Ecology, physiology, and proximal sensing of upland plants in response to environmental change

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DEDICATION

To my parents, who laid the foundation for my journey in life, education, and research. Your endless support, sacrifice, and love have not only made me the person I am today but also made this journey possible. Your life lessons were my first education, and they continue to guide me.

To my beloved wife (Tahany), who has been my rock and safe harbour throughout this challenging journey. Your understanding, patience, and unwavering belief in me have made it possible to persevere during the toughest times.

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This achievement is not mine alone. It belongs to all of us. This thesis is not just a testament to my work, but it is also a symbol of your love, sacrifices, and unwavering faith in me.

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Thesis summary

Upland ecosystems in the UK, including grasslands and heather moorlands, make up approximately one-third of the UK's land area. In addition, the UK uplands are of substantial national and international importance due to their biodiversity, with unique plant assemblages adapted to the specific climate and soil conditions. They also serve important ecosystem services, such as carbon sequestration, clean water provision and fodder production. At present, plants in upland ecosystems, including grasslands and heather moorlands, are experiencing several significant environmental challenges, including increased levels of atmospheric carbon dioxide (CO_2), nitrogen (N) pollution, and mismanagement caused by human activity. These could potentially hinder the ability of upland ecosystems to adapt to upcoming environmental changes or maintain ecosystem services. In particular, the effects of elevated CO_2 (eCO_2) and N pollution on phosphorus-limited grasslands are poorly understood. It is also not clear how *Calluna vulgaris* (the species that dominates heather moorland) varies ecologically, physiologically, and biochemically across its different life phases.

This thesis uses an empirical methodology to investigate the consequences of eCO_2 and N pollution, and their combination for P-limited grassland. It accomplishes this using a CO_2 fumigation experiment (miniFACE) on P-limited grassland taken from a long-term nutrient manipulation experiment. It investigates the impacts of eCO_2 and nutrient loading (singly and in combination) on plant physiology, leaf biochemistry, and species biodiversity. In addition, this thesis uses field methodology to investigate the ecological, physiological, and biochemical variation through *Calluna vulgaris* (*C. vulgaris*) life stages in heather moorland. Also, it explores the ability of remote sensing applications to detect the changes in P-limited grassland plants and *C. vulgaris* life stages.

It was found that in P-limited grassland, eCO_2 affected leaf gas exchange and stimulated photosynthesis; there was also evidence of acclimation, and N addition alleviated the magnitude of acclimation. eCO_2 also changed leaf biochemistry, and increased biodiversity and species richness, allowing more species to coexist, but N addition reduced biodiversity. eCO_2 and N created different plant communities. Leaf-level hyperspectral reflectance differed with eCO_2 combined with N. In addition, *C. vulgaris* varied considerably ecologically, physiologically, and biochemically across its life phases, with variations in leaf and canopy levels of hyperspectral reflectance. In conclusion, this thesis contributes to the need to comprehend the responses of P-limited ecosystems to a future of increased CO_2 and N availability, and the understanding of *C. vulgaris* variations across life stages.

Declaration

I, Abdulssalam Hasan Khafsha, confirm that the Thesis is my own work. I am aware of the University's Guidance on the Use of Unfair Means (www.sheffield.ac.uk/ssid/unfair-means). This work has not been previously presented for an award at this, or any other, university.

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Chapter 1

General introduction

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1 General introduction

Upland ecosystems in the UK encompass a variety of habitats, including grasslands, heather moorlands, and bogs, covering about a third of the UK land surface (Reed et al. 2009). The UK uplands hold significant national and international value for their biodiversity with unique plant assemblages adapted to the specific climatic and soil conditions, in addition to being a vital source of essential ecosystem services, such as carbon sequestration, water filtration and fodder production (Bonn et al. 2011; Holden et al. 2007; Thompson et al. 1995). Upland ecosystems are large carbon sinks, sequestering huge amounts of carbon in their soil and vegetation (Reed et al. 2009), and consequently upland soils are the largest carbon store in England (Baggaley et al. 2021). Upland soils play an important role in mitigating anthropogenic emissions and therefore climate change, by lowering the amount of carbon dioxide in the atmosphere. However, once these habitats are degraded, they can become sources of greenhouse gases (Albertson et al. 2010). Currently and in the future, upland ecosystems are facing a variety of environmental challenges including increased atmospheric carbon dioxide (CO_2), nitrogen (N) pollution, and mis(management) (Allen et al. 2016; Wang et al. 2019; You et al. 2017), which could potentially impact their ability to adapt and respond to future environmental change or continue to provide ecosystem services.

The current geological era has been renamed the "Anthropocene" to reflect the considerable human influence on the environment (Waters et al. 2016). As a result of the combined effects of both the agricultural and industrial revolutions, atmospheric CO_2 concentrations are today higher than they have been in the previous 3 million years (Masson-Delmotte et al. 2021; Willeit et al. 2019), and for the first time in history, human sources of N have exceeded all-natural sources together such as biological nitrogen fixation, atmospheric deposition, and decomposition & mineralization (David et al. 2013). Such dramatic changes in atmospheric CO_2 and N deposition disrupt the geochemical cycle of C, N, and P and expose the Earth including upland ecosystems in the UK- to climatic and environmental conditions never previously experienced (Steffen et al. 2015). In addition, heather moorlands in the UK, face several challenges due to mismanagement practices (Allen et al. 2016).

Terrestrial ecosystems play an important role in mitigating the negative anthropogenic impacts on the environment. For instance, terrestrial ecosystems sequester up to a third of the world's total anthropogenic CO_2 emissions (IPCC et al. 2013), and some grassland ecosystems can absorb as much as 76% of the pollutant N that is deposited on them (Phoenix, Leake, et al. 2004). Grasslands account for around a fifth of the global terrestrial net primary production (NPP) (Chapin 2011) and are the most widespread terrestrial ecosystem on Earth, present on every continent except Antarctica (Ali et al. 2016). In temperate regions, grasslands are also the most widespread phosphorus (P) limited environment (Watson et al, 2011). However, it is uncertain how P-limited ecosystems, will respond to future increases in CO_2 and N availability, including how effective they will be in mitigating CO_2 increases or their sensitivity to N pollution. Broadly, we know very little about ecosystem responses when productivity is strongly limited by P limitation to the single and combined increases in atmospheric CO_2 and N deposition.

Moorlands are extraordinary ecosystems that host significant plant communities both in the UK and global contexts (JNCC and Defra, 2010). The UK Biodiversity Action Plan and Annex I of the EU Habitats Directive both provide protection for these ecosystems because of their importance to wildlife. The UK is home to three-quarters of the world's remaining moorlands, with moorland encompasses around 25% of the UK's upland areas, including heather moorland, which is dominated by a single species *C. vulgaris*, which has 4 distinct life phases (Allen et al. 2016; Sands and Gimingham 1975). The moorlands of the UK are also a substantial carbon sink, storing in the region of 3000 Mt (Smith et al. 2007). The capacity of moorlands to store carbon can significantly fluctuate depending on management practices such as grazing, drainage, and prescribed burning (Allen et al, 2013; Holden et al. 2007). Understanding *C. vulgaris* including through its four distinct life stages, is crucial for management, protecting and improving the condition of moorlands nationally and globally, and preserving the ecosystem services they provide.

This thesis aims to expand our understanding of how UK upland ecosystems are responding to different types of environmental change, using a combined ecological, physiological and remote sensing approach. In particular, how P-limited grasslands may respond to a future increase in CO_2 (eCO_2) and nutrient pollution from N deposition, expand our understanding of the variations throughout *C. vulgaris* life stages, and determine the ability to detect these changes in P-limited grassland plants and *C. vulgaris* life stages using remote sensing approach.

1.1 Elevated CO_2 (eCO_2)

Elevated CO_2 (eCO_2) refers to an increase in the concentration of CO_2 in the Earth's atmosphere above its natural levels, which is primarily caused by human activities. Atmospheric CO_2 concentrations have now exceeded 420 ppm (Friedlingstein et al. 2022), and they are expected to more than double from their pre-industrial levels by 2100 (Masson-Delmotte et al. 2021). The effects of these shifts in atmospheric CO_2 concentration can cause a variety of complex and multi-directional responses in plants, including changes in photosynthesis, growth, and leaf stoichiometry (Ainsworth and Rogers 2007a; Du et al. 2019; Leakey et al. 2009; Teng et al.

2006), as well as changes in biodiversity and ecosystem function (Grünzweig and Körner 2001; Kleynhans et al. 2016; Taylor 2021; Tilman et al. 2014). Yet, we know very little of the impacts of eCO_2 in ecosystems where productivity is limited by P.

1.1.1 Effects on photosynthesis

eCO_2 can stimulate the photosynthesis rate in many plant species, particularly those that use the C3 photosynthesis pathway. Photosynthetic efficiency can increase (Ainsworth and Long 2005, 2021), allowing plants to produce more organic matter for a given amount of light energy. Studies have shown that eCO_2 can increase the rate of photosynthesis by 30-50% or more (Ainsworth and Long 2005, 2021). This can lead to increased growth and productivity, which can have benefits for ecosystem services such as carbon sequestration. Additionally, eCO_2 can increase water use efficiency (WUE) (Li et al. 2017; Pazzagli et al. 2016; da Silva et al. 2017; Yan et al. 2017), allowing plants to grow and thrive in drier conditions. This occurs because eCO_2 has been widely observed to decrease stomatal conductance (g_s) (Ainsworth and Long 2005, 2021) as well as modifying the stomata morphology (Haworth et al. 2016). Consequently, plants can maintain a greater leaf water content (Tausz-Posch et al. 2015; Wullschleger et al. 2002). In addition, as plant transpiration decreases, soil water availability can increase, and in water-limited or semi-arid grasslands, this can promote productivity (Dijkstra et al. 2008; Dijkstra et al. 2010).

Furthermore, plants rely on the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) to fix CO_2 from the atmosphere and convert it into organic compounds. However, Rubisco can also catalyse the reaction with oxygen, a process known as photorespiration, which can reduce the efficiency of photosynthesis. Therefore, under eCO_2 conditions, plants can more easily obtain the carbon they need for photosynthesis, which can reduce the amount of photorespiration and increase the efficiency of Rubisco (Ainsworth and Long 2005; Ainsworth and Rogers 2007a).

Some plant species that are growing under eCO_2 conditions can photosynthetically acclimate to eCO_2 (Pastore et al. 2019), which means the photosynthesis rate decreases in plants grown in eCO_2 compared to plants grown in ambient CO_2 (aCO_2) when both are measured at the eCO_2 concentration. Acclimation can occur in as little as a few days or weeks (Grulke et al. 1993; Sage 1994; Thomas and Strain 1991; Tjoelker et al. 1998) or could take as long as years (Griffin et al. 2000; Warren et al. 2015). Acclimation to eCO_2 is typically seen in low nitrogen (N) conditions (Ainsworth and Rogers 2007a) because photosynthetic rates are highly associated with leaf N status (Reich et al. 1997). C supply could exceed demands under eCO_2 when N is limiting (Ainsworth and Rogers 2007; Drake, Gonzàlez-Meler et al. 1997) because low N supply could decrease C sink strength, defined as the plant's capacity to utilize photosynthate. Photosynthesis acclimation occurs when leaves accumulate nonstructural carbohydrates in response to sink limitation (Stitt and Krapp 1999). Carbohydrate accumulation, in particular, acts as an indicator for the downregulation of Rubisco synthesis and/or activity, resulting in a reduction in maximum carboxylation capacity (V_{cmax}) (Drake et al. 1997; Rogers and Humphries 2000; Stitt and Krapp 1999; Urban 2003). Reduced chlorophyll content and foliar N concentration are two further physiological changes correlated with acclimation (Stitt and Krapp 1999; Urban 2003).

1.1.2 Effects on plant communities

eCO_2 reduces stomata conductance. As plant transpiration reduces, the availability of soil water increases, which may enhance productivity in water-stressed or semi-arid grasslands (Dijkstra et al. 2008; Dijkstra et al. 2010). Increased microbial mineralization of N may occur as microbial activity relies on soil moisture (Schimel 2018). In N-limited grasslands, this may then promote the growth of plants (Dijkstra et al. 2008), At the community scale, such responses can encourage the growth of certain functional types, such as graminoids, and result in canopy shading of other plants (Polley et al. 2012), or they could extend the growing season for dominant species, all of which may potentially cause a decline in biodiversity (Zavaleta et al. 2003).

The impact of eCO_2 on plant growth, as well as the resulting changes in soil moisture and nutrient cycling, can vary greatly depending on the species of plant (Dijkstra et al. 2008; Dijkstra et al. 2010; Hanley et al. 2004; Stöcklin et al. 1998). For example, in an eCO_2 experiment on calcareous grassland, some species, like *Carex flacca* (a sedge), increased above-ground biomass by 120% with eCO_2, while others, like legumes, increased by a modest 4%, and some grasses, like *Bromus erecta*, declined (Stöcklin et al. 1998). This is because eCO_2 accelerated the transition from xerophytic to mesophytic grasses and legumes.

While numerous studies have been conducted on semi-arid or N-limited grasslands, few investigate the effects of eCO_2 (and nutrient enrichment) on P-limited grasslands. Plant communities may be less likely to be affected by eCO_2 compared to N-limited systems since the impacts of eCO_2 are expected to be stronger for water and N availability than for P (Dijkstra et al. 2008; Dijkstra et al. 2010). None the less, plant community composition and diversity may undergo shifts as a result of eCO_2 and its interactions with nutrient cycling, especially P (Jin et al. 2015), which may be highly species- and functional-type-specific.

Previous work on the limestone grassland used in this thesis, investigated the changes that occurred in the community after three years of CO_2 fumigation at the same experiment site (Taylor 2021). It was discovered that in the limestone grassland, eCO_2 had a significant effect on the community composition by creating communities that were dissimilar from each other, but it had no significant effect on diversity, species richness, or functional composition after three years of CO_2 fumigation (Taylor 2021). This is discussed more in Chapter 3.

1.1.3 Effects on leaf nutrient content

The impact of eCO_2 on plant nutrition, nutrient demand, and absorption can be investigated using tissue stoichiometry. Tissue C:P and C:N ratios are increased by eCO_2 in many ecosystems, most of which are expected to be N-limited (Du et al. 2019; Novotny et al. 2007), implying that increases in C supplies are surpassing any potential uptake of extra nutrients, or that eCO_2 is constraining nutrient uptake. The former is an example of the 'carbon dilution' effect, in which a rise in the relative C content of biomass results from the stoichiometric flexibility of plant tissues reflecting changes in C availability (Gifford et al. 2000). However, in P-limited habitats, the productivity response to eCO_2 may be as much as 50% lower than when P is in an abundant supply (Edwards et al. 2005), and this occurs most frequently in ecosystems where the biomass of plants is stimulated by eCO_2.

Consistently, researchers have discovered that eCO_2 has a significant effect on plant N content, typically resulting in decreased tissue N concentration (and subsequent increases in C: N)

(Taub and Wang 2008). Plants are able to downregulate Rubisco synthesis (Long et al. 2004) since eCO_2 decreases photorespiration by as much as a third (Wujeska-Klause et al. 2019) and increases the availability of CO_2 to Rubisco active sites. Since this enzyme is found in photosynthetic tissue, its decline is anticipated to result in significant reductions in foliar N content in comparison to other tissues (Luo et al. 2021). The decrease in transpiration due to increased CO_2 levels could lead to less nitrogen being acquired through mass flow, as suggested by Mcgrath and Lobell (2013). Phosphorus uptake, on the other hand, is generally determined by direct contact between roots and the element and through active diffusion (Barber et al. 1963; Gerke 2015). Therefore, alterations in transpiration are less likely to influence phosphorus uptake by plants.

In ecosystems where C, as well as N, are abundant supply, plant phosphorus content can also increase (Huang et al. 2015) because all of the P acquisition requires some kind of C (and N) input by the plant. Thus, eCO_2 has been proposed as a possible way of liberating plants from P limitation by enhancing the efficiency of P uptake (Jin et al. 2015). For instance, root exudation may increase in response to eCO_2 in order to accelerate the rate of phosphorus (P) chelation and mobilisation (Jin et al. 2015), an impact that is expected to vary with plant functional type (Phoenix, Booth, et al. 2004) and conditions of the soil (Tyler and Ström 1995).

In a comprehensive meta-analysis encompassing 112 studies, Deng et al. (2015) found that plants under eCO_2 levels resulted in increased P absorption. This was due to a 19.3% increase in phosphatase activity, which enhanced the availability of labile P by 4.2% (Deng et al. 2015). Furthermore, a plant that invests additional carbon resources into its arbuscular mycorrhizal (AM) associations may boost mycorrhizal growth, P acquisition, and phosphorus usage efficiency, albeit to a lower amount if P is more limited (Jakobsen et al. 2016).

The potential beneficial impacts of eCO_2 on P cycling may be nullified in P-limited grassland soils, if microbial competition for P is particularly strong (Schneider et al. 2017; Taylor et al. 2021). This is because any newly liberated P may be quickly and fully immobilised by the microbes (Bünemann et al. 2012). It, therefore, becomes crucial to have an understanding of the possible significance of eCO_2 on P nutrition in grassland plants in order to accurately forecast how their productivity might react to future climatic scenarios.

1.2 Nitrogen deposition

Atmospheric N deposition involves the transfer of nitrogen from the atmosphere to the Earth's surface. While it occurs naturally, anthropogenic nitrogen deposition refers to the addition of N to the environment by this process as a result of human activities. Agriculture and the combustion of fossil fuels are the primary contributors to the anthropogenic atmospheric N deposition (Schlesinger 2009). These two sources produce different types of N pollution: reduced and oxidized.

Since the beginning of the industrial and agricultural revolutions, global rates of nitrogen deposition may have tripled (Fowler et al. 2004). As a result, humans currently contribute over 210 Tg yr^{-1} of fixed nitrogen, greater than the amount of fixed nitrogen that natural forms contribute by 7 Tg N yr^{-1} (David et al. 2013). Even though a historical deposition is beginning to decrease in north-west Europe (Tomlinson et al. 2021), rates are still high, and the accumulated loading of nitrogen to soils persists in having negative effects on biodiversity and ecosystem functioning (de Schrijver et al. 2011). Such large quantities of nitrogen introduced into ecosystems can have various effects on the plants and soils within those ecosystems (Phoenix et al. 2012). Some of these effects include shifts in the carbon sequestration (Tipping et al. 2017), plant photosynthesis (Shen et al. 2019), plant communities (Bobbink et al. 2010), and leaf biochemistry (Stevens et al. 2018).

1.2.1 Effects on plant photosynthesis

Nitrogen is the most important macronutrient within terrestrial ecosystems that affects the growth and development of plants (Elser et al. 2007; Mo et al. 2020; Zheng et al. 2020). N play an essential role in the production of chlorophyll and photosynthetic enzymes (Mu and Chen 2021). The impact of N deposition can cause a range of complex, multi-directional responses in plants, including in photosynthesis and growth (Crous et al. 2019; Esmeijer-Liu et al. 2009; Shen et al. 2022).

Increased N availability enhances plant growth and photosynthetic capacity. Nitrogen is a key component of chlorophyll, the major pigment involved in absorbing light energy, as well as the enzyme Rubisco (Mu and Chen 2021). Therefore, N has a strong connection with photosynthetic capacity due to the fact that the photosynthetic apparatus, as well as proteins associated with the Calvin cycle and thylakoids, are primarily made up of N elements (Kattge et al. 2009; Pasquini and Santiago 2012; Westoby et al. 2004). Hence, an increase in N levels frequently drives improved photosynthetic efficiency in plants by encouraging the creation of certain essential chemicals. Previous studies have demonstrated that moderate additions of N can increase the photosynthetic capacity of plants and boost grassland productivity (Bai et al. 2010; Suding et al. 2005). In addition, previous studies suggested that high N concentrations alleviated photosynthesis adaptation to eCO_2 (Ainsworth and Long 2005; Ruiz-Vera et al. 2017). This is due to the fact that under eCO_2 circumstances, Rubisco suppression is associated with leaf N reallocation from a non-limiting source to other limiting photosynthetic components, such as electron transport enzymes or the light-harvesting complex (Evans 1989; Kanno et al. 2017).

1.2.2 Effects on plant community and stoichiometry

Nitrogen deposition can cause shifts in ecosystem biodiversity. Some grasslands, such as 68 acidic grasslands in the UK, lost on average one species per 2.5 g N m^{-2} yr^{-1} (in a 4 m^2 area) due to nitrogen deposition, which may have a negative impact on ecosystem services that rely on diversity (Isbell et al. 2011). This can happen either directly, as nutrients can increase production, or indirectly, as plants become more sensitive to secondary stresses such as herbivory, frost, and drought as a result of N-induced acidification (Phoenix et al. 2012). Since the 1800s, species richness in the UK has dropped by as much as 39% (Edward Tipping et al. 2021). Non-vascular plants, like bryophytes that take in nutrients through their surface tissues, can find the extra N toxic (Arróniz-Crespo et al. 2008). Species better suited to eutrophic environments, may experience the quickest growth stimulation (Bobbink et al. 2010), especially and most pervasively (but not exclusively) in N-limited systems. This may prevent other plants from growing (Hautier et al. 2009). As a result, slower-growing species, or those incapables of tolerating the secondary impacts of N deposition, may be competitively excluded (Stevens, Thompson, et al. 2010).

Since the input of N has less of an impact on the productivity of grasslands that are P-limited, the secondary implications (i.e. not via productivity) of N deposition could prove quite important. Soil acidification is one such effect; it can hinder the growth of some species, cause carbonates to dissolve (Raza et al. 2020), and reduce the availability of base cations (Horswill et al. 2008), all of which can contribute to the extinction of forbs that require a high pH soil.

Additionally, it can influence plant recruitment by diminishing the number of flowers produced (Basto et al. 2015), causing harm to higher trophic levels like pollinators (Stevens et al. 2018). It can also reduce seed coatings’ C: N ratio, enhancing seed decomposability and reducing diversity of seedbanks (Basto et al. 2015). Similarly, changes to foliar C: N ratios can increase plants' vulnerability to herbivores and pathogens (Phoenix et al. 2012), in addition to altering litter quality and, as a result, soil nutrient cycling (Bontti et al. 2009).

Much research has been done on the effects of N deposition on grasslands, and the consensus among researchers is that biodiversity and species richness decrease because many forbs and bryophytes are replaced by graminoids, such as grasses and sedges, even in P-limited grasslands (Bobbink et al. 2010; Lu et al. 2021; Payne et al. 2017; Stevens et al. 2004). As a result, N deposition has been highlighted as the third largest threat to global biodiversity after climate and land-use change (Sala et al. 2000). However, future grasslands will certainly experience a scenario in which N deposition is combined with eCO_2, but we know very little about how plant communities might change in response to this combined scenario. Significant ramifications for plant communities may result from these potential interactions between the C, N, and P cycles (Davies et al. 2016), which cannot be foreseen from the impacts of N deposition or eCO_2 separately.

