to habitat/community heterogeneity are a distinct methodological approach. The moderator interaction term between the 'leaf–canopy' and 'sampling season' was also significant (see Table S2). There was no relationship between spectral variance and biodiversity for leaf-level studies outside the summer season, whereas, for canopy-level studies, the relationship held for non-summer sampling. This indicates that summer sampling is more critical for leaf-level than for canopy-level approaches and that the Spectral Variation Hypothesis, at the canopy scale, may be successfully used during the spring and autumn when non-mature or senescing vegetation is present. The results of the interaction model with 'leaf–canopy' and 'site type' as terms suggest that experimental sites, rather than natural grasslands, have larger effect sizes for leaf-level estimates compared to canopy-level and vice versa. At the canopy level, the effect of higher levels of species richness was very slightly positive compared to the leaf level, where there was no effect. This result does not support our hypothesis that, in data sets with high numbers of species, our ability to estimate diversity using the Spectral Variation Hypothesis decreases.

The low influence of outliers on the results of the moderator models further suggests that most of the methodological concerns associated with testing the hypothesis seem to be systematically unimportant across existing studies. The exception is perhaps the study by [30] when testing the moderating variable 'site type'. By removing this study, the difference between the two site types (natural or experimental) became significant (but only just at $p = 0.032$). This study stands out, as it is the only example where repeat sampling was carried out across a season at both a natural and an experimental site.

High levels of heterogeneity were observed across all the models. This may reflect what is known in meta-analyses as the 'apples and oranges' effect, where we are not strictly comparing like for like [98]. High heterogeneity is, however, common in ecological meta-analyses [99], and values between 60 and 90% are usual. The high level of heterogeneity attributable to within-study variance, compared to between-study, indicates that the choice of data processing approaches within studies is responsible for more effect size variations than the study-level variables, such as site geographical location and instrumentation choice.

4.2. Limitation in the Scope of Studies

All studies included in the meta-analysis were carried out in the Northern Hemisphere. Evidence from the Southern Hemisphere and tropical and arid grasslands is notably absent. This reflects, in part, the lack of funding for experimental work in the developing world [100]. However, our exclusion of studies that dealt with partially wooded environments at the landscape scale, such as savannahs and chaparrals, impacted the scope. We predict that isolated trees in otherwise grass- and forb-dominated landscapes will probably increase the spectral diversity due to the inclusion of two very different land cover types. Other studies have shown good outcomes for the estimation of tree covers in these types of communities [101,102], and we may be able to utilize these estimates as covariates alongside the Spectral Variation Hypothesis within these systems to separate out pixels that include trees and those that capture only grassland.

An observation from this meta-analysis is that, despite the phenological dynamism of grassland systems, there are only a few instances of multitemporal testing of the hypothesis. Explicit testing of temporal stability was only examined in three cases [29,30,94], with all studies reporting instability across time when using the same instrumentation and analytical approaches. Most other studies focused on a mid-summer assessment. The results from the interaction models suggest that this is a good choice, at least when dealing with spectral data captured at the leaf level.

There are likely to be some additional sources of study bias that we were not able to explore within this meta-analysis. For example, the quality of the spectral data between and within studies due to the variability in terrain variables. Rugged terrain creates shadows that affects reflectance [103]. This could be especially problematic when assessing the hypothesis across large-scale landscapes using satellite data. However, terrain effects can also be observed within high spatial resolution data sets, collected using unmanned aerial vehicle technology. In future analyses, more attention should be given to validate reflectance data that could be affected by the terrain.

Although we did not detect any significant publication bias in this meta-analysis using funnel plots, this result should be treated with caution, as methods for testing publication bias with dependent data sets are still under development [78]. While the non-publication of negative data is a well-known phenomenon amongst scientists [104,105], within this synthesis, we found that there was a range of both negative and positive results reported, which perhaps indicates that this phenomenon is not as prevalent in this research field as in others.

4.3. Spectral Variation as a Covariate in More Complex Models

The high level of heterogeneity in the models presented in this study imply that species diversity prediction using spectral variation is likely to require the consideration of additional covariates. Within the reviewed studies, more complex relationships were examined that incorporated biomass levels [95], vertical sward complexity [83] and the proportion of the canopy at a mature phenological stage [30]. Spectral variance has also been found to be related to ecosystem productivity in grasslands [106], and spectral diversity, captured by satellites, has been shown to be principally influenced by the land cover type [107]. Combining reflectance data with structural characteristics, such as the tree height from LiDAR [108], has also proven promising in mapping species, suggesting that different types of remotely sensed variables can be combined to predict diversity.

4.4. Approaches to the Spectral Variation Hypothesis Outside This Meta-Analysis

While examining the literature on the Spectral Variation Hypothesis, we noted emerging approaches that expand on the traditional definition, which relates to the spectral variation in space. For example, some authors have looked at the spectral variance of a pixel or cluster of pixels over time [109–111]. This is based on the idea that plant species or community-specific responses to temperature, rainfall, day length and soil conditions can be exploited for diversity estimations. One step further is to combine temporal and spatial spectral variations into a composite measure [94]. Spectral variance has also been used to estimate plant functional diversity [112,113]. In addition, relationships have been found between phylogenetic and spectral distances among species [114]. It is evident that, as the field of biodiversity estimations from spectral data expands, these newer approaches will require scrutiny.

5. Conclusions

The results of this study indicate that there is some promise for the use of the Spectral Variation Hypothesis to estimate biodiversity in grasslands but that more work is needed before we can exploit the method with confidence. A diverse assemblage of approaches is in use by analysts, making this an exciting and active field of research. However, this also creates challenges when synthesizing results from studies. We encourage more work in extensive natural systems, especially in tropical and arid regions, and in the Southern Hemisphere. In addition, the repetition of experiments across phenological cycles and between years will also help increase our understanding of the stability of the hypothesis across time. Hyperspectral imaging sensors that capture data at very small scales and enable scaling up to the field level (while keeping all other site and analysis variables stable) are an important link in understanding the future possibilities and limitations of this approach.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/rs15030668/s1: Table S1: The search terms used in the literature search. Figure S1: The PRISMA flow chart for standardized literature reviews and synthesis. Table S2: The results of the three-level models with the interaction terms. Figure S2: The results of the outlier analysis for the three-level models displaying the Cook's distance metric clustered at the study level. Study outliers are those studies with values above the dotted line representing 0.2. Table S3: The results of the three-level model results after removal of the outliers at the study level. Figure S3: Funnel plot for the basic three-level model with this study as a cluster, showing (A) the raw Fisher's Z plotted against the standard error and (B) the model residuals plotted against the standard error.

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Intra-annual taxonomic and phenological drivers of spectral variance in grasslands

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ABSTRACT

According to the Spectral Variation Hypothesis (SVH), spectral variance has the potential to predict taxonomic composition in grasslands over time. However, in previous studies the relationship has been found to be unstable. We hypothesise that the diversity of phenological stages is also a driver of spectral variance and could act to confound the species signal. To test this concept, intra-annual repeat spectral and botanical sampling was performed at the quadrat scale at two grassland sites, one displaying high species diversity and the other low species diversity. Six botanical metrics were used, three taxonomy based and three phenology based. Using uni-temporal linear permutation models, we found that the SVH only held at the high diversity site and only for certain metrics and at particular time points. We also tested the seasonal influence of phenological stage dominance, alongside the taxonomic and phenological diversity metrics on spectral variance using linear mixed models. A term of percentage mature leaves, alongside an interaction term of percentage mature leaves and species diversity, explained 15-25% of the model variances, depending on the spectral region used. These results indicate that the dominant canopy phenology stage is a confounding variable when examining the spectral variance-species diversity relationship. We emphasise the challenges that exist in tracking species or phenology-based metrics in grasslands using spectral variance but encourage further research that contextualises spectral variance data within seasonal plant development alongside other canopy structural and leaf traits.

1. Introduction

1.1. Grassland monitoring

An important criterion when assessing field-level grassland condition is the complexity of the plant community, often summarised as the number of taxonomic units co-existing within the sward and their spatial distribution. These surveys are typically targeted at mid growing season when most plants are flowering, a time referred to as peak phenology (Stohlgren, 2006), with few data having been collected outside what is considered to be this 'optimal' window (Magurran, 2007). Plant communities can be dynamic in terms of the number of taxa present at a single time point during a growing season (Mellard et al., 2019; Wang et al., 2016), however, repeat intra-annual botanical surveys are very time-consuming and so little is understood about these community dynamics.

1.2. Spectral variation as a proxy for species diversity

An option to increase our understanding is to utilise remote sensing (Ali et al., 2016; Wachendorf et al., 2017) and in particular hyperspectral reflectance data (Fava et al., 2010; Möckel et al., 2016; Wang and Gamon, 2019). The Spectral Variation Hypothesis (SVH) proposes that the variance in spectral reflectance within a given area can be used as a proxy for plant taxonomic diversity. The concept of reflectance variance as an ecological surrogate was first described by Palmer et al. (2002). Rocchini et al. (2010) provide a review of the concept and the challenges to its implementation. Evidence to support the hypothesis has been gathered at the landscape scale (Hall et al., 2010) using broad-band satellite data products, down to the leaf-level with close-range imaging spectrometers (Wang et al., 2018). In some studies, however, the SVH been found to be unstable (Schmidtlein and Fassnacht, 2017; Torresani et al., 2019) and context dependent (Imran et al., 2021).

Convergent optical properties of photosynthetically active material

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alongside the impact of environmental drivers, such as water availability, may prove obstacles to species differentiation (Asner et al., 2009; Ollinger, 2011). Furthermore, spectral distance between species may be affected more by functional variation rather than by the number of taxonomic units (Schweiger et al., 2018). This perspective is intrinsic to the 'optical type' theory (Ustin and Gamon, 2010), where, in the context of high spatial resolution reflectance data, 'diversity' can be framed as a product of leaf traits at the individual level (Leaf Mass Area, Nitrogen, Chlorophyll, Carotenoids, Lignin) rather than taxonomic variation (Ma et al., 2020).

1.3. Measures of spectral variance

One of the complications in assessing the SVH and prior findings in this field, is that measures of spectral variance are calculated in different ways. Sophisticated approaches have been employed to deal with the multi-variate data sets produced from hyperspectral data sensors, for example, the 'Spectral Angle Mapper' (Gholizadeh et al., 2018), k-means clustering (Rocchini et al., 2005) and Partial Least Squares regression (Möckel et al., 2016; Polley et al., 2019). It is arguable, however, that descriptive statistical approaches, such as taking dispersions around the mean value for a single wavelength or index value, are more useful at this stage to evaluate the hypothesis (Torresani et al., 2019; Wang et al., 2018) as they allow the strength of the relationship to be more effectively compared between study findings. The coefficient of variation (CoV) is an often-used metric when dealing with hyperspectral data (Aragón et al., 2011; Blanco-Sacristán et al., 2019; Lucas and Carter, 2008; Wang et al., 2018) as it normalises the dispersion against the mean reflectance for each wavelength, thus accounting for the differences in magnitude between spectral regions.

1.4. The performance of the SVH over time

Another obstacle is that the relationship between spectral variance and taxonomic diversity when examined over time has been shown to be inconsistent (Schmidtlein and Fassnacht (2017)). Inter-annual studies with similar sampling dates in temperate systems (Gholizadeh et al., 2020) suggest this inconsistency is not merely a product of 'time of year' but may be due to a complex relationship between reflectance and seasonally dynamic leaf and canopy traits (Feilhauer and Schmidtlein, 2011; Feilhauer et al., 2017). High spatial resolution hyperspectral data, to our knowledge, has been collected on multiple dates in grasslands for only a couple of studies (Feilhauer and Schmidtlein, 2011; Gholizadeh et al., 2020), both of which reported varying relationships over time between taxonomic diversity and spectral reflectance. These observations could be due to the dynamic nature of grassland canopies, in terms of their responses to rainfall and management (Li et al., 2013) and phenological variability (Ustin and Gamon, 2010).

1.5. The impact of phenological stage on the spectral variation

Hyperspectral reflectance data are usually collected at peak biomass or growth when assessing taxonomic diversity, and there is good theoretical basis for this decision. At these times, grassland canopies are generally less affected by dead plant tissue and exposed soil, which are significant additional sources of spectral variation (Asner, 1998). When these sources are minimised, leaf intracellular structure and chemical traits drive variation in leaf reflectance (Ollinger, 2011; Ustin et al., 2009) and it is this variation that has been shown to be strongly correlated with the species present (Asner and Martin, 2011, 2016). In addition, leaf traits and so reflectance, and derived vegetation indices, alter with leaf age (Chavana-Bryant et al., 2017) which, if not accounted for, could be confounded with, among others, a taxonomic signal. On the other hand, the effect of leaf age and plant life cycle stage on reflectance could be exploited when the aim is to map single species or functional types. For example, in temperate deciduous woodlands, species specific timing of leaf emergence and senescence, accompanied with species specific leaf colouring, have been instrumental in distinguishing between tree species (Fassnacht et al., 2016; Hill et al., 2010; Voss and Sugumaran, 2008).

The spatial scale of data acquisition is highly significant when assessing the SVH (Gamon et al., 2019). At the leaf level, phenological stage affects reflectance through the process of leaf maturation (the development of palisade and spongy mesophyll and increase in chlorophyll cell number) (Noda et al., 2021), followed by senescence (reallocation of resources away from the leaf to over-wintering or reproductive structures). At the canopy scale, the quantity and developmental stage of leaves affect reflectance through increases in parameters such as leaf area index (LAI) and total canopy chlorophyll (Jacquemoud et al., 2009). Non-leaf plant architecture (buds, flowers, seeds) will also influence reflectance as these parts of the plant are generally not photosynthesising (Asner, 1998).

The number of differing phenological stages present will therefore be an additional driver of spectral variation alongside taxonomic diversity. The extent to which individuals within plant communities exhibit phenological stages at simultaneous or staged phases is known as phenological synchrony or asynchrony (Rathcke and Lacey, 1985; Forrest and Miller-Rushing, 2010) and the number of co-occurring phenological stages can be understood as phenological diversity (Lasky et al., 2016; Ramos et al., 2014). These properties may, however, be difficult to estimate as they are likely to vary between years and within a growing season (Mazer et al., 2013; Tansey et al., 2017) due to individual-based responses to environmental conditions (Wolkovich et al., 2014).

1.6. Study aims

This study uses intra-annual repeat taxonomic and phenological observations alongside the variance of high-resolution spectral reflectance data collected at two grassland sites, with differing levels of species diversity, soil type and management regime, to test the following:

1) The temporal stability of the SVH across a growing season in relation to plant taxonomic metrics and to determine the best time of year for biodiversity surveys using this method.
2) The extent to which the phenological diversity of the canopy drives spectral variance.
3) The relative impact of phenological and taxonomic diversity and phenological stage dominance on spectral variance across the growing season.

2. Methods

2.1. Grassland site description and sampling campaign overview

Two sites in the south east of England were used for the collection of remote sensing and botanical data. The first site is Dawcombe nature reserve, Betchworth, Surrey, UK (51.259, −0.261). It is an example of medium quality chalk grassland situated on the scarp slope of the North Downs and is managed for biodiversity conservation. From hereon, this site will be referred to as 'Dawcombe'. The second site is a long-term experimental grassland managed by Imperial College London and called 'Nash's Field' located at Silwood Park, Sunningdale, Berkshire, UK (51.413, −0.645). It is composed of acid grassland plots that have undergone varying soil nutrient and grazing treatments (Crawley et al., 2005). The range of treatments have created a site with high levels of community variation within a small area. This site will be referred to as 'Silwood'. Both sites undergo annual late summer or winter biomass removal through mowing and grazing but are not managed during the spring and summer months when the sampling was undertaken. However, they are subject to some low-level grazing throughout the year by wild herbivores (deer and rabbits) and invertebrates. We selected the sites for their strong gradients of community composition. They are also grasslands where plant species undergo their reproductive cycles without significant interruption, unlike in many agricultural grasslands where high grazing pressures can occur throughout the year.

Twenty 50 × 50 cm ($0.25 m^2$) quadrats sub-divided into twenty-five 10 × 10 cm subquadrats, using a botanical grid as a guide, were measured over the 2019 growing season. Ten of these were situated at Dawcombe and ten at Silwood (see Fig. 1). Quadrats were used as they represent the most common approach to monitoring vegetation in grasslands. At Dawcombe, quadrat locations were randomly chosen along a slope gradient, intended to capture maximum variation in the plant community, and were simply labelled 1–10. At Silwood, the quadrats were chosen to represent a range of nutrient and grazing applications, to the same aim. They were labelled to reflect the experimental plot (L), whether they were subject to grazing (UF = unfenced) or not (F = fenced) and the nutrient treatment (N = nitrogen, K = potassium, P = phosphate, All = all nutrients, None = control). The quadrats were marked with pegs and geolocated to sub-cm accuracy by use of a differential GPS so the same areas could be revisited. The botanical sampling grid was painted matt black to avoid interference with the reflectance observations. Botanical observations were made at six time points at Silwood and ten at Dawcombe. Hyperspectral sampling events totalled five at Silwood and seven at Dawcombe. Details of botanical and spectral sampling are to follow.

2.2. Plant community metrics

We calculated two sets of metrics, which are listed with their respective equations in Table 1. The first set is based on *taxonomic* units and metrics were calculated per time point per quadrat. The second set is designed to evaluate the impact of plant phenological stage on spectral diversity. These metrics are based on *phenological* observations associated with the recorded taxa and were also calculated per time point per quadrat.

2.2.1. Taxonomic metrics

Plant taxonomic complexity can be described and summarised by using a range of metrics, each of which present a different aspect of, or approach to, diversity measurement (Magurran and McGill, 2011; Morris et al., 2014). The following three metrics were calculated (Table 1): 'species richness', which is a count of species occurrence and is the basic measure in biodiversity assessment; the 'Simpson evenness index', also known as relative abundance (Smith and Wilson, 1996) which describes the dominance distribution of the species present; and the 'Simpson diversity index', a composite measure which incorporates both richness and evenness. Visual estimations of percent cover per species, are often used in botanical assessments, but this measure is very subjective, especially in more complex species-rich quadrats. So, instead, the proportion of sub-quadrats in which the species occurred was used to calculate relative abundance and subsequently derive the Simpson's diversity and Simpson's evenness indices.

Because proximal remote sensing instruments are generally set to

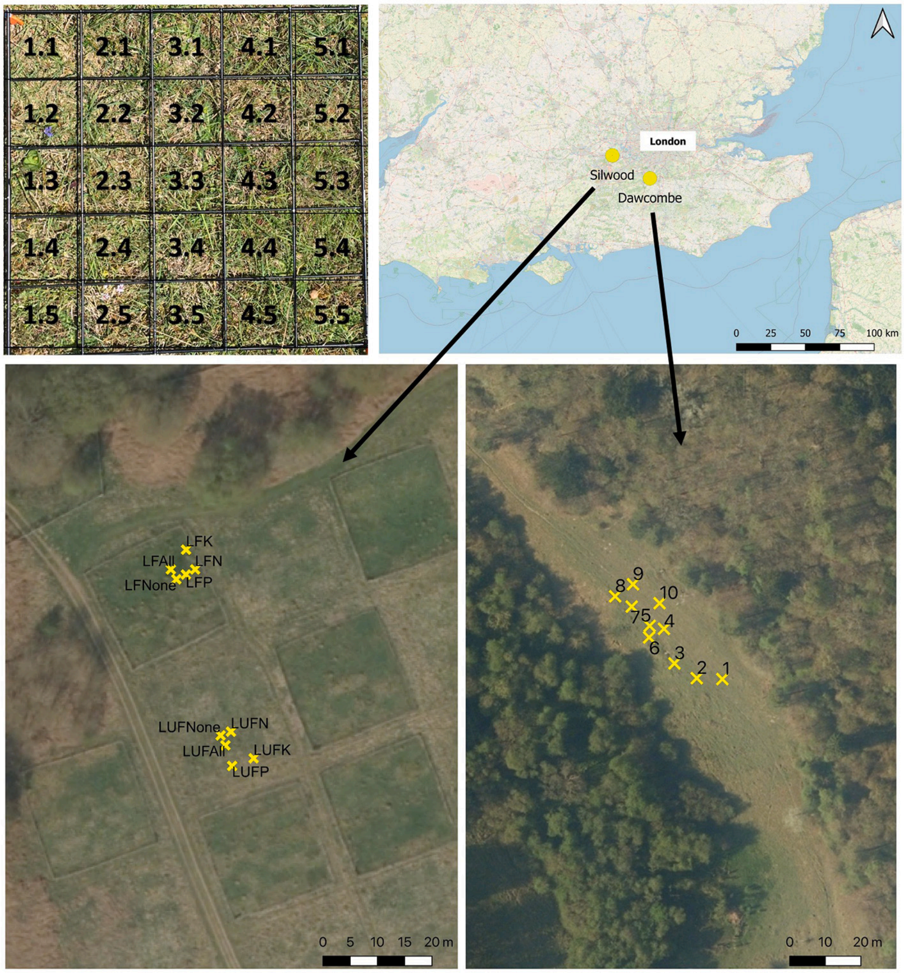

Fig. 1. The sampling sites and the quadrat locations at Silwood and Dawcombe. Top left: the sampling grid used to acquire both spectral and botanical samples for each of the 20 quadrats at each time point.