1.3 Phosphorus

1.3.1 Effects on plant photosynthesis

Phosphorus is an essential macronutrient for plants and is involved in various physiological and biochemical processes in which it plays an essential role (Roch et al. 2019). Phosphorus plays a pivotal role in plant energy transfer and metabolism as a constituent of ATP (adenosine triphosphate) that is used in photosynthesis and respiration to transfer energy within the plant (Lambers and Plaxton 2015; Stigter and Plaxton 2015; Zhang et al. 2009). In addition, nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), nucleic acids, and phospholipids all play essential functions in plant growth, production, signal transduction, and photosynthesis (Hammond and White 2008; Kayoumu et al. 2023; Lu et al. 2023). Approximately thirty percent of the world's arable soils are considered deficient in P and could benefit from fertilisation with this element to improve yields (MacDonald et al. 2011). Plants in natural and agricultural ecosystems are commonly susceptible to P nutritional stress due to the restricted availability of P in the soil as well as the limited movement of P (MacDonald et al. 2011).

The production of energy by photosynthesis is crucial for plant growth. Photosynthetic efficiency depends on several factors, including photosynthetic pigments, photosystems, electron-transport systems, gas-exchange mechanisms, and enzymes involved in carbon metabolism (Ashraf and Harris 2013; Li et al. 2022). P deficiencies often reduce photosynthesis since P plays a role in various cellular functions, including energy conservation, regulating metabolism, and signal transmission (Carstensen et al. 2018). A deficiency of P could reduce the stomata’s opening, resulting in less CO_2 being taken in and converted to triose phosphate. This leads to a significant reduction in the recycling of ATP and NADPH, which in turn reduces the photosynthetic capacity and growth of plants (Neocleous and Savvas 2019). In an environment of a P deficiency, photosynthesis often decreases due to inhibitory feedback driven by decreased growth of leaves. These photosynthesis reductions may reduce leaf mass per unit leaf area and improve photosynthetic P-use efficiency (Ghannoum et al. 2010). The ability of different plant species and even different genotypes of the same species to maintain photosynthetic activity in the face of P limitation varies significantly. Under low P availability, there is considerable interspecific and intraspecific variability in photosynthesis P-use efficiency (Pang et al. 2018; Wen et al. 2022).

Photosynthetic pigments are essential for light absorption and energy transmission (Kume et al. 2019). Phosphorus deficiency causes a reduction in the amount of photosynthetic pigments found in the leaf, including chlorophyll (Chl) and carotenoids (Niu et al. 2022). Previous studies demonstrated that a deficiency of phosphorus reduces the amount of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity in cotton (*Gossypium hirsutum L.*) and diatoms (*Phaeodactylum tricornutum*) (Alipanah et al. 2018; Liu et al. 2021). According to many studies, P deficiency causes a reduction in the amount of net CO_2 assimilation, which, in most cases, leads to a reduction in the stomatal conductance (Nguyen, Palmer, and Stangoulis 2022). Phosphorus deficiency diminishes the capacity for photosynthesis by interfering with the activities of enzymes that are involved in the Calvin cycle as well as ATP and NADP (H) (Chu et al. 2018; Schlüter et al. 2013). This reduces Calvin-cycle activity, which in turn reduces the need for reduction of NADP (H) and ferredoxin Fd_{red}. Phosphorus deficiency also inhibits the electron transport chain, which, when associated with an increase in lumen pH acidification caused by a decrease in the production of ATP, leads to the accumulation of reduced electron carriers such as plastoquinone. In addition to their negative effect on Calvin-cycle enzyme expression, they also negatively regulate the expression of photosynthesis and electron transport chain components (Michelet et al. 2013).

1.3.2 Phosphorus limitation

Ecosystems' responses to alterations in biogeochemical cycling, such as those caused by eCO_2 and N deposition, are in a significant way determined by their limiting nutrient status (Fleischer et al. 2019; Yang et al. 2014; Zaehle et al. 2015). For instance, if N is limiting productivity, it might drastically impair future C sequestration by up to 58 percent in terrestrial ecosystems (LeBauer and Treseder 2008; Zaehle et al. 2015). It is concerning; however, most of our knowledge comes from ecosystems where N is the limiting nutrient (LeBauer and Treseder 2008), because numerous ecosystems globally, including grasslands, are constrained by P or are simultaneously limited by N and P (Fay et al. 2015). Additionally, as N deposition from the atmosphere escalates, the degree and intensity of P limitation may also be on the rise (Goll et al. 2012). According to Du et al. (2020), as many as 82% of terrestrial ecosystems may be constrained, at least partially, by the availability of P, either through its single limitation or combined limitation with N. This stands in contrast to a mere 18% of ecosystems that are limited solely by N. Given the significant disparities in N and P cycles, ecosystems limited by these elements likely will not react identically to N deposition and eCO_2. Therefore, it is essential to gain a distinct understanding of P-limited ecosystems.

Contrasted with the highly eroded soils of tropical regions, P limitation is relatively uncommon in the post-glacial soils found in the temperate ecosystems of Northern Europe (Vitousek and Farrington 1997; Walker and Syers 1976). Nevertheless, in the instances where it does happen, such as in certain grasslands within the Peak District National Park in the UK (Morecroft et al. 1994), P limitation is a significant determinant of biodiversity (Phoenix et al. 2020). In fact, a substantial number of the Earth's most diverse ecosystems are found predominantly in soils that are limited by phosphorus (Laliberté et al. 2013; Lambers et al. 2013). In these instances, the heightened interspecies competition for P has driven the evolution of a wide array of plant adaptations for P acquisition (Péret et al. 2014; Vance et al. 2003). This promotes the coexistence of diverse species due to niche differentiation (Ceulemans et al. 2017; Phoenix et al. 2020) and complementarity (Teste et al. 2014). The association between P-limited availability, biodiversity, and ecosystem function could be disrupted by changes in the availability of other nutrients like N or increased carbon resources (due to eCO_2) that plants may utilise to secure additional P (Jin et al. 2015).

1.3.3 Acquisition of phosphorus through plants

Phosphorus is recognised as the second most crucial macronutrient for plants (following N) because of its integral role in the formation of ATP (adenosine triphosphate), nucleic acids, and phospholipids (Schachtman et al. 1998). Moreover, P is a vital nutrient in photosynthetic processes, aiding in the regulation of enzyme activities linked with carbon fixation and alterations in the availability of CO_2 (Pandey et al. 2015). As a consequence, a deficiency in P can hinder growth and the assimilation of CO_2, as well as limit the processes essential for effective photosynthetic responses to elevated levels of CO_2, such as the regeneration of RuBP (Ribulose-1,5-bisphosphate), carboxylation, and stomatal conductance (Reich et al. 2009).

While plants have the capacity to obtain N in various forms, encompassing inorganic nitrogen supplied by deposition and organic forms like amino acids (Näsholm, Kielland, and Ganeteg 2009), P can only be directly assimilated as orthophosphate ions (PO_4^{3-}) (Péret et al. 2014). Compared to other forms of P, phosphates often exist in extremely low concentrations and can be transformed into one of over 170 insoluble organic or inorganic physicochemical forms that are inappropriate for plant uptake (Holford 1997). Phosphates are most accessible in soils with a neutral pH. In contrast, in both acidic and alkaline soils, P can be quickly adsorbed to soil colloids like Al/Fe oxides or Ca-P complexes, respectively, or it can be immobilised within soil organic matter (Devau et al. 2009; Lambers et al. 2008). Plants have developed numerous physiological and morphological adaptations to extract and dissolve P from both mineral and organic forms, converting it into phosphate (Vance et al. 2003).

To access mineral P forms, plants can exude organic acids and protons which can make P mobile by locally reducing the pH of the soil surrounding the root. This, in turn, speeds up the release of P from encrusted minerals (Lambers et al. 2008). Dauciform roots of *Carex* sedges (Shane et al. 2006) and proteoid cluster roots of lupins (Vance et al. 2003) are two instances of species with specific root structures which are vulnerable to this type of exudation. In P-limited soils, where the competition for P among microbes is high (Bünemann et al. 2012; Schneider et al. 2017), these strategies are successful, possibly because of their ability to surpass microbial competition (Phoenix et al. 2020).

Changes in root morphology can indicate an increased allocation to belowground structures by the plant in response to the availability of P in the soil, consistent with the theory of functional equilibrium (Poorter and Nagel 2000). This theory suggests that plants adjust to alterations in resource availability by directing resources to the organs most proficient in its acquisition. In scenarios of nutrient limitation, these organs are the roots (Poorter et al. 2012; Poorter and Nagel 2000). Hence, root systems under P-limited conditions often exhibit more branching, extended root and epidermal cells, and a higher density of root hairs (Péret et al. 2014; Shen et al. 2011; Vance et al. 2003).

In addition, the colonisation of plant roots by fungal partners can boost the amount of P that the plant is able to absorb (Smith and Read 2008). As many as 74% of flowering plant species form mutually beneficial relationships with arbuscular mycorrhizae (AM) (Brundrett 2009), especially grassland species, although the level of mycorrhizal colonisation in plants can be extremely specific to the species (Phoenix, Booth, et al. 2004). Such symbiotics expand the root systems to allow plants to access a greater quantity of soil, which may include pore spaces that are too tiny for plant roots (Aleklett et al. 2021). Additionally, they accelerate the chemical process known as the weathering of mineral P and the hydrophilic breakdown of organic P (Hodge 2014; Quirk et al. 2012). In exchange for enhancing P uptake in plants, the fungus obtains carbon resources, which are traded through arbuscules integrated into the roots of plants (Smith and Read 2008).

It is possible that organic forms of phosphorus, such as those included within soil organic matter and litter, account for between 30 and 50 percent of the total P found in soil (Plante 2006). Organic forms of P can be a significant source of P, particularly in soils with limited availability of inorganic P (Janes-Bassett et al. 2020). In a procedure known as biochemical mineralisation, the release of enzymes such as phosphatases and phytases may trigger the hydrolysis of phosphorus from organic forms (Shen et al. 2011; Wang et al. 2007). If nutrient scarcity also curtails microbial activity, the excess carbon seepage due to eCO_2 levels may speed up the breakdown of soil organic matter (a process known as priming), thereby unlocking access to nutrients (Kuzyakov 2002). This process could also influence the availability of C and N, as well as their potential leakage from the soil (R. Chen et al. 2014). However, such a scenario is more likely to occur in N-limited soils than in those lacking P (Dijkstra et al. 2013). As a consequence, atmospheric CO_2 concentrations and rates of N deposition can have a significant impact on the cycling of carbon and nutrients in the soil by virtue of the impacts it has on the demand for P from plants and soil microbial organisms.

Numerous such strategies exist in grassland ecosystems (Phoenix, Booth, et al. 2004), and their efficacy in acquiring a specific form of P over another varies (Phoenix et al. 2020). As a result, their effectiveness is contingent on the soil type and the prevailing conditions. Moreover, every one of these adaptations likely demands distinct relative contributions of carbon and nitrogen from the plant (Lynch and Ho 2005). As a result, changes in the availability of C and N, such as those caused by eCO_2 and nitrogen deposition, could alter the efficacy of these adaptations and potentially affect the survival of the species that rely on them.

1.4 Grassland ecosystems

Grasslands provide an ideal environment to examine the interconnected impacts of eCO_2 levels, nitrogen deposition, and phosphorus limitation on biogeochemical cycles. In terms of practicality, grasslands are empirically manageable; entire plant-soil structures can be moved with minimal disruption to more suitable locations for field experiments. They are essential on a worldwide scale due to the fact that they are the most abundant terrestrial ecosystems on Earth (Ali et al. 2016), they can be found on all continents other than Antarctica, and they are the most ubiquitous P-limited ecosystems in temperate climates (Watson et al. 2011).

In addition, grasslands are of tremendous value not just from an ecological point of view but also in terms of their role in the regulation of global C and cycles of nutrients. Grasslands have the potential to contain more than a quarter of the world's soil organic C stocks (FAO and ITPS 2020), account for a fifth of terrestrial production (Chapin et al.2011), and have the potential to absorb deposited N, so, significantly lowering the amount of N that is polluting watercourses (Phoenix, Leake, et al. 2004). Grasslands are also among the most biodiverse habitats in temperate areas (Carbutt et al. 2017), with nearly a fifth of Europe's endemic vascular plant species being exclusive to these ecosystems (Habel et al. 2013). This impressive species diversity is a testament to the variety of environmental conditions and resource availability within these ecosystems, further highlighting their significance for biodiversity conservation. Finally, grasslands are among the most globally endangered biomes (Hoekstra et al. 2005), partially due to the ongoing impact of N deposition (Stevens, Dupr, et al. 2010). Within the UK specifically, these ecosystems have been dramatically lost, with 97% of species-rich meadows disappearing since the 1930s (Fuller 1987; Hooftman and Bullock 2012). This rapid decline underscores the urgent need for conservation measures to protect these diverse and critical habitats.

1.5 Heather Moorland and *Calluna vulgaris* (heather)

Heather moorlands, also referred to as *Calluna*-dominated moorlands, represent a unique type of upland habitat primarily located in the UK (Holden et al. 2007). They offer a distinct ecosystem with significant conservation value, in addition to the ecosystem services provided such as carbon sequestration (Holden et al. 2007; Ramchunder et al. 2009). Heather moorlands are dominated by a single species *Calluna vulgaris* (*C. vulgaris*), which we know little about the ecological, physiological, and biochemical changes throughout its life stages (Holden et al. 2007; Reed et al. 2009).

C. vulgaris is a low-growing, evergreen dwarf shrub which produces densely packed branches with purple flowers (Gimingham 1960; Gordon et al. 1999; Sands and Gimingham 1975). As a result of its extremely competitive and hardy nature, it is able to preserve its dominant status. Based on the research of (Sands and Gimingham 1975; Watt 1955) identified four distinct stages in the life cycle of *C. vulgaris*. These phases are associated with variations in biomass and shoot production (Society 1971) as well as variations in the plant's immediate surroundings (Barclay-Estrup and Gimingham 1969; Sands and Gimingham 1975).

I. Pioneer phase: Characterized by small individuals with limited lignification, resulting in a patchy distribution pattern with a lush green color appearance. Flowering is rare, occasionally missing, and the patch is open, with a large number of bryophytes and vascular plants growing beneath.

II. Building phase: The dense, green canopy of larger individuals entirely covers the ground, with little light reaching the ground level. Flowering is vigorous, with a lack of other plants developing under *C. vulgaris*.

III. Mature phase: Plants keep expanding, although at a slower rate. A less thick canopy with more gaps is the result of this development type, with abundant flowering and the beginning of other plants growing beneath.

IV. Degenerate phase: Due to the collapse of the major branches, the canopy gaps produced during the mature phase get larger, and other plant species expand rapidly; the lichen *Parmelia physodes* grow on the stems during the degenerate phase (Gimingham et al. 1981; Sands and Gimingham 1975).

As a result, each of these phases has an association with distinct physiology, morphology, and ecological composition (Gimingham et al. 1985; Sands and Gimingham 1975). *Calluna vulgaris* is found in a wide range of habitats due to its excellent tolerance to a wide range of environmental conditions, including moorlands, heathlands, acidic grasslands, and woodland understories (Damgaard 2019; Holden et al. 2007).

Calluna vulgaris is a distinctive plant in *Calluna*-dominated moorlands and heaths with high nature conservation value, and it is adapted to the nutrient-poor, acidic soils of open habitats. Changes in N-deposition, and (mis)management practices, such as overgrazing, poor burning, and artificial drainage (Allen et al. 2016; Chapman et al. 2010; Clarke et al. 1995; Gimingham 1960) have all contributed to declining heathland habitats across Europe including the UK (Damgaard 2019; Holden et al. 2007). In *Calluna*-dominated environments, rotational prescribed burning has been the primary prevalent management approach (Måren et al. 2010; Velle et al. 2012). However, recent research (Garnett et al. 2000; Ramchunder et al. 2009) indicates that burning could have negative consequences on ecosystem services. As a result, cutting is being explored as an alternative management option to fire (Cotton and Hale 1994; Sanderson et al. 2020). In *Calluna*-dominated habitats, researchers have largely investigated heather's ability to produce new shoot biomass to sustain sheep grazing or high numbers of game such as red grouse (*Lagopus lagopus scoticus Latham*) and red deer (*Cervus elaphus L.*).

Traditional and existing techniques for monitoring the growth of *C. vulgaris*, such as manual ecological surveys carried out on-site, are considered to be labour-intensive, restricted in their spatial coverage, and sometimes too qualitative (Mac Arthur and Malthus 2012). Hence, the scientific community has become interested in alternative near-Earth remote sensing technologies for categorizing *C. vulgaris* growth and canopy morphology on a sub-meter scale (Nichol and Grace 2010). This includes leaf-level and canopy-level hyperspectral reflectance (Neumann et al. 2020; Nichol and Grace 2010). However, because of the high level of accuracy and resolution that is necessary, this has proven to be difficult. As a result, very few successful and repeatable solutions have been discovered up to this point, if any at all.

1.6 Remote sensing

1.6.1 General principles

Remote sensing is the process of detecting and monitoring an object’s biochemical or structural characteristics by measuring radiation reflected or emitted from the object without coming in to contact with it (Blackburn 1998; Daughtry et al. 2000; Weiss et al. 2020). Reflectance is the ratio of the amount of incident light reflected from a surface, such as a leaf to the amount of incident light that strikes that surface (Milton 1987). For instance, a perfect white surface reflects 100% of the incident light, while an ideal black surface absorbs 100%. The fundamental mechanisms of reflectance can be divided into three primary interactions between light and a plant: i) Absorption, which occurs when light is absorbed by the leaf, and the amount and wavelength of light absorbed depends on the leaf characteristics and structure (Anderson et al. 2009; Milton 1987). ii) Reflection, this is when light bounces off a surface, and the amount of reflected light depends on the leaf characteristics, structure, and angle of incidence (Anderson et al. 2009; Milton 1987). iii) Transmission is when light passes through a leaf and like absorption, transmission depends on the properties of the light and on the leaf characteristics and structure (Anderson et al. 2009; Milton 1987).

Remote sensing is an increasingly important tool for the assessment of plant physiological and biochemical parameters (Eitel et al. 2014) due to its time and resource efficiency, and its ability to capture spatially-continuous data over large spatial extents. Remote sensing technology can be used to measure various plant biochemical, physiological, and structural traits (Assmann et al. 2019; Dash and Curran 2007; Nichol and Grace 2010). These include leaf chlorophyll content, leaf N content, plant water content, plant height and photosynthetic capacity

(Blackburn 1998; Blackburn 2007; Daughtry et al. 2000). Hyperspectral sensors sample reflectance at hundreds of narrow wavelength bands compared to multispectral sensors that capture data within a small number (up to 10) of broader spectral bands (Blackburn 2007), allowing the extraction of information from a greater number of absorption features, which are often narrow in width. Therefore, in comparison with multispectral data, hyperspectral data provides a much more detailed spectral profile, allowing for the extraction of more precise or subtle information about the plant (Gerhards et al. 2019). For instance, hyperspectral data can often detect subtle changes in a plant's health before they become apparent in multispectral data such as changes in chlorophyll content, water status, or the presence of certain diseases (Cheng et al. 2006; Clevers and Gitelson 2013; Zhang et al. 2019). However, hyperspectral sensors are less common and less readily available than multispectral sensors, especially on satellite and drone platforms. This is mainly due to the complexity and cost of hyperspectral sensors and the large amount of data they produce, which can be challenging to process and analyze (Gerhards et al. 2019; Milton 1987; Prananto et al. 2020). There is frequently a trade-off between temporal, spectral, and spatial resolution, and this trade-off is driven mostly by technological and practical limits in sensor design, data storage and processing capabilities, and data applications (Hassler et al. 2019; Milton 1987; Weiss et al. 2020).

However, there are also challenges and limitations associated with capturing and using remote sensing data. For instance, canopy-level reflectance can be affected by atmospheric conditions, soil background reflectance, and the angle of the sun, among other factors (Homolová et al. 2013; Ollinger 2011; Serbin and Townsend 2020). From a leaf-level reflectance perspective, measurements can be affected by the internal structure of the leaf, including broadleaf and needle leaves (Croft et al. 2014; Serrano 2008; Zarco-Tejada et al. 2004). Additionally, interpreting remote sensing data can be complex and requires specialized knowledge.

1.6.2 Leaf level reflectance acquisition

Leaf-level hyperspectral reflectance is an effective technique that has been increasingly utilized in plant leaf biochemistry and physiology (Huemmrich et al. 2022). To precisely measure leaf reflectance, requires a leaf clip, a reference standard, and a light source (Huemmrich et al. 2022; Lewis and Disney 2007). The leaf clip is attached to a spectrometer and contains an aperture through which the light source illuminates the leaf, and the detector captures the reflected light (Gitelson et al. 2003). To avoid interference, the leaf clip blocks out external light and has a narrow field of view, allowing for detailed, localized measurements of reflectance from a single leaf rather than a larger, potentially heterogeneous area (Gitelson et al. 2003; Huemmrich et al. 2022). The reference standard, a white reference or calibration panel, is used to calibrate the spectrometer before measurements are taken (Anderson et al. 2009; Milton 1987). A consistent, stable, uniform illumination and a controlled light source are crucial for accurate leaf reflectance measurement; often, a halogen, LED, or quartz tungsten halogen lamp is used (Sims and Gamon 2002). The principle of fundamental leaf-level hyperspectral reflectance is that different plant species and different environmental conditions within a plant species produce unique patterns of reflectance across the electromagnetic spectrum (Blackburn 1998; Huemmrich et al. 2022; Milton 1987). Using leaf-level hyperspectral reflectance spectra to model leaf biochemistry and physiology is a promising alternative to traditional measurements since reflectance can be measured quickly and efficiently with spectrometers (Huemmrich et al. 2022; Zarco-Tejada et al. 2004). The number of wavelengths included in a reflectance spectrum can range anywhere from several hundred to thousands, depending on the spectral resolution and the range of wavelengths that are measured (Callaghan et al. 2011). A full-range spectroradiometer would typically encompass these wavelengths, which include the visible (VIS, 380–700 nm), blue (450-495 nm), green (495-570 nm), and red (620-750 nm), near-infrared (NIR, 700–1100 nm), and short-wave infrared (SWIR, 1100–2500 nm) ranges, which have been used as a rapid and non-destructive method for plant biochemistry and physiology analysis (Blackburn 2007; Prananto et al. 2020).

1.6.3 Canopy reflectance acquisition

Canopy-level hyperspectral reflectance is a powerful approach in plant physiology and ecology, which allows for the monitoring of vegetation status on a larger scale compared to leaf-level measurements (Serbin and Townsend 2020). This approach involves measuring the reflectance from the entire, or part of the, canopy of a plant community rather than individual leaves (Assmann et al. 2019; Dash and Curran 2007; Kovar et al. 2019). Canopy reflectance data can be collected using a variety of approaches, such as ground-based, drone, and satellite platforms (Ač et al. 2009; Homolová et al. 2013; Myneni et al. 1997; Sun et al. 2021). Ground-based acquisition usually involves handheld or tripod-mounted spectrometers, which can provide detailed, high-resolution accurate information data over small areas from a certain distance, and the process can be labor-intensive (Malenovský et al. 2009). The drone-based acquisition can carry multispectral (common) or hyperspectral sensors (not common and expensive) and capture data from above the canopy, allowing for the collection of data over larger areas than ground-based methods, while still providing a relatively high spatial resolution (Roslim et al. 2021; Sun et al. 2021). In addition, drones can be more adaptable and cost-efficient compared to satellite techniques, offering data as needed. However, operating them still demands substantial time and expertise. Moreover, drone flights frequently encounter regulatory limitations (Assmann et al. 2019; Hassler et al. 2019; Sun et al. 2021). The satellite-based acquisition provides the widest coverage and captures data in several broad spectral bands (Gao and Zhang 2021; Royimani et al. 2019). While satellite data often lack the spatial detail provided by ground or drone sources, primarily due to the substantial distance between the sensor and the canopy, it facilitates reliable, long-term surveillance of vast regions (Bansod et al. 2017). Nonetheless, satellite data can be influenced by weather conditions, and the schedule for data collection is dictated by the satellite's orbit, rather than the user's specific requirements.