Table 1
Definitions of the taxonomic and phenological metrics used in this study.

Name of Metric	Description	Category	Equation
Species Richness (S)	The total number of species observed for each time point (*t*)	Taxonomic	S_t
Species Diversity (Simpson's diversity inverse or reciprocal) (D)	The inverse of the probability that two species drawn from the same sample will belong to the same species. Index ranges from 1 to infinity, where 1 represents a community with a single species and the higher the number the more complex the community.	Taxonomic	$D_t = \frac{1}{\sum_{i_t}^{S_t}\left(\frac{N_{it}}{N_t}\right)^2}$ Where: N is the total species abundance*;* N_i is the abundance of species *i; t* is time point *t*
Species Evenness (Simpson's Evenness) (E)	Describes the distribution of individuals among classes and is therefore a measure of relative abundance. *E* is expressed as a proportion of the maximum value which *D* could assume if individuals in the community were completely evenly distributed (which equals *S*). The metric takes a value between 0 and 1 where 1 is complete evenness.	Taxonomic	$E_t = \frac{D_t}{S_t}$
Phenological Richness (P)	The number of phenological stages observed for each time point (*t*)	Phenology	P_t
Phenological Diversity (PhenD)	Index ranges from 1 to infinity, where 1 represents a community with a single phenological stage present and higher values indicate a greater number of phenological classes and a more complex distribution of classes among species.	Phenology	$PhenD_t = \frac{1}{\sum_{i_t}^{P_t}\left(\frac{M_{it}}{M_t}\right)^2}$ Where M is the total number of phenology stages observed and M_i is the total number of phenology stages observed for species *i. t is time point t*
Phenological Evenness (PhenE)	As definition for *E but for phenological stage*	Phenology	$PhenE_t = \frac{Phen\ D_t}{P_t}$

nadir viewing and so are limited to sensing the top part of canopies, only species within the quadrat that were observed when looking directly down were considered.

2.2.2. Phenology metrics

Firstly, for each taxonomic unit observed in in the quadrat, an observation of phenological stage was made according to the definitions in Table 2. Some species displayed multiple stages at a single time point. The number of observations in each phenological category were then summed and weighted to produce a measure of phenology stage dominance for each quadrat at each time point.

Table 2
Descriptions of the phenology stages used to calculate the phenological diversity metrics.

Phenology stage code	Stage name	Stage description
SEN(1)	Senescent	Plant material in senescence (brown, lacking in chlorophyll) when quadrat was first examined in the spring (principally from last years' growth season).
YOU(2)	Young	Leaf material is thin/downy – displays colours (bright green) not in line with those expected from mature leaves.
MAT(3)	Mature	Leaf material is thickened / some cases waxy – displays colours in line with those expected from mature leaves
BUD(4)	Budding flowers	Plant has the beginnings of reproductive organs - flower buds for broadleaved herbs, or sheathed heads for grasses
FLO(5)	Flowering	Plant is in flower; flower heads emerged
SEE(6)	Seeding	Plant has seeds or seed encapsulating organs visible
SEN(7)	Senescent	The current growth season's plant material in senescence (brown or red/brown discoloured leaves).

Measures are available that describe the timing of plant phenology stages, such as frequency, regularity, amplitude, synchrony and duration (Newstrom et al., 1994; Denny et al., 2014). However, to evaluate the impact of plant phenological stage on spectral variance we required metrics that capture the phenology stage diversity observed at any moment in time. As far as we are aware, these do not exist. We therefore adopted the above taxonomic metrics to produce the following phenological metrics: 'phenological richness', the number of different phenology stages present at a given time in a given quadrat'; phenological evenness', a measure of the relative abundance of phenology stages present; and 'phenological diversity' which was designed to reflect the diversity of phenology stages present at any moment in time within a quadrat, as a product of the species richness and abundance. For full definitions of the metrics and the calculations used to produce them see Table 1.

2.3. Spectral data capture and calculation of spectral variance

The Coefficient of Variance (CoV) from hyperspectral reflectance observations was selected as the spectral variance metric. The next sections outline the in-situ instrument setup and hyperspectral data preprocessing steps taken to ensure a robust dataset for reliable derivation of reflectance CoV.

2.3.1. Hyperspectral field radiometry setup

Hyperspectral reflectance measurements (350 nm – 2500 nm) were collected for each sub-quadrat (25) of each quadrat (20). We used two SVC non-imaging spectrometers (SVC HR2024i spectroradiometers, Spectra Vista Corporation, USA) in a Dual Field Of View (DFOV) mode (Maclellan, 2017; Punalekar et al., 2018), to simultaneously record irradiance and reflected radiance. This approach is recommended when data is collected under fluctuating illumination conditions (which is often the case in the UK) and is expected to deliver more accurate observations, which are particularly important when, as in most vegetation studies, spectral distance between target classes is small. Before target sampling began, both spectrometers were mounted on tripods pointing at their respective Spectralon panels and reference readings were taken concurrently. The instrument measuring down-welling radiation was then set to timed-mode while the instrument measuring upwelling radiation was used on a boom held at nadir 70 cm above the grassland canopy, resulting in a sample spot size of 10 cm. Each grassland quadrat measured 50 × 50 cm and was subdivided into twenty-five 10 × 10 cm sub-quadrats using as a guide, the same matt black grid that was used in the botanical sampling. For each sub-quadrat one reading was taken. The target spots were intended to be non-overlapping but spatially correlated in order to emulate the effect of pixels from an imaging sensor. All measurements were taken between the hours of 10 am and 3 pm local time (BST). Twenty-five measurements were taken of each quadrat at each time point resulting in 250 measurements per sampling date for each site, totalling 3000 spectral samples.

2.3.2. Hyperspectral data pre-processing

Pre-processing of the spectrometry data involved calibration of each sub-quadrats' reflected radiance spectrum against its respective Spectralon white reference panel spectrum to produce reflectance. Parts of the spectrum affected by water absorption and scattering were removed (339–399 nm, 1900–2051 nm, 2450–2519 nm) and a Savitzky-Golay smoothing filter was applied. The spectrum was binned by 10 nm increments. Smoothing and binning was carried out with the package HSDAR (Lehnert et al., 2019) in R (R Core Team, 2021).

Spectrometry data can suffer from erroneous measurements caused by slight changes in viewing angle and subject illumination (Wehrens, 2011). It is vital to ensure that the inclusion of these measurements is minimalised as we are dealing here with variance measures from a mean or a centroid value. A common practice is to carry out repeat measures of the same target and take an average. Due to the number of measurements required per day this process was not feasible. Instead, thorough data cleaning and pre-processing was carried out to identify the erroneous readings. Two principal sources of measurement error were considered; 1) time stamp mismatch between the two spectrometers (one measuring the quadrats, the other the white reference panel), especially in rapidly changing conditions and 2) changes in reflectance caused by variations in viewing and sun angle. To minimise these sources of error, we used 'Robust Principal Component Analysis' (ROBPCA) (Hubert et al., 2005; Hubert, 2020) which was applied to the spectra grouped by time-point and quadrat (amounting to 120 data sets). Outliers are computed using 'projection pursuit' techniques and the Minimum Covariance Determinant (MCD) method (Hubert and Debruyne, 2010). The ROBPCA approach can be used to compute PC scores that are outlier resistant, but also to detect the outliers themselves. The level of data cleaning changed with the α parameter (0.5–0.9); lower values indicate more 'robust' outlier detection, with more samples being removed from the analysis. Data sets produced with five values of α (0.5, 0.6, 0.7, 0.8 and 0.9) were used to help assess the stability of the model fits for the uni-temporal data sets (Section 2.5.1). For the rest of the analysis, we used the ROBPCA corrected data with an α value of 0.8 resulting in a total sample size of 2561 spectra. For sample sizes, the sampling dates and their corresponding day of year (DoY) see Table 3.

2.3.3. Coefficient of variation

The coefficient of variation (CoV) was used as the spectral diversity metric and was calculated for each waveband *i* as follows:

$$CoV_i\,(\%) = \frac{\sigma_i}{\mu_i}\,x\,100 \tag{1}$$

where μ_i equals the mean reflectance of the 25 subplots and σ_i equals the standard deviation. Wang et al., 2018 used the mean of the band specific CoV values across spectral regions as a summary measure of hyperspectral variance and found strong positive correlations with taxonomic diversity metrics. Here we follow this method in order to compare findings. Firstly, the band specific measures of CoV were averaged across the full visible to short wave infra-red spectrum and then, secondly, across three spectral regions; the visible (400–699 nm), the near infrared (700–1299 nm) and the short wave infra-red (1300–2519 nm). These averages are referred to as 'mean-CoV', 'vis-mean-CoV', 'NIR-mean-CoV' and 'SWIR-mean-CoV', respectively. Although the exact values of these regional cut-off points are somewhat arbitrary, spectral variation within these three chosen spectral regions (visible, NIR and SWIR) has been shown, through use of radiative transfer models and global sensitivity analysis, to be driven by different leaf or canopy traits (Li and Wang, 2011; Xiao et al., 2014). At the leaf level, use of the PROSPECT model (Jacquemoud and Baret, 1990) shows that global spectral variation is dominated by variation in chlorophyll concentration in the visible region (400 nm – 699 nm) and the leaf structural parameter (N) in the NIR (700–1299 nm). Although the influence of N is still relevant at certain spectral sub-regions beyond 1300 nm, equivalent water thickness (Cw) becomes the principal contributor to spectral variance throughout the SWIR region (1300– 2500 nm). Similarly, at the canopy scale, the PROSAIL model (Jacquemoud et al., 2009) shows that these spectral regions retain their discrete importance. Variation in reflectance in the visible region is driven by chlorophyll content and by water throughout the SWIR. In contrast to the leaf level, at this scale, spectral variation in the NIR is mainly driven by dry matter content (Cm) and leaf area index (LAI). We hypothesise that, during the growing season, different leaf and canopy traits will be dominant in driving spectral variance and summarising data by these regions will help with interpretation of results.

Table 3
Sample sizes and dates for the hyperspectral data set.

Site	Date	Day of Year	Time point	Spectrometer sample size(n=)	
				All data	ROBPCA screen
Dawcombe	18th April 2019	108	1	250	216
	1st May 2019	121	2	250	215
	16th May 2019	136	3	250	209
	31st May 2019	151	4	N/A	N/A
	11th June 2019	162	5	N/A	N/A
	27th June 2019	178	6	N/A	N/A
	13th July 2019	194	7	250	215
	24th July 2019	205	8	250	217
	8th August 2019	220	9	250	211
	20th August 2019	232	10	250	213
Silwood	29th April 2019	119	1	250	210
	21st May 2019	141	2	250	217
	5th June 2019	156	3	250	218
	20th June 2019	171	4	N/A	N/A
	12th July 2019	193	5	250	210
	29th July 2019	210	6	250	210

2.4. Satellite NDVI to contextualise findings

A time-series of satellite derived NDVI values obtained from the Sentinel-2 mission at 10 m spatial resolution was used to contextualise the findings of the field observations in terms of the main growing season periods: green-up, peak biomass and senescence (Fig. 2). For each available time-point, cloud free MSI pixels corresponding with site quadrats locations were extracted and a site-specific mean NDVI (and standard error) was calculated. Seven pixels over 31 dates were used to construct the time-series for Dawcombe and five pixels over 19 dates for Silwood.

The NDVI time-series were divided into three phenology stages, which we call "Pre-NDVI max" (representing 'green-up' of the site vegetation), "NDVI max" (the plateaux of maximum NDVI which we assume to coincide with the vegetation being at maximum growth stage) and "Post-NDVI max" (where vegetation begins to senescence). The period of peak growth (NDVI max) corresponded to 25 days either side of the highest NDVI value, although this value was more difficult to ascertain at Silwood, as the site exhibited cloudy conditions at this time

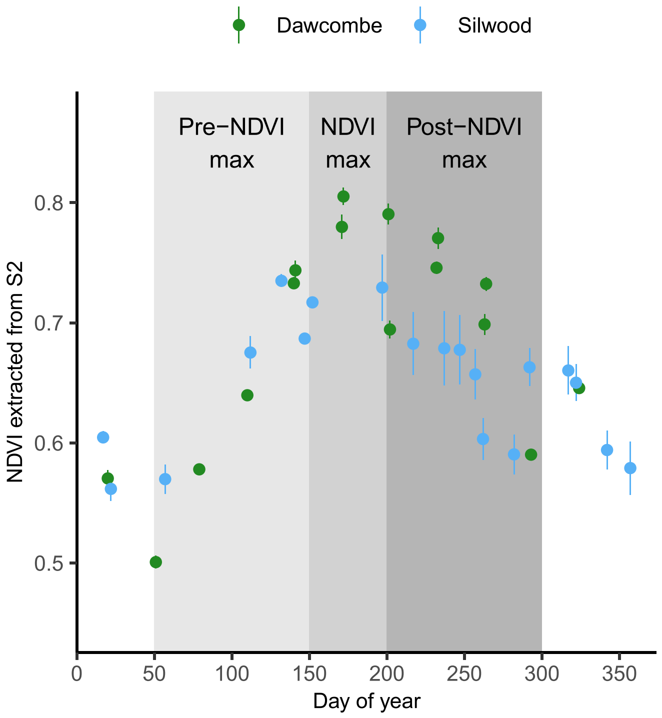

Fig. 2. The three phenology stages (Pre-NDVI max, NDVI max and post-NDVI max) derived from Sentinel-2 MSI NDVI time-series for two grassland sites.

of year. Pre-NDVI max covers the months of March, April and May (DoY 50 to 150), NDVI max covers June and the first half of July (DoY 150 to 200) and Post-NDVI max covers late July, August and September (DoY 200 to 300).

2.5. Statistical analysis of spectral variance and taxonomic and phenological metrics

The key aims of this study are to test the temporal stability of the SVH in relation to taxonomic metrics and to assess the extent to which phenological diversity drives spectral variance. In order to test these hypotheses two types of modelling were carried out. The first consisted of simple linear models which assessed the strength of the relationship between spectral variance and the three taxon and three phenology based metrics at each sampling event at each site. The second utilised mixed models to evaluate the consistency of these same relationships over all sampling points and across both sites.

We also used mixed modelling to investigate the third aim of the work which was to assess the relative impact of taxonomic and phenological diversity, alongside phenological stage dominance on spectral variance over all sampling points.

2.5.1. Simple linear models

Simple linear models were used to test the relationship between each narrow band value (the hyperspectral approach), as well as mean-CoV, vis-mean-CoV, NIR- mean-CoV and SWIR-mean-CoV, (the spectral regions approach) and the three species-based and three phenology-based diversity measures. For the spectral regions models, 288 uni-temporal model runs were carried out (Dawcombe: 7 time-points x 6 diversity metrics x 4 spectral regions =168 and Silwood: 5 time-points x 6 diversity metrics x 4 spectral regions = 120). Our data sets are small, when considered for each time point and site, so a permutation modelling approach was applied (LaFleur and Greevy, 2009), where *p* values for each linear model are assessed for stability using imputation, and the resulting adjusted r^2 values are reported.

2.5.2. Linear mixed models

One of the challenges associated with the data set collected is its structure, which includes temporal and spatial auto-correlation. Each quadrat was revisited several times so within-quadrat samples could be more similar to each other than to the data from other quadrats. It is also possible that samples taken at similar times of year will be more similar to each other. With this in mind, all data were modelled using a mixed model (Zuur et al., 2009), where the fixed effect is the taxonomic or phenological metric and the random effects, the quadrat and sampling time point (Pinheiro and Bates, 2000).

The package *lme4* (Bates et al., 2015) in R was used for the mixed model analysis. The model random effects structure was determined following the procedure outlined in Barr et al. (2013). The model fitting was performed using restricted maximum likelihood (REML) and the most complex random structure that would converge, used sampling event (day of year) and quadrat as random effects, producing an intercept only model with two random terms. Site was added as a fixed effect, because it only has two levels (the recommended minimum number of levels in a random effect is five (Zuur et al., 2009)). Examination of model residuals displayed heteroscedasticity, so spectral variance was converted to the natural log. This brought the residuals into an acceptable distribution. Application to the model residuals of a first order autocorrelation function revealed no significant temporal autocorrelation (Mitchell et al., 2020).

We also used mixed modelling to investigate the sources of spectral variance over time and used spectral variance as the response variable. Before modelling, all predictor variables are scaled from −1 to +1 and centred to allow interaction effects to be suitably assessed. The maximal model, containing the same random effects structure as in the first modelling stage, was fitted by Maximum Likelihood (ML) with all six of the taxonomic and phenology-based community variables and the percentage canopy stages as predictors with interaction terms included. The most parsimonious model, assessed using Akaike Information Criterion and Bayesian Information Criterion, included the terms % Mature leaves (MAT(3)) and species diversity and a term of their interaction.

For both stages of mixed modelling, reported coefficients, confidence intervals and *p*-values for fixed effects were obtained by fitting the models using Restricted Maximum Likelihood (REML) and by use of the Swatterwaite post-hoc test. Two pseudo r^2 were calculated to assist with the evaluation of the models: the marginal r^2, which is the fixed effects variance divided by the total variance (fixed + random + residual) and the conditional r^2, which is the fixed and random effects variance divided by the total variance. The marginal r^2 indicates the percentage of the total model variance explained by the fixed effects and the conditional r^2 indicates how much of the model variance is explained by the complete model (Nakagawa and Schielzeth, 2013). These values enable assessment of the relative impact of the spatial (quadrat) and temporal (sampling time) grouping variables and the fixed-effect predictor variables.

3. Results

3.1. Plant taxonomic and phenological diversity between sites and over time

The two sites were very distinct in terms of their species and phenology-based community composition (Fig. 3a). Throughout the season, relatively speaking, species richness is low to medium at Silwood (1–10 species) and medium to high at Dawcombe (9–24 species). Dawcombe shows very high levels of quadrat evenness (0.4–0.6) in all quadrats at all times, meaning there is no single dominant species. Silwood displays a range of quadrat evenness from 1.0 (only one species present – so completely even) to levels comparable with Dawcombe for more uneven quadrats (0.4).

In terms of phenological richness, Dawcombe shows higher values, partly reflecting the fact that the site has more species, so is more likely

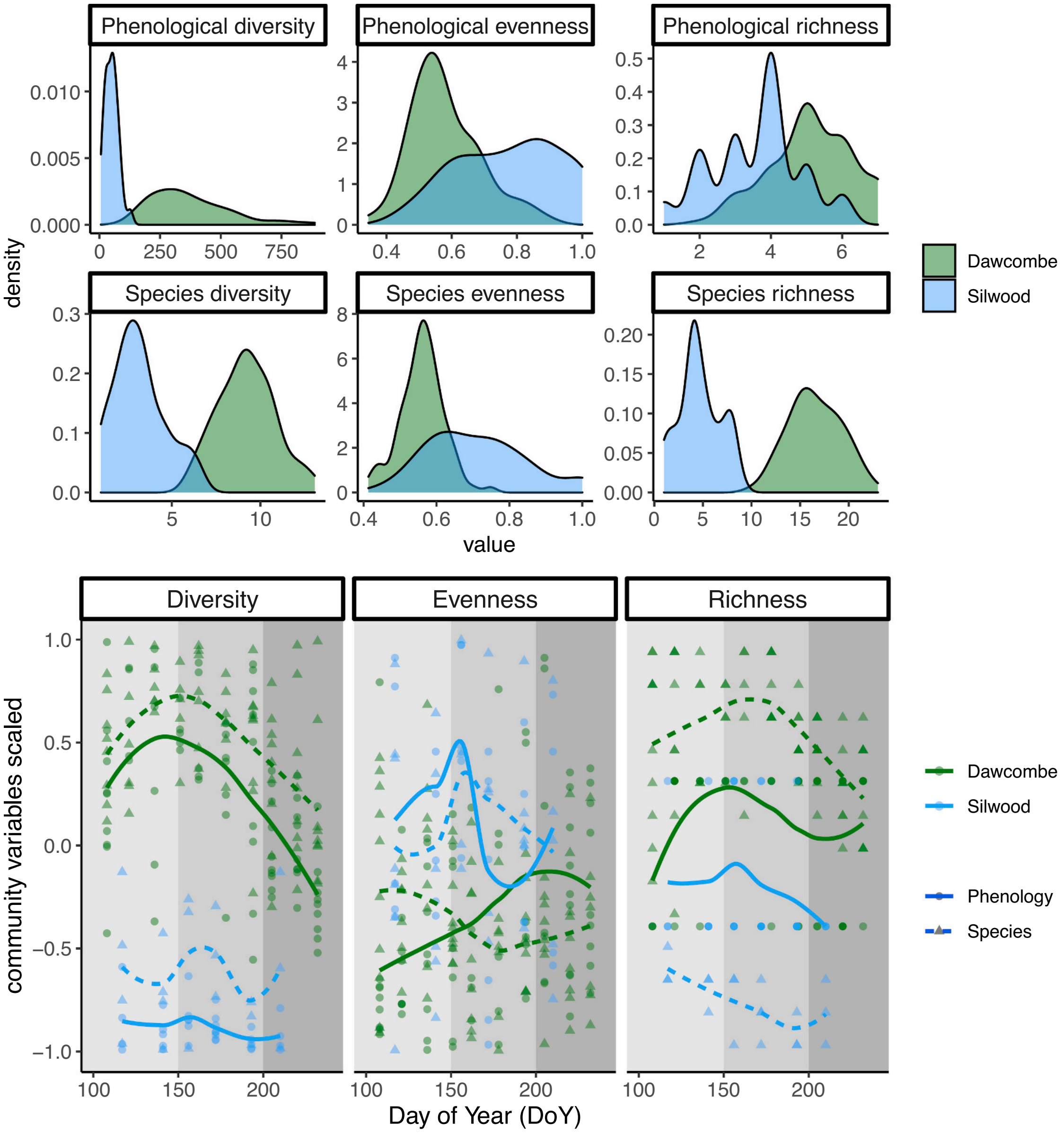

Fig. 3. a (top): Density distributions of the community metrics across the two sites for all times. b: (bottom): Community metrics over time at the two sites. All metrics have been scaled so they can be displayed together and the inter-site differences can be emphasised. A lowess smoother has been applied to emphasise any seasonal data trends.

to have many phenological stages occurring at one time. Results for phenological evenness concur with species evenness, with Silwood having more phenologically homogenous swards compared to Dawcombe. All quadrats at Silwood have low phenological diversity, whereas at Dawcombe there is a large spread in the values of this metric with some quadrats displaying different species' specific phenological states simultaneously.

In terms of seasonal patterns (Fig. 3b), at Dawcombe phenological richness and diversity follow species richness and diversity, with a sharp build up at pre-NDVI max stage followed by a peak at around DoY 150, and then a decline into post-NDVI max. Both species and phenology diversity metrics at Silwood, the species poor site, peak slightly later than at Dawcombe, and less strongly, coinciding more with NDVI-max. These results suggest that if we want to capture the full extent of species and phenological diversity we should sample just before and during NDVI-max. We speculate that both spring and summer emerging species are occurring simultaneously at this time, thus maximising measures of both species and phenological diversity.

When comparing the community metrics for each site using pair-wise correlation (Fig. 4), high intra-site positive correlation between species richness and species diversity can be observed (Pearson's correlation coefficient of 0.83 at Dawcombe and 0.93 at Silwood). The strength of the pairwise correlations between the two types of community metrics (species and phenology based) is generally greater at Silwood than at Dawcombe. This result indicates that at the species poor site (Silwood), phenological traits of the community over the whole season are more closely aligned to species community traits and in contrast, at the more species rich site (Dawcombe), phenological and species traits are more divergent.

Phenological stage dominance was determined by use of the seven phenological stage definitions and weighted percentages of total canopy coverage per stage per quadrat were calculated (Fig. 5). YOU(2) (young material) dominated the swards at both sites in early spring sampling (DoY 108 and 121 at Dawcombe and DoY 119 at Silwood). By DoY 156 at Silwood no new material was emerging, except in very small amounts in three quadrats (LUFN and LUFNone and LUFP). In contrast, at

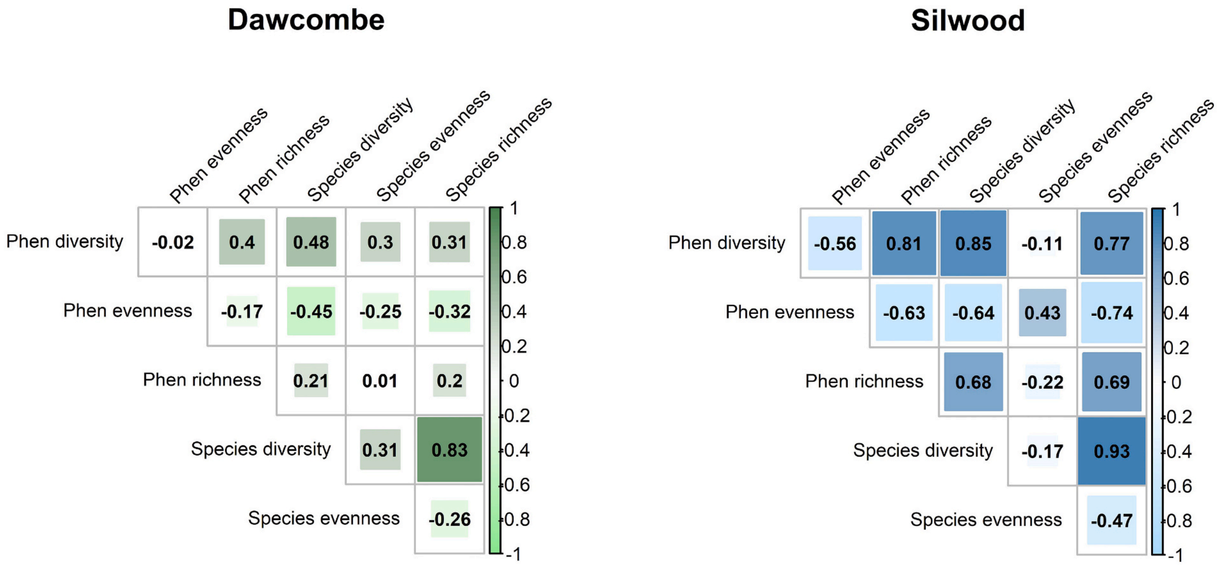

Fig. 4. Correlation heat map of the taxonomic (Species diversity, evenness and richness) and phenology (Phenological diversity, evenness and richness) metrics over all sampling times. Pearson's correlation coefficients are shown. Light colours indicate a negative correlation; dark colours a positive correlation.

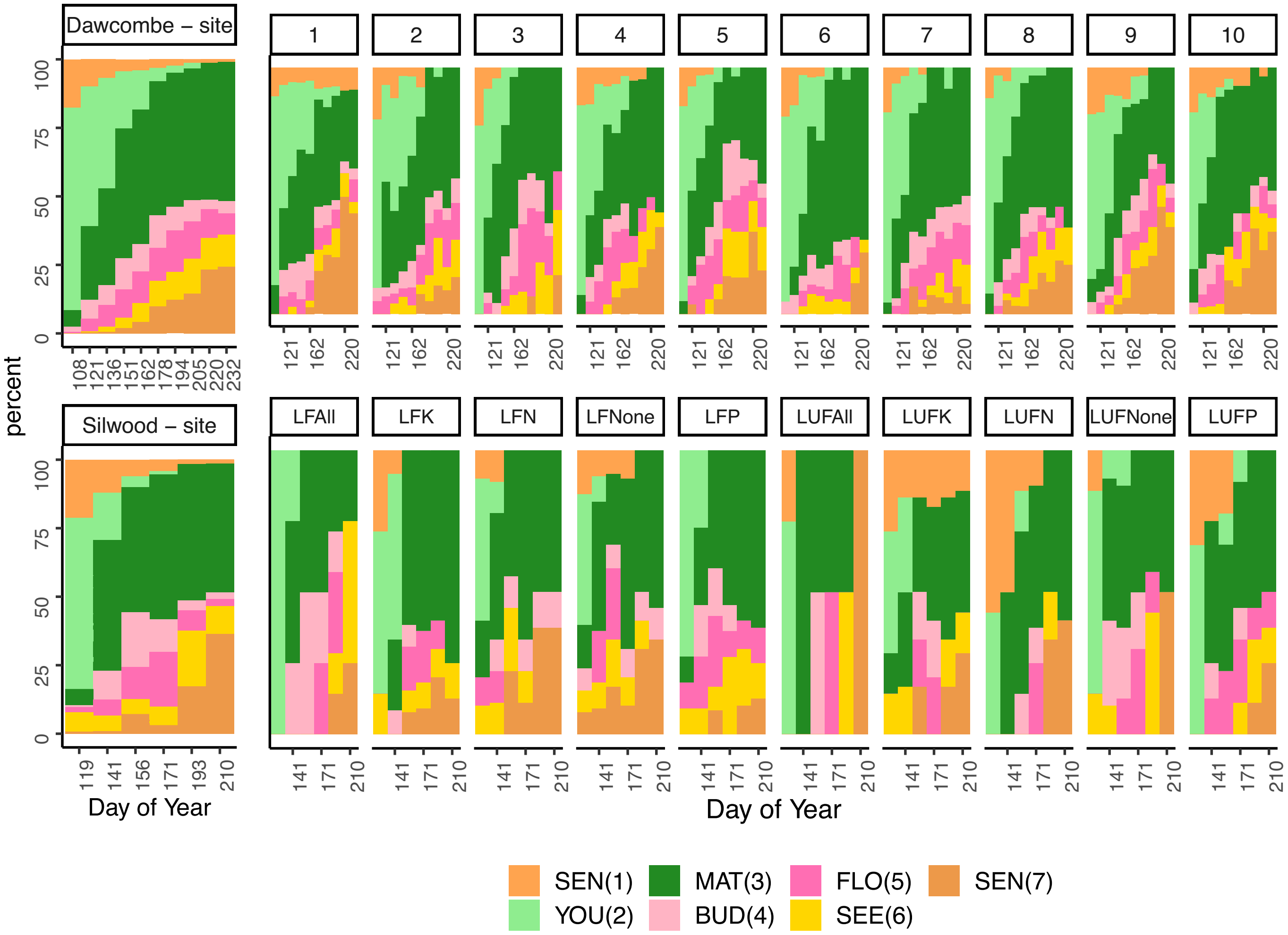

Fig. 5. The percentage of the canopy dominated by each of the seven documented phenological stages at each time point over the growing season at the site and the quadrat level.

Dawcombe, young material was still emerging in all quadrats up to DoY 194 and 205, towards the end of the phenology period NDVI-max. YOU (2) material was absent in all quadrats during the last two sampling points (DoY 220 and 232). Peak MAT(3) was reached on DoY 151 at Dawcombe and occurred at the cusp of the two satellite derived phenology stages (pre-NDVI-max and NDVI-max). At Silwood, peak MAT(3) was recorded at DoY 171, well into the NDVI-max satellite period. The percentage of the sward in stages BUD(4), FLO(5) or SEE(6) (bud, flowering or seed respectively) was very variable between quadrats at any one time.

3.2. Spectral CoV over time

Mean reflectance values per quadrat, per sampling time are shown in Fig. 6a and 6b alongside changes in the spectral variance for each wavelength. Mean reflectance for some quadrats (quadrat 5 at Dawcombe and quadrat LUFP at Silwood for example) remained very stable throughout the season whereas other quadrats displayed clear seasonal shifts (quadrat 4 at Dawcombe and quadrat LUFAll at Silwood). The largest magnitude in changes is observed in the NIR part of the spectrum. Seasonal patterns in CoV also changed dramatically in some quadrats but not in others. The temporal change in spectral variability were evaluated by the slope of a linear regression model CoV = f(DoY) for each quadrat (See Supplementary Material B, Table B1). Within quadrat rates of change were not very different between the spectral regions and the extent of change was principally a cross spectra phenomenon, therefore, only the mean-CoV is reported here. At Dawcombe quadrats 2, 3, 5 and 8 remained stable in time (model slope close to zero) whereas quadrats 1, 4, 6, 7, 9, and 10 increased over time (model slopes $> +0.1$, the fastest changing quadrat was quadrat 6 at $+0.34$). At Silwood, quadrats LFK and LUFK were stable, whereas all other quadrats at this site increased in spectral variability as the season progressed (with the maximum rate of change found at quadrat LFNone, model slope $+$ 0.47).

At the site level, mean-CoV followed the same overall trajectory at both sites, starting at a low level and increasing as the season progressed

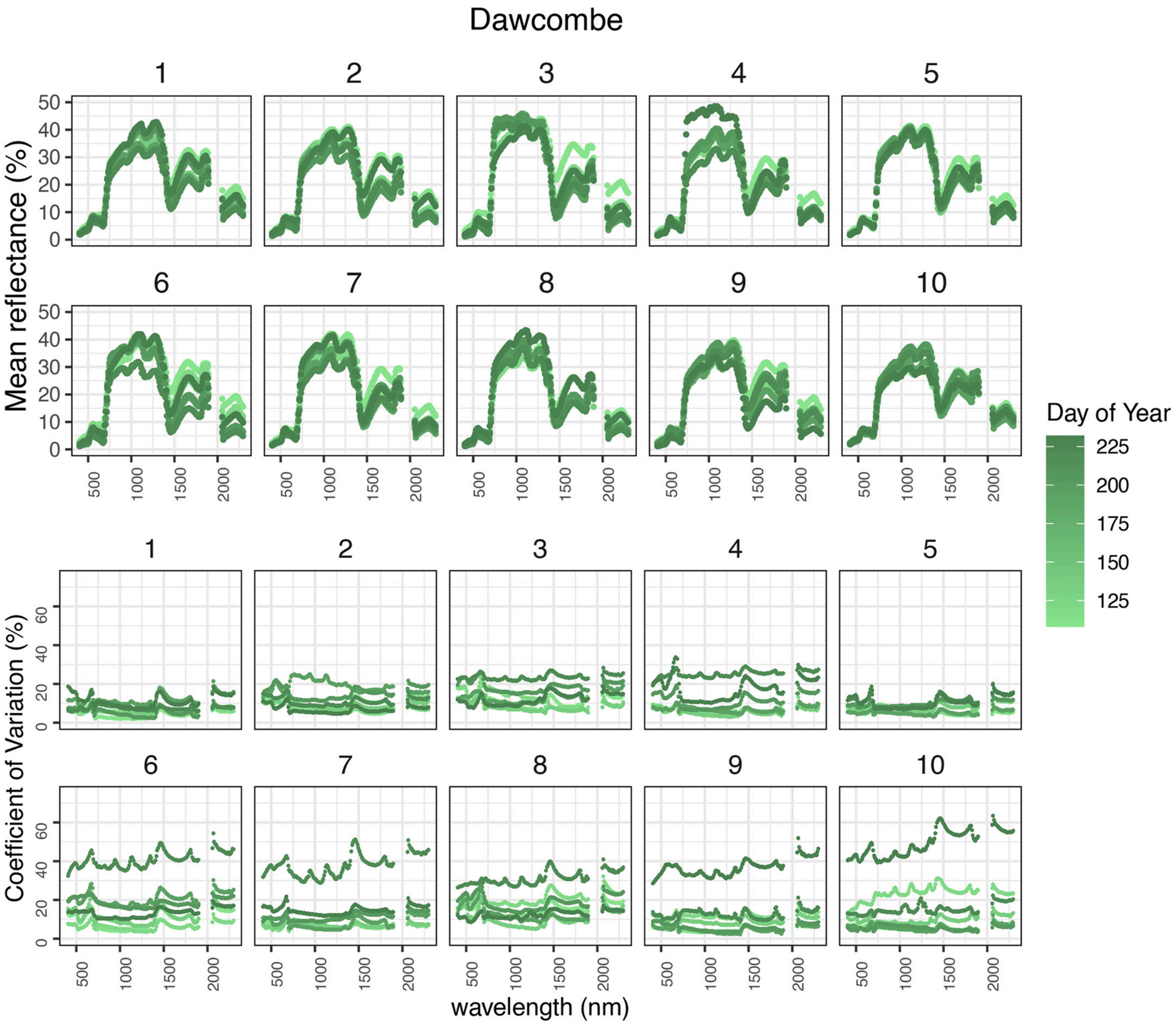

Fig. 6. a: Mean spectral reflectance and Coefficient of Variation (CoV) per quadrat per wavelength over the sampling days from the quadrat-based spectrometry data for Dawcombe. b: Mean spectral reflectance and Coefficient of Variation (CoV) per quadrat per wavelength over the sampling days from the quadrat-based spectrometry data for Silwood.

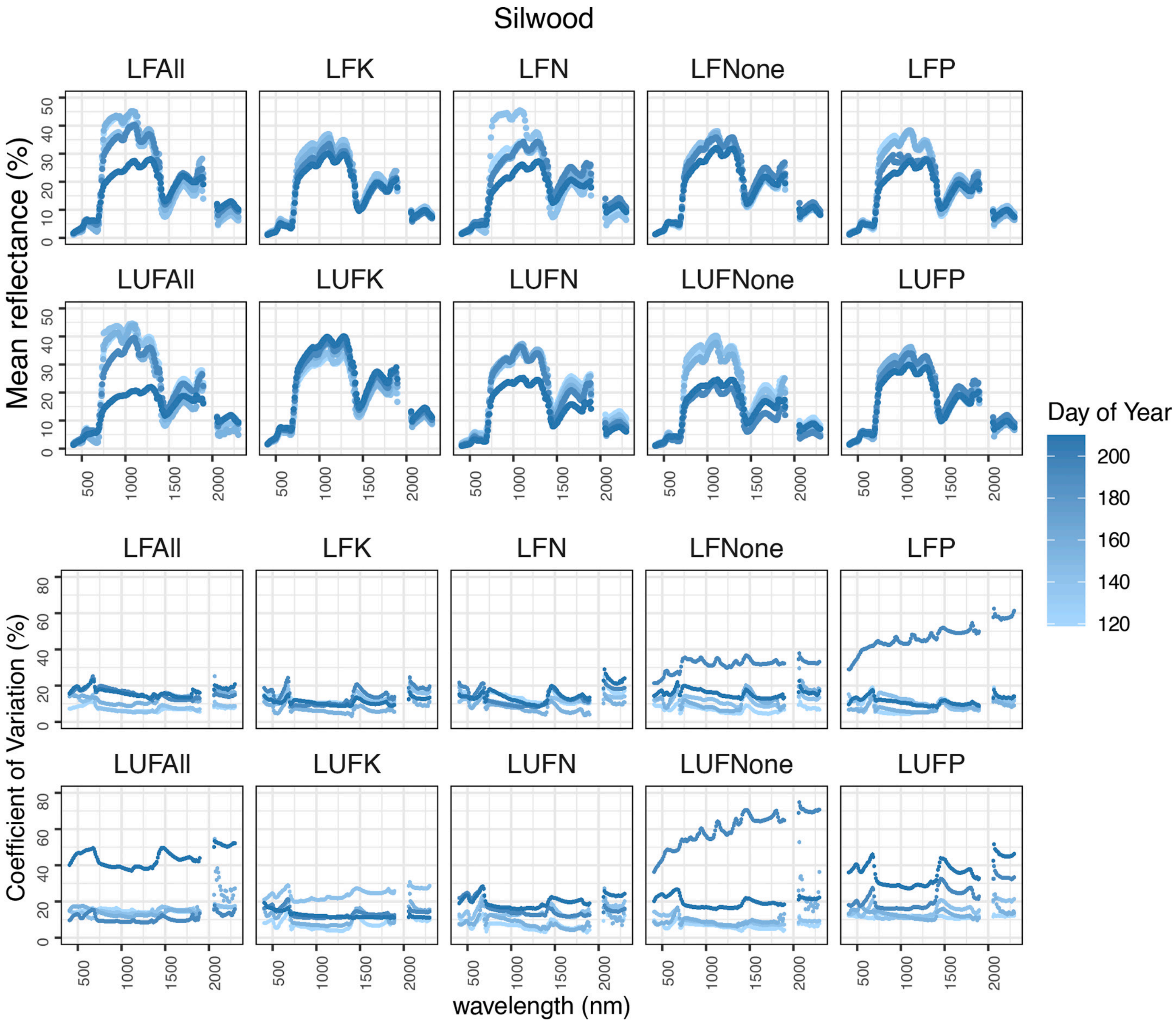

Fig. 6. (*continued*).

through pre-NDVI max and NDVI max, with the highest values occurring in late summer during post-NDVI max (Figs. 6 and 7). The extent to which the CoV changed over time is expressed as the slope of a linear regression model (Dawcombe $r^2 = 0.18$, $p = 0.00021$ (2sf), slope $= +0.08$ and Silwood $r^2 = 0.20$, $p = 0.000069$, slope $= +0.15$). The rate of change was slightly higher at Silwood in all spectral regions compared to Dawcombe.