The reflectance at the canopy level integrates information from all the elements in a given field of view – including leaves at different angles, branches, understory, and even bare soil, and as a result, provides a more comprehensive understanding of the vegetation's status and the overall ecosystem's status (Blackburn 2007; Ustin et al. 2004). This can provide insights into the productivity and nutrient status of the ecosystem (Croft et al. 2017). For instance, hyperspectral reflectance can be used to monitor changes in vegetation biochemistry and physiology in response to environmental changes or disturbances, including eCO_2 and N deposition (Liu et al. 2021; Schlerf and Atzberger 2006; Zhu et al. 2008). In addition, it can also be utilized to estimate carbon sequestration in plants, which is critical for understanding the C cycle and perhaps managing carbon emissions. The reflectance observed at the canopy level is influenced by various factors, including leaf structure, leaf area index (LAI), aggregation of leaves, distribution of leaf angles, population density, and elements of the canopy that do not partake in photosynthesis (Croft et al. 2014; Demarez et al. 2000; Simic et al. 2011). Furthermore, the solar and viewing geometry, ground coverage, and vegetation in the understory also have an impact (Broge and Leblanc 2001).

1.6.4 Leaf functional traits and optical reflectance

Leaf functional traits are various characteristics of a leaf that can influence a plant's growth, survival, reproduction, and overall performance within its environment (Yan et al. 2022; Zhang et al. 2021). These traits can be morphological such as leaf shape, size and thickness, physiological such as the photosynthetic or transpiration rate, and biochemical such as nutrient content and pigment concentrations (Barker et al. 1997; Croft et al. 2014; Croft et al. 2017; Zhang et al. 2008). These traits can have a significant impact on a leaf's optical reflectance, which refers to the fraction of incoming light that the leaf reflects across various wavelengths. For instance, the reflectance of a leaf is determined by the presence of foliar elements, such as leaf chlorophyll concentration, leaf N concentration, and water content (Fig 1.1) (Cheng et al. 2006; Dash and Curran 2007; Ustin et al. 2004). The following are some of the functional characteristics of leaves that could significantly impact optical reflectance in various ways.

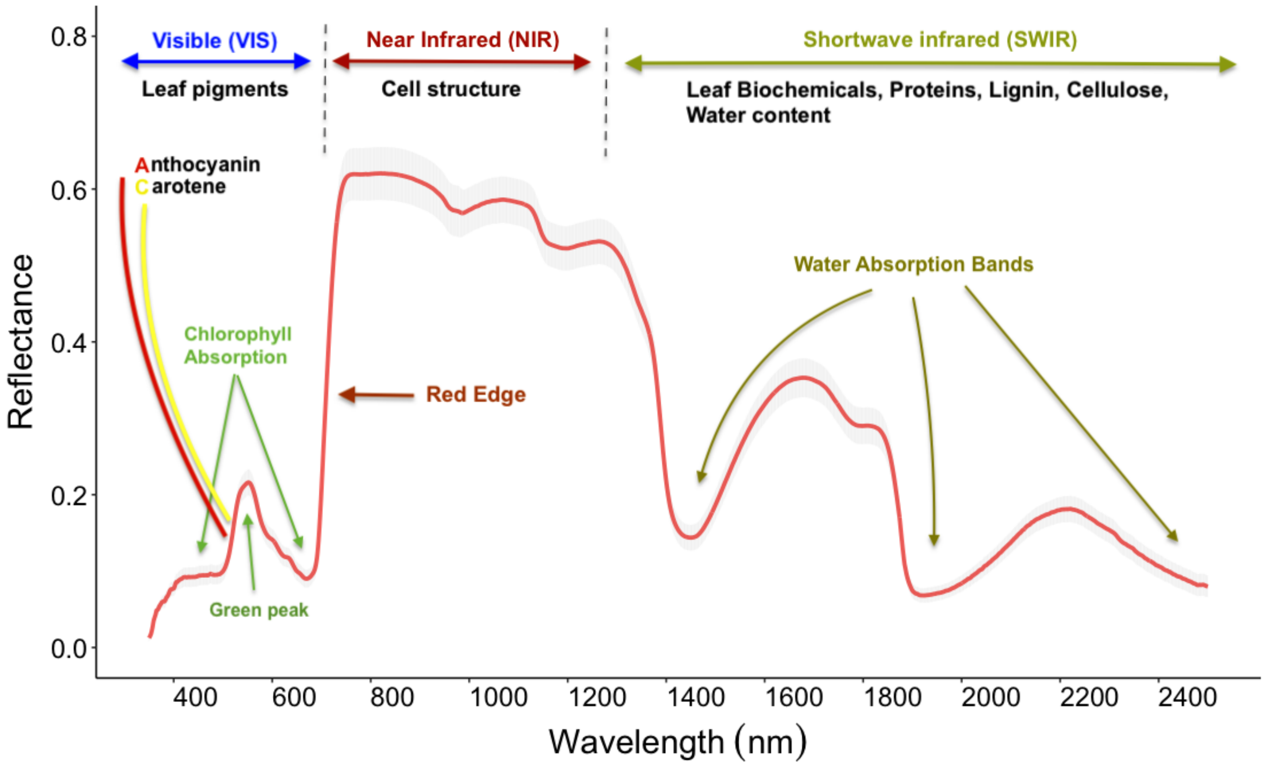

Figure 1.1: A reflectance spectra curve with the major absorption features of plants indicated.

Chlorophyll

Chlorophyll, the main photosynthetic pigment in plants, plays a vital role in how light is absorbed and reflected by leaves and has a significant absorption in the red and blue spectral regions in the visible spectrum (Blackburn 2007; Dash and Curran 2007; Heenkenda et al. 2015). Chlorophyll molecules absorb light most strongly in the blue (around 400-500 nm) and red (around 600-700 nm) regions of the spectrum, with the maximum absorbance occurring between 660 and 680 nm, and less strongly in the green (around 500-600 nm) regions, with the maximum reflectance occurring in the green wavelength range (560 nm) (Fig 1.1) (Blackburn 1998, 2007; Croft et al. 2014; Dash and Curran 2007).

Nitrogen

The concentration of nitrogen that is present in a leaf can have an effect on the way that it reflects light across the spectrum, especially in the visible (VIS, 400-700 nm) region of the spectrum (Blackburn 2007; Xue et al. 2004). Since nitrogen is such a crucial component of chlorophyll, an increase in the nitrogen content of the leaf typically results in an increase in the chlorophyll concentration in the visible spectrum (Blackburn 2007; Filella et al. 1995; Wang et al. 2007; Xue et al. 2004). As a result of such, the reflectance can be reduced in the blue (about 400-500 nm) and red (approximately 600-700 nm) regions (where chlorophyll absorption is strong), but it can be slightly increased in the green (around 500-600 nm) regions (Fig 1.1) (Eitel et al. 2014; Homolová et al. 2013; Kattge et al. 2009).

In addition, since nitrogen is a key component of amino acids, proteins, and nucleic acids, some studies have found a correlation between leaf nitrogen content and reflectance in certain NIR and shortwave infrared (SWIR, 1300-2500 nm) wavelengths (Herrmann et al. 2010). However, the relationship between leaf nitrogen and reflectance is more complex and can be influenced by other factors, such as leaf structure and water content (Fig 1.1) (Herrmann et al. 2010; Rotbart et al. 2013).

Water

The infrared region of the spectrum is where water is the most effective in absorbing light, particularly in the near-infrared (NIR) and shortwave infrared (SWIR) regions of the spectrum (Quemada et al. 2021). Water begins to absorb light in the Near-Infrared (NIR) Region (700-1300 nm); however, not as strongly as in the SWIR Region (Damm et al. 2018). An increase in water can reduce NIR reflectance. In the Shortwave Infrared (SWIR) region, spanning from 1300-2500 nm, water exhibits a strong light absorption (Cheng et al. 2006). Multiple water absorption features exist in this region, with the most notable ones usually found around 1450 nm, 1940 nm, and 2500 nm (Cheng et al. 2006; Damm et al. 2018; Quemada et al. 2021). Consequently, an increase in the leaf's water content can result in a marked reduction in reflectance within these bands (Fig 1.1) (Quemada et al. 2021).

Leaf structure

The structure of a leaf significantly affects the way light interacts with the leaf's surface, thus impacting the absorption, transmission, and reflection of light (Homolová et al. 2013; Serrano 2008b). The structure of the leaf's external surface, such as its roughness, hairs, and waxy cuticle, can alter how light is scattered when it reaches a leaf, thus affecting the amount of light that can be absorbed (Serrano 2008a; Sims and Gamon 2002). The internal structure of a leaf also influences how much incident light is absorbed, scattered, and reflected through the upper epidermis, owing to refractive discrepancies between the air spaces within cells and the cell walls (Blackburn 2007; Serbin and Townsend 2020). Cellulose and lignin, the main structural elements of plant cell walls, greatly contribute to the leaf's overall structure, and the NIR and SWIR are where these compounds absorb the most light (Jacquemoud et al. 1996; Tamary et al. 2019; Westoby et al. 2004). The absorption features typical of cellulose and lignin are often positioned around 2100-2300 nm (Homolová et al. 2013; Jacquemoud et al. 1996; Stéphane et al. 2019). In addition, different leaf types, such as broadleaf and needle leaf, have different reflectance spectra even with the same amount of chlorophyll (Croft et al. 2013; Croft et al. 2014). Broadleaf plants possess a slender epidermal layer, extended palisade cells, and a plethora of air spaces surrounding their spongy mesophyll cells (Croft et al. 2014; Serrano

2008a). In contrast, needle-like leaves display a dense and undifferentiated mesophyll with thick cell walls (Ollinger 2011). The ratio of the surface area of mesophyll cells to the space between them has been shown to control NIR reflectance (Serrano 2008a). The light-absorbing and scattering features change in parallel as the structure and chemical composition of leaves change with species, age, environment, and stress (Wu et al. 2017; Yang et al. 2016).

1.6.5 Spectral vegetation index (VIs)

Spectral vegetation Indices (VIs) that are generated using remotely sensed reflectance data are fairly simple approaches that are highly beneficial for qualitative and quantitative assessments of plant leaf biochemistry, physiology and foliar pigments such as leaf chlorophyll concentration, leaf nitrogen concentration, and leaf water content (Blackburn 2007; Croft et al. 2014; Dash and Curran 2004, 2007; Osborne et al. 2002). These indices can be obtained from canopy-level or leaf-level reflectance and can leverage the distinct absorption properties of vegetation in various light spectrum ranges, leading to their extensive use in a variety of applications (Xue and Su 2017). Vegetation indices are formed through the combination of wavelength ratios that are sensitive to certain leaf pigments or spectral regions where scattering is primarily caused by either a leaf's internal structure or canopy structure (Blackburn 2007). Although the vast majority of VIs are calculated using reflectance from wavelengths in the visible, NIR, and around the red edge, certain VIs also comprises only the visible wavelength (Filella et al. 1995; Gitelson et al. 2002). Leaf surface reflectance was identified as a crucial factor in weak connections between VIs and chlorophyll concentration by Sims and Gamon (2002), who assessed almost 400 samples of leaves from 53 species. Le Maire et al. (2004) discovered that a modified simple ratio, mSR705, could account for surface scattering on both an experimental database (consisting of 53 leaves) and a simulated database (consisting of more than 11,000 spectra), despite the fact that the VI had a dependency on the amount of water in the leaf (Serrano 2008a). While there has been a substantial number of studies committed to obtaining statistical correlations between leaf optical properties and chlorophyll, nitrogen concentrations and water content, these relationships have frequently been developed and tested using a small number of closely related species at the leaf scale and in a laboratory setting under strict control (Blackburn 1998; Chou et al. 2017; Croft et al. 2014; Gamon and Surfus 1999; Gitelson et al. 2003).

Despite the significant progress achieved with VIs applications, it remains essential for researchers to apply and examine these VIs to a variety of ecosystems and plant species from leaf-level to canopy-level reflectance, including in the investigation of eCO_2 and N deposition impacts on P-limited grassland.

1.7 Thesis aims and scope

The preceding sections have underscored the role that different environmental drivers have on shaping the structure and function of UK upland ecosystems, and how remote sensing may be used to derive information on how these structural and functional plant traits vary. In this thesis, I focus specifically on addressing: 1) our limited understanding of how P-limited grasslands might respond to simultaneous eCO_2 and N additions. It emphasizes the need for further research to gain a deeper understanding of the potential ecological consequences. These biogeochemical alterations are expected to cause crucial effects on the carbon sequestration, leaf physiology, community composition, and leaf biochemistry of phosphorus-limited grasslands, with unknown implications for the ecosystem functioning. We cannot assume that what we know about N-limited ecosystems applies to P-limited ecosystems because of the interactions that occur among the C, N, and P cycles. Hence, an integrated experimental approach that uses both eCO_2 and a long-term nutrient manipulation experiment on P-limited grassland may prove to be particularly effective at exploring this knowledge gap.

2) *Calluna vulgaris* (*C. vulgaris*) is plant species that dominates most British moorland environments and is highly vulnerable to climate change (Albert et al. 2011; Gordon et al. 1999). Although it is a well-studied species, we still know very little about the ecology, physiology, and biochemistry of the different *C. vulgaris* life stages, which is crucial for protecting and managing moorland in the face of challenges such as poor management and climate change.

This thesis, therefore, aims:

(i) To determine the physiological and biochemical responses of *Plantago lanceolata* to eCO_2 after three years of eCO_2 exposure and long-term (>25 years) nutrient additions of N and P impact leaf's response to eCO_2. (Chapter 2)

(ii) To quantify *Plantago lanceolata* leaf biochemical and physiological traits changes resulting from the different eCO_2 and nutrient treatments using leaf-level hyperspectral reflectance. (Chapter 2)

(iii) To determine responses of plant community and biodiversity to five years of eCO_2 exposure and long-term nutrient manipulation over (>27 years) of N and P additions. (Chapter 3)

(iv) To review and advance measurements of the ecological variations among *C. vulgaris* life stages while investigating new ecological traits, and determine further comprehend the physiological and biochemical variations among growth stages, and determine if hyperspectral reflectance from the leaf and canopy levels can detect the different life stages. (Chapter 4)

1.7.1 Field site description

Bradfield site description

The University of Sheffield's Bradfield Environmental Laboratory (53°25N 1°35W) is at 390 meters above sea level (Fig. 1.2), with mean annual maximum and minimum air temperatures of 13.8 and 6.9 ˚C, respectively. The average annual precipitation is 893 mm. It is mainly a mix of heathland dominated mainly by *Calluna vulgaris*; and acidic grassland typically mainly consisting of grasses *Deschampsia flexuosa*, *Festuca ovina*, and *Nardus stricta* and the forb *Galium saxatile*.

Figure 1.2: Bradfield laboratory site, the yellow-border area. At the top left, the main building. Green rectangle, the miniFACE experiment site. Orange circles *C. vulgaris* blocks, with a total of eight blocks, each block has four different life stages. The map on the left shows the location of the Bradfield laboratory site in the United Kingdom. Scale label and compass directions at the bottom right.

The miniFACE experiment at Bradfield

At Wardlow Hay Cop (in the Peak District) is a long-term nutrient modification experiment on limestone and acidic grassland that was started in 1991 (plots no longer in use) and 1995 (plots utilized in this study) to investigate the effects of atmospheric N deposition and elimination of P constraint on grassland ecosystems (Johnson et al. 1999; Morecroft et al. 1994).

In the year 2017, complete monoliths of plant and soil sized 35 cm by 35 cm were taken from Wardlow nutrient manipulation plots and brought to the Bradfield station. The monoliths were then relocated into polypropylene boxes with free draining, with a depth of 15 cm for the limestone samples and 20 cm for the acidic grassland samples (Fig. 1.3). To better simulate field conditions in the limestone mesocosms; the bottom 5 cm layer consists of limestone chips, derived from the same limestone found in Wardlow. The depth of the acidic mesocosms encompasses the rhizosphere soil, which is usually confined to the top 15 cm, and it includes both the organic and mineral horizons found in the acidic profile. These mesocosms were given time to recuperate from the initial disruption of relocation before exposure to eCO_2 fumigation the next spring.

From each of the two grassland types, ten replicates of the nutrient treatment were extracted to create a total of eighty experimental mesocosms. Mesocosms that received the same nutritional treatment were paired up based on their productivity (which was established by a biomass harvest), and then they were divided into treatments of either ambient or elevated CO_2. This method of pairing was employed to minimize the chance that any variances in reaction to elevated CO_2 were due to intrinsic discrepancies in grassland productivity. For the eCO_2 treatments, a mini free air CO_2 enrichment (miniFACE) system was utilised to raise the ambient

CO_2 concentration to 600 ppm (Miglietta et al. 2001). From the beginning of April through the end of October (Fig. 1.4), eCO_2 is supplied during daylight hours.

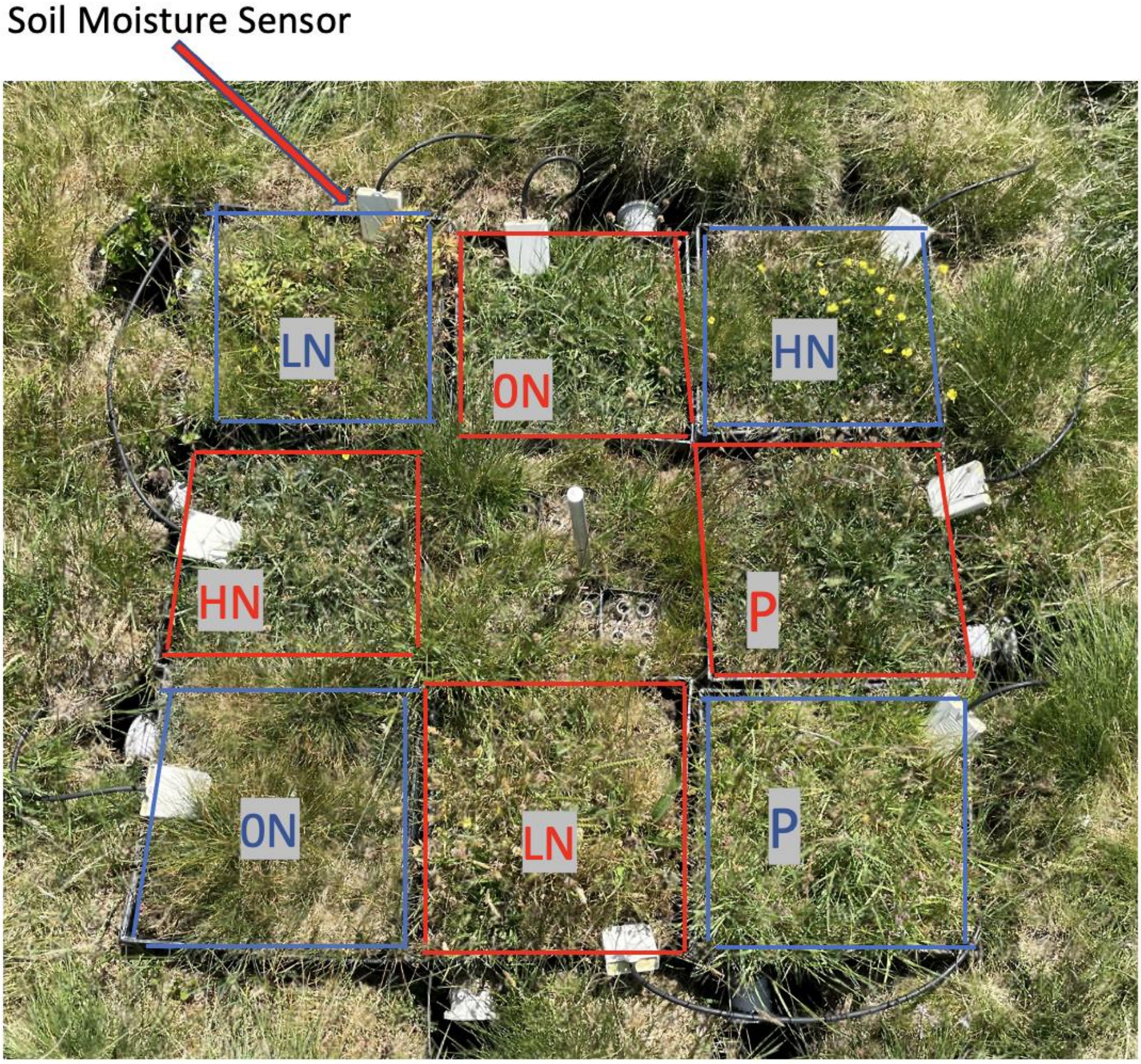

Figure 1.3: An eight mesocosms block of ambient CO_2 treatment. The mesocosms with red writing represent the limestone grassland, and the blue writing represents the acidic grassland. Treatment codes (0N) control, (LN) low nitrogen, (HN) high nitrogen, and (P) phosphorus.

Figure 1.4: The top image of a block of eCO_2 mesocosms. MiniFACE ring with laser-drilled holes provides CO_2 to grasses from a connecting pipe. In order to achieve the target concentration (600 ppm), the central computer receives information from the CO_2 sensor located in the ring's center. This is modified based on the results of a weather station located on the site, considering both the speed and the direction of the wind. The image at the bottom of the page displays an eCO_2 block in the middle of the active experiment, as well as the automated CO_2 flux chamber (Skyline system). Bottom image credit (Ben Keane)

1.7.2 Thesis structure

I specifically focus on addressing:

Interactive effects of elevated CO_2 and nutrient inputs on Plantago lanceolata physiology in a phosphorus (P) limited grassland (chapter 2)

Elevated CO_2 (eCO_2) has been commonly found to increase plant photosynthetic rate (A) while decreasing maximum carboxylation capacity (V_{cmax}), maximum rate of electron transport (J_{max}), stomatal conductance (g_s) and changing stomatal morphology (Haworth et al., 2016). As a result, plants are able to preserve more water in the leaves (Tausz-Posch et al., 2015; Wullschleger et al., 2002) and enhance their water use efficiency (WUE) (da Silva et al., 2017; Li et al., 2017; Pazzagli et al., 2016; Yan et al., 2017). Currently, human activity has increased atmospheric CO_2 concentrations to more than 420 ppm (Friedlingstein et al., 2019; Willeit et al., 2019) and are estimated to contribute more N to the global N cycle than all natural sources combined (Fowler et al., 2013). Grasslands can be found on every continent other than Antarctica (Ali et al., 2016), making them the most widely spread terrestrial ecosystem on Earth. Plants can have a wide variety of complicated, multi-directional reactions to variations in atmospheric CO_2 concentration and N deposition, including changes in photosynthesis, growth, and leaf stoichiometry (Crous et al. 2019; Esmeijer-Liu et al. 2009). However, we know very little about the effects of eCO_2 and N availability, either alone or in combination, in habitats where P limits production. This is critical since up to 82% of global terrestrial production may be P-limited or P-limited with N and P (Du et al. 2020).