3.3. The relationship between spectral diversity (CoV) and taxonomic and phenological diversity using linear permutation models

3.3.1. The spectral regions approach

The strength of the relationship between spectral variance averaged across spectral regions and each of the six uni-temporal plant community metrics (species richness, species evenness, species diversity, phenological richness, phenological evenness and phenological diversity) was very variable across time (Fig. 8), indicating that at the quadrat level spectral variance does not track changes in these metrics over a season. Values of adjusted r^2 for 209 out of 288 of the models were less than 0.1, meaning that at the majority of sampling points and for most community variables very little variation, if any, was explained by the metrics. Twenty-five out of 288 of the models were significant at $p < 0.05$. In eight of these models, mean-CoV was the predictor variable, in four, vis-mean-CoV, in seven, NIR-mean-CoV and in six, SWIR-mean-CoV. Three of the significant models predicted well values of phenological diversity, six phenological evenness, five phenological richness, six species diversity, one species evenness and four species richness (see Table 4). The sampling times when spectral variance best predicted taxonomic diversity (highest r^2 values and significant models) was at the end of pre-NDVI max (DoY 136) and post-NDVI-max (DoY 220 and 232) for Dawcombe and during NDVI-max (DoY 156) for Silwood.

The stability of the model r^2 also depended on the level of data cleaning imposed by the alpha parameter in the ROBPCA (Supplementary Material, Section A Fig. A1 and A2). At some time points, model r^2 steadily increased with more robust data cleaning. For example, at Dawcombe, Phenological diversity at DoY 194, during NDVI-max and Phenological evenness and diversity at DoY 136, during pre-NDVI-max, displayed this behaviour. Other model r^2 values remained constant, despite the level of data cleaning, for example for species evenness and species diversity at DoY 108. These results suggests that, at times, the

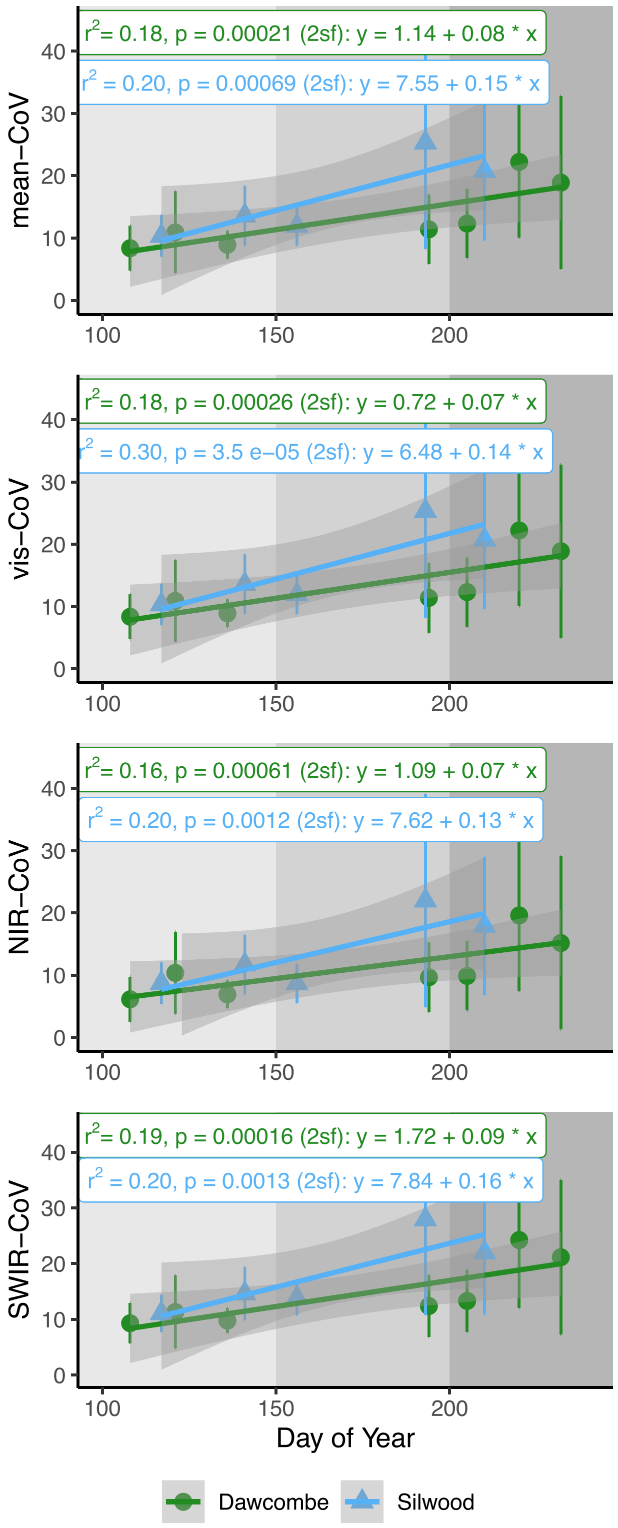

Fig. 7. Mean-CoV and spectral-regions-CoV over time, with linear model results describing the temporal increase at the site level.

quadrat level CoV can depend on a few reflectance outliers caused by, for example, bare soil, or a single plant dominating a sub quadrat or measurement errors such as un-intended off-nadir viewing. Our confidence in the SVH should increase with models that remain stable or improve in fit with data cleaning.

3.3.2. The hyperspectral approach

The linear permutation models were also applied to spectral variance at the hyperspectral level. The adjusted r^2 of these models is reported in Fig. 9. At Dawcombe at DoY 220 and 232, the whole of the spectrum displayed strong correlations to the metrics, however, at DoY 136 only narrow regions of the spectrum were correlated. Examination of the model fits from the hyperspectral approach demonstrated that the spectral regions approach was largely effective at picking up the best sampling times and metrics of interest.

3.4. Mixed models: relationships between variables over time

All the spectral data, summarised as spectral regions (the spectral regions approach), was included in a series of mixed models, allowing for temporal and spatial pseudo-replication. In the first stage of mixed modelling, which tested the ability of the CoV of spectral variance to predict taxonomic or phenological metrics over all sampling times and both sites, none of the models contained significant terms (see Supplementary Material C, Table C1). A large amount of variance in these models was explained by the random terms. Values of the Intra-class Correlation Coefficient (ICC) (the ratio of the between group variance to the total variance) (Nakagawa et al., 2017) ranged from 0.32–0.43 (these are considered high values and validify the use of the mixed model approach). The random term, quadrat, had a much smaller impact on the model, with estimates of around 10% that of sampling-time. These results further support the results from the uni-temporal models, that the strength of the relationship between spectral variance and these metrics is heavily time dependent.

During the second stage of mixed modelling, differing interaction effects of percent phenology stage dominance (SEN(1), YOU(2), MAT(3), BUD(4), FLO(5), SEE(6), SEN(7)) and taxon and phenology-based community metrics on spectral variance were tested. A significant effect of MAT(3) mature stage (slope = 0.19, p = 0.003) alongside a significant interaction effect of MAT(3) and species diversity (slope = 0.12, p = 0.014) was found for mean-CoV (Fig. 10) with similar results for the other spectral regions (see Supplementary Material C, Table C2 for full model results). NIR was the spectral region with the highest marginal r^2, with around 25% of the variance explained by the fixed terms, and an effect size of 0.25 for the mature term and 0.15 for the interaction term mature and species diversity. The model using vis-mean-CoV as the response variable displayed the largest values of conditional r^2 with 43% variance explained, 16% of which was explained by the fixed terms.

4. Discussion

4.1. Relationships between spectral variance and taxonomic and phenology metrics over time

The uni-temporal models at the site level were able to predict gradients of both taxonomic and phenology-based community metrics. However, the predictive ability of the models varied over time indicating that tracking these metrics across a growing season using spectral variation is problematic. The highest correlations between spectral and community metrics tended towards late pre-NDVI-max and early NDVI-max at both sites, suggesting that late spring (around DoY 150) is optimal for estimation of taxonomic and phenological traits in these grassland systems. These dates coincided with maximum species and phenological diversity at both sites. Late summer sampling (DoY 220 and 232 during post-NDVI-max) also proved productive at Dawcombe, although data was not collected on comparable dates for Silwood due to the site management regime. Using the mixed model approach, we found that none of the six metrics displayed a consistent relationship to spectral variance over time, further confirming that there is a temporal dependence in the relationship.

However, at the low species diversity site, Silwood, the best models (DoY 156) consistently predicted a negative relationship between the

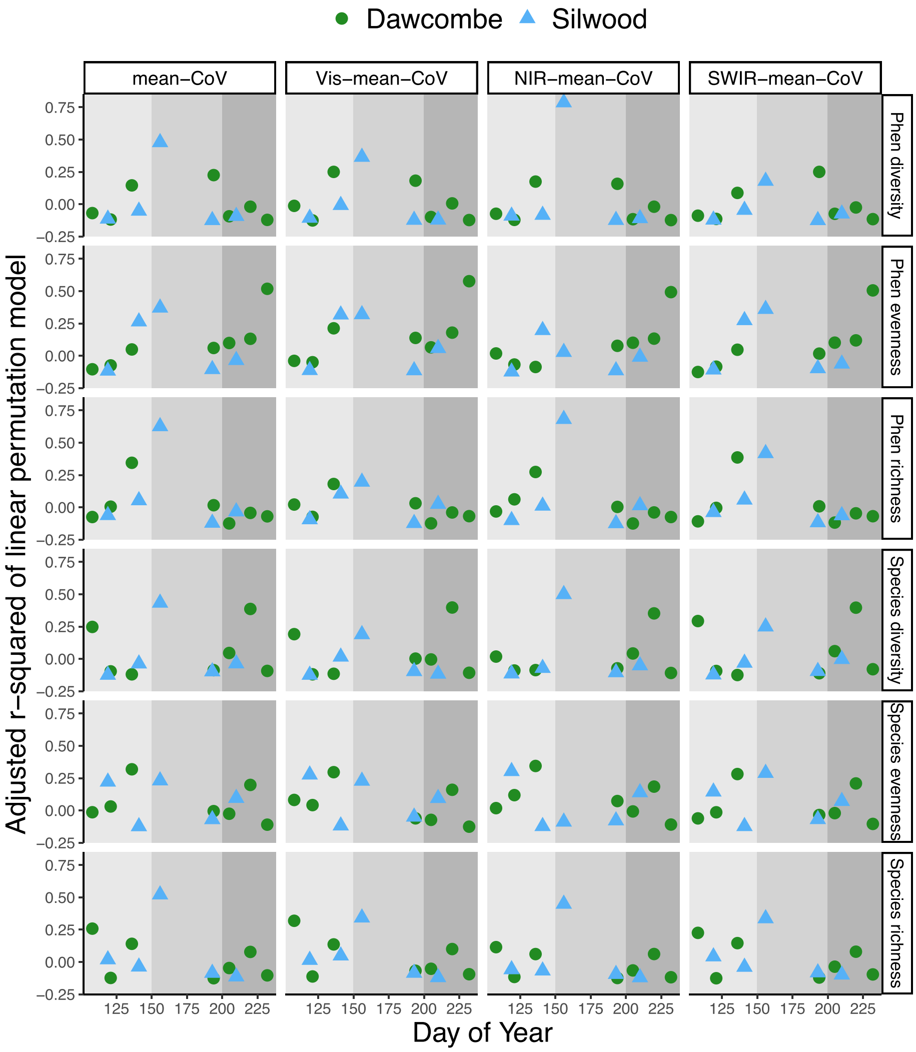

Fig. 8. Adjusted r^2 of the simple uni-temporal linear permutation models (spectral variance = f(diversity metric) using the spectral regions approach.

metrics and spectral variance, for five out of six of the metrics (none of the models predicted well Species Evenness). At Dawcombe, regardless of the sampling time, for the best models, there was always a positive relationship between spectral variance and the metrics; a result that supports the SVH (note that species and phenological evenness should under the hypothesis display a negative relationship, as low measures of evenness represent more varied communities).

We proposed that grassland community phenological dynamics could be responsible for some of the variation in spectral variance. We tested this hypothesis using our own phenological metrics and looked for interactions between these metrics and the species-based metrics in a mixed modelling approach. However, we found no significant interaction terms, implying that phenology-based spectral signals are not operating systematically across the growing season either to detract from species-based signals or to enhance them.

When considering the overall trend in spectral variance between sites, despite Silwood and Dawcombe displaying low and high taxonomic and phenological diversity, respectively, the mean-CoV values at the site level were marginally higher at Silwood (Fig. 7) clearly demonstrating that, in this instance, the site with the higher diversity did not have a higher spectral variance.

4.2. Taxonomic and phenological dynamics between sites

The trends in taxonomic and phenology metrics show how grasslands can display diverse temporal dynamics in terms of the seasonal development of their community composition which may have effects on our ability to monitor them using remote sensing techniques and the SVH. Judging from these results if we want to capture the full extent of taxonomic and phenological diversity we should sample just before and during NDVI-max. We speculate that both spring and summer emerging species are occurring simultaneously at this time, thus maximising measures of both species and phenological diversity. We observed that at Silwood the phenology and taxonomic metrics were more strongly correlated across the season than at Dawcombe (Fig. 4). This suggests that at Silwood phenological diversity follows seasonal species turnover whereas at Dawcombe there is a more complex relationship. This complexity could be a direct result of the higher species diversity of the site or the type of species present. It could also reflect other phenomena such as assortative mating and the development of discrete subpopulations that over time become reproductively isolated (Elzinga et al., 2007).

4.3. Mature leaves as drivers of spectral variance

Across the growing season, at the site level an increase in spectral variance was observed, which was found to be independent of the taxonomic and phenological based metrics. Rather spectral variance was found to be partly driven by the occurrence of plant parts in MAT(3) phenology stage. There are different possible interpretations of this

Table 4
Results of the significant uni-temporal permutation models at $p < 0.05$. Results that do **not** support the SVH are highlighted in **grey**.

Site	Time Point	DoY	Satellite derived phenology stage	Community metric	Spectral variable	(Coefficient) Intercept	(Coefficient) Slope	adjusted r^2	p value
Dawcombe	3	136	Pre-NDVI-max	Species evenness	NIR-mean-CoV	8.652	-13.775	0.345	0.043
Dawcombe	9	220	Post-NDVI-max	Species diversity	mean-CoV	31.025	7.716	0.387	0.032
Dawcombe	9	220	Post-NDVI-max	Species diversity	NIR-mean-CoV	28.028	6.770	0.353	0.041
Dawcombe	9	220	Post-NDVI-max	Species diversity	SWIR-mean-CoV	33.774	8.797	0.397	0.030
Dawcombe	9	220	Post-NDVI-max	Species diversity	vis-mean-CoV	27.855	6.006	0.398	0.030
Dawcombe	3	136	Pre-NDVI-max	Phenological richness	mean-CoV	11.055	2.129	0.345	0.043
Dawcombe	3	136	Pre-NDVI-max	Phenological richness	SWIR-mean-CoV	12.307	2.676	0.387	0.032
Dawcombe	10	232	Post-NDVI-max	Phenological evenness	mean-CoV	27.107	-129.600	0.519	0.011
Dawcombe	10	232	Post-NDVI-max	Phenological evenness	NIR-mean-CoV	23.407	-115.058	0.493	0.014
Dawcombe	10	232	Post-NDVI-max	Phenological evenness	SWIR-mean-CoV	29.626	-144.397	0.507	0.013
Dawcombe	10	232	Post-NDVI-max	Phenological evenness	vis-mean-CoV	26.114	-109.356	0.578	0.006
Silwood	3	156	NDVI-max	Species richness	mean-CoV	13.237	-0.803	0.521	0.011
Silwood	3	156	NDVI-max	Species richness	NIR-mean-CoV	9.453	-0.712	0.449	0.020
Silwood	3	156	NDVI-max	Species richness	SWIR-mean-CoV	15.341	-0.892	0.337	0.046
Silwood	3	156	NDVI-max	Species richness	vis-mean-CoV	13.791	-0.688	0.342	0.044
Silwood	3	156	NDVI-max	Species diversity	mean-CoV	13.237	-1.029	0.433	0.023
Silwood	3	156	NDVI-max	Species diversity	NIR-mean-CoV	9.453	-1.024	0.499	0.013
Silwood	3	156	NDVI-max	Phenological richness	mean-CoV	13.237	-1.528	0.626	0.004
Silwood	3	156	NDVI-max	Phenological richness	NIR-mean-CoV	9.453	-1.490	0.682	0.002
Silwood	3	156	NDVI-max	Phenological richness	SWIR-mean-CoV	15.341	-1.707	0.418	0.026
Silwood	3	156	NDVI-max	Phenological evenness	mean-CoV	13.237	9.911	0.371	0.036
Silwood	3	156	NDVI-max	Phenological evenness	SWIR-means-CoV	15.341	12.890	0.360	0.039
Silwood	3	156	NDVI-max	Phenological diversity	mean-CoV	13.237	-0.051	0.478	0.016
Silwood	3	156	NDVI-max	Phenological diversity	NIR-mean-CoV	9.453	-0.059	0.787	0.000
Silwood	3	156	NDVI-max	Phenological diversity	vis-mean-CoV	13.791	-0.046	0.365	0.038

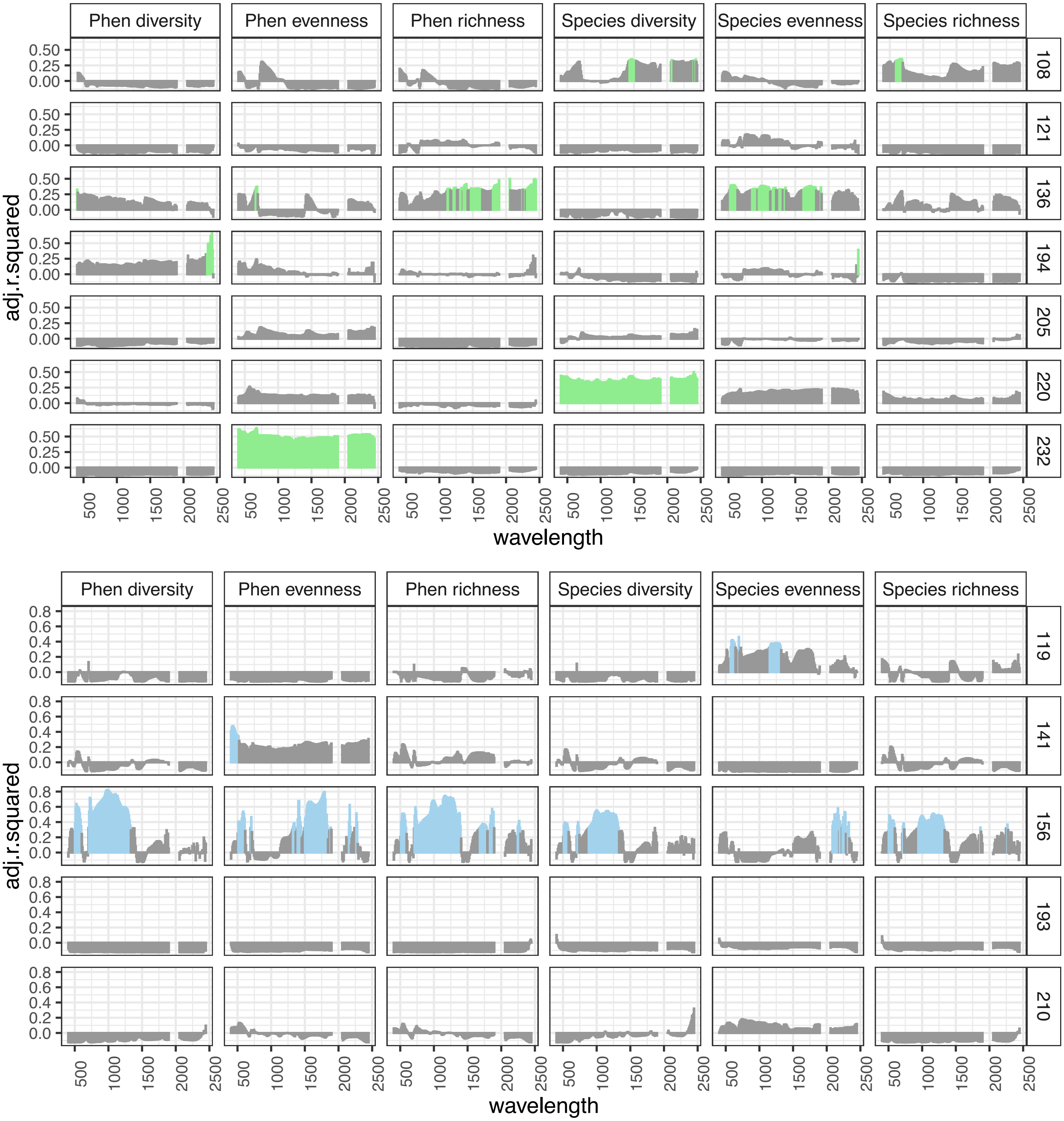

Fig. 9. Adjusted r^2 of the simple uni-temporal linear permutation models (spectral variance = f(metric) using the hyperspectral approach. Significant wavelengths are shown in colour; non-significant in grey.

result. Firstly, mature leaves of plants could, in fact, be more spectrally variable than other leaf growth stages. Another perspective is that when plants are in their mature stages canopy structural attributes contribute to spectral variance through self-shading. This problem is difficult to eliminate in mixed 'pixel' situations, but if the pixel sizes were small enough (i.e., those obtained through drone acquisition), this problem could be reduced through removal of low NDVI 'shade' pixels, in a similar way to soil correction techniques (Gholizadeh et al., 2018). Additionally, as canopies develop over time, they could become more spectrally variable due to vertical complexity (Conti et al., 2021). The mixed model with the highest explained variance by the fixed terms (MAT(3) and species diversity) was in the NIR spectral region. This suggests that canopy traits such as LAI and leaf angle distribution could be influential.