Detecting any climate-induced biophysical or physiological changes in leaf characteristics is thus critical for accurately estimating plant performance at many spatial and temporal scales (Roosjen et al. 2018; Varvia et al. 2018). Hyperspectral remote sensing techniques have the potential to provide a precise, quick, and scalable evaluation of dynamic biophysical changes caused by changes in atmospheric CO_2 concentration or nutrient availability (Eitel et al. 2014). The presence of leaf pigments such as chlorophyll, N, and water content influences leaf reflectance (Ustin et al. 2004). However, the efficacy of hyperspectral techniques in detecting plant acclimatization to eCO_2 circumstances and long-term nutrient additions N and P in a P-limited ecosystem, on the other hand, has yet to be investigated.

In a novel miniFACE experiment at Bradfield, this chapter provides a detailed study of the biochemical and physiological acclimation of a common grassland forb *Plantago lanceolata* to eCO_2 and various N and P treatments in P-limited limestone grassland. The aim of this chapter is to evaluate the physiological and biochemical responses of *Plantago lanceolata* to eCO_2 after it has been exposed to eCO_2 for three years, as well as the impact of long-term (>25 years) supply of N and P on the leaf's response to eCO_2.

Impacts of five-year-CO_2 enrichment and long-term nutrient additions on phosphorus-limited limestone grassland biodiversity (chapter 3)

Anthropogenic activity has raised atmospheric CO_2 concentrations to more than 420 ppm (Friedlingstein et al. 2019), a level that is unprecedented in human history (Willeit et al. 2019), and human activities are now estimated to contribute more N to the global N cycle than all natural sources combined (Fowler et al. 2013). To yet, however, investigations on ecosystems where N is the limiting nutrient have provided much of our understanding of the effects of rising CO_2 and increasing atmospheric N deposition. However, P availability may be limiting production on around 82% of land areas, either independently due to P constraint or as a co-limiter with N and P (Du et al., 2020). To date, very little is known about the response of P-limited ecosystems, such as grasslands, to simultaneous changes in atmospheric carbon dioxide and nitrogen deposition.

Hence, chapter 3's aim is to provide a comprehensive analysis of the effects of simultaneous eCO_2 and nutrient treatment on plant community in a limestone P-limited grassland using the miniFACE experiment at Bradfield. Variations in community composition, species richness, diversity, and functional composition are quantified in order to achieve a deeper comprehension of how plant communities respond to altering levels of C, N, and P availability after five years of eCO_2 exposure, as well as long-term (>27 years) N and P additions.

Ecological and Physiological Comparisons and Contrasts between Calluna vulgaris (Heather) life stages (Chapter 4)

In terms of biogeography and carbon cycling, British moorlands are distinctive and valuable ecosystems of national and international importance (Chapman et al. 2010; Harris et al. 2011; Natural England 2015; Allen et al. 2016). These ecosystems are being degraded, and their environmental resilience to climate change is being reduced as a result of continuing and historical (mis)management practices such as overgrazing, harmful burning, and artificial drainage (Gimingham 1960; Clarke et al. 1995; Chapman et al. 2010; Allen et al. 2016). It is essential for conducting long-term, reproducible monitoring of moorland areas in order to determine the level and trajectory of environmental change (MacArthur and Malthus 2012; Knoth et al. 2013; Chapman et al. 2010). A single plant species, the shrub *Calluna vulgaris* (*C. vulgaris*), dominates most British moorland environments and is highly vulnerable to climate change (Gordon et al., 1999; Albert et al., 2011). Understanding the ecology, physiology, and biochemistry of *C. vulgaris* is crucial for protecting and managing moorland in the face of challenges such as poor management and climate change. However, this is complicated by the fact that *C. vulgaris* goes through four distinct life stages, each one having a distinct physiology, leaf biochemistry, and hyperspectral reflectance, which are much less known.

Hence, Chapter 4 is carried out at the Bradfield site, which is dominated by *C. vulgaris*, and has grown naturally for a long time. In addition, the work enhances understanding of the ecological variations among *C. vulgaris* life stages while investigating new ecological traits, the physiological and biochemical variations among growth stages, and also determine if hyperspectral reflectance from the leaf and canopy levels can be used to detect the different life stages.

Chapter 2

Interactive effects of elevated CO_2 and nutrient inputs on *Plantago lanceolata* physiology in a phosphorus (P) limited grassland

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2 Abstract

Grasslands are the most widespread terrestrial ecosystem in the world and are responsible for around 20% of worldwide terrestrial net primary productivity (NPP). Grasslands also represent the most abundant type of phosphorus (P)-limited ecosystem found in temperate zones. However, there is considerable uncertainty in how grasslands will physiologically respond to elevated CO_2 (eCO_2) and nutrient additions (N, P) in P-limited ecosystems due to a lack of empirical data. FACE (Free Air CO_2 Enrichment) experiments provide a unique opportunity to measure plant photosynthetic combined responses to eCO_2 and N and P. We investigated the biochemical and physiological acclimation of the common grassland forb, *Plantago lanceolata*, to eCO_2 (600 ppm) after 3 years of eCO_2 fumigation and Long-term (>25 years) of N and P treatments in a P-limited limestone grassland at the University of Sheffield's Bradfield Environmental Laboratory, within a mini-FACE experiment. In addition, we assess the ability of hyperspectral reflectance to discriminate between *Plantago lanceolata* acclimated to different CO_2 and nutrient treatments, and selected vegetation indices were regressed against measured leaf traits. We found out that eCO_2 significantly stimulated photosynthesis rate (A) by 14% on average and increased water use efficiency (WUE) by 34% compared to ambient CO_2 (aCO_2). Importantly, despite being a P-limited ecosystem, N addition combined with eCO_2 had the greatest effect on photosynthesis which alleviated the acclimation. Conversely, eCO_2 significantly reduced V_{cmax} by 15%, stomatal conductance (g_s) by 26%, leaf nitrogen content by 16%, and leaf chlorophyll content by 13%, compared to aCO_2. With nutrient addition, N increased leaf N and leaf chlorophyll, whereas P increased leaf P. Selected vegetation indices were regressed against measured leaf traits, among all treatments, the strongest relationship was found between leaf chlorophyll content and the MERIS terrestrial chlorophyll index (MTCI) ($R^2 = 0.92$). The strongest relationship was found between MTCI and leaf nitrogen concentration ($R^2 = 0.86$) out of all the indices examined. Our work, therefore, suggests that increased productivity of P-limited grasslands may be limited due to the acclimation of photosynthesis. Despite being a P-limited ecosystem, N addition combined with eCO_2 had the greatest impact on leaf physiology (leaf gas exchange), biochemistry and hyperspectral reflectance. These findings are crucial for comprehending how plants in P-limited ecosystems respond physiologically to eCO_2 and how eCO_2 and N/P nutrition availability impact plants' ability to sequester C in a high CO_2 in the future.

2.1 Introduction

Grasslands are the most widespread terrestrial ecosystem on the planet, existing on all continents except Antarctica (Ali et al. 2016), and contribute to approximately a fifth of global terrestrial net primary production (NPP) (Chapin 2011). Grasslands also represent the most prevalent phosphorus (P) limited habitat in temperate climates (Aspinall et al. 2011) and are vulnerable to nutrient cycle perturbations, including increases in atmospheric CO_2 concentrations (Lee et al. 2003; Zhang et al. 2011) and atmospheric nitrogen (N) deposition (Phoenix et al. 2012). However, to date, there is little understanding of how P-limited ecosystems, including grasslands, will respond to eCO_2 or how responses may be modified by nutrient inputs.

Anthropogenic activities have contributed to atmospheric CO_2 concentrations exceeding 420 ppm (Friedlingstein et al. 2019); the highest level of the last three million years (Willeit et al. 2019), and by the year 2100, atmospheric CO_2 concentration may more than double that of pre-industrial times (Collins et al. 2013). Furthermore, the global cycling of N has doubled since the outset of the industrial revolution (e.g., anthropogenic contributions to the annual global N cycle of 210 Tg of fixed N are significantly larger compared to natural sources by 7 Tg N yr^{-1}) (David et al. 2013). The impact of these changes in atmospheric CO_2 concentration and N deposition can cause a range of complex, multi-directional responses in plants, including photosynthesis, growth and leaf stoichiometry (Crous et al. 2019; Esmeijer-Liu et al. 2009), and fluctuation in biodiversity (Grünzweig and Körner 2001; Kleynhans et al. 2016; Reich 2009; Southon et al. 2013; Stevens, Dupr, et al. 2010). However, we have very little understanding of the impacts of eCO_2 and N availability, either individually or in combination, in ecosystems where productivity is limited by P. This is critical because up to 82% of global terrestrial productivity may be limited by P, either being P-limited or co-limited by N and P (Du et al. 2020).

Elevated CO_2 (eCO_2) has been widely observed to enhance plant photosynthesis rate (*A*), but decrease stomatal conductance (g_s) (Ainsworth and Long 2005, 2021), as well modifying stomata morphology (Haworth et al. 2016). Consequently, plants are able to maintain a greater leaf water content (Tausz-Posch et al. 2015; Wullschleger et al. 2002) and to improve water use efficiency (WUE) (Li et al. 2017; Pazzagli et al. 2016; da Silva et al. 2017; Yan et al. 2017). If there is a sufficient nutrient and water availability (Brodribb et al. 2020), this initial stimulation of net photosynthesis under eCO_2 can be retained during a longer-term exposure (Davey et al. 2006). For example, in a 10-year grassland FACE experiment, Ainsworth et al. (2003a) found that photosynthesis rates increased continuously with growth at eCO_2 and remained higher than at ambient CO_2. This occurred despite significant decreases in Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity, maximum carboxylation capacity (V_{cmax}) and maximum rate of electron transport (J_{max}), and a decline in stomatal conductance (g_s). Exposure to eCO_2 is expected to increase net photosynthesis (A_{net}), with the greatest photosynthetic enhancement expected under higher light conditions if the rate of Rubisco carboxylation is limited (Sage et al. 2008). In contrast, declines in A_{net} under eCO_2 have frequently been linked to nutrient supply constraints such as N, P and water content (Ainsworth and Rogers 2007a; Nowak et al. 2004). Because most of the leaf N is associated with proteins of the Calvin cycle and thylakoids, photosynthetic capacity is correlated with the leaf N content (Evans 2016; Reich et al. 1997). However, under eCO_2, a reduction in leaf N concentration has been observed, which can impact carbohydrate metabolic processes and leaf photosynthesis (Li et al. 2016; Sanz-Sáez et al. 2010; da Silva et al. 2017). This phenomenon has been attributed to N dilution by increased biomass and altered root structure or decreased root N absorption attributed to reduced transpiration-induced mass flow, caused by partial stomatal closure (Loladze 2002; Mcgrath and Lobell 2013; Taub and Wang 2008). Nitrogen use efficiency (NUE) is frequently observed to increase under eCO_2, resulting from the decreased N and increased C concentration in dry matter (Reddy et al. 2010). On the other hand, plants growing at eCO_2 for longer periods (months to years) are often observed to acclimate to eCO_2 by decreasing the photosynthesis rate (Ainsworth and Rogers 2007b). The extent to which plants acclimate under eCO_2 varies between plant functional types and according to the photosynthetic pathway (Ainsworth and Long 2005; Pastore et al. 2019). In addition, leaf P can constrain photosynthesis and modify the relationship of N with photosynthesis. (Reich et al. 2009) showed that across biomes, plants with low leaf P have a low A_{max} and reduced sensitivity of A_{max} to leaf N. Similarly, (Walker et al. 2014) showed in a global analysis that decreasing leaf P decreased the sensitivity of V_{cmax} to leaf N.

Monitoring any climate-induced biophysical or physiological changes in leaf traits is important for accurately modelling plant performance over a range of spatial and temporal scales (Gamon et al. 2019; Sun et al. 2023; Varvia et al. 2018). Hyperspectral remote sensing techniques offer the potential for precise, rapid, and scalable evaluation of the dynamic biophysical changes that may occur as a result of changes in atmospheric CO_2 concentration or nutrient availability (Eitel et al. 2014; Liu et al. 2021). Leaf reflectance is controlled by the presence of foliage pigments such as chlorophyll, N, and water content (Ustin et al. 2004). Various methods exist for modelling these leaf traits from remotely sensed data, including spectral vegetation indices and radiative transfer models (Croft et al. 2017; Heenkenda et al. 2015; Main et al. 2011). However, the efficacy of hyperspectral techniques for detecting plant acclimation to eCO_2 conditions and long-term nutrient additions N and P in a P-limited ecosystem have yet to be investigated.

We, therefore, explore the biochemical and physiological acclimation of a common grassland forb *Plantago lanceolata* to eCO_2 and variable N and P treatments in a P-limited limestone grassland within a novel FACE experiment. This paper aims to investigate the physiological and biochemical response of *Plantago* to eCO_2 after 3 years of eCO_2 exposure and, additionally, how long-term (>25 years) nutrient additions of N and P impact leaf response to eCO_2. In addition, to investigate the ability of hyperspectral reflectance to distinguish between *Plantago lanceolata* acclimation to different CO_2 and nutrition treatments (N and P) and if the adjustments in leaf physiological and biochemical traits in response to CO_2 and nutrient treatment can be quantified remotely via hyperspectral reflectance, using vegetation indices against measure leaf traits. Thus, this study addressed the following research questions: (i) Does atmospheric CO_2 enrichment stimulate plant photosynthesis after 3 years of eCO_2 exposure in a P-limited ecosystem? (ii) How do N deposition and P enrichment affect plant photosynthesis and leaf biochemistry content in a P-limited ecosystem? (iii) Can changes in leaf biochemical and physiological traits resulting from the different eCO_2 and nutrient treatments be detected using hyperspectral reflectance?

2.2 Methods

2.2.1 Field experiment

The research was conducted at the University of Sheffield, Bradfield Environmental Laboratory (53°25N 1°35W), 390 m above sea level, with mean annual air temperatures of 10.35 ^{o}C (Fick and Hijmans 2017). The mean annual precipitation is 893 mm (Fick and Hijmans 2017). In 2017, grassland mesocosms of intact soil-turf monoliths (35 cm by 35 cm) were extracted from a long-term nutrient addition experiment established in 1995 at Wardlow Hay Cop (Johnson et al. 1999) in the Peak District, UK (53°15'N 1°44'W). Grassland monoliths were then transferred to the Bradfield Environmental Laboratory and set up within the mini-FACE experiment (Fig. 2.1).

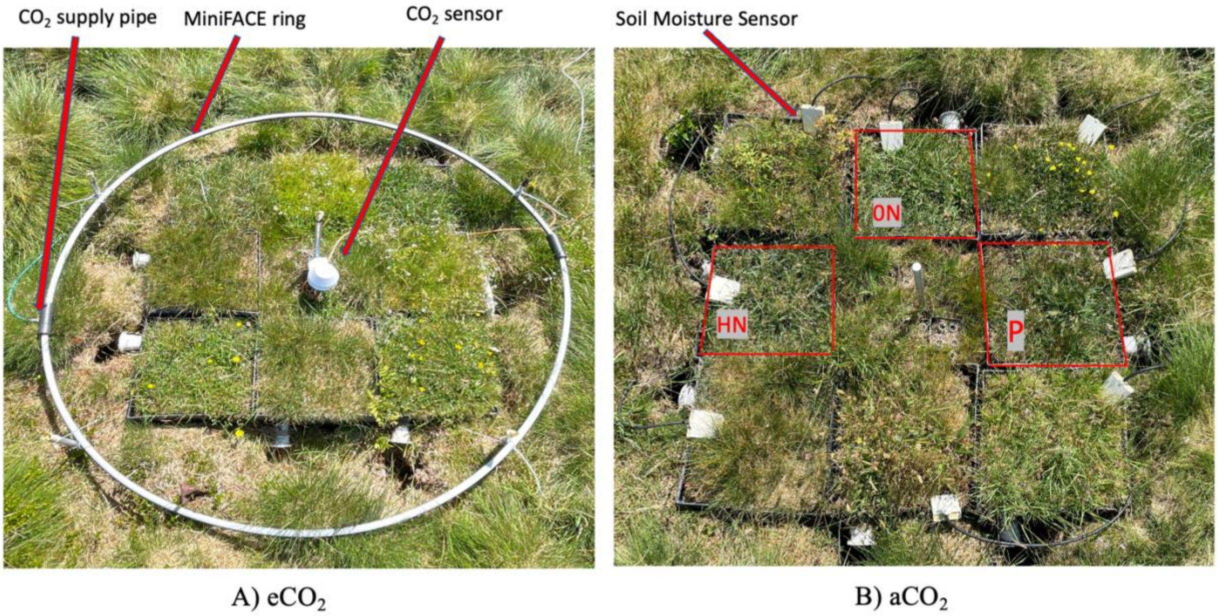

Figure 2.1: Experimental set-up: (A) elevated CO_2 ring (eCO_2) (at 600 ppm) and (B) ambient CO_2 (aCO_2). Both have eight grassland mesocosms. Highlighted limestone grassland mesocosms in (B) show those used in this study (0N, HN, and P). There are 8 mesocosms in total because the full experiment (Keane et al. 2020) also has a low-N treatment, and acidic grassland mesocosms not used in this study.

The mesocosms were exposed to either ambient CO_2 (aCO_2, approximately 410 ppm) or elevated CO_2 (eCO_2, 600 ppm) (Keane et al. 2020; Miglietta et al. 2001). Within each CO_2 ring, the mesocosms also receive one of three nutrient treatments, added as solutions of distilled water and applied as fine spray using a pumped hand-held mister: i) Zero Nitrogen (0N) - distilled water control; ii) High Nitrogen (HN) - NH_4NO_3 applied at 14 g N m^{-2} yr^{-1}; and iii) Phosphorus (P) - NaH_2PO_4 applied at a rate of 3.5 g P m^{-2} yr^{-1} (Keane et al. 2020; Morecroft et al. 1994). This study focussed on a common and widespread grassland forb, *Plantago lanceolata*, which was also the most abundant forb in the mesocosms. Paired measurements of gas-exchange, hyperspectral reflectance and leaf biochemical analyses were conducted on the same leaves from each plant.

2.2.2 Gas-exchange measurements

Gas exchange (photosynthesis rate (*A*), stomatal conductance (g_s), maximum carboxylation capacity (V_{cmax}) and maximum rate of electron transport (J_{max}), and CO_2 response curves; ACi) were measured using a portable photosynthesis system (LI-6800, LI-COR, Inc., Lincoln, NE, USA) with a 6 cm^2 leaf chamber. CO_2 curves were taken at the following concentrations 400, 300, 200, 100, 50, 400, 400, 600, 800, 1000, 1200, 1500, and 1800 ppm. Before commencing measurements, the leaves were acclimated inside the leaf chamber for approximately 20–40 min until steady-state conditions were reached, measurements were performed on one representative leaf per mesocosm. Leaf gas exchange was measured between 11:00 am to 14:00 pm; this is for accurate measurement as the plants are at their maximum levels of activity. For all measurements, PAR was maintained at 1600 µmol m^{-2} s^{-1} via red (680 nm) and blue (430 nm) LEDs integrated into the leaf chamber fluorometer (LI6800). Photosynthesis rate measurements were taken at 400 ppm on ambient CO_2 (aCO_2) plots and at 600 ppm on eCO_2 plots. The chamber temperature was set to 25 ^{o}C. Leaf area was calculated using ImageJ (1.23a, Wayne Rasband, NIH, USA). V_{cmax} and J_{max} were modelled using the Plantecophys package (Duursma 2015) via R (R Studio, R Core (Team 2021). Instantaneous water use efficiency (WUE, mmol mol^{-1}) was determined as the ratio of A (µmol m^{-2} s^{-1}) to transpiration rate (T_r) (mmol m^{-2} s^{-1}) (Hatfield and Dold 2019). Specific leaf area (SLA) was calculated as the ratio of leaf area to leaf dry mass (cm^2 g^{-1}) (Vile et al. 2005).

2.2.3 Biochemistry measurements

A single leaf of each mesocosm was sampled (the same leaf used for gas exchange measurements) and kept at ~0 °C in dark conditions during transport back to the University of Sheffield (~10km away) for laboratory analysis. To extract leaf chlorophyll, three equally sized leaf samples (leaf disks) were taken using a hole puncher with a 6-mm diameter (28.3 mm^2). Each leaf sample was then immediately transferred into N, N-dimethylformamide (DMF) for foliar chlorophyll extraction following the methods of (Moran 1982), and the absorbance was subsequently measured at 663.8, 646.8 and 480 nm (Wellburn 1994) using a Spectrophotometer (Cecil CE1020 100 series spectrophotometer, Spectronic). Leaf N, C and P concentrations were determined from fresh leaves (Due to the restriction on the amount of leaf material for nutrient analysis, Leaf N and P analysis were collected on adjacent plants from the same mesocosm) that were oven dried at 80 ^{o}C prior to grinding by tissue-lyser (FastPrep-24TM 5G bead beating grinder and lysis system, MP biomedical). To assess tissue concentrations of N, C, and P, approximately 2 – 4 mg of finely ground plant material was placed inside a tin capsule and sealed with tweezers for subsequent analysis by an isotope-ratio mass spectrometer (ANCA GSL 20-20 Mass Spectrometer, Sercon PDZ Europa).

2.2.4 Hyperspectral reflectance measurements

A Spectral evolution PSR+ 3500 field spectrometer (Spectral Evolution Inc., MA, USA) was used to measure leaf-level hyperspectral reflectance from each mesocosm (the same leaf of gas exchange measurement and leaf biochemistry). The PSR+ 3500 has a spectral range of 350-2500 nm and a spectral resolution of 2.8 nm at 700 nm. Leaf level reflectance was measured on individual leaves using a leaf clip attachment with an internal light source. Spectral on panel (Spectral Evolution Inc., MA, USA) was used as a calibration reference panel to convert reflected radiance to reflectance. Several spectral vegetation indices were selected according to their sensitivity to different structural, biochemical, or physiological traits (Table 2.1).

Table 2.1: Details of all published spectral vegetation indices tested in this study.