Irrespective of the ultimate driver of spectral variance associated with the presence of mature leaves, the observed underlying seasonal increase in spectral variance needs to be taken into account when sampling across dates for the purposes of taxonomic diversity evaluation as it will likely confound the desired signal. The observed interaction effect of species diversity and mature leaves implies that the usefulness

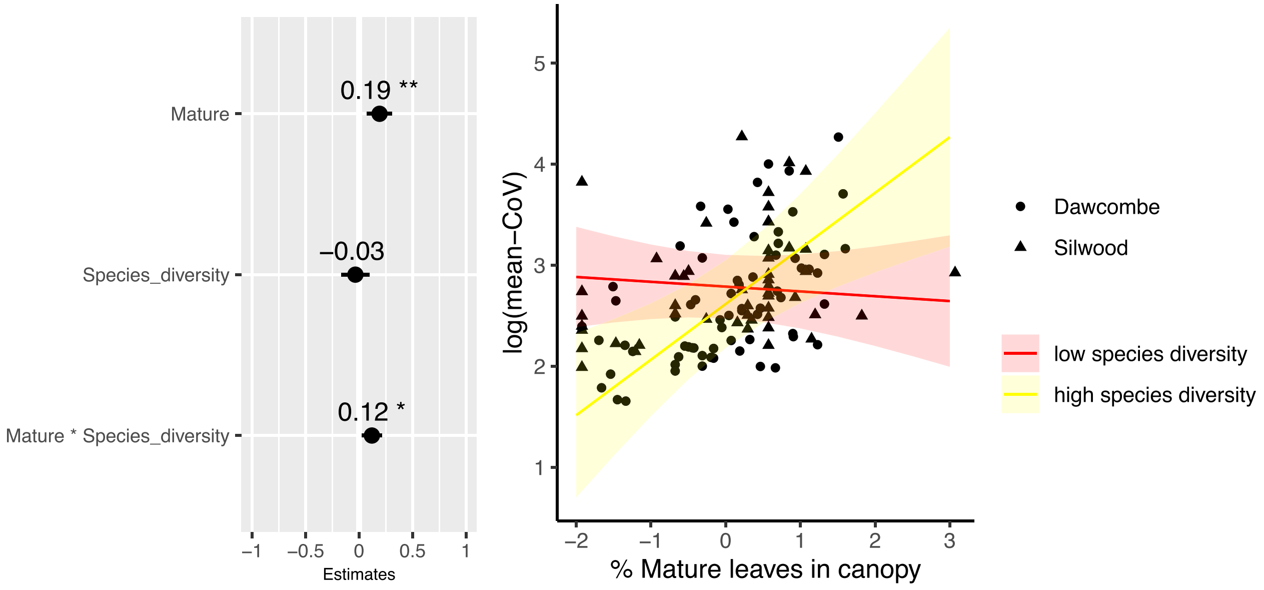

Fig. 10. Left: Forest plot showing the standardised effect sizes of the fixed terms in the mixed model; percent mature leaves (MAT(3)) species diversity and their interaction term.
Right: Significant interaction effect of MAT(3) and species diversity on mean spectral variance. Prediction lines with confidence intervals show values of species diversity at extremes of the data set ($D_t = 1$ and 13). MAT(3) is scaled with values ranging from $-2 = 0\%$ and $+3 = 100\%$).

of spectral variance as a surrogate for species diversity is dependent on the extent to which plant species are synchronous in terms of their display of mature leaves.

4.4. Issues of scale in estimations of species diversity

One of the major challenges in testing the SVH is that the strength of findings may rely on both the temporal *and* spatial scale of the observations. Here we investigated whether the species diversity of small grassland plots could be predicted using very high resolution (10 cm^2) simulated pixels. A similar study in grasslands showed significant correlations between spectral variance and species diversity at single points in time (Wang et al., 2018) and demonstrated that spectral variance calculated using the smallest pixels (1mm^2) had the strongest relationship to taxonomic-based metrics, with the relationship declining as pixel size increased and 10 cm^2 pixels being the largest size at which the relationship held. A possible explanation for this decline in the relationship with increased scale is that species diversity metrics per quadrat may not be well aligned to the spectral data. Consider the situation where one quadrat has many species but they are small and evenly distributed throughout the quadrat. This situation is typical of the quadrats at Dawcombe in the species rich calcareous grassland. The spectral diversity of this quadrat at 10 × 10 cm pixel resolution could be very low, as each of the pixels are very similar. Compare this to a quadrat with only two species that are distributed in clumps and spectrally dissimilar. In contrast the spectral diversity of this quadrat could be high. To adequately assess community complexity using reflectance data our plant diversity metrics need to be robust in light of this type of dilemma with consideration given to the appropriate pixel size scaling to the community at hand.

We tested the SVH using both the spectral regions approach and the hyperspectral approach. In this instance, the models fitted using the spectral variance of very narrow wavelengths did not perform better, or provide more insight, than using broad spectral bands (regions) in terms of the timing of sampling nor the taxonomic or phenological metrics. Wang et al., 2018 also showed that summary measures of variance taken across the spectrum were sufficient to predict species diversity. However, other previous studies have demonstrated that species discrimination is possible only by small differences in reflectance in narrow bands (Kokaly et al., 2003; Schmidt and Skidmore, 2003). These results suggest that high spectral resolution data may be less important for diversity studies than for detecting species classes.

4.5. Challenges and further study: other sources of spectral variance in grasslands

Diversity in temperate grasslands has been shown to be a product of structural lack of species dominance in the canopy and light ‘sharing’ (Borer et al., 2014; Pulungan et al., 2019). Diverse grasslands by consequence have sparser canopies, are lower in absolute biomass and are usually found in soils lower in nutrients (which determines the absence of nitrophiles, that tend to dominate the canopy) (Crawley et al., 2005; Silvertown et al., 2006). Grasslands that follow this definition may be detectable by virtue of their canopy structural parameters such as height and LAI (Stenzel et al., 2017). It is possible that the negative relationship between spectral variance and the diversity metrics at Silwood is linked to these variables. Self-shading or vertical complexity as a source of spectral variation in high biomass swards could be additional sources of variation at this small scale. At this site, high spectral variance was found in high fertilizer addition plots with single species (LUFAll at DoY 210) alongside a large seasonal growth in CoV (model slope 0.23–0.29 depending on spectral region, see supplementary material table B1). In this instance, we could say that high levels of intra-specific spectral variation are displayed as this change is not associated with changes in species composition.

The principal challenge in interpreting the results of this study is that we don’t know the relative importance of leaf and canopy traits in driving spectral variance over time. A future option would be to monitor biomass variation both between sampling points and within a sampling unit. It is obviously impossible to monitor changes in biomass within a quadrat using destructive sampling techniques. However, biomass models using non-destructive measures of LAI and NDVI, in partnership with radiative transfer modelling, have been shown to provide reasonably accurate time-series of fluctuations (Punalekar et al., 2018). Some traits could therefore be simulated from spectral data. Future studies into the relationship between spectral variance and diversity metrics should attempt to incorporate at least some other leaf and canopy traits.

Temporal variability in the relationship between floristic patterns and spectral response in grasslands have been demonstrated in other studies using multi-temporal hyperspectral sampling and the physical model, PROSAIL (Feilhauer et al., 2017). In this case, the driver of spectral variability was found to be local resource stressors (i.e., leaf dehydration) and had little to do with changes in the actual canopy composition. In other multi-temporal studies, seasonal burning of the sward was proposed to be responsible for the failure of spectral variance to predict species diversity in some years (Gholizadeh et al., 2020). Large scale disturbance events could be associated with a re-setting of phenological niche partitioning that drives phenological diversity causing the relationship between spectral variance and plant community diversity to break down.

The observation that the amount of data cleaning changed the strength of the relationship between spectral variance and the taxonomic and phenology metrics also deserves further investigation. We may expect that in the early part of the growing season bare soil may be present in certain sub-quadrats. By recording total vegetation cover per plot it would be possible to infer if reflectance measurements were being affected by the present of bare ground. Later in the season, some plants with erect growth forms could cast shadow on other plants that display a more recumbent habit. Alongside erroneous data, these are the kinds of spectra that require filtering from the dataset. Ensuring the correct level of data cleaning and the most appropriate methods remain significant challenges.

5. Conclusion

Results of this study suggest that spatial variability in reflectance fails to hold across space and time as a predictor of species diversity in grasslands. It appears that at a single point in time stochastic combinations of species and/or phenological traits of canopies can drive spectral diversity. This may explain the instability of previous studies that examine similar questions. We observe that for these grasslands the canopy stage MAT(3) is positively correlated with canopy spectral variance over the season and that if this canopy stage is accounted for there may be an opportunity to predict well species diversity using these data. The full reasons for these observations remain unclear and we highlight the need for simultaneous collection of some leaf and canopy traits in future similar studies to help determine the cause.

The fact that species and phenological properties of canopies were comparably estimated in the uni-temporal models suggests that spectral variance may be *at least as* suitable for looking at phenological properties as taxonomic ones. Establishing a link between spectral variance and phenological patterning of grassland communities would be an important addition to the study of plant phenology and conservation biology (Morellato et al., 2016) as well as furthering our understanding of the effects of climate change on species phenological partitioning.

Under current knowledge, application of the SVH to within-site monitoring of taxonomic diversity should be approached with caution. More studies are required that incorporate multiple sampling dates, at differing spatial scales, to determine if the relationship is stable enough to be useful in ecological evaluations. However, verifying the results of this study by expanding the geographical extent of detailed multi-temporal studies will remain a significant challenge due to the time-consuming nature of repeat botanical and spectral sampling at a gradient of spatial resolutions.

Authorship contribution statement

Rachael Thornley: conceptualisation, spectral and botanical data collection, data analysis and writing. France Gerard: conceptualisation, review and editing. Kevin White: conceptualisation, data collection, technical guidance on instruments, review and editing. Anne Verhoef: conceptualisation, data collection, review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rse.2022.112908.

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Article

The Feasibility of Leaf Reflectance-Based Taxonomic Inventories and Diversity Assessments of Species-Rich Grasslands: A Cross-Seasonal Evaluation Using Waveband Selection

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Abstract: Hyperspectral leaf-level reflectance data may enable the creation of taxonomic inventories and diversity assessments of grasslands, but little is known about the stability of species-specific spectral classes and discrimination models over the course of a growing season. Here, we present a cross-seasonal dataset of seventeen species that are common to a temperate, dry and nutrient-poor calcareous grassland, which spans thirteen sampling dates, a week apart, during the spring and summer months. By using a classification model that incorporated waveband selection (a sparse partial least squares discriminant analysis), most species could be classified, irrespective of the sampling date. However, between 42 and 95% of the available spectral information was required to obtain these results, depending on the date and model run. Feature selection was consistent across time for 70 out of 720 wavebands and reflectance around 1410 nm, representing water features, contributed the most to the discrimination. Model transferability was higher between neighbouring sampling dates and improved after the “green-up” period. Some species were consistently easy to classify, irrespective of time point, when using up to six latent variables, which represented about 99% of the total spectral variance, whereas other species required many latent variables, which represented very small spectral differences. We concluded that it did seem possible to create reliable taxonomic inventories for combinations of certain grassland species, irrespective of sampling date, and that the reason for this could lie in their distinctive morphological and/or biochemical leaf traits. Model transferability, however, was limited across dates and cross-seasonal sampling that captures leaf development would probably be necessary to create a predictive framework for the taxonomic monitoring of grasslands. In addition, most variance in the leaf reflectance within this system was driven by a subset of species and this finding implies challenges for the application of spectral variance in the estimation of biodiversity.

Keywords: semi-natural grasslands; biodiversity; hyperspectral; species classification; multi-temporal; partial least squares discriminate analysis; spectral variation hypothesis (SVH)

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1. Introduction

The conservation and management of species-rich semi-natural grasslands require temporally and spatially detailed information on community composition [1–3]. However, these data are very difficult and expensive to collect using traditional field-based surveys. It is now possible to create very high-resolution hyperspectral maps of grasslands due to advances in airborne remote sensing, with pixel sizes that are comparable to leaf sizes. Analyses of species-specific leaf and canopy spectra in herbaceous habitats have demonstrated that there is the potential for mapping taxonomic units [4–6], phylogenetic groups [7] and plant functional types [8,9].

However, large variances in intra-specific leaf reflectance have been reported [10,11], corroborating concerns about whether hyperspectral data can be used toreliably discriminate between taxonomic units [12]. There has also been mounting evidence that the biophysical drivers of spectral reflectance vary significantly over time as they are influenced by the phenological stage of the plant [13] and/or leaf age [14]. In addition, variation in leaf traits across environmental gradients, such as soil water availability [15], climate [16] and soil fertility [17] have been found. These results suggest that when using spectral data to predict species classes, both the temporal dimensions of the sampling campaign and the environmental context of the plant community need to be considered. As a consequence, the ability of spectral reflectance at specific wavelengths to predict species may be unstable and the relative positions of species within spectral space could vary over the course of a growing season. It seems likely that the temporal and spatial configurations of field campaigns will affect our ability to monitor species in varied and complex ways [18]. Certainly, the use of models that are built using data that capture evolving leaf states could improve our understanding of the spectral spaces that taxonomic classes occupy [19] and allow the determination of optimal temporal windows within leaf phenology for taxonomic assessments.

There is also an important link between the spectral separability of taxonomic units and the spectral variation hypothesis (SVH), which proposes a positive correlation between spectral variance and the number of taxonomic units or functional classes that are present within an area at the leaf or plant scale. Variations in leaf-level spectral reflectance have been successfully correlated with the number of species that are present [20] and functional diversity [21]. In forests ecosystems, where more research has been conducted, direct linkages have been found between spectral diversity and the diversity of the biochemical properties of leaves within taxonomically complex stands [22]. However, Feret and Asner [23] demonstrated that the ability of spectral variation to predict species diversity and taxonomic classes becomes saturated with a higher number of species. Recent studies on grasslands have also demonstrated the scale [20] and temporal dependence of the SVH [24,25]. Different grassland types have displayed positive and negative relationships with spectral variance [26], independent of space and time. Thus far, there has been a limited understanding of these results. It is probable that spectral variation is unevenly influenced by differing leaf and canopy properties, depending on the spatial scale of the data acquisition and the trait space that is occupied by the community in question.

Hyperspectral data have a particular structure and contain many highly correlated bands. These types of data have been described as having "the curse of dimensionality" and several approaches have been used to deal with this challenge within the context of species differentiation, namely decision trees [27], support vector machines [28,29], partial least squares discriminant analysis [30] and neural networks [31]. Most methods used for class determination involve projection to latent variables and/or data splitting. Some processing chains also include an assessment of the importance of the variables, which is followed by variable selection [32]. As the number of species classification studies has increased, it has become possible to determine whether any consistencies in waveband selection can be observed [33]. Although feature selection has been analysed in terms of spatial scale (leaf or canopy) and plant group (woody or herbaceous) [34], to date, to our knowledge, the temporal dependence of waveband selection has not been assessed.

In this study, we collected the leaf-level hyperspectral reflectance spectra of a complex community of herbaceous species, which is characteristic of UK calcareous grasslands, throughout a growing season. Our principal aims were to:

(1) Determine whether the species within the community could be separated using classification models and to what extent the classification of these species changed over time;

(2) Explore the temporal stability of band selection during classification and test the transferability of classification models across sampling dates;

(3) Test whether the species that were more easily classified displayed particular leaf traits or were more phylogenetically distant from other species within the community;
(4) Examine the importance of the biochemical traits of a leaf in classification over time.

2. Materials and Methods

2.1. Experimental System

A species-rich ancient grassland with a calcareous rendzina soil type, which is called "Wrotham Water" and is situated in the North Downs in Kent, southeast England (51°19′15″ N, 00°20′04″ E), was selected as the study site. Plants within this system are either specialists that have adapted to low nutrient and water regimes or more plastic species that undergo dwarfism. To characterise the site, we used the Ecological Flora of the British Isles database [35], which contains the ecological traits of species, to acquire Ellenberg's indicator values. These values can be interpreted as follows: species light demand from low to high (1–9); moisture demand from low to high (1–12); soil pH from very acid to very alkaline (1–9); and nitrogen demand from the least to excessive (1–9). These values provide evidence of the environmental niche within which these species are typically found. We also used the CRS (competitor/ruderal/stress-tolerator) functional strategy framework that was developed by Grime [36]. Thirteen out of the seventeen species in this study have been provided with autecological accounts [37]. We used these accounts to understand the extent to which the species were obligate stress tolerators or more plastic species that had adapted to this environment.

To situate our sampling dates within a temporal context in terms of precipitation and seasonal vegetation development, we used the Enhanced Vegetation Index (EVI), surface soil moisture values (both of which were derived from Copernicus Sentinel data) and regional precipitation data. All three time series were created for the period day of year 90 to 260. A site-based EVI time series was obtained from Sentinel-2 to describe the green-up trajectory. The EVI was derived of 60 pixels at a 10-m resolution over 10 cloudless dates. A time series of surface soil moisture derived from Sentinel-1 Synthetic Aperture Radar data at a 1-km pixel resolution was also created. The temporal resolution of the product was between 2 and 5 days and resulted in 99 measurements. Daily regional precipitation records were also sourced from the UK Met Office Hadley Centre observations database [38].

2.2. Leaf Spectra Acquisition and Pre-Processing

Seventeen species that are typical of the habitat were selected from the grassland (Figure 1A). Starting in the spring, on day of year 119 (29 April 2021), bi-directional leaf-level reflectance spectra were collected using a spectrometer that was fitted with a fibre optic cable and leaf clip over the visible, NIR and SWIR regions of the spectra (SVC HR2024i spectroradiometers, Spectra Vista Corporation, Ploughkeepsie, New York State, USA). Data were collected approximately every seven days over three months of the growing season until day of year 204 (23 July 2021). The intention was to capture the period of leaf thickening and maturation but avoid the period of the year in which leaves begin to senesce. In total, 13 dates were sampled, which represented a multi-temporal spectral signature for each species. On each sampling date, a single leaf from five separate plants that were situated along transects was cut for each of the 17 species. Leaves that were trampled, insect damaged or otherwise unhealthy were avoided, as were shaded plants. Within a few minutes of the leaves being collected, three leaf clip readings were taken for each sample and the average of these readings was used in the analysis. The spectra were examined after capture and filtered for erroneous measurements [39]. Reference readings were taken regularly throughout the sampling campaigns using a Spectralon white panel. In three instances, less than five acceptable mean spectra were available (*Inula conyza* $n = 2$ and *Fragaria vesca* $n = 4$ on DoY 174 and *Brachypodium sylvaticum* $n = 4$ on DoY 126). We included these data in the analysis but the results for these dates and species must be treated with caution. The sampling campaign resulted in 1100 averaged leaf spectra.

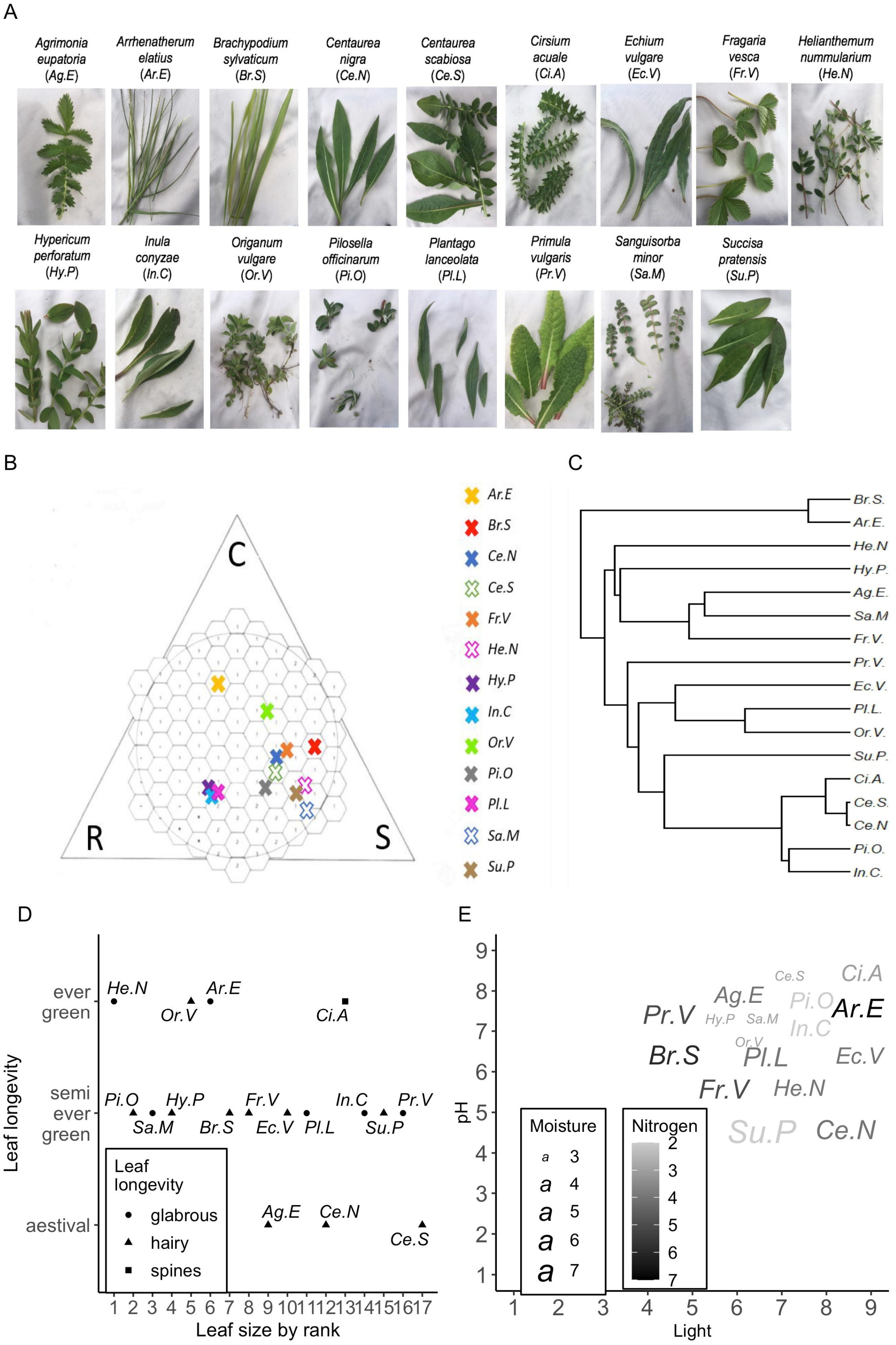


Figure 1. (**A**) The 17 grassland species that were involved in this study; (**B**) a plot of 13 of those species within the Grime strategy space, where data were available; (**C**) the phylogenetic relationship between species; (**D**) the morphological and phenological characteristics of the leaves; (**E**) Ellenberg's indicator values for light, moisture, pH and nitrogen.

The spectra were pre-processed through the removal of sensor overlap using SVC HR-1024i PC data acquisition software. They were then smoothed using a Savitzky–Golay filter. Different filter lengths were applied to the spectra and the optimal smoothing was obtained using a filter length of 55. The spectra were trimmed to 340–2500 nm and resampled to a 3-nm resolution (720 wavebands). The nominal bandwidth of the spectrometer was $\leq$1.5 nm in the region of 350–1000 nm, $\leq$3.8 nm in the region of 1000–1890 nm and $\leq$2.5 nm in the region of 1890–2500 nm. 3 nm was chosen so as to exploit the maximum spectral information without overly replicating information in neighbouring bands. All pre-processing was carried out using the HSDAR package in R [40]. Example spectra at each stage of pre-processing are provided in the Supplementary Materials, Figure S1.

2.3. Spectral Dissimilarity within and between Species

The spectral distances between pairs of mean spectra were measured using two different algorithms: the Spectral Angle Mapper [41] and the Euclidean distance. We wanted to ascertain whether the distance between pairs of intra-specific spectra was generally smaller than the distance between pairs of spectra from our target species and the other species (inter-specific distance) at certain times of the year. The two chosen distance metrics represent slightly different things: SAM measures the differences in angles for a pair of spectra and, therefore, minimises the effects of illumination and albedo; the Euclidean distance is calculated as the square root of the sum of the squared differences between two vectors. The distribution of the intra-specific distances was compared to the distribution of the inter-specific distances for each species at each time point (see Supplementary Materials S1 and S2 for the distributions). A two-sided Kolmogorov–Smirnov test [42] was performed on the two distributions and the statistic D was reported to ascertain whether the two distributions were likely to be made up of samples from the same population. Lower levels of D indicated that the distributions were the same and higher values indicated that the distributions were likely to be different. The p values for the test were also calculated.

2.4. Sparse PLS-DA for the Class Determination of Species

To establish how easily species could be separated from each other, we used a sparse partial least squares discriminant analysis (sPLS-DA), which is a supervised version of the classic partial least squares regression. In the sPLS-DA approach, a sparsity assumption is made that only a limited number of variables (wavebands within this context) arenecessary for the classification of samples [43]. Non-sparse PLS models tend towards the creation of independent latent variables (also known as components), which each contain very small amounts of information from multiple original variables. The sparse approach ensures that variables that makevery small contributions to the model are excluded from the analysis, which is in line with other so-called “lasso” approaches [44]. In the context of leaf-level hyperspectral reflectance, variability in optical leaf traits has a cross-spectral effect [45]; however, reflectance at neighbouring wavelength values is highly correlated, which makes much hyperspectral data redundant. The minimum waveband selection from the sparse approach had several advantages within this context. Firstly, it enabled a wavelength selection comparison across the sampling dates, which was vital for the aims of this study. Secondly, it has been demonstrated more generally that the ratio of samples to variables affects the performance of PLS-DA models [46]. Hence, by reducing the number of wavebands, we minimised the magnitude of this ratio and increased the likelihood of producing more reliable results. Thirdly, hyperspectral imaging devices that are capable of very high spatial resolution often require prior band selection. This is because of the time that is needed to capture many simultaneous bands. Therefore, results from the sparse approach are more useful for transferability to imaging systems.

A sPLS-DA was performed for each of the thirteen sampling dates in the dataset for each of the seventeen species classes. The classes were dummy coded and linear combinations of the Y classes and X variables (the spectral data matrix) were created to maximise the co-variance. Each model was tuned, whereby both the number of latent variables (components) and the number of wavebands that were required for classification were minimised. To tune the model, three criteria were required: (1) the optimal distance metric for the assignment of new samples into classes during the cross-validation process (a choice of maximum distance, Mahalanobis distance or centroids distance); (2) the number of components; and (3) the number of wavebands to be used in each component (more generally, the minimum number of X variables that were necessary to explain the variance in the Y classes). The optimal number of components was selected by observing the stabilisation of the error after the introduction of an increasing number of latent variables. The waveband selection was based on the stability and frequency of the wavebands that were selected during model permutations. The distance metric was selected by the optimisation of the model error that was achieved by the use of the three metrics. One of the main limitations of PLS models is that they are prone to overfitting [47]. Therefore, this model optimisation was achieved by M-fold cross-validation and an evaluation of the RMSE of the model. The number of folds was selected as the number of classes plus two (17 + 2 = 19) and 50 runs were performed within each model. When the specified number of folds was too large, the number of folds was reduced until cross-validation became possible. The whole process was repeated 10 times (over 10 model runs) for each sampling point. The sPLS-DA, model tuning and performance assessment were executed using the mixOmics package [48] in R [49]. Detailed instructions on the procedure for the above approach can be found in Lê Cao et al. 2011 [50].

2.5. Assessment of Waveband Selection and Model Stability

To assess the stability of the wavelength selection at each time point, the frequency with which each waveband was selected in the 10 model runs was determined. Wavebands that were consistently selected, both between runs and between times, could be said to have cross-seasonal importance for discrimination. Other wavebands that were consistently selected within a sampling point for all model runs but were not always selected for all sampling dates could be said to have temporally dependent importance.

To assess the extent to which models that were trained using data from a single time point were over-fitted, we used the model that was trained using one time point to predict species from the data that were collected on the other sampling dates. By examining the mean model error of the 10 model runs, we could determine whether the wavelength selections were temporally dependent. When models performed better on neighbouring data than on data that were further away in time, we could say that the relative position of the species within spectral space was evolving with leaf age and phenology.

2.6. Grounds for the "Ease" of Species Separation

We defined a "well-classified" species as a species for which a classification error rate of less than 0.1 (10%) was obtained. Each species was assigned a value at each time point, which was based on the number of latent variables that were required to achieve this classification accuracy (see Supplementary Materials Figure S4). We equated this value to the "ease" of the classification of a species within our framework. In some cases, it was not possible to classify species to this level of accuracy, so those classes were dummy coded with a value of 25 so that they could be included in the analysis. The mean and standard error of these values across the time points were also calculated.

To assess the possible causes of the "ease" of the classification of a species, we tested several hypotheses:

(1) Species that were taxonomically or phylogenetically more distinctive were easier to classify;
(2) Species with smaller, and therefore harder to measure, leaves were harder to classify (due to increased noise within the leaf clip dataset);
(3) The leaf longevity that is typical of this species affected the ease of species classification;
(4) The leaf surface defence mechanisms affected the ease of species classification;
(5) The amount of bi-directional leaf reflectance affected the ease of species classification;

(6) The spectral distance between pairs of species-specific spectra compared to interspecific spectral distances (as denoted by the Kolmogorov–Smirnov statistic *D*) was a good predictor of the ease of species classification.

To test Hypothesis 1, a phylogeny for the 17 species was generated using the phylomaker software in R [51]. From this phylogeny, a relative measure of phylogenetic distance was created for each species within the community. To test Hypothesis 2, the relative leaf sizes of the species were judged according to observations in the field and ranked from smallest (1) to largest (17). It has been shown that leaf surface properties can be contributing factors to reflectance [52]. To test Hypotheses 3 and 4, we used the Ecological Flora of the British Isles database [35] to access species traits on leaf longevity (whether leaves were evergreen, semi-evergreen or spring emerging (aestival)) and leaf surface properties that are related to defence (whether the leaves are glabrous, hairy or covered in spikes). Sims and Gamon [52] observed that reflectance at 445 nm is almost entirely driven by leaf surface properties. Here, reflectance at 445 nm was used as a proxy for leaf specular reflectance and these values were used to test Hypothesis 5. Hypothesis 6 was tested using the data that were mentioned in Section 2.3. For all hypotheses, a linear regression model was used to test the proposed relationship and when the dependent variable was categorical, Tukey post-hoc tests were used to determine the differences between the groups.

2.7. Use of the PRO-COSINE Radiative Transfer Model to Understand the Biochemical Basis of Shifting Waveband Importance

As the PROSPECT model [53] was developed for use with hemispherical reflectance data that were measured with an integrating sphere, it may not be appropriate for understanding wavelength selection in bi-directional reflectance data that were collected using a leaf clip. PRO-COSINE offers an approach for unifying the PROSPECT-4 model with data that were collected using a leaf clip to enable a mechanistic understanding of the results [54]. The principal additional factor that needed to be accounted for was the specular reflection of the leaves through the b_{spec} parameter. The b_{spec} ranges in value from −0.2 to 0.6 (unitless) and increases in value with increased specular reflectance, which influences reflectance in strong absorption regions (around 400 nm and at 1930 nm and 2500 nm). Studies so far have shown that specular reflectance can be explained to some extent by the species [55,56]. It has also been demonstrated that the impact of specular leaf properties on reflectance is relatively small compared to the variance within and between individuals of the same species [10]. Values ranging from 0 to 0.10 were used as the parameters for the b_{spec} input of the model. N was constrained to the range of 1–2, following the method of Jacquemoud and Baret [53], which are the values that are suitable for healthy leaves that are not in senescence. The additional model inputs of chlorophyll content (Cab), leaf mass area (LMA) and equivalent water thickness (EWT) were not parameterised.

We wanted to understand the biochemical relevance of the wavelength selections across time. Traditionally, leaf chemical assays have been used to determine variance partitioning in conjunction with radiative transfer models [23]. However, this approach is time and effort prohibitive and has only been attempted for woody species and never over time. Here, we used an alternative method: we performed a global sensitivity analysis (GSA) of PRO-COSINE using the Saltelli method and the ARTMO toolbox V1.14 in MATLAB [57]. The total sensitivity effects (the first-order effect plus interactions with other input variables) were calculated for each of the model input variables for each spectral band. We then used the waveband selection of each of the sPLS-DA models, which were trained using data from each time point, to extract from the results of the GSA, thus representing the probability of relative trait importance for each of the first six components per sampling point.

3. Results

3.1. Ecological Context of the Plant Community and Timing of Sampling Campaign

The species-specific CSR strategies revealed a community comprising of mainly stress tolerating specialists. A few species were more competitive (*Arrhenatherum elatius* and

Origanum vulgare) or more ruderal in their preferences (*Plantago lanceolata, Inula conyzae* and *Hypericum perforatum*). In terms of the four Ellenberg's indicators, the species were all light demanding and suited to either neutral or high pH soils. Their preferences for water and nitrogen were more variable (Figure 1).

The start of the sampling season (DoY 119) was preceded by very low rainfall in the region and low surface soil moisture (Figure 2A,B). Later in the season, the peaks and troughs in surface soil moisture were driven by precipitation events throughout the sampling period and there was evidence of the repeated wetting and drying of the soil. A likely consequence of the very dry conditions in the spring was the slowing of the green-up. The first five sampling dates (DoY 119, 126, 132, 140 and 147) appeared to be during the green-up period of the grassland prior to the period of peak biomass (Figure 2C). Unfortunately, due to frequent cloud cover during 2021, the Sentinel-2 time series was sparse; so, the end of the green-up period was speculative but appeared to occur around DoY 160. The remaining eight sampling events took place during peak biomass.

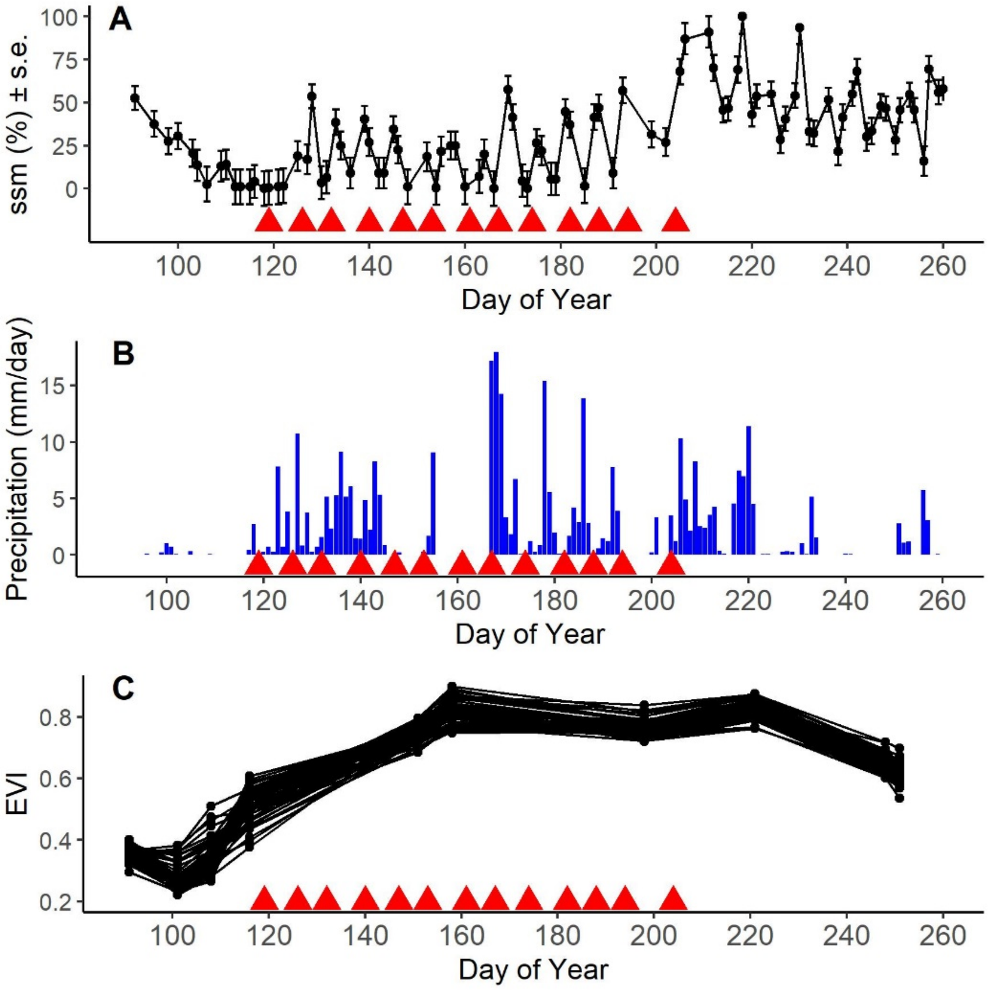


Figure 2. (**A**) Satellite-derived time series of surface soil moisture (Sentinel-1) at a 1-km resolution; (**B**) regional daily precipitation averages; (**C**) the site-based green-up trajectory using EVI (Sentinel-2) at a 10-m resolution. The 13 field sampling dates are shown as red triangles.

3.2. Spectral Distance over Time

The lowest cumulative Euclidean distance and SAM value between pairs of spectra across all species occurred on day of year 174 (13 July 2021) and the highest occurred on day of year 204 (2 June 2021). There was a moderate to strong correlation between the pairwise spectral distances, whether calculated using SAM or the Euclidean distance (Spearman's rank correlation = 0.7142857; p value = 0.008143).

The mean intra-specific distances for each species and time point were smaller than the mean inter-specific distances for both distance metrics (bar *Sanguisorba minor* at DoY 153, 194 and 204). This indicated that the leaf samples that shared the same species were generally more spectrally similar (see Supplementary Materials Figures S2 and S3 for the distributions and means of the distances). The Kolmogorov–Smirnov test statistic (D) was used to determine whether the distribution of the intra-specific distances was significantly different from that of the inter-specific distances for each species at each time point. The values of D and their associated p values are presented in Figure 3. The values of D for five of the species (*Primula vulgaris*, *Inula conyza*, *Fragaria vesca*, *Cirsium arvense* and *Agrimonia eupatoria*) were always significant, regardless of the sampling point or distance metric. The values of D that were calculated using SAM were more stable in two of the species (*Brachypodium sylvaticum* and *Cirsium arvense*) than those that were calculated using the Euclidean distance. However, overall, there appeared to be no advantage to using either metric in terms of species separability from the single sampling point perspective. In contrast, the value of D was equivalent or larger for SAM than the Euclidean distance across all sampling dates for all species except *Primula vulgaris* and *Inula conyza*. So, cross-seasonally, SAM may be a more useful metric to use for species discrimination problems.