Index	**Name**	**Equation**	**Reference**
MTCI	MERIS terrestrial chlorophyll index	(R754 − R709)/ (R709 − R681)	(Dash and Curran 2004)
NDRE	Normalized Difference red edge index	(R790−R720)/(R790+R720)	(Fitzgerald et al. 2006)
MNDVI1	Modified NDVI	(R755-R745)/ (R755 + R745)	(Mutanga and Skidmore 2004)
MNDVI8	Modified NDVI	(R755-R730)/ (R755 + R730)	(Mutanga and Skidmore 2004)
RVII	Ratio Vegetation Index I	R810/R660	(Zhu et al. 2008)
RVIII	Ratio Vegetation Index II	R810/R560	(Xue et al. 2004)
$CI_{red\ edge}$	Red edge chlorophyll index	(NIR/Red Edge) − 1	(Gitelson et al. 2005; Gitelson et al. 2003)
RWC	Relative Water Content	(R1451/ R1263)	(Yu et al. 2000)
P_1080_1460	Phosphorus index	(R1080 - R1460)/ (R1080 + R1460)	(Pimstein et al. 2011)
P_1645_1715	Phosphorus index	(R1080 - R1460)/ (R1080 + R1460)	(Pimstein et al. 2011)
P_2015_2195	Phosphorus index	(R2015- R2195)	(Mutanga and Kumar 2007)
P_550_750	Phosphorus index	((R550- R750)	(Mutanga and Kumar 2007)
WBI	Water Band Index	(R970/ R900)	(Penuelas et al. 1993)
WCI	water content index	(R686- R955)/ (R955- R548)	(Mertens et al. 2021)
WPI	Water Poverty Index	(R665 - R715)/ (R715)	(Mertens et al. 2021)

2.2.5 Statistical analysis

Statistical analyses were carried out using R (R Studio, R Core (Team 2021)). Two-way ANOVA models were used to assess the main factor effects and interactions of CO_2 and nutrient treatment. If there were significant overall differences among treatment groups, the Tukey multiple comparison of means was used to determine between which pairs of treatment differences occurred. The (userfriendlyscience) (Peters 2018) and (spectrolab) (Jose et al. 2023) packages were used for reflectance data analysis for calculating both the indices and for determining whether the life stages had different reflectance features. (lm) function was used to calculate the linear regression among leaf physiology and biochemistry. All the plots were performed using the (ggplot2) function (Wilkinson 2011).

2.3 Results

2.3.1 Leaf gas exchange, leaf biochemistry and water use efficiency (WUE)

The effect of CO_2 and nutrient treatments and the interactions between CO_2 and nutrient treatments on *Plantago lanceolata* biochemistry and physiological parameters are shown in Figure 2.2 and S.2.1.

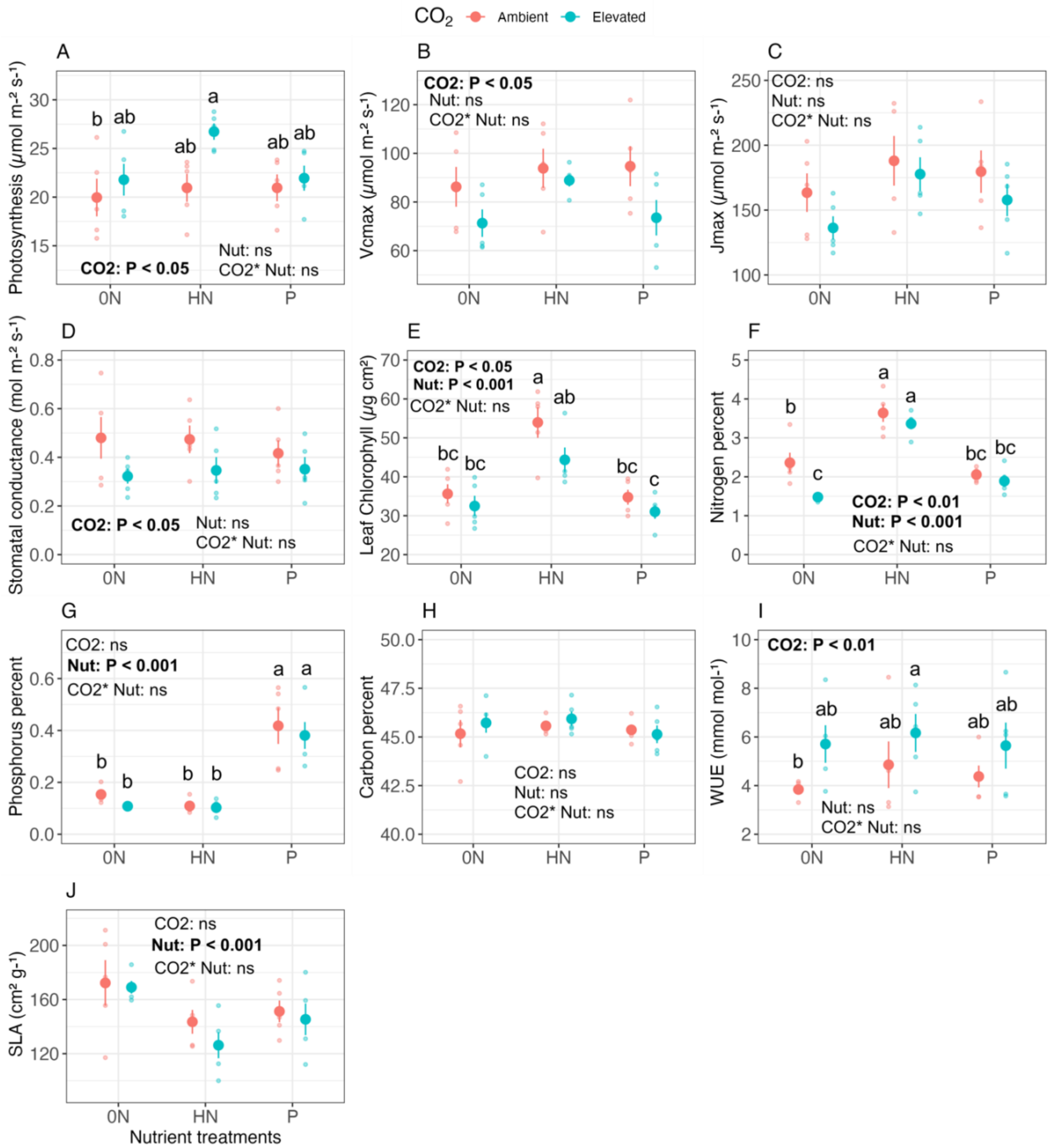

Figure 2.2: Effects of CO$_2$, and nutrient treatments on A) Photosynthesis rate (*A*), B) Maximum carboxylation capacity V_{cmax}, C) maximum rate of electron transport J_{max}, D) Stomata conductance (g_s), E) leaf chlorophyll content, F) leaf nitrogen content, G) leaf phosphorus content, H) leaf carbon content, I) Water use efficiency (WUE), and J) Specific leaf area (SLA). Large points with standard error bars indicate the mean, and the smaller points are individual data points. Overall main factor effects of CO$_2$ and nutrients and their interactions are indicated in each graph (2-way ANOVA). Pairs of treatments that do not share a letter are significantly different (Tukey, $P < 0.05$).

Elevated CO_2 (eCO_2) significantly increased the photosynthesis rate (*A*) by 14% compared to aCO_2 (Fig. 2.2A; *F* = 5.814, *df* = 1, 24, $P < 0.05$), and water use efficiency (WUE) by 34% compared to aCO_2 (Fig. 2.2I; *F* = 6.088, *df* = 1, 24, $P < 0.05$). Interestingly, photosynthesis was higher in eCO_2-HN than in all other treatments. On the other hand, in comparison to aCO_2, eCO_2 significantly reduced V_{cmax} by 15% (Fig. 2.2B; *F* = 5.800, *df* = 1, 24, $P < 0.05$), stomatal conductance (g_s) by 26 % (Fig. 2.2D; *F* = 6.211, *df* = 1, 24, $P < 0.05$), leaf chlorophyll content by having 13 % less leaf chlorophyll (Fig. 2.2E; *F* = 5.956, *df*= 1, 24, $P < 0.05$), and leaf nitrogen content by having 16 % less leaf nitrogen content (Fig. 2.2F; *F* = 10.417, *df*= 1, 24; $P < 0.01$). However, CO_2 treatment had no significant effect on J_{max} (Fig. 2.2C; *F* = 2.789, *df* = 1, 24; $P > 0.05$), leaf phosphorus content (Fig. 2.2G; *F* = 0.979, *df* = 1, 24, $P > 0.05$), leaf carbon content (Fig. 2.2H; *F* = 0.399, *df* = 1, 24; $P > 0.05$), and specific leaf area (SLA) (Fig. 2.2J; *F* = 1.032, *df* = 1, 24, $P > 0.05$).

Among nutrient treatments, the HN treatment significantly increased leaf chlorophyll and leaf nitrogen content compared to the control 0N and P treatments, with no significant differences between 0N and P treatments. (Fig. 2.2E; *F* = 21.686; *df* = 2, 24; $P < 0.001$; and Fig. 2.2F; *F* = 57.629; *df* = 2, 24; $P < 0.001$), but significantly reduced SLA compared to the control 0N, with no significant differences between 0N and P treatments (Fig. 2.2J; *F* = 5.802; *df* = 2, 24; $P < 0.01$). The P treatment significantly increased leaf phosphorus content compared to the control 0N and HN treatments (Fig. 2.2G; *F* = 19.013; *df* = 2, 24; $P < 0.001$). Interestingly, nutrient treatments had no significant effect on leaf gas exchange, such as photosynthesis rate, V_{cmax}, J_{max}, and stomatal conductance (g_s), and had no significant effect on leaf carbon content and WUE (Figure 2.2).

There were no significant interactions between CO_2 and nutrient treatments in leaf gas exchange, leaf biochemistry, WUE, and SLA (Figure 2.2).

2.3.2 Regression between leaf gas exchange and leaf nutrients

Leaf chlorophyll content showed a positive relationship with photosynthesis for all treatments (Fig. 2.3A). Interestingly, HN under eCO_2 showed a higher rate of photosynthesis with a similar amount of leaf chlorophyll compared to HN under aCO_2 (Fig. 2.3A).

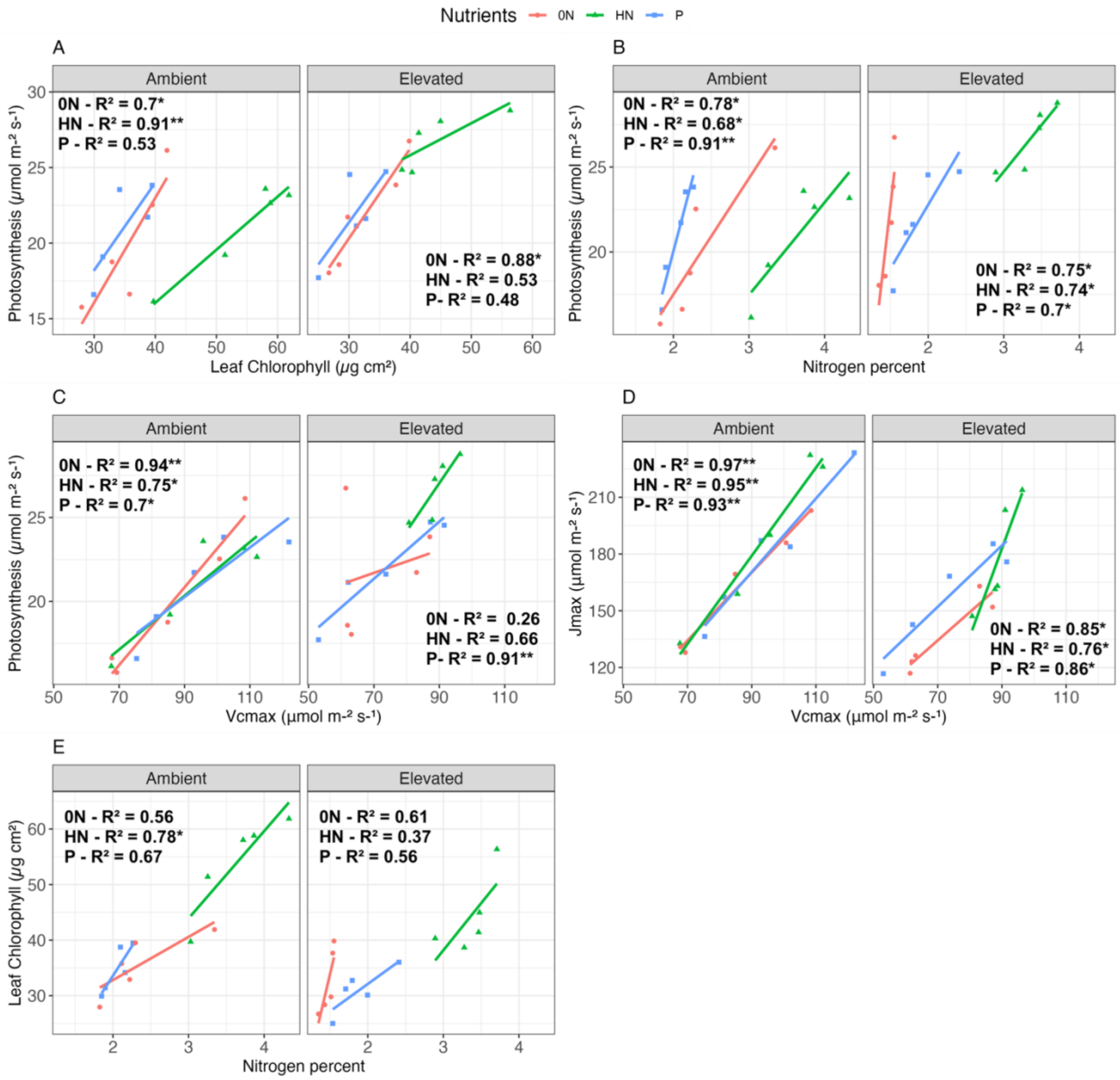

Figure 2.3: Regression analysis between photosynthesis rate and A) leaf chlorophyll (overall regression coefficient of $R^2 = 0.203$; $p < 0.01$), B) leaf nitrogen content (overall regression coefficient of $R^2 = 0.214$; $p < 0.05$), C) V_{cmax} (overall regression coefficient of $R^2 = 0.287$; $p < 0.01$), D) between V_{cmax} and J_{max} (overall regression coefficient of $R^2 = 0.861$; $p < 0.001$), and E) between leaf chlorophyll and leaf nitrogen content (overall regression coefficient of $R^2 = 0.778$; $p < 0.001$), * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

Leaf nitrogen content and V_{cmax} showed positive relationships with photosynthesis (Fig. 2.3B and 3C). V_{cmax} was lower under eCO_2 compared to aCO_2, and V_{cmax} under eCO_2 showed high photosynthesis with lower amount of V_{cmax}. Also, the strong relationship between V_{cmax} and J_{max} was apparent as V_{cmax} showed a positive relationship with J_{max} (Fig. 2.3D); also, V_{cmax} and J_{max} were less under eCO_2, and the slope gradient of HN under eCO_2 showed a slight increase compared to other treatments. Leaf chlorophyll content showed a positive relationship with leaf nitrogen content (Fig. 2.3E); Overall, N additions increased leaf chlorophyll content, although the greatest increase occurred under aCO_2, where chlorophyll concentrations are at their highest. In addition, leaf chlorophyll content was also lower in eCO_2 than in aCO_2.

2.3.3 Hyperspectral reflectance signatures for Plantago lanceolata in different CO_2 and nutrition treatments

The ability of hyperspectral reflectance to discriminate between *Plantago lanceolata* acclimated to different CO_2 and nutrient treatments was investigated (Figure 2.4). eCO_2_HN showed a higher reflectance in the visible and infrared regions compared to aCO_2_HN (Fig. 2.4A and 2.4B), and the HN treatment showed the lowest reflectance under aCO_2 compared to aCO_2 0N and P (Fig. 2.4A). Interestingly, HN treatments showed a variation in reflectance under the different CO_2 treatments. The P treatment remained the same under aCO_2 and eCO_2; also, 0N almost remained the same under aCO_2 and eCO_2 with a slightly higher reflectance in the peak of visible light under eCO_2 (Fig. 2.4A and 2.4B), also, 0N and P separate in the green peak and after 1400 nm for aCO_2 but not for eCO_2.

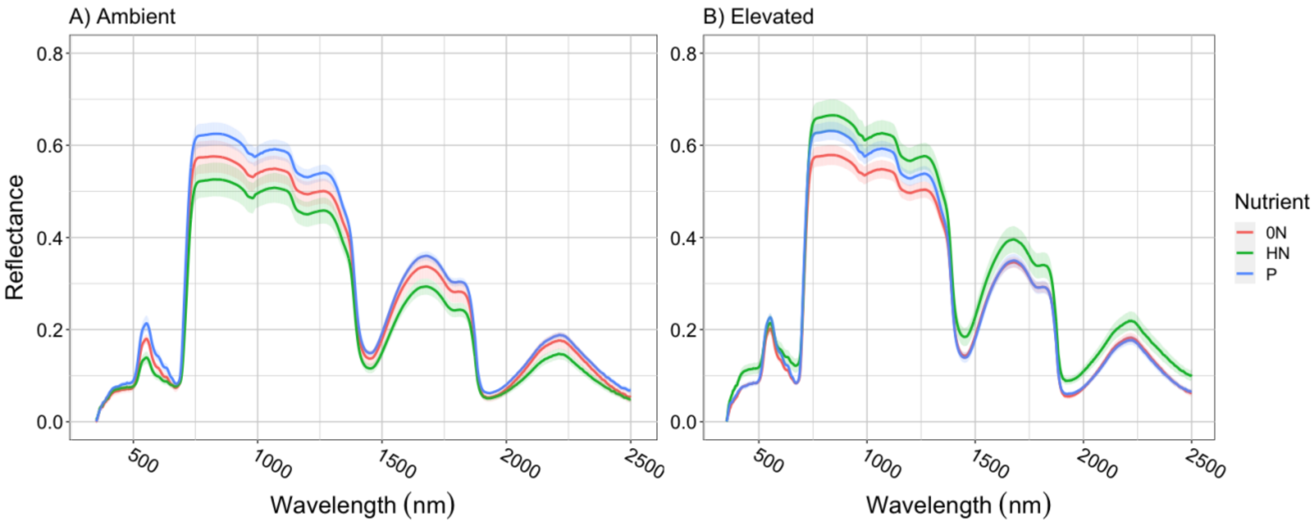

Figure 2.4: Spectral reflectance among CO_2 treatments and nutrient treatments. (A) The reflectance of the nutrient treatments under aCO_2 for all the reflectance regions; (B) The reflectance of the nutrient treatments under eCO_2 for all the reflectance regions.

2.3.4 Leaf chlorophyll, leaf nitrogen, and leaf phosphorus content and vegetation indices at the leaf level

To investigate if the adjustments in leaf physiological and biochemical traits in response to CO_2 and nutrient treatment (seen in Figure 2.2) can be quantified remotely using hyperspectral reflectance, selected vegetation indices are regressed against measured leaf traits (Figure 2.2 and S.2.3).

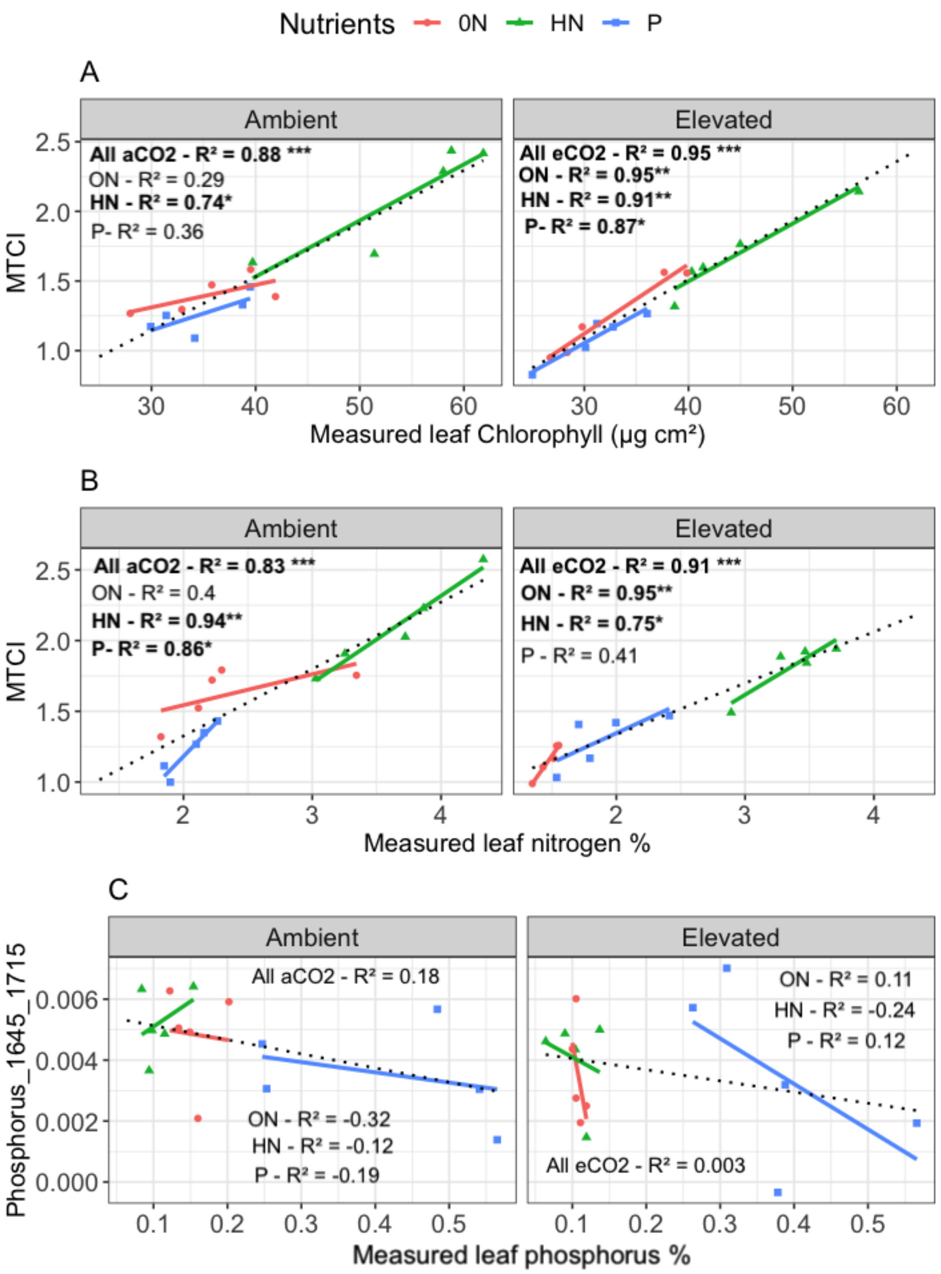

Figure 2.5: VIs at the leaf level for (A) MERIS terrestrial chlorophyll index (MTCI; R^2 = 0.92, $p < 0.001$); B) MERIS terrestrial chlorophyll index (MTCI; R^2 = 0. 85, $p < 0.001$); C) P_1645_1715 phosphorus index (P_1645_1715; R2 = 0.11, $p > 0.05$). R^2 values for linear trend lines are shown on each plot. * and ** with bold indicate significance at $p < 0.05$ and $p < 0.01$, respectively. The dotted lines represent the overall regression among each CO$_2$ treatment.

For all treatments together, leaf chlorophyll content had the strongest relationship with MERIS terrestrial chlorophyll index (MTCI), with a regression coefficient of $R^2 = 0.92$ ($p < 0.001$) (Fig. 2.5A). From all the indices tested, leaf nitrogen content also presented the strongest relationship with MTCI; $R^2 = 0.86$, $p < 0.001$) (Fig. 2.5B). Repetition of four spectral indices were sensitive to leaf phosphorus four indices were tested; however, none of the indices displayed a significant relationship with the measured phosphorus content. The strongest one was P_1645_1715, with a regression coefficient of $R^2 = 0.11$ ($p > 0.05$) (Fig. 2.5C).