3.3. Performance of PLS-DA over Time: Waveband and Model Stability

The sPLS-DA models at each time point performed well, with overall model errors ranging from 0.02 on DoY 174 (23 June) to 0.12 on DoY 182 (1 July) (Table 1). The number of independent components that were required to obtain these low errors was quite high, ranging from 15 components on DoY 140 (20 May) to 21 components on several of the other dates. The number of wavebands that were used to obtain this level of classification ranged from 300 to 683, with 42–95% of available bands being exploited. In other words, even when using the sparse approach, a large proportion of the spectra was required to classify the 17 species for some time points and model runs.

Table 1. A summary of the results of the cumulative spectral distances and sPLS-DA models for each sampling date.

Sampling Date	Date	Day of Year (DoY)	Cumulative Distance (Euclidean)	Cumulative Distance (Spectral Angle Mapper)	Model Error (Range of 10 Runs; 2 d.p.)	Number of Components (Range of 10 Runs)	Number of Unique Wavelengths (Range of 10 Runs)
1	29 April	119	12,398,267	4970	0.1–0.11	18–20	467–576
2	6 May	126	12,504,256	4874	0.09–0.1	20–21	444–541
3	12 May	132	11,961,457	4889	0.07–0.11	18–20	518–663
4	20 May	140	13,740,155	5087	0.07–0.11	15–21	438–630
5	27 May	147	13,645,126	5041	0.04–0.04	16–17	439–554
6	2 June	153	12,610,071	4940	0.08–0.11	20–21	436–555
7	10 June	161	11,830,265	4778	0.08–0.08	19–20	442–658
8	16 June	167	12,367,520	4824	0.04–0.08	18–20	493–621
9	23 June	174	11,581,843	4691	0.02–0.05	20–21	582–683
10	1 July	182	12,825,589	5014	0.08–0.12	16–19	403–545
11	7 July	188	12,582,159	5119	0.04–0.08	19–20	463–574
12	13 July	194	12,329,164	5104	0.05–0.08	19–21	583–641
13	23 July	204	13,851,285	5146	0.05–0.07	19–20	300–593

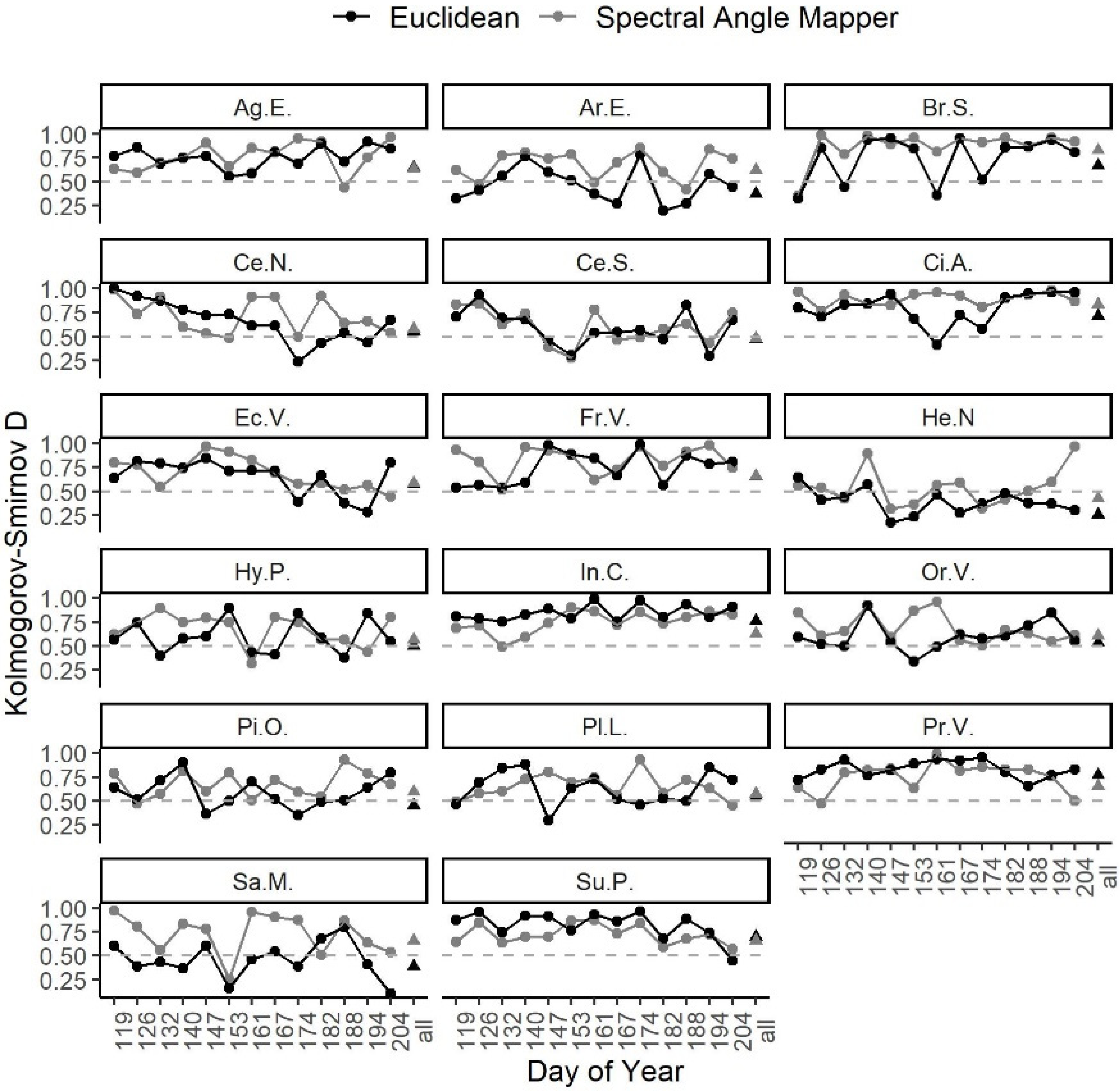


Figure 3. The value of *D* (the Kolmogorov–Smirnov statistic): a test of whether the distributions of the intra-specific and inter-specific distances were different from each other at each time point and across all sampling dates for each species class. The results are shown for both the Spectral Angle Mapper and the Euclidean distance. The values of *D* ranged from 1–0, with higher values representing distributions that were more distinct. A *p* value = 0.01 for the test is shown by a dashed line. Values above the line denote significantly different distributions.

Within each time point, the variable selection across the 10 model runs was consistent for some wavebands but not for others (Figure 4). There were also multiple different solutions for the model at any one time in terms of waveband selection.

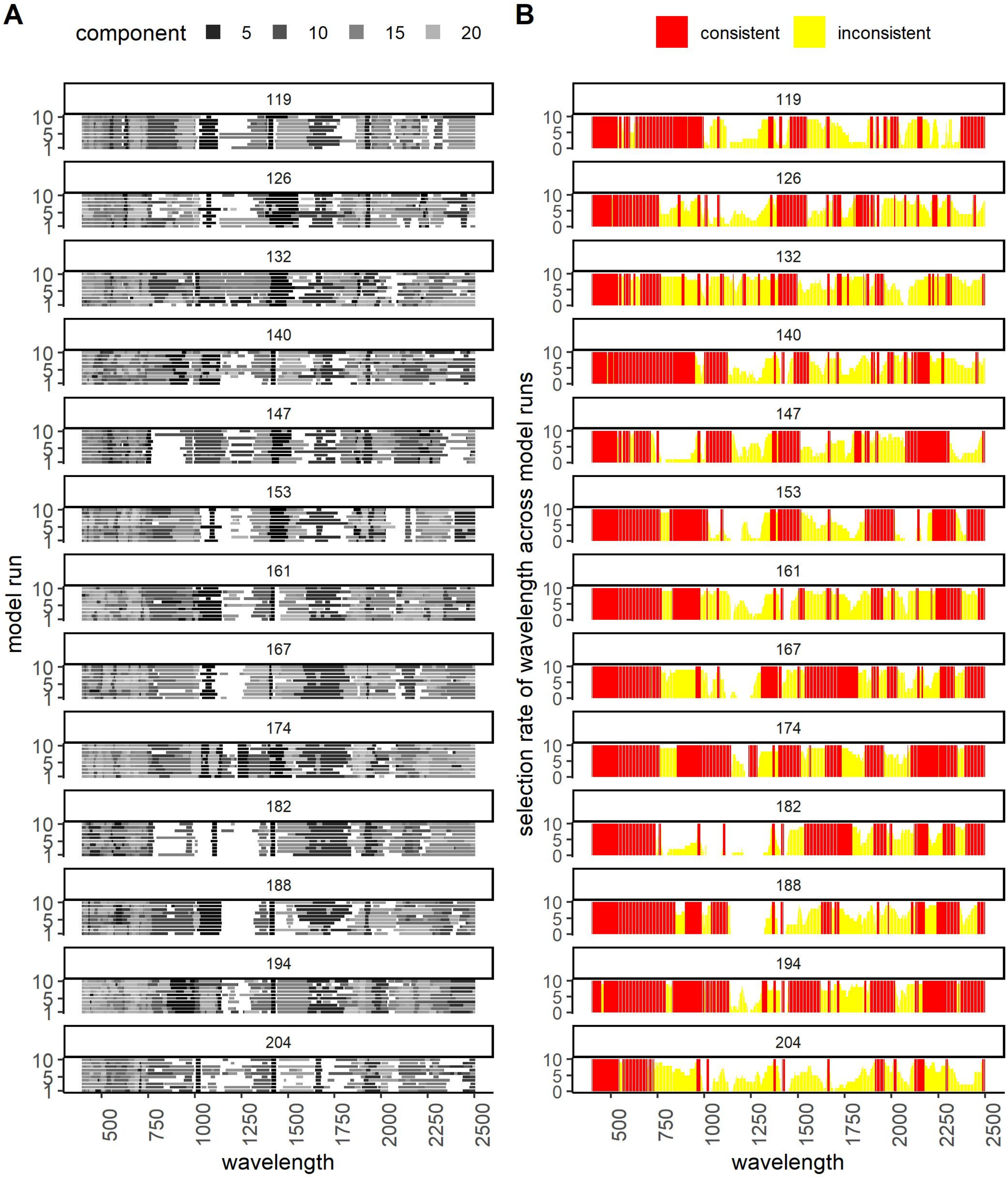


Figure 4. (**A**) The position within the spectra of components (latent variables) that were used for species–class determination for the 13 dates (day of year presented in the banner header). The darkest greys indicate components that captured more variations in the spectral data. (**B**) The selection rate of wavebands for model runs within each sampling date. Red bars represent wavelengths that were consistently selected in 10/10 runs; yellow bars are those that were only selected for some of the model runs.

The wavebands that were consistently selected in all 10 models runs within a time point are shown in Figure 5. The number of times that these same wavebands were selected out of the 13 sampling dates is also shown. In total, 70 wavebands were selected in all model runs and time points (i.e., in 13 × 10 = 130 models) and 65 of these were in the visible part of the spectrum. The overlaid example spectrum in Figure 5 reveals the consistent general importance of wavelengths in both the visible and red-edge regions. Other important features can be seen at 1000 nm, the minimum points of reflectance in the SWIR at 1400 nm, 1950 nm and 2500 nm, the peak of 1800 nm in the SWIR and the slopes on either side of the peak at 2200 nm. The conformity of selection in the rough locations of important spectral features can also be observed. In contrast, there was a large variability in the exact location of band selection between sampling dates. Figures 4 and 5 show the need to exploit much of the spectra to classify the taxonomic units.

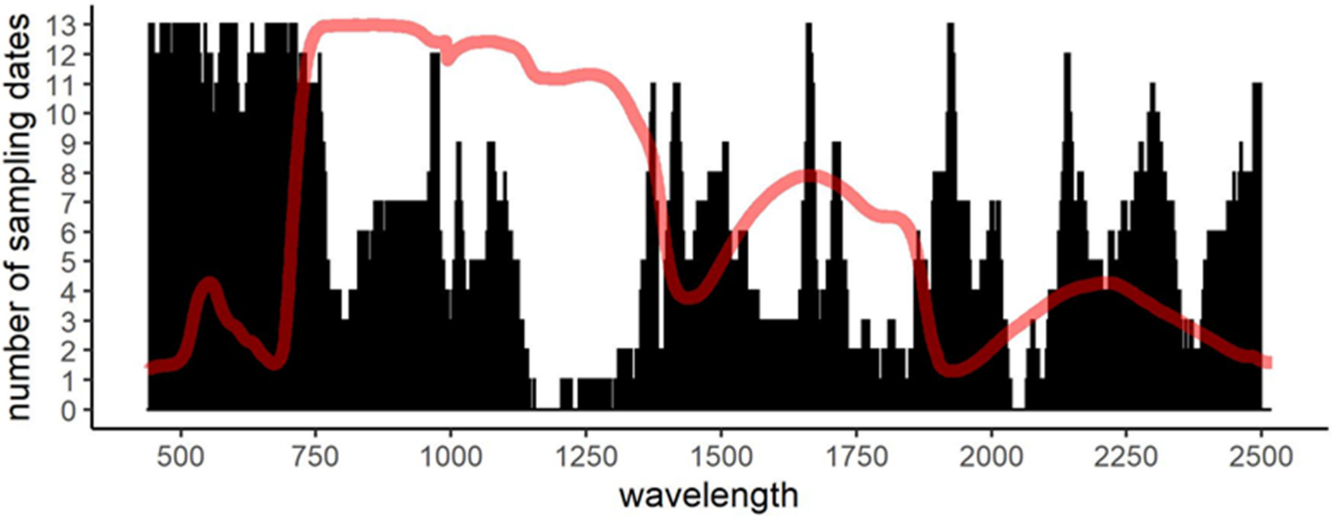
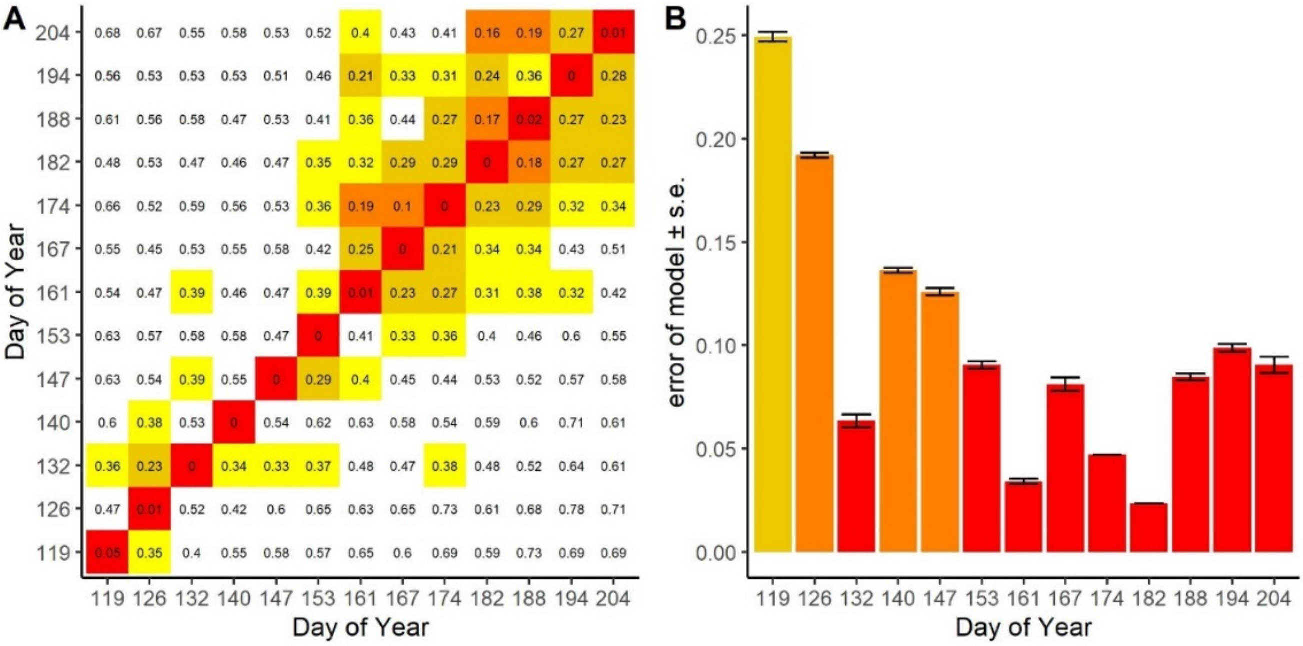


Figure 5. The number of times within each of the 13 sampling dates that wavebands were consistently selected in all model runs. A reference leaf spectrum (red line) is superimposed on the plot for contextualisation.

To assess model transferability across time, we tested the ability of the models that were trained using data from each sampling date to predict species using data from each of the other sampling dates (Figure 6A). We also used the model that was trained using all of the data to predict the species for each individual date (Figure 6B). In both cases, there was an observable increase in temporal dependence in the models after DoY 153. This stabilisation correlated with the end of “green-up” (see Figure 2C). When using the model that was trained using the cross-temporal data, the error rates were noticeably lower in the second half of the sampling campaign, which further indicated the stabilisation of waveband selection for species classification later in the growing season.



Day of Year	119	126	132	140	147	153	161	167	174	182	188	194	204
204	0.68	0.67	0.55	0.58	0.53	0.52	0.4	0.43	0.41	0.16	0.19	0.27	0.01
194	0.56	0.53	0.53	0.53	0.51	0.46	0.21	0.33	0.31	0.24	0.36	0	0.28
188	0.61	0.56	0.58	0.47	0.53	0.41	0.36	0.44	0.27	0.17	0.02	0.27	0.23
182	0.48	0.53	0.47	0.46	0.47	0.35	0.32	0.29	0.29	0	0.18	0.27	0.27
174	0.66	0.52	0.59	0.56	0.53	0.36	0.19	0.1	0	0.23	0.29	0.32	0.34
167	0.55	0.45	0.53	0.55	0.58	0.42	0.25	0	0.21	0.34	0.34	0.43	0.51
161	0.54	0.47	0.39	0.46	0.47	0.39	0.01	0.23	0.27	0.31	0.38	0.32	0.42
153	0.63	0.57	0.58	0.58	0.47	0	0.41	0.33	0.36	0.4	0.46	0.6	0.55
147	0.63	0.54	0.39	0.55	0	0.29	0.4	0.45	0.44	0.53	0.52	0.57	0.58
140	0.6	0.38	0.53	0	0.54	0.62	0.63	0.58	0.54	0.59	0.6	0.71	0.61
132	0.36	0.23	0	0.34	0.33	0.37	0.48	0.47	0.38	0.48	0.52	0.64	0.61
126	0.47	0.01	0.52	0.42	0.6	0.65	0.63	0.65	0.73	0.61	0.68	0.78	0.71
119	0.05	0.35	0.4	0.55	0.58	0.57	0.65	0.6	0.69	0.59	0.73	0.69	0.69

Figure 6. (**A**) Confusion matrix of the mean errors of the 10 model runs that were trained using data from single sampling dates and tested using data that were collected on the other sampling dates. The temporal dependence of the data was higher after DoY 153. (**B**) The error of the model that was trained using data from all sampling dates and tested using data from single sampling dates. The error bars show the standard error of the mean model error after 10 runs.

3.4. Ease of Species Separability

We noted that 99% of the spectral variance in the single date models was explained by only six independent components (see Figure 7A, the "scree plot" of the models). This was the case in all model runs and at all time points. The species classification error was examined for each species across time. With the recommended number of components in the model, all species achieved a satisfactory error rate (<0.1) for at least seven of the sampling dates. Three of the species (*Cirsium acaule*, *Fragaria vesca* and *Sanguisorba minor*) were well classified at all time points (Figure 7C). A very high error rate was found for *Inula conyza* on DoY 174. This was due to the low number of samples ($n = 2$) that was obtained for this species on this date. The class-based error rate of the 99% spectral variance and the six components was very stable across model runs within time points but overall, it was very temporally dependent (Figure 7B). Using this reduced number of components, almost all species (apart from *Centaurea scabiosa*) were well classified at certain times, but none of the species were consistently well classified, irrespective of the time point. The classification error was high for most species, which suggested that very small differences in spectral reflectance were responsible for most of the class differentiation of species within this community throughout the season.