2.4 Discussion

2.4.1 Summary of results

In this study of P-limited grassland using the common forb *Plantago lanceolata* as a study species, we have determined how leaf gas exchange and biochemistry respond to elevated atmospheric concentrations of CO_2 and nutrient enrichment in a P-limited grassland. We found out that, after three years of eCO_2 fumigation stimulated photosynthesis rate (*A*) by 14% on average and increased water use efficiency (WUE) by 34% compared to ambient CO_2 (aCO_2). On the other hand, eCO_2 significantly reduced V_{cmax} by 15%, stomatal conductance (g_s) by 26 %, leaf nitrogen content by 16 %, and leaf chlorophyll content by 13 % less compared to aCO_2. In addition, HN treatment significantly increased leaf chlorophyll and leaf nitrogen content compared to the control 0N and P treatments, and P treatment significantly increased leaf phosphorus content compared to the control 0N and HN treatments.

2.4.2 Effects of eCO_2 and nutrient treatment on photosynthesis and leaf gas exchange: N addition alleviates photosynthetic acclimation to eCO_2

In this study, after three years of CO_2 enrichment, we observed an increase of photosynthesis rate (*A*) under eCO_2, by 14%. This photosynthesis increase is attributed to a higher rate of carboxylation and a reduction of the rate of the competing oxygenation reactions in Rubisco due to the increased CO_2 concentration (Long et al. 2004). This increase in photosynthesis under eCO_2 is consistent with previous studies, including a meta-analysis where eCO_2 was found to increase photosynthesis by 28% on average among FACE experiments (Ainsworth and Long 2005, 2021). A longer-term grassland FACE study on *Lolium perenne* observed that the photosynthesis rate increased by an average of 36% over a 10-year period, despite 18%

decreases in V_{cmax}, 9% decrease in J_{max} and a 30% decline in g_s (Ainsworth et al. 2003b). However, our findings demonstrated a lower photosynthetic response with eCO_2 in comparison to other studies and model predictions, which could be due to photosynthesis acclimation after three years of eCO_2 exposure. Photosynthesis acclimation occurs as a result of the suppression of Rubisco gene expression by nonstructural carbohydrate accumulation, which is accelerated under eCO_2 (Byeon et al. 2021; Jacob et al. 1995). Therefore, in this study, when the plants grown at aCO_2 and eCO_2 were both measured at the eCO_2 concentration (600 ppm), there was a 15% decrease in the photosynthesis rate of eCO_2 plants compared to aCO_2 (Fig. S.2.2). In addition, in our results, photosynthetic acclimation was evident, with a significant decrease in Rubisco activity (V_{cmax}) of 15% at 600 ppm for eCO_2 plants compared to aCO_2 plants. The reduction in V_{cmax} at eCO_2 is attributed to Rubisco reduction, consistent with many other studies that have shown submaximal photosynthetic responses in plants acclimated to eCO_2 may be caused by lower V_{cmax} if photosynthesis becomes more limited by ribulose1,5bisphosphate (RuBP) regeneration and less by Rubisco as CO_2 concentration increases (Ainsworth and Long 2005; Ainsworth and Rogers 2007a; Maier et al. 2008; Pastore et al. 2019; Rogers and Humphries 2000; Tissue et al. 1999). While J_{max} was not statistically significantly affected by CO_2, it followed the same trend as V_{cmax} and was consistent with the N addition. Ainsworth and Rogers (2007) also showed that V_{cmax} is reduced by about twice that of J_{max} in response to long-term eCO_2. These photosynthesis and leaf gas exchange findings suggest that past findings may also apply to P-limited ecosystems even though they have been studied little.

Interestingly, despite being P-limited grassland, N addition had a bigger effect on photosynthesis than P addition under eCO_2 (Fig. 2.2A). Previous studies have also suggested that photosynthesis acclimation to eCO_2 is alleviated at high N concentration (Ainsworth and

Long 2005; Ruiz-Vera et al. 2017). This is because, under eCO_2 conditions, Rubisco suppression is related to leaf N reallocation from a non-limited source to other limiting photosynthetic components, for instance, electron transport enzymes or the light-harvesting complex (Evans 1989; Kanno et al. 2017). This shift in N allocation can give a chance to improve photosynthetic N usage efficiency (Leakey et al. 2009), which is defined as the net C assimilation rate per unit leaf N and relates to Rubisco's increased efficiency under eCO_2 due to the suppression of photorespiration (Gifford et al. 2000; Wujeska-Klause et al. 2019). Additionally, in P-limited ecosystems, N addition can reduce P limitation due to the stimulation of phosphatase enzymes or dissolution of mineral P (Chen et al. 2020; Wang et al. 2021).

Stomata conductance g_s and water use efficiency WUE

In our study, eCO_2 reduced g_s by 26 % on average, attributable to the increase in intercellular CO_2 concentration under eCO_2 sensed by guard cells to maintain intercellular CO_2 between 20 to 30% lower than the CO_2 concentration at the leaf surface (Ainsworth & Rogers, 2007), also due to g_s decreased with eCO_2, WUE increased by 34%. Other FACE experiments have also shown an eCO_2 decrease in g_s by 22%, which allows plants to conserve water and increase water use efficiency (Chater et al. 2015). Over longer time periods of eCO_2, new leaves may develop with lower stomatal densities (Gray et al. 2000). In addition, g_s reduction is one of the primary findings which indicate photosynthesis acclimation to eCO_2, consistent with other studies (Pastore et al. 2019) and shows that *Plantago lanceolata* has undergone photosynthetic acclimating to eCO_2 after three years exposure, which in turn could potentially limit C sequestration in response to eCO_2. Our findings suggested that past findings from different ecosystems may also apply to P-limited ecosystems even though they have been studied little.

2.4.3 Effects of eCO_2 and nutrient treatment on biochemistry and leaf traits

Across all nutrient treatments, leaf nitrogen content was observed to decrease by 16 % on average under eCO_2 compared to aCO_2, and there are various potential mechanisms for eCO_2-induced N reduction (Taub and Wang 2008). These include a reduced demand for the N-rich Rubisco enzymes (Luo et al. 2021), a reduction in photorespiration, which leads to a decrease in the reductant supply necessary for NO_3 reductase activity (Wujeska-Klause et al. 2019); or a decrease in transpiration, which results in a decrease in the mass flow of nitrogen (Taub and Wang 2008). Interestingly, our findings on leaf N are consistent with a number of meta-analyses involving a large number of N-limited ecosystems (Du et al. 2019; Wang et al. 2021; Xu et al. 2020). In addition, across all nutrient treatments, leaf chlorophyll content was observed to decrease on average by 13% under eCO_2. This is attributable to the reduction in leaf N by 16% under eCO_2 and by the reduction in Rubisco (Loladze 2002; Mcgrath and Lobell 2013; Taub and Wang 2008). In addition, as expected, N addition increases the leaf chlorophyll concentration due to N being an essential element in the chlorophyll (Evans 1989; Marschner 2011; Taiz et al. 2015).

SLA was unaffected by CO_2, under eCO_2, in general, any reallocation of biomass to thicker leaves or additional carbohydrate storage in the leaves would tend to increase leaf mass more than leaf area, therefore decreasing SLA. This finding suggests that the additional carbohydrate has been stored elsewhere, possibly by allocating the C below ground as organic acids and root exudates to enhance the nutrient capture (Ma et al. 2022). However, N enrichment reduced the SLA, attributable to the N enrichment increase in leaf chlorophyll alleviating photosynthesis acclimation.

2.4.4 Effects of CO_2 and nutrient treatment on leaf-level hyperspectral reflectance

The changes in the leaf-level hyperspectral reflectance among eCO_2 and aCO_2, driven by the alterations in leaf physiological and biochemical traits of *Plantago lanceolata*, that are affected by CO_2 treatment (Cha et al. 2017; Zheng et al. 2020). eCO_2 can change leaf water content due to the stomata closure, which leads to a higher water use efficiency in plants, as it was also detected in our results, meaning they lose less water per unit of carbon gained (Ainsworth and Rogers 2007b; Nelson et al. 2004). As a result, leaves might have higher water content under eCO_2 (Tausz-Posch et al. 2015; Wullschleger et al. 2002). As water also affects the light absorption and reflectance properties of leaves, changes in leaf water content could influence the hyperspectral reflectance, especially in SWIR water absorption bands around 1450, 1940, and 2500 nm (Junttila et al. 2022; Kovar et al. 2019). Therefore, as leaf water content increases, absorption increases, and reflectance decreases. In addition, eCO_2 levels can also change in leaf structure, which leads to changes in the physical structure of leaves, making them thicker and denser (Prior et al. 2004). This is because eCO_2 often promotes growth and leads to a higher rate of carbon assimilation (Ainsworth and Long 2005, 2021). This increase in leaf thickness and density can alter the light-scattering properties of leaves, which can influence reflectance patterns, particularly in the near-infrared part of the spectrum (700-1300 nm), so thicker leaves scatter more NIR light and reflect more light back to the sensor, increasing reflectance (Stéphane Jacquemoud and Ustin 2019; Pauli et al. 2017). This is due to an increase in cell layers and air spaces that light can interact with. Also, eCO_2 can often lead to changes in the chlorophyll content of leaves, the changes in chlorophyll concentration can alter the reflectance patterns in the visible part of the spectrum, where chlorophyll absorbs visible light strongly, particularly in the blue (430-450 nm) and red (640-680 nm) wavelengths, with maximum absorbance between 660 and 680 nm, while reflecting green light (500-570 nm), with maximum reflectance in green wavelengths at (560 nm) (Clevers and Gitelson 2013;

Gitelson et al. 2003). In addition, eCO_2 can affect the production of other biochemicals like carotenoids or anthocyanins, which can also influence spectral reflectance, especially in the visible region of the spectrum, where carotenoids absorb light in the blue and blue-green regions of the spectrum (around 400-500 nm), and anthocyanins absorb light in the green region (around 500-550 nm), which can lead to lower reflectance in these wavelengths (Byeon et al. 2021). However, it is also important to consider that these changes might not always be consistent and can be influenced by other factors such as nutrient availability, plant species, and physiological conditions (Croft et al. 2013; Croft et al. 2014; Crous et al. 2019).

In this study, MTCI at the leaf-level hyperspectral reflectance showed a strong relationship with the measured leaf chlorophyll (Clevers and Gitelson 2013; Dash and Curran 2004, 2007). The ability to estimate chlorophyll content at the leaf and canopy levels is primarily due to chlorophyll's unique ability to absorb and reflect light. Importantly, chlorophyll also reflects light in the near-infrared (NIR) and shortwave-infrared (SWIR) regions of the electromagnetic spectrum. MTCI is a red-edge position-based index that utilizes the advantage of the distinct transition (red-edge) between red light, which chlorophyll strongly absorbs, and near-infrared light, which chlorophyll reflects (Clevers and Gitelson 2013; Dash and Curran 2004, 2007). This transition allows the index to capitalize on the distinct difference between these two types of light. MTCI tool assesses, in particular, the steepness of this red-edge transition, which has been found to be related to the chlorophyll concentration (Clevers and Gitelson 2013; Dash and Curran 2004, 2007). In addition, we can utilize the leaf chlorophyll index as an indicator of leaf nitrogen status because of the strong relationship between leaf chlorophyll and leaf nitrogen (Clevers and Gitelson 2013). As we found, MTCI showed a strong relationship with the measured leaf N in the lab (Clevers and Gitelson 2013; Dash and Curran 2004, 2007). The results were promising, suggesting that existing indices can be used to forecast leaf chlorophyll and N in P-limited ecosystems, providing useful indications of plant production and leaf biochemical condition.

aCO_2-HN showed the lowest leaf level reflectance in the visible wavelength region compared to all other treatments due to the greater leaf chlorophyll. The spectral band between 680 and 760 nm is the region in which the reflectance of vegetation changes sharply due to the transition from chlorophyll absorption in the red region to cell scattering in the near-infrared region (Dawson and Curran 1998). Also, in visible wavelengths, chlorophyll absorbs strongly in red and blue spectral regions, with maximum absorbance between 660 and 680 nm and maximum reflectance in green wavelengths (560 nm).

However, in this study, we tested the utility of hyperspectral reflectance to estimate the phosphorus concentration of *Plantago lanceolata* in a P-limited grassland using leaf-level hyperspectral reflectance data. While the remote sensing of foliar P has received very little attention compared to N and chlorophyll, it plays an important role within physiological processes, leaf biochemistry, and biodiversity in P-limited ecosystems (Mutanga and Kumar 2007; Mutanga et al. 2004; Osborne et al. 2002). Even though studies on field spectra have pinpointed numerous absorption characteristics linked to a variety of biochemicals (G. A. Blackburn 1998; Clevers and Gitelson 2013; Fitzgerald et al. 2006), our understanding of the significance of these absorption features, particularly their specific bands for predicting P, remains limited (Mutanga and Kumar 2007; Mutanga et al. 2004; Osborne et al. 2002). In simpler terms, there's a significant lack of knowledge about which parts of the electromagnetic spectrum are beneficial for forecasting P leaf concentration. Selecting the appropriate wavelengths is a critical process in developing a functional model for estimating leaf P levels using remote sensing data. Considering P received less attention, the indices we evaluated were weak compared to the N and Chlorophyll indices, and the results were unimpressive. In addition, we only had a small number of replication measurements, which made developing our own index challenging.

2.5 Conclusion

We found that after three years of eCO_2 exposure, eCO_2 stimulated photosynthesis, though to a lesser extent than often seen in other studies and model predictions, with evidence of clear photosynthesis acclimation to eCO_2. In addition, eCO_2 reduced V_{cmax} and g_s, which are also strong indicators for photosynthesis acclimation. Our work, therefore, suggests that increased productivity of P-limited grasslands may be limited due to the acclimation of photosynthesis. Furthermore, our work shows that responses in leaf photosynthesis and biochemistry to eCO_2 in P-limited grasslands are similar to those observed in other work that has often been conducted on N-limited ecosystems. Therefore, despite the limited amount of research on eCO_2 in P-limited ecosystems, understanding from N-limited ecosystems might be used in the absence of work in P-limited ecosystems when developing leaf photosynthesis models. Consistent with this, our work also shows that despite the grassland being P-limited, it was still N that had the greatest impact on photosynthesis, not P, extending further to P-limited ecosystems the strong control of N on photosynthesis compared to P. These findings are important for understanding the physiological responses of plants in P-limited ecosystems to eCO_2, and how their capacity to sequester C in a high CO_2 world will be impacted by that eCO_2 and by N and P nutrient availability.

2.6 Appendix

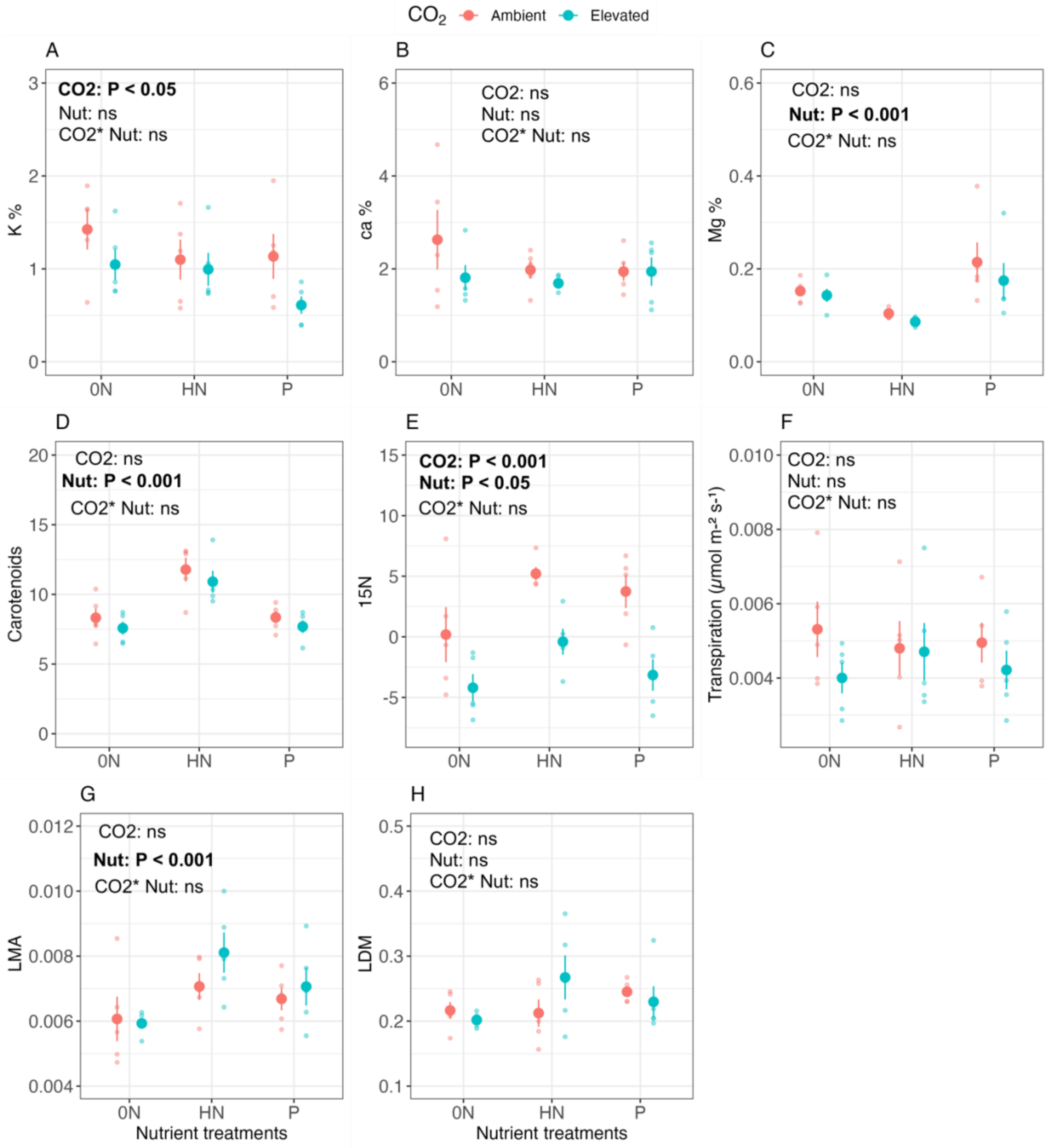

Figure S.2. 1: Effects of CO_2, and nutrient treatments on A) Potassium, B) Calcium, C) Magnesium, D) Carotenoids, E) 15N, F) Leaf transpiration, G) Leaf mass per area, H) leaf dry matter content. Large points with standard error bars indicate the mean, and the smaller points are individual data points. Overall main factor effects of CO_2 and nutrients and their interactions are indicated in each graph (2-way ANOVA).

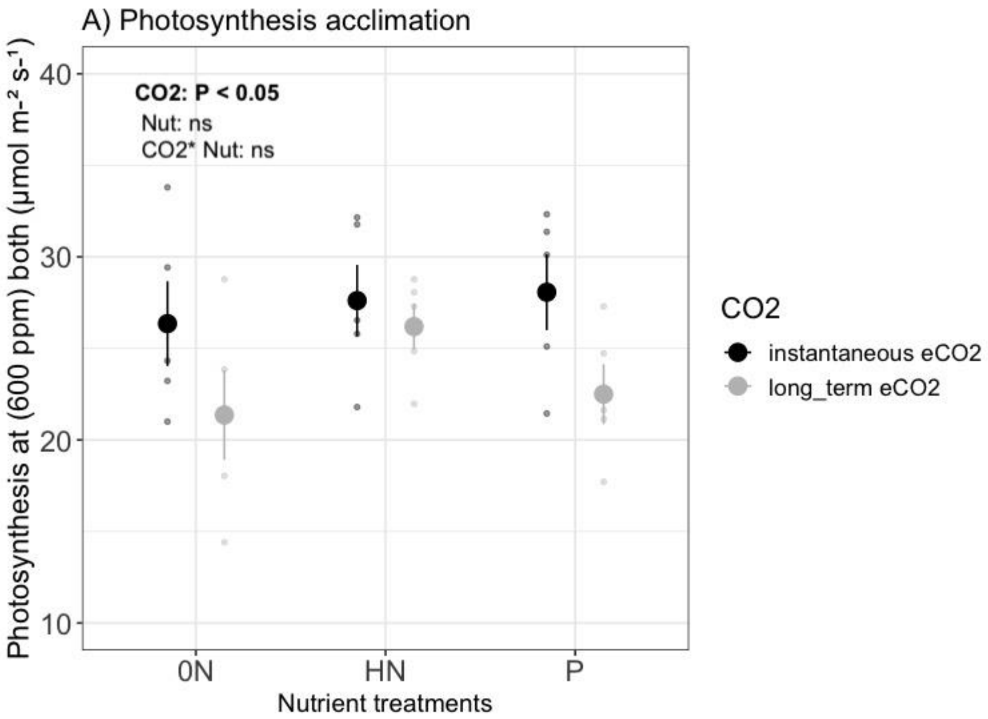

Figure S.2. 2: Effects of CO_2, and nutrient treatments on A) Photosynthesis rate (A), both aCO_2 and eCO_2 measured at eCO_2 concentration (600 ppm), where instantaneous means the plants grown at aCO_2, and long-term eCO_2 means plants grown at eCO_2. Large points with standard error bars indicate the mean, and the smaller points are individual data points. Overall main factor effects of CO_2 and nutrients and their interactions are indicated in the graph (2-way ANOVA).

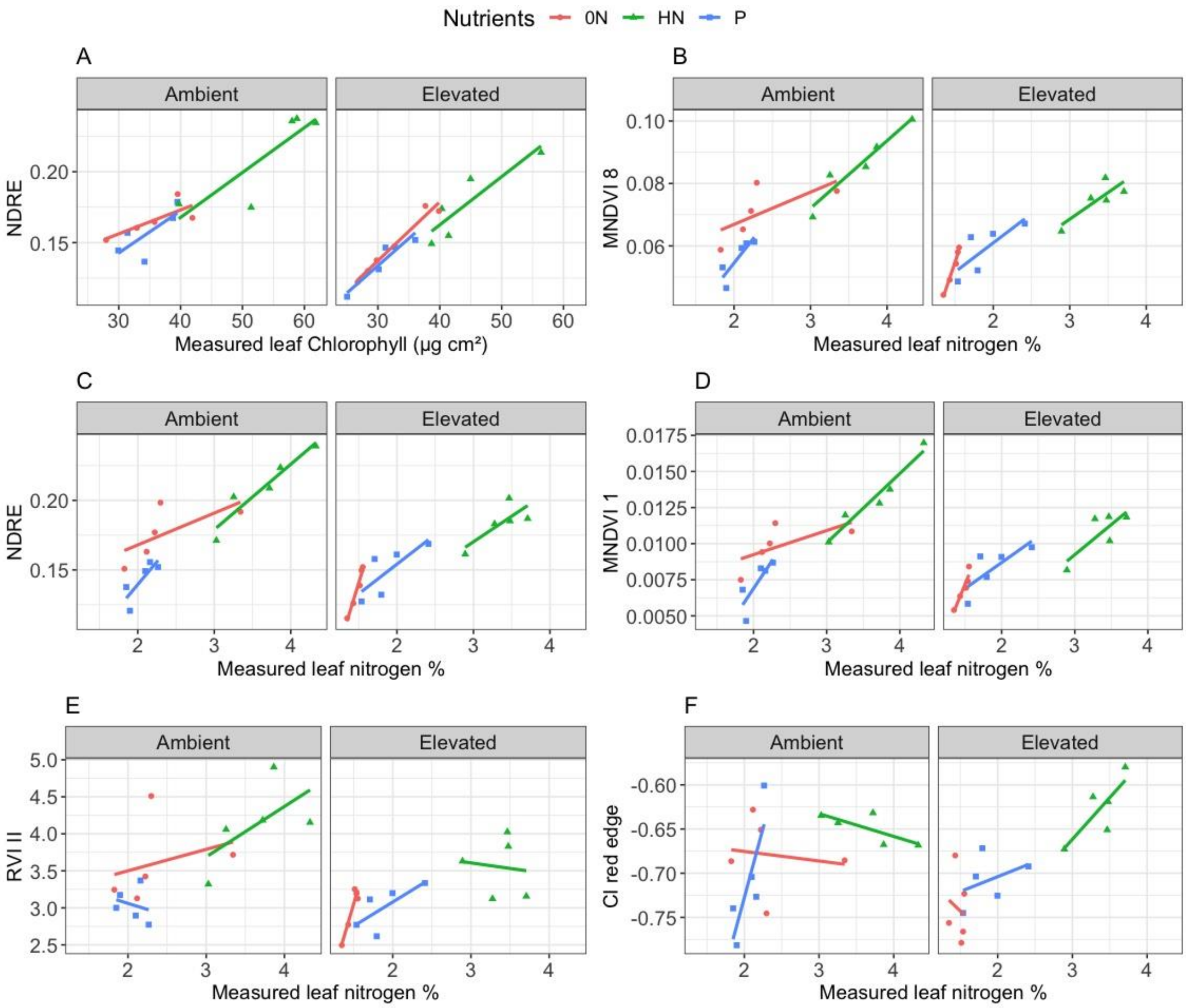

Figure S.2. 3: VIs (chlorophyll and nitrogen indexes) at the leaf level for; A) Normalized Difference red edge index (NDRE) (NDRE; $R^2 = 0.8639$, $p < 0.001$); B) Modified NDVI 8 (MNDVI 8; $R^2 = 0.\ 816$, $p < 0.001$); C) Normalized Difference red edge index (NDRE) (NDRE; $R^2 = 0.795$, $p < 0.001$); D) Modified NDVI 1 (MNDVI 1; $R^2 = 0.\ 771$, $p < 0.001$); E) Ratio Vegetation Index II (RVI II; $R^2 = 0.\ 511$, $p < 0.001$); F) Red edge chlorophyll index ($CI_{red\ edge}$; $R^2 = 0.\ 435$, $p < 0.001$).

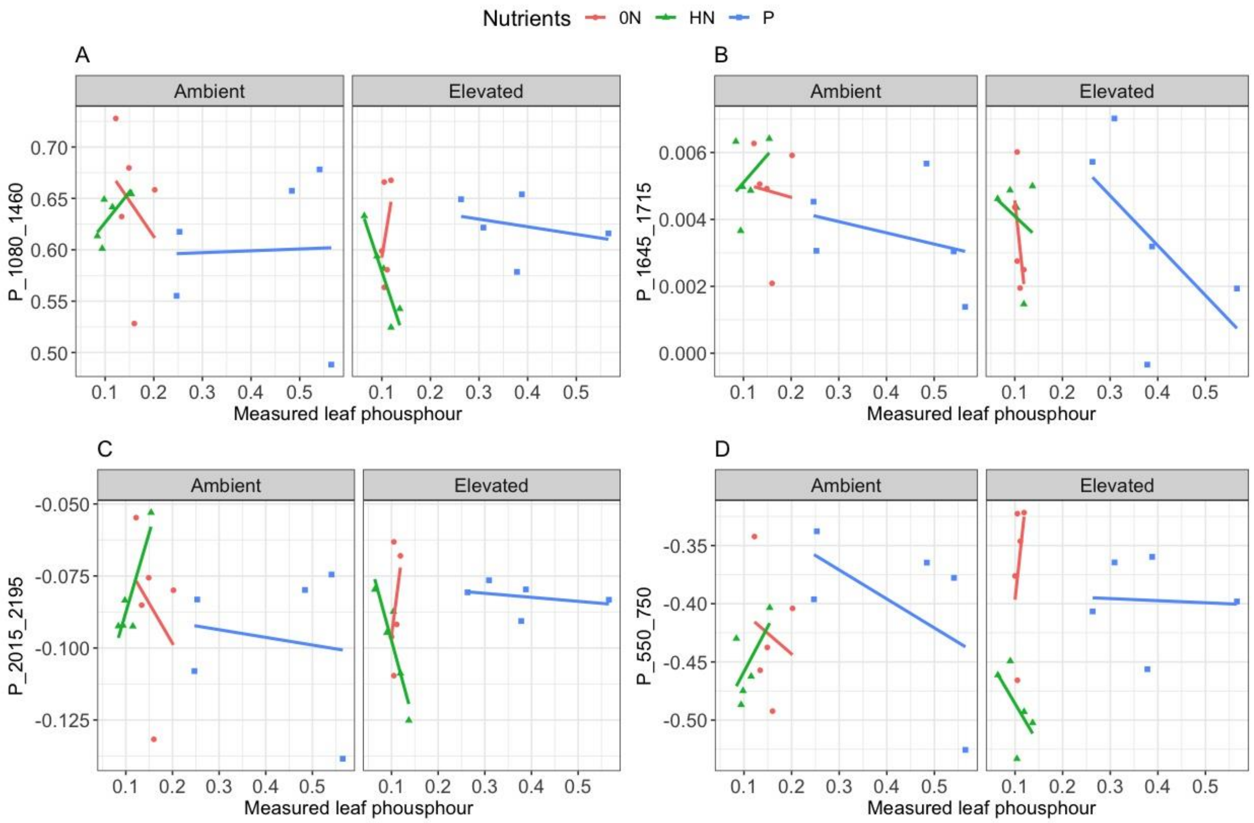

Figure S.2. 4: VIs (phosphorus index) at the leaf level for (A) P_1080_1460 ($R^2 = 0.002$, $p > 0.05$); (B) P_1645_1715 ($R^2 = 0.111$, $p > 0.05$); C) P_2015_2195 ($R^2 = 0.006$, $p > 0.05$); D) P_550_750 ($R^2 = 0.022$, $p > 0.05$).

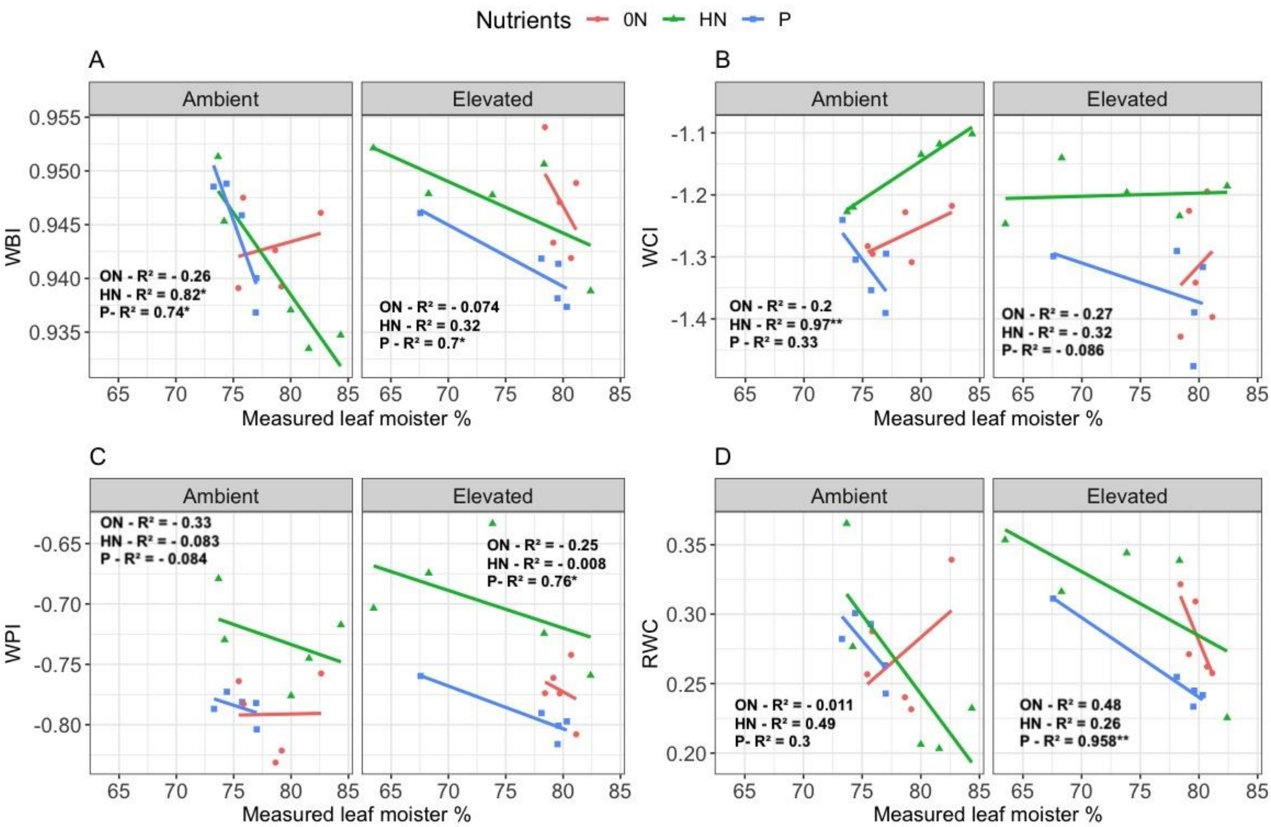

Figure S.2. 5: VIs (water index) at the leaf level for (A) Water Band Index (WBI; $R^2 = 0.265$, $p < 0.001$); (B) Water content index (WCI; $R^2 = -0.036$, $p > 0.05$); (C) Water Poverty Index (WPI; $R^2 = 0.137$, $p < 0.05$); (D) Relative water contents (RWC; $R^2 = 0.313$, $p < 0.001$). R^2 values for linear trend lines are shown on each plot. * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

Chapter 3

Impacts of five-year-CO_2 enrichment and long-term nutrient additions on phosphorus-limited limestone grassland biodiversity

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3 Abstract

Humans have altered the carbon (C) and nitrogen (N) cycles significantly through the industrial and green revolutions, with negative consequences for plant biodiversity in many ecosystems due to rising atmospheric N deposition. Increased levels of atmospheric carbon dioxide (eCO_2) in ecosystems could potentially have an effect on plant communities either directly or indirectly through impacts on N and phosphorus (P) cycling. Although research has been conducted on how N deposition affects plant communities, the potential interconnections of eCO_2, N deposition, and nutrient limitation on plant communities are still poorly understood. This is particularly true in habitats where the limiting nutrient is P. In this study, we used a mini-Free Air CO_2 Enrichment (miniFACE) system to expose grassland mesocosms from a long-term (> 27 years) nutrient manipulation experiment on P-limited limestone grasslands in the Peak District, UK, to eCO_2 (600ppm) for five years. This was in factorial combination with a phosphorus treatment (P, 3.5 g m^{-2} yr^{-1}), a low nitrogen treatment (LN, 3.5 g m^{-2} yr^{-1}), a high nitrogen treatment (HN, 14 g m^{-2} yr^{-1}), and a distilled water control (0N). Changes in plant community composition, biodiversity (species richness and diversity), and functional composition were all assessed. Species richness and diversity of species were found to be increased by eCO_2. The functional composition changed as eCO_2 increased the dominance of legumes, especially in combination with P addition, while decreasing the abundance of grass and bryophyte PFTs. Nutrient treatments (N and P) also strongly impacted community composition, diversity, species richness, and functional composition. N additions considerably reduced species richness and diversity, especially under HN, and P-treated communities were also significantly less diverse than 0N. The addition of N and P changed the functional composition by decreasing forb abundance and increasing grass abundance. The N treatment also increased sedge abundance, while the P treatment decreased this. Sedges therefore replaced grasses as the dominant plant functional type under elevated CO_2 and N conditions, potentially due to their P-acquisition strategy benefiting more than that of mycorrhiza-dependent grasses, which may be less able to acquire more of the limiting P resource with increased C investment. Under the future scenario of rising CO_2 and N pollution, species shifts in grassland communities may reduce the value of the ecosystem services they provide as a result of their combined impact on plant competition.

3.1 Introduction

Disruption to the carbon (C), nitrogen (N), and phosphorus (P) cycling can have large impacts on the structure and function of terrestrial ecosystems and the ecosystem services they provide (Jones and Donnelly 2004; Phoenix et al. 2004a, 2012). Anthropogenic activity has increased atmospheric CO_2 concentrations to over 417.2 ppm (Friedlingstein et al. 2022), an unprecedented level in human history (Willeit et al. 2019), while it is estimated that human activities now contribute more N to the global N cycle than all natural sources combined (David et al. 2013). To date, however, much of our understanding of the impacts of elevated CO_2 and increased atmospheric N deposition has come from studies on ecosystems where N is the limiting nutrient. Yet, the productivity of approximately 82% of the land's surface may be limited in some way by P availability, either alone through P limitation or by co-limiting with N and P (Du et al. 2020). To date, there is very little understanding of how P-limited ecosystems, including grasslands, will respond to concurrent perturbations in eCO_2 and N deposition.

Grasslands are one of the most extensive P-limited ecosystems in temperate regions (Aspinall et al. 2011) and the most widespread terrestrial ecosystem on Earth (Ali et al. 2016). They provide a variety of essential services, including the storage of large amounts of soil C, and represent about 20% of terrestrial net primary productivity (NPP) (Chapin 2011; Jones and Donnelly 2004) and the reduction of nutrient pollution leaching into watercourses (Phoenix, Leake, et al. 2004). Furthermore, in temperate regions, species-rich grasslands are among the most biodiverse habitats (Carbutt et al. 2017) and in Europe, they host 1 in 5 endemic vascular plant species (Habel et al. 2013). Grassland biodiversity is critical to the provision of ecosystem services (Isbell et al. 2011), yet temperate grasslands are one of the world's most threatened biomes (Hoekstra et al. 2005).

Elevated CO_2 (eCO_2) enhances photosynthesis and promotes productivity (Ainsworth and Long 2005, 2021), including P-limited limestone grassland (Keane et al. 2023; Chapter 2), though this effect is strongly dependent on species as well as the functional type (Poorter and Navas 2003), including in calcareous grasslands – the study system in this chapter (Hanley et al. 2004; Stöcklin et al. 1998). Species which can make better utilization of additional carbon resources may increase in size and population at the expense of others (Polley et al. 2012). The effect of eCO_2 is not only on increasing photosynthesis and growth, but also the plant may use the extra C to capture more nutrients, such as phosphorus (Phoenix et al. 2020). In addition, eCO_2 can, through the reduction of stomatal conductance, lead to reduced evaporative water loss and increased water use efficiency (Leakey et al. 2009). As a consequence, increases in soil water availability could stimulate N mineralization and enhance plant growth (Dijkstra et al. 2010) or by extending the dominant graminoids growing period, which may therefore shade out late-season growing plants and reduce diversity (e.g. as seen in California grassland ecosystem) (Zavaleta et al. 2003).

The interactions between eCO_2 and nutrient availability would become more important, which is anticipated in environments where nutrients rather than water availability are limiting productivity, such as P-limited temperate grasslands (Grünzweig et al. 2003; Reich et al. 2001). eCO_2 has the potential to disturb soil nutrient relationships significantly; for instance, eCO_2 can affect soil nutrients by altering the activity of soil microorganisms, such as bacteria and fungi, which play a key role in the cycling of nutrients in the soil (Wang et al. 2020; Wang et al. 2019). eCO_2 levels can alter the composition and activity of soil microbial communities, potentially leading to changes in nutrient availability (Taylor 2021; Wang et al. 2020; Wang et al. 2019). In addition, eCO_2 can affect the chemistry of soil organic matter, which can impact nutrient availability and hence affect plant biodiversity and community composition because of the close couplings of the C, N, and P cycles (Davies et al. 2016) and the potential roles of C for N and P acquisition.

Plant diversity depends on the nutrient availability in the soil (Lambers et al. 2008), and high levels of plant diversity are often associated with low levels of availability P, with the most diverse ecosystems being found disproportionate in highly weathered soils that are low in P (Laliberté et al. 2013; Lambers et al. 2013). As a result of P limitation, several adaptations have evolved in plants to acquire P (Vance et al. 2003), with some acting on particular groups of P compounds in soil to liberate the phosphate (PO_4^-) ion for uptake (Ceulemans et al. 2013, 2014; Phoenix et al. 2020). Acquisition of P from different chemical forms in the soil enables distinct species or functional types to utilize complementary niches for P, therefore reducing interspecific competition for the limiting P resource and facilitating the establishment of diverse plant communities (Phoenix et al. 2020). Therefore, alterations in nutrition availability may uncouple these interactions and decrease biodiversity. For instance, increasing PO_4^- in a P-limited ecosystem may reduce the need for niche complementarity and reduce biodiversity because most P-demanding species will have ready access to PO_4^-.

Carbon is invested in numerous ways by plants to acquire phosphorus. These include trading metabolites with mycorrhizal partners such as most forbs and grasses (Smith and Read 2008), growing highly branching root systems such as some non-mycorrhizal forbs (Li et al. 2017), or secreting acidic exudates to mine P from soil (P-mining) (Shane et al. 2006), and growing specialist roots such as dauciform roots in sedges that release organic acids and chelators in spatially limited bursts to overwhelm soil microbes and facilitate plant P acquisition (Shane et al. 2006). Various 'carbon costs' are incurred by each of these P-acquisition strategies (Lynch and Ho 2005). Therefore, because various species and functional types employ different P-acquisition strategies, the rise in atmospheric CO_2 and the additional C supply could alter how plants acquire the limiting P resource and hence impact grassland community composition and biodiversity (Lambers et al. 2008; Phoenix, Booth, et al. 2004).

Extensive research into the effects of increased N availability on terrestrial ecosystems, including grasslands, has shown that N deposition has a negative impact on plant diversity, leading to communities that are more graminoid-dominated (Bobbink et al. 2010; Lu et al. 2021; Phoenix et al. 2003; Stevens et al. 2004; Stevens, Dupr, et al. 2010). In fact, N deposition is the third most dangerous factor threatening biodiversity worldwide (Payne et al. 2017; Sala et al. 2000). The rapid expansion of the most nitrophilous species has been linked to N deposition in N-limited environments, potentially causing local extinction of more sensitive and slower-growing species (Bobbink 1991; Bobbink et al. 2010). Although P-limited grasslands are susceptible to the negative effects of N deposition (Basto et al. 2015; Carroll et al. 2003; Horswill et al. 2008), little is known about how they may react when additionally subjected to high CO_2.

Prior to the study reported in this chapter, community changes after 3 years of CO_2 fumigation in the same experiment found that in the limestone grassland, eCO_2 had no significant effect on diversity, species richness, and functional composition (Taylor 2021). However, nutrient treatments (N, P) had a significant effect on diversity, species richness, and functional composition, where N enrichment, as well as P additions to a lesser extent, resulted in less diversified graminoid-dominated communities with lesser forb species. In addition, community composition was affected by both eCO_2 and nutrient treatments with the interactions between CO_2 and nutrient treatments. Hence, this study chapter examines the community changes after more years (five years total) of CO_2 fumigation and nutrient treatments; therefore, a greater response is expected.

To investigate this, we examine how a P-limited limestone grassland ecosystem responds to eCO_2 and long-term N and P additions. Into a Mini-Free Air CO_2 Enrichment (MiniFACE) facility, intact plant-soil monoliths (mesocosms) were transferred from a long-term (> 25 years) N and P addition field experiment. The N treatment simulates atmospheric N deposition; the P addition alleviates the P-limitation. We surveyed plant communities in the limestone grassland mesocosms to explore how five years of eCO_2 and the long-term N and P treatments impacted biodiversity, species richness, community composition, and functional composition.

We hypothesized that:

1) Higher CO_2 concentrations will enhance species richness and diversity compared to aCO_2, as species benefit from increased C supplies to gain the limiting nutrients.
2) Grassland biodiversity and species richness will decline, and graminoid functional types will dominate plant communities as a result of long-term nutrient treatments (N and P).
3) eCO_2 combined with P enrichment will increase the abundance of legumes since legumes are co-limited by C and P.
4) Long-term N addition will increase the abundance of the graminoids grass and sedges at the expense of the abundance of forbs.

3.2 Methods

3.2.1 Field site description

Wardlow Hay Cop grassland (original nutrient treatment site)

The limestone grassland is naturally P- limited and is located at the Wardlow Hay Cop long-term experiment in the Derbyshire Dales National Nature Reserve, UK (Morecroft et al. 1994). The plant community (National Vegetation Classification *Festuca-Avenula*, CG2d; (Rodwell 1992)) grows in a shallow ranker soil, pH ca. 6.8 (Horswill et al. 2008), that extends no more than 10 cm to the limestone below (Keane et al. 2020). The community is speciose and predominantly calcicolous. In 1995, replicate 9 m^2 experimental plots were established (Johnson, Leake, and Lee 1999). These plots have been subjected to one of the following nutrient treatments: a control of distilled water (0N); a low nitrogen treatment (LN, 3.5 g m^{-2} yr^{-1}), which is roughly equal to the background N deposition at peak 1980s levels; and a high nitrogen treatment (HN, 14 g m^{-2} yr^{-1}) simulating very high N loading. A phosphorus treatment (3.5 g m^{-2} yr^{-1}) relieves the natural P limitation. For the N and P treatments, solutions of NH_4NO_3 or NaH_2PO_4 are applied in solution as a fine mist using a backpack sprayer. Since 1995, the limestone grassland has received nutrient treatments once a month and then every two months since 2017. However, phosphorus (P) treatments were suspended between 1996 and 2011 after their initial application due to a large productivity response. The N treatments in the first years increased P limitations and caused reductions in limestone grassland productivity (Carroll et al. 2003; Johnson et al. 1999; Phoenix, Booth, et al. 2004). However, in the long-term, the stimulation of P-acquiring enzyme activity appears to have alleviated P limitation in line with evidence from other systems receiving long-term N loading (Chen et al. 2020; Taylor 2021).

Bradfield FACE experiment (elevated CO_2)

The MiniFACE experiment is described in Keane et al. (2020), with the general system set-up also described in (Miglietta et al. 2001). Briefly, in 2017, 35 cm by 35 cm intact soil-turf monoliths were extracted from each nutrient treatment of the limestone grassland (10 replicates per nutrient treatment, including controls, 40 monoliths in total). To capture the complete soil profile, monoliths were extracted to a depth of 10 cm (Keane et al. 2020). These monoliths were moved into free-draining polypropylene mesocosm boxes. In the bottom of the mesocosms, 5 cm of limestone chippings of the same limestone type as at Wardlow were added since plant roots are often in contact with the bedrock at the Wardlow grassland. Monthly nutrient treatments, as used in the Wardlow experiment, were also applied to the experimental mesocosms.