We used the number of components that were required to achieve a classification error of less than 0.1 as an indicator of the "ease of classification" for each species. The mean value of the standard errors across all time points and models runs ($n = 130$) per species is presented in Figure 8A. *Fragraia vesca* and *Cirsium acaule* were clearly the easiest to classify according to our criteria. The other species all showed large standard errors around the mean, which implied that the ease of classification was more temporally dependent. The same evaluation was carried out for the sPLS-DA model that was trained using the cross-seasonal data (Figure 8B). These results provided a clearer picture, with six species requiring under 10 components to be well-classified, five species requiring between 10 and 20 components and the remaining six species being impossible to classify to the desired level of error. When the species were ranked from the easiest to hardest to classify, the means of the results from the single time points and the model that was trained using the cross-seasonal data were well correlated (Spearman's rank correlation = 0.8). In the further analyses, the classification "ease" metric from each of the single time point models was used.

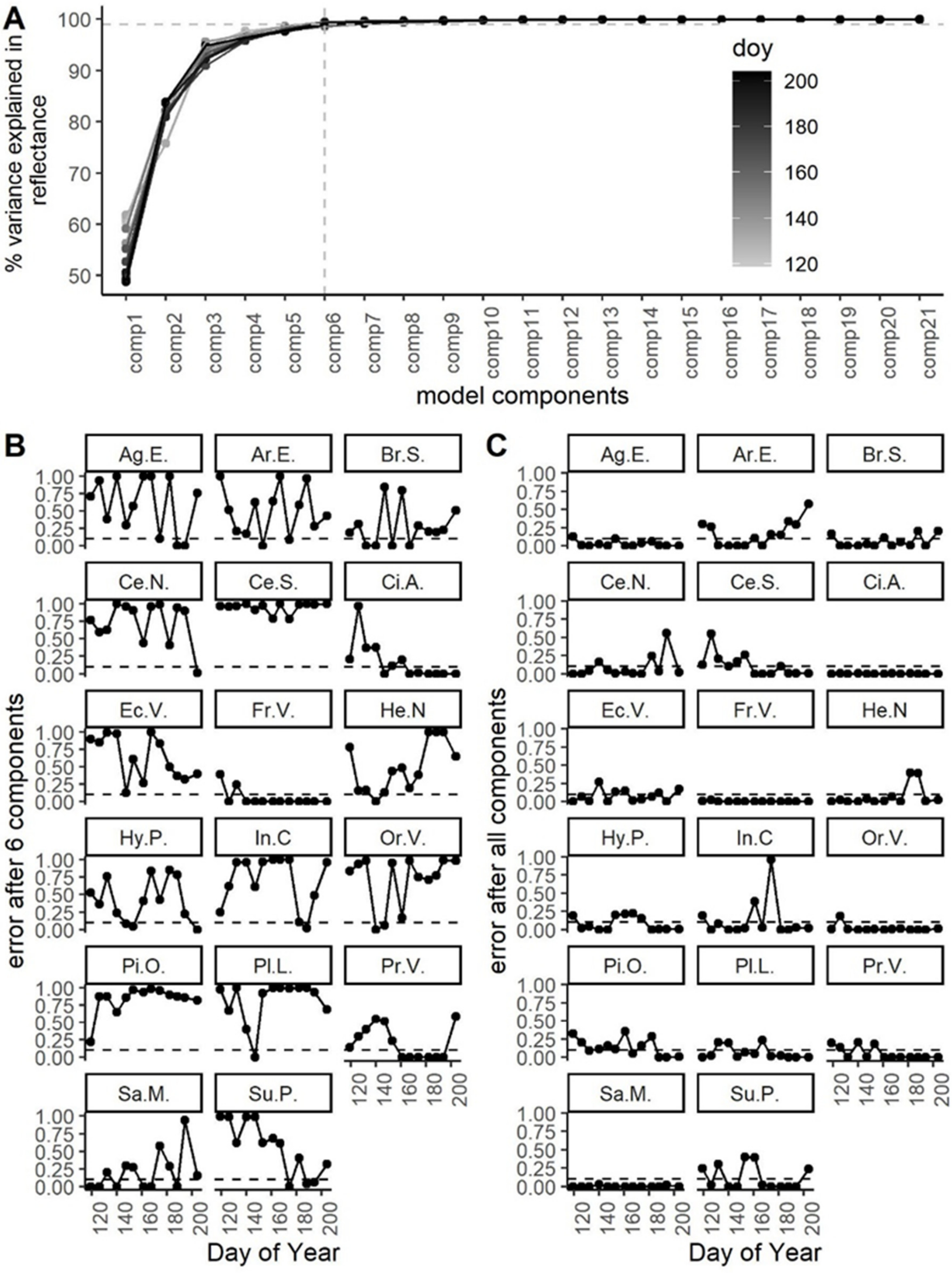


Figure 7. (**A**) The "scree plot" of the models at each time point, i.e., the variance in the X variable as explained by the model latent variables/components. The grey reference line represents the 99% variance in the X variable that was captured by six components, irrespective of sampling time; (**B**) species classification error over time with six components; (**C**) species classification error with the chosen number of components (i.e., the final model for each time point). Mean error is shown for each time point over the 10 model runs (the S.E. of the model runs was very small and is not shown).

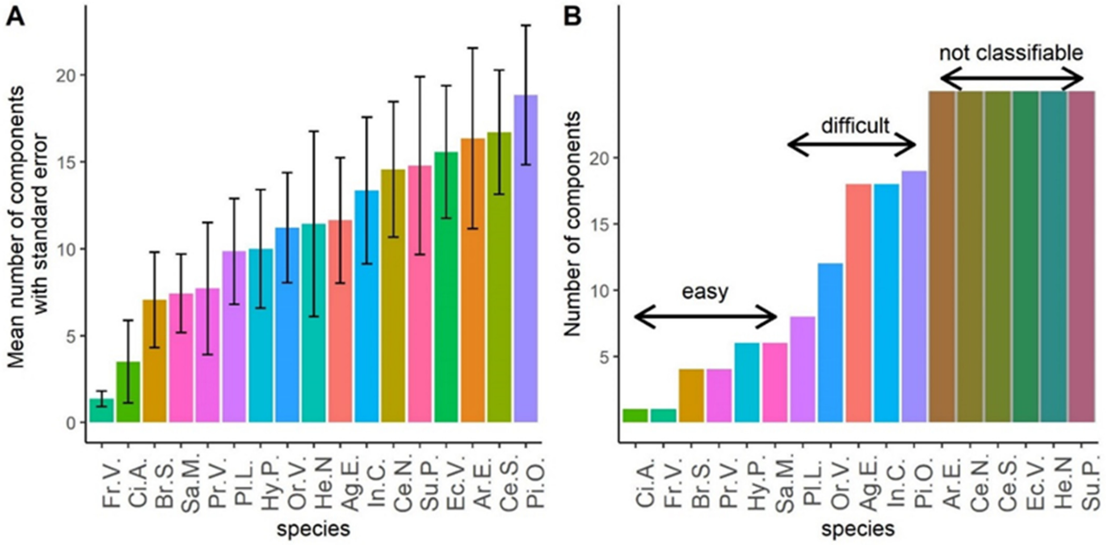


Figure 8. The "ease" of classification, defined as the number of components (latent variables) produced from the sPLS-DA models that were required to classify a species to a <10% error rate. Species are ranked from easiest to hardest to classify (left to right); (**A**) the mean and SE of the models across sampling dates; (**B**) the results from the model that was trained using the cross-seasonal dataset. Shaded bars show the species that were not classifiable to the required error rate.

3.5. Phylogenetic and Morphological Drivers of Species Separability

We used linear models to test whether the ease of classification was related to the phylogenetic and morphological aspects of the community (see Figure 1C–E). Firstly, we tested whether smaller phylogenetic distances between pairs of species made them more difficult to separate. We found that phylogenetic distance was very weakly correlated with the ease of classification within this community ($r^2 = 0.05$, slope = 0.03, $p = 0.00287$), with species that had smaller evolutionary distances being slightly harder to classify. We proposed that species with smaller leaves would be harder to measure using the leaf clip and that the measurements of these leaves would be subject to increased noise. However, we found no effects of leaf size on the ease of classification. We found bi-directional leaf reflectance at 445 nm to be very weakly correlated with the ease of separation; however, this finding was driven by two species (*Helianthenum nummularium* $r^2 = 0.36$, slope = -119, $p < 0.001$ and *Sanguisorba minor* $r^2 = 0.168$, slope = -98, $p < 0.001$). The more specular the reflectance, the easier these two species were to classify. We performed an ANOVA and a paired Tukey test to test whether leaf longevity or leaf surface mechanisms had any effects on classification ease. We found that aestival (spring emerging) leaves were harder to classify than evergreen and semi-evergreen leaves (ANOVA: F = 4.445, $p < 0.05$); the post hoc Tukey test showed that aestival leaves differed significantly from the other two groups at $p = 0.03$ and $p = 0.01$. We also found that species with spines were easier to classify than those with glabrous or hairy leaves (ANOVA: F = 8.552, $p < 0.0001$); post hoc Tukey test showed that spines differed significantly from the other two groups at $p < 0.0001$ and $p < 0.001$. However, this latter result should be treated with caution as only one species in the community had spines (*Cirsium acaule*).

By using the GSA of PRO-COSINE and the waveband selections from the sPLS-DA models, we were able to understand which leaf traits were likely to be the principal drivers of spectral variations within the plant community (Figure 9A). The consistent results for Component 1 (Figure 9B) highlighted the importance of the SWIR water feature in explaining the variances between species. Regardless of the sampling date, the wavebands that accounted for the largest amount of independent variation (between 49–61%) were situated in the region of Cw maximum, around 1410 nm. The second most variable region (21–35%) was represented by wavelength selections in the NIR at all time points, except for one (DoY 132). This is the region where the structural parameter of the leaf, N, is most strongly expressed. Component 3 represented variations in the visible region and hence, the region of chlorophyll expression. In the second half of the sampling season (DoY 161, 174, 182, 188 and 204), specular reflectance (b_{spec}) also became an important trait for certain sampling dates. Components 4–6 captured variations in Cm that only represented between 1 and 5% of the total spectral variance.

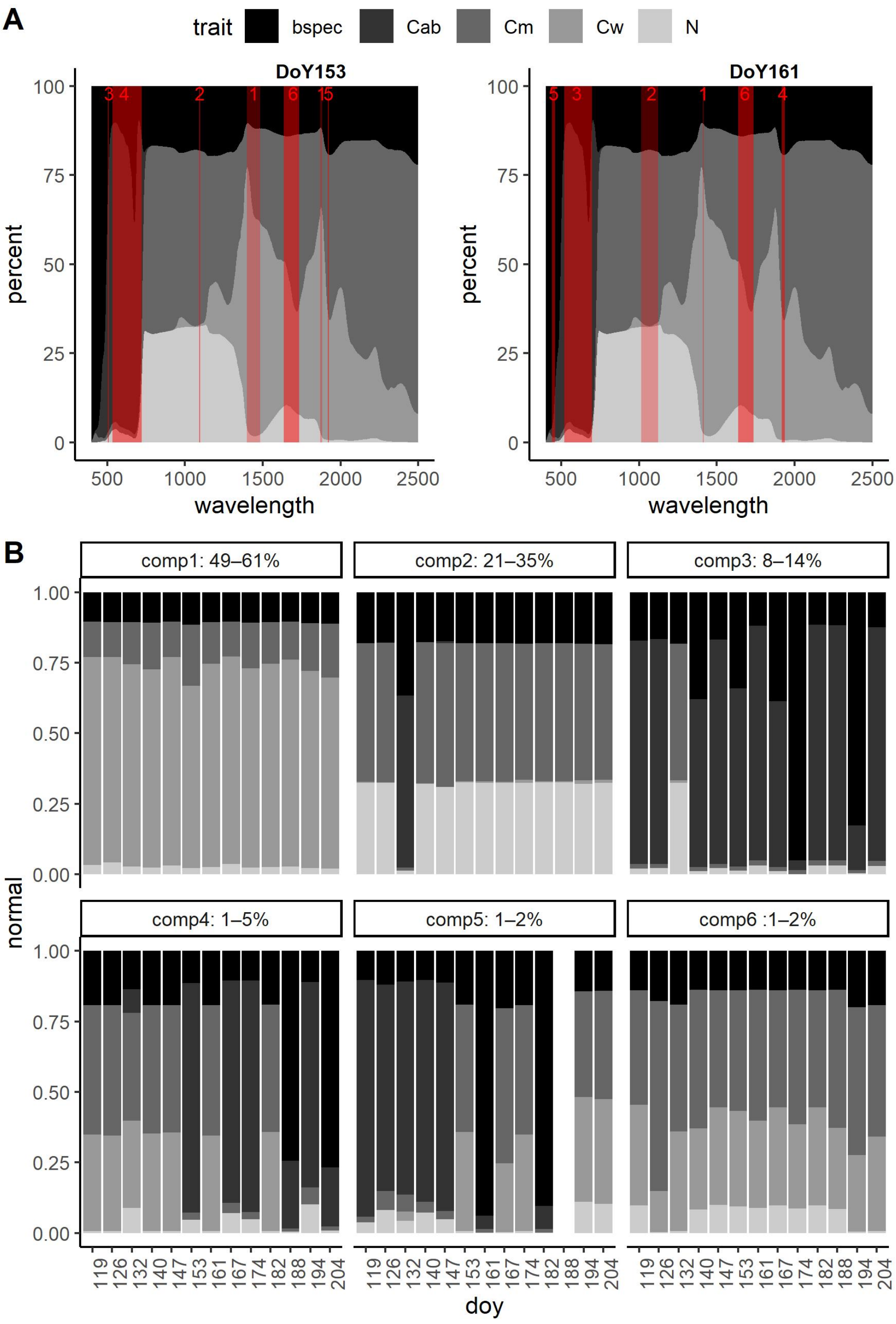


Figure 9. (**A**) The global sensitivity analysis of the radiative transfer model PRO-COSINE for leaf clip data with overlaid waveband selection for the first six components for two example time points (DoY 153 and 161); (**B**) the probability of the importance of traits for each of the six components over time using the wavelength selection from the best performing sPLS-DA models for each sampling date. The range of variance between model runs for each model component is presented in the panel header.

4. Discussion

Using the sparse PLS-DA approach with leaf clip data, species were classifiable to a very good error rate of 0.1% in most cases across the season. This result was obtained using a small sample size (*n* = 5) per species per time point and it was possible to collect these samples for 17 species within a single sampling day. However, the models that were produced were complex and required between 15 and 21 components, depending on the sampling date and model run. These results suggest that species classification within complex communities will not be an easy task. In addition, 99% of the spectral variance for any one of the sampling dates was explained by only six model components. All model runs and sampling dates were very consistent in this respect. After the application of six components, most species displayed an unsatisfactory error rate for any single sampling date. This meant that a large amount of the discriminatory ability of reflectance data for the species was based on extremely small differences between spectra, which probably resulted from the complex co-varying relationships between the leaf optical traits.

The results from across the growing season showed that some species were consistently easy to classify using a small number of components. Another group was possible to identify but required more components, most of which represented a very small amount of the total spectral variance. The final group of species was impossible to classify to the desired error rate of 10% across time, but at certain time points, the species were well classified. For sampling campaigns in which data are collected during a single day, there is the possibility that species discrimination results from sampling errors and instrumental noise when it is based on very small differences in leaf spectra. It has been shown through simulation studies that when there are more than twice as many classes as samples, the PLS-DA readily finds a hyperplane that is stochastic in nature [43]. We showed that for species that are easy to classify, the model that was produced from cross-seasonal sampling merely confirmed the results of the models that were produced from single time points; however, for species that are more difficult to classify, it could provide confidence when discriminating between noise and biological signals.. We may be able to understand the reasons for the variations in classification error over time in some cases. For example, we saw that for two of the species examined here (*Helianthenum nummularium* and *Sanguisorba minor*), variations in specular reflectance over the course of a growing season strongly affected the ease of classification.

The result that 99% of the spectral variance classified only six species to less than a 10% error rate across time suggested that the ability of the SVH to hold at the leaf-level in single date sampling campaigns depended on the extent to which the community was composed of species that were “easy” to detect. SVH, as an unsupervised form of biodiversity assessment, assumes that cross-spectral variance in reflectance can account for the diversity of taxonomic units. However, from the results that are presented here, we could not infer that spectral variance was necessarily correlated with species numbers or their abundances.

The global variance decomposition that resulted from the radiative transfer modelling, alongside the waveband selections that were required for each model run, revealed that leaf EWT was the most important and consistent driver of spectral variance that was related to species classification, followed by N, Cab, b_{spec} and LMA (although the relative importance of these traits was more temporally dependent). The importance of the wavebands that related to EWT did not vary with sampling date nor seasonal soil moisture content, as simulated by Sentinel-1. Grime’s CSR strategy and Ellenberg’s indicator values for the species that were examined here revealed a plant community that was dominated by stress tolerators and adapted to high pH soils. However, the moisture and nitrogen demand of these species was more variable. Similar sampling campaigns that involve the collection of leaf-level water content alongside leaf-level reflectance may help us to better understand why this feature is so important and whether this is limited to this type of stress tolerator system. The transferability of the models during the sampling period (day of year 161 to 204) could also coincide with the trait stabilisation of the leaves and, in turn, the stabilisation of the spectral representation of traits (waveband selection). Yang et al. [13] found that in tree leaves, LMA and chlorophyll a/b content increase with green-up and then remain steady until leaf fall.

The detection of leaf traits using reflectance data is optimal when using a leaf clip and integrating sphere [58], which provides both reflectance and transmittance. In contrast, bi-directional reflectance data that are obtained using a leaf clip result in the over-estimation of cross-spectral reflectance due to surface reflectance of the leaves. The application of the results of this study (and others that take a similar approach) in close range imaging spectroscopy requires a consideration of additional sources of variation that relate to anisotropy (light incident angle and illumination zenith angle). These variables can also be modelled using the COSINE radiative transfer model [54] but would be additional sources of uncertainty in species determination. Reflectance variance in grasslands at the very high-resolution canopy scale has already been attributed to non-taxonomic properties, such as the vertical complexity of the sward [59], the presence of mature leaves [25] and pixels containing soil [60]. In this work, we avoided sampling plants that were growing in shaded environments, but there is also evidence that chlorophyll levels vary between leaves that are in the sun and those that are in the shade [61]. These additional sources of variation are likely to further increase the difficulty of species discrimination using close range imaging spectroscopy.

Feature selection and classification model specifications over time could also be affected by methodological choices in the analysis. Here, we applied the sPLS-DA approach to data that were pre-processed using a Savitzky–Golay filter. When utilising close range imaging spectroscopy, spectra are likely to contain more noise than when using a leaf clip. Therefore, the type and optimal amount of spectral smoothing need to be examined in more detail and within differing instrumental contexts. Here, spectra were resampled to a 3-nm bandwidth; however, when optimising classification, the bandwidth choice within differing spectral regions could vary. Finally, sPLS-DA is only one modelling approach for classification and feature selection. In order to develop more robust species discrimination models over time, it is likely that more advanced methods would also need to be tested and compared [62,63].

5. Conclusions

To date, species discrimination tasks using hyperspectral data have generally been focused on woody species. Despite their conservation status and importance, herbaceous species are less studied and when they are, observations are mostly confined to the dominant species rather than attempting to capture the full botanical composition of the sward. Plant trait studies have shown that the spectral determination of the leaf properties of herbaceous species may be more difficult to obtain than that of the leaf properties of woody species [64]; therefore, we should practice caution when applying results from studies that are performed in forests to grasslands and we should instead conduct similar workon grassland communities.

In this study, we found that some species within a community framework were easier to discriminate across the season than others. This pointed to a relative distinction in their leaf reflectance properties. Other species that were more difficult to discriminate required complex waveband combinations, which fluctuated across time. Cross-seasonal sampling, even with small sample sizes, could help to verify which species are driving measures of spectral diversity. Studies that explore species-specific chemical and structural leaf properties and relate these to leaf spectral signatures [65] are needed to help us to explain with more certainty why some species are easier to distinguish than others and to create a predictive framework for species monitoring and diversity assessment using leaf reflectance. We recommend further studies that explore functional trait frameworks when making predictions of species classes and exploit GSMs and RTMs, alongside biochemical assays, to estimate the importance of traits across different scales and instruments.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/rs14102310/s1, Figure S1: Spectra at each pre-processing stage prior to inclusion in the sPLS-DA models, Figure S2: Differences in distributions using Euclidean Distance, Figure S3: Differences in distributions using SAM, Figure S4: Ease of classification based on the number of components.

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Data Availability Statement: The data presented in this study are openly available through the NERC's Centre for Environmental Data Analysis at http://dx.doi.org/10.5285/8a798466b9f74b3da500004a94ee5fee (accessed on 18 March 2022).

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