The Bradfield Environmental Laboratory research station where the FACE experiment is located is in the Peak District (ca. 350 m asl), which is geographically close to Wardlow (~20 km) and has a climate and altitude similar to that of Wardlow (ca. 390 m asl). Mesocosms were embedded into the ground at Bradfield to provide a soil temperature buffer (Keane et al. 2020). Mesocosms were either exposed to ambient CO_2 (aCO_2, approximately 410 ppm) or elevated CO_2 levels (eCO_2, target of 600 ppm using the miniFACE system). Following a full year after extraction to allow the mesocosms to settle, fumigation began in April 2018 and continued during the daylight hours between the beginning of April and the end of October each year to capture the growing season.

Plant diversity data collection

In the 5th year of CO_2 fumigation (this also being the 27th year of nutrient manipulation), community composition surveys were performed to determine the percentage cover of each plant species. Each mesocosm was divided into a quadrat, which consisted of nine replicate squares of the same size (~12 cm by 12 cm). Each species' percentage cover in each square was estimated by eye, and the overall coverage of all species was allowed to exceed 100%.

Bare soil and dead plant cover were also quantified but not analyzed in this chapter. For further analysis, each species was allocated to one of the following plant functional types (PFT): forb (non-legume), grass, sedge, legume, bryophyte, rush or unknown. Bryophytes were not identified to species level due to time constraints, and comparable congeners of common species were aggregated. This was the situation with *Agrostis spp*., where the distinction between *Agrostis capillaris* and *Agrostis vinealis* was not made. Only one species is included within the rush functional type: *Luzula campestris*. Although *Helianthemum nummularium* and *Thymus praecox* include some woody tissue, they have been classified as forbs due to their functional similarity to other forbs. To avoid attributing unexpectedly additional percentages of cover to the incorrect PFT, an unknown PFT was included in the analysis. The unknown plant community represented a very small percentage of the entire mesocosm cover, accounting for an average of 0.1% of the total mesocosm cover.

3.2.2 Statistical analyses

Community composition

Statistical analyses were carried out using R (R Core Team 2022), primarily in the ‘tidyverse’ environment (Wickham et al. 2019). Non-metric Multi-Dimensional Scaling (NMDS) was used to assess the variations in community composition by analysing species abundance data: in this, a greater NMDS axis value dissimilarity indicates greater differences in plant communities (Anderson 1971). NMDS is a powerful technique for data that may not fulfil the assumptions of other ordination methodologies due to its rank-based approach. For the purpose of this study, the Bray-Curtis dissimilarity index was used, as it is the standard of choice for ecological data in which numerous rare species coexist (Anderson 1971; Klupar et al. 2021).

Prior to NMDS analysis, data were min-max normalised to ensure that each variable contributed equally to the NMDS. The 'metaMDS' function of the 'vegan' package in R was utilised to conduct NMDS studies (Dixon 2003). Using 100 model iterations, the NMDS reduced the data dimensionality to two dimensions and generated stress values below the 0.2 thresholds (Klupar et al. 2021). Permutational Multivariate Analysis of Variance (PERMANOVA) was employed to assess the effects of CO_2 treatment, nutrients, and their interactions on plant communities. PERMANOVA is a non-parametric statistical test that distributes variability across multivariate data, and it's capable of utilizing a Bray-Curtis dissimilarity matrix (Anderson 2017), making it useful in conjunction with an NMDS analysis. A significant result from a PERMANOVA implies that treatment groups are considerably dissimilar, as it tests the null hypothesis that the centroid of each treatment's ordination in the dissimilarity matrix is not different (Anderson 2017). Each mesocosm's plant community's

Bray-Curtis dissimilarity matrix was analysed using the 'adonis2' function from the vegan package, calculated from 999 permutations.

Species associations

To visualize the relative association of each species to the community of a particular treatment combination, principal component analyses (PCA) were used. The PCA analyses presented here assist in visualizing the data, as the approach is a more restrictive parametric ordination method that implies the linearity of variables. Hence, statistical analyses on the PCA results were not performed. Following min-max normalization on the data, PCAs were performed using the 'PCA' function from the 'FactoMineR' package in R (Lê et al. 2008).

Grassland diversity

The species richness was analyzed by summing the number of species recorded in each mesocosm and performing a two-way ANOVA. CO_2 treatment, nutrient treatment, and their interaction were included as modelled fixed effects with species richness as the response variable. Where the entire ANOVA model indicated a significant impact, Tukey multiple comparisons of means *post-hoc* test were used to determine pairwise treatment differences.

Simpson's diversity was calculated using the same statistical analyses as were used for species richness. The Simpson's diversity index is a measure of community diversity that considers species richness and relative abundance, allowing for the assessment of community evenness and dominance (Simpson 1949). As our grassland community is relatively rich but contains multiple rare species with low abundance, we deemed the Simpson's diversity index to be more appropriate than the more commonly used Shannon index (Shannon 1948; Spellerberg and Fedor 2003). The Simpson's index value (D) assesses the possibility of two independently collected individuals belonging to the same species; thus, D decreases as diversity increases. Thus, we employ 1-D to facilitate intuitive interpretation so that greater values signify greater diversity. D was calculated with the 'diversity' function in the 'vegan' package by R.

Plant functional composition

The mean total percentage cover of a PFT in each mesocosm was computed by summing the percentage cover of each species that shared a functional type across quadrat squares and dividing it by 9 (number of quadrat squares per mesocosm). In order to better fit the complete statistical models, zero-loaded PFTs were removed from the analyses. As a result of the data's positive skewness, transformations were required to improve residuals' adherence with the appropriate statistical test assumptions. The data were changed using the natural log + 1 transformation.

Linear mixed effect models (LMEs) are effective statistical tools that are resistant to deviations in distributional assumptions typical of complex data structures like ours (Schielzeth et al. 2020). The models were fit to data where the total PFT cover was the response variable and where PFT, CO_2, and nutrient treatment were categorical fixed effects with factorial interaction terms. The models' output was not affected by the inclusion of the pair and mesocosm ID random intercept terms; thus, we eliminated them. In order to perform the model fitting, the 'lmer' function was used from the lme4 package in R (Bates et al. 2015). Tukey's multiple comparisons of means tests were used to classify the data into distinct functional types, and the 'emmeans' function from the emmeans package (Lenth et al. 2022) was utilized to obtain these classifications.

3.3 Results

3.3.1 Community composition (grassland NMDS)

Both eCO_2 and the nutrient treatments created plant communities that were significantly dissimilar from one another (CO_2: PERMANOVA, df = 1, 39, F = 2.4, $p < 0.05$; Nutrients: PERMANOVA, df = 3, 39, F = 6.1, $p < 0.001$) (Fig. 3.1). However, there was no significant interaction between CO_2 and nutrient treatments (PERMANOVA, df = 3, 39, F = 0.4, $p > 0.05$) though eCO_2 appeared to have a stronger effect on plant communities in the LN and HN treatments compared to the 0N and P treatments (Fig. 3.1). Communities treated with different nutrients (N or P) exhibited distinct characteristics compared to the 0N control, following different paths in the ordination space. This underscores that communities treated with N and P also showed differences from one another.

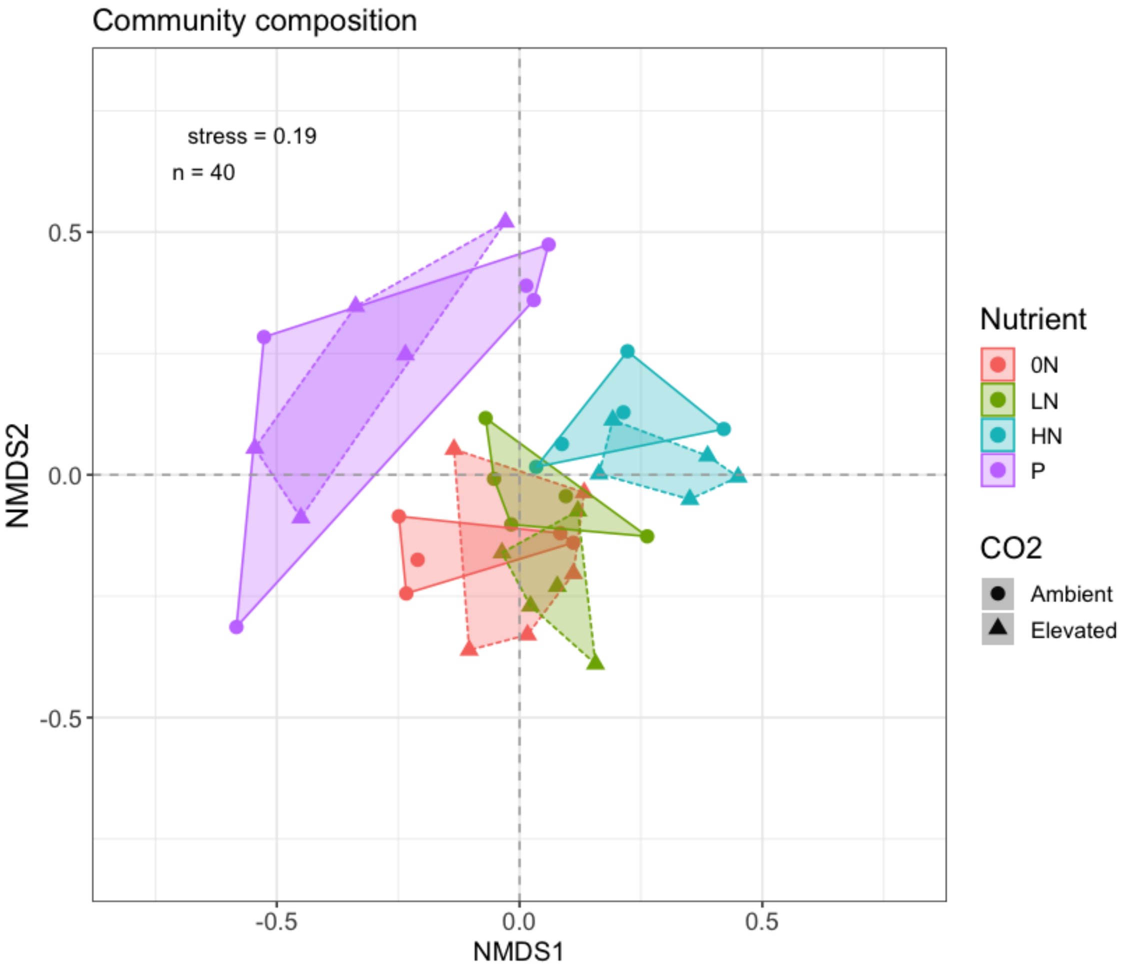

Figure 3.1: The limestone grassland community composition is mapped using (NMDS) for each of the 40 experimental mesocosms, depicted as individual data points. The NMDS plot depicts the (dis)similarity among plant communities subject to varying CO_2 (ambient and elevated) and nutrient (0N, LN, HN and P) treatment combinations. Every treatment combination claims a unique area within the ordination plot, indicated by the shaded polygons. The more distance there is between these polygons, the greater the dissimilarity among the plant communities.

3.3.2 Species associations (grassland PCA)

The PCA indicated that some species have strong associations with specific CO_2 and nutrition treatment combinations. Many species were strongly associated with 0N and LN when combined with eCO_2, especially a large number of forbs, including *Bellis perennis, Sanguisorba minor, Succisa pratensis, Helianthemum nummularium, Ranunculus repens, Gentianella amarella*, *Leontodon hispidus* and sedge *Carex caryophyllea*, and to lesser extent the forbs *Cirsium acuale* and *Scabiosa columbaria*, the legume *Lotus corniculatus*. The forbs *Plantago lanceolata*, and *Thymus praecox* were associated with 0N when combined with eCO_2. Fewer species were strongly associated with N addition (HN and LN), especially when combined with eCO_2, the grass *Avenula pratense,* forb *Potentilla erecta, Polygala vulgaris,* and sedges *Carex flacca* and *Carex panicea*, and to less extent Forb *Euphrasia officinalis* and *Cirsium acuale* (Fig. 3.2). P addition was associated with many grasses, including *Koeleria macrantha*, *Anthoxanthum odoratum*, *Danthonia decumbens, Briza media, Holcus linatus,* along with the rush *Luzula campestris*, and forbs *Veronica officinalis* and *Galium verum* (Fig. 3.2).

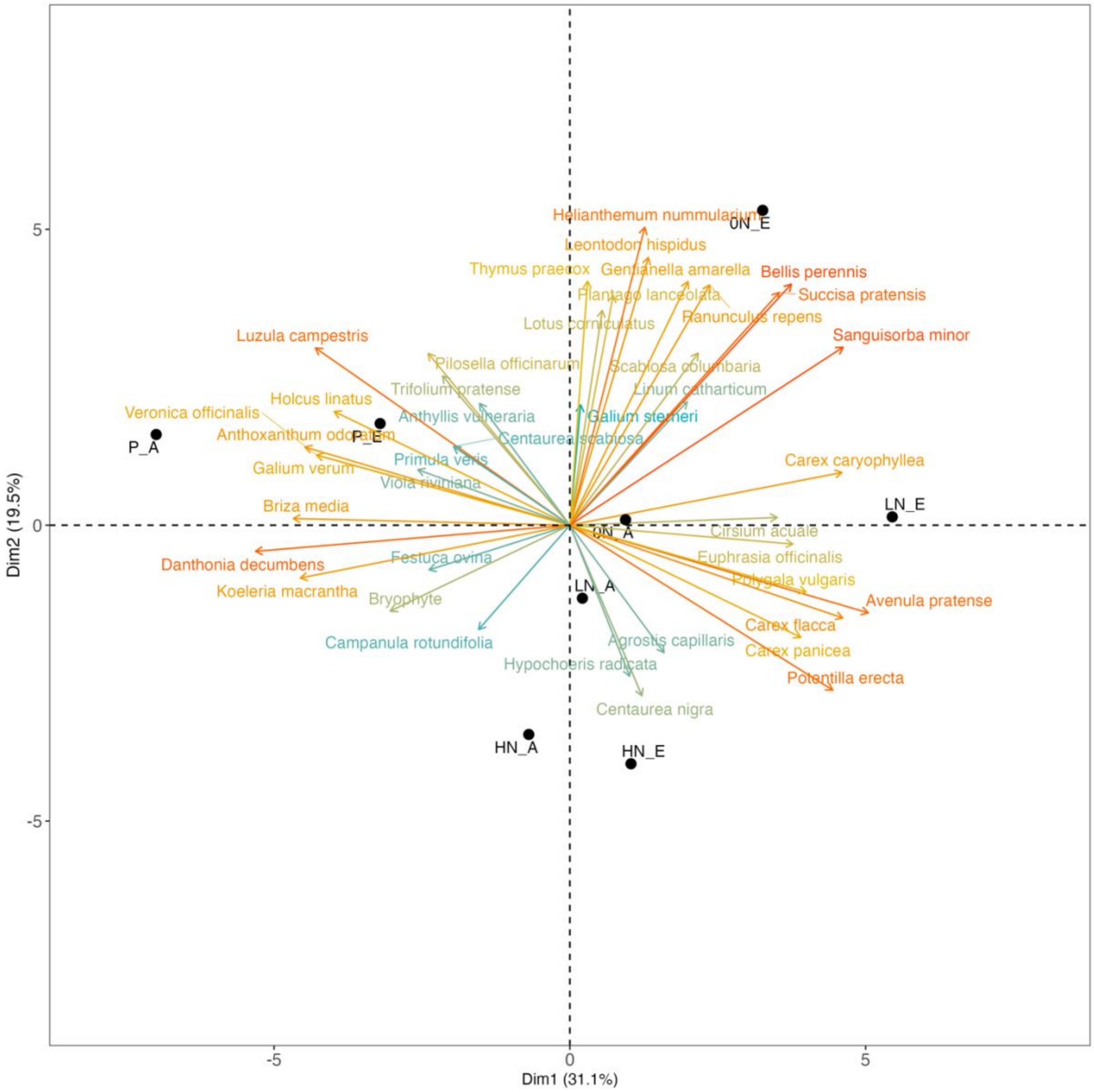

Figure 3.2: A PCA biplot represents the relationship between each species of limestone grassland to the average community composition of CO_2 and nutrient treatment combinations. Here, A and E represent aCO_2 and eCO_2, respectively, while the same nutrient identifiers (0N, LN, HN, and P) are utilized. Every recorded species has an arrow that is positioned in the ordination space in the direction of that species' most potent association with CO_2 and nutrient conditions. The size and warmth of the arrow's colour are proportionate to the strength of a species' relationship. The average community composition for each combination of treatments is shown.

3.3.3 Grassland diversity

Species richness

eCO_2 increased species richness by 11%, with 4 more species on average compared to aCO_2 (ANOVA, Fig. 3.3A, *df* = 1, 32; *F* = 7.2; $p < 0.01$). Species richness was significantly affected by nutrient treatment (ANOVA, Fig. 3.3A, *df* = 3, 32, *F* = 5.0; $p < 0.001$), and there was an interaction between CO_2 and the nutrient treatments (ANOVA, Fig. 3.3A, *df* = 3, 32; *F* = 3.0; $p < 0.05$). The HN treatment significantly reduced richness by five species compared to the 0N (Tukey, $p < 0.05$), but not LN and P treatments (Fig. 3.3A). Among all treatment combinations, eCO_2-HN had significantly lower species richness than eCO_2-0N (Tukey, $P<0.05$). In addition, eCO_2-HN, aCO_2-HN and aCO_2-P tended to decrease species richness compared to eCO_2-0N; also, eCO_2-P increased species richness compared to eCO_2-HN (Fig. 3.3A). Interestingly, eCO_2, when combined with HN addition, reduced species richness compared to eCO_2-0N and eCO_2-P (Fig. 3.3A).

Simpson's diversity

The effects of CO_2 and nutrient treatments on Simpson's diversity closely resembled the species richness results. Simpson's diversity was significantly affected by CO_2 enrichment (ANOVA, Fig. 3.3B, *df* = 1, 32, *F* = 5.8, $p < 0.05$), where eCO_2 communities were significantly more diverse than aCO_2 communities. Also, there were significant effects of nutrient treatment (ANOVA, Fig. 3.3B, *df* = 3, 32, *F* = 9.0, $p < 0.0001$): HN and P-treated communities were significantly less diverse than 0N overall (Tukey $p < 0.05$), and for each treatment combination aCO_2-P was significantly lower than all 0N and LN treatments of both CO_2 levels (Tukey $p < 0.05$). There was no significant interaction between CO_2 and nutrients (ANOVA, Fig. 3.3B, *df*

= 3, 32, $F = 1.3$, $p > 0.05$). In addition, the Shannon diversity index has been provided in the appendix even though it closely resembles Simpson's diversity results.

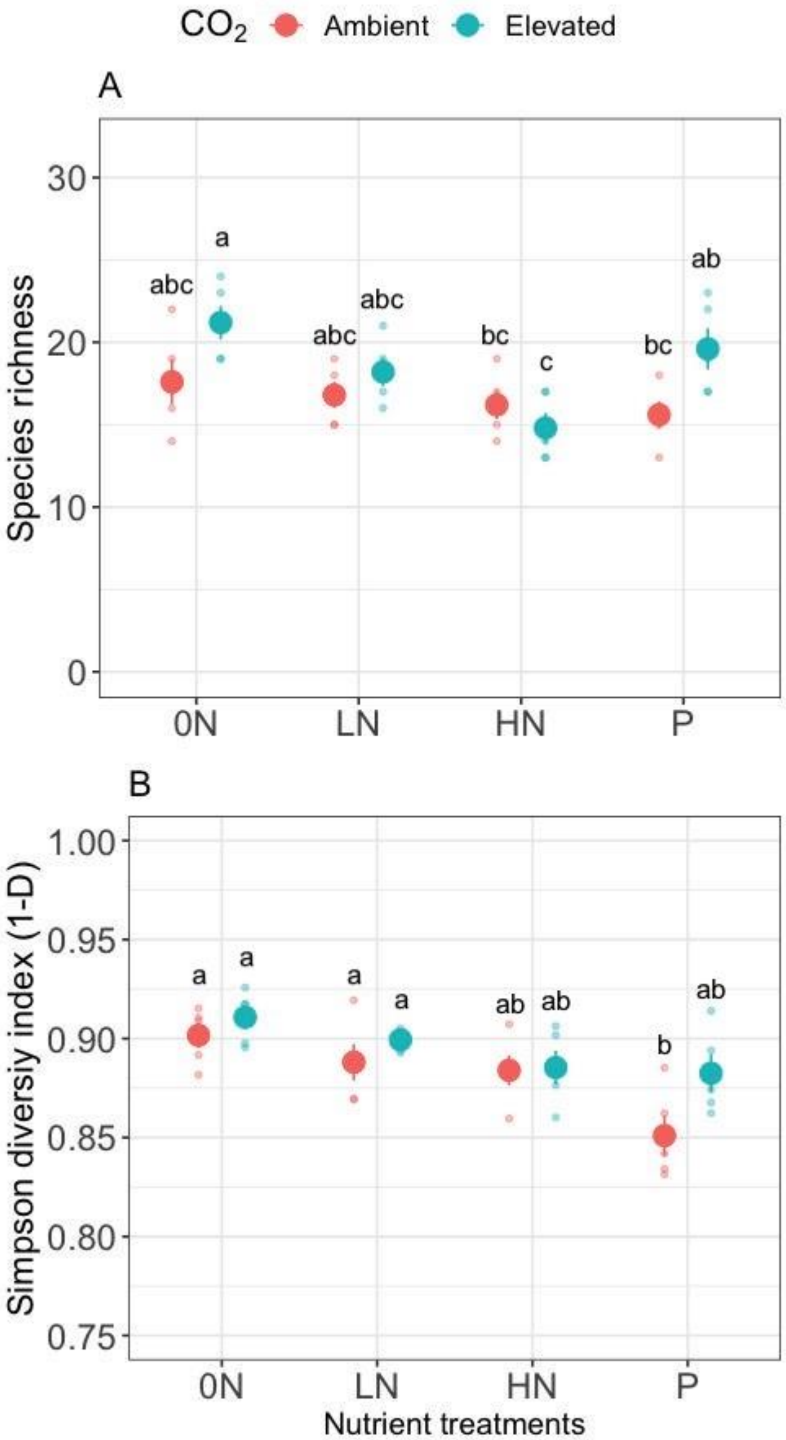

Figure 3.3: A) Species richness and B) Simpson's diversity across CO_2 and nutrient treatments. The smaller transparent points represent the richness and diversity of each individual mesocosm, respectively. The larger points, which are accompanied by their respective standard errors, show the mean richness and diversity across all mesocosms with the same treatment. Points sharing the same letter don't have a significant statistical difference ($p < 0.05$). Simpsons is shown a 1-D so that a greater Simpsons value intuitively indicates a greater diversity.

3.3.4 Plant functional composition

The abundance of overall plant functional types was significantly affected by CO_2 treatment, where eCO_2 reduced the abundance of all plant functional types by 4% on average compared to aCO_2 overall (LME, $df = 1, 240$; $F = 5.5$; $p < 0.001$) and the abundance of specific plant functional types was significantly affected by nutrient treatment (LME, $df = 3, 240$; $F = 3.6$; $p < 0.001$), generally HN-treatments reduced the abundance of all plant functional types by 7% on average and P treatments by 10% on average compared to 0N. However, there were no significant interactions between CO_2 and nutrients (LME, $df = 12, 240$; $F = 1.6$; $p > 0.05$) (Fig. 3.4). As individual functional types responded to CO_2 and nutrients to different extents, the functional composition changed considerably.

Forb abundance was unaffected by CO_2 treatment (LME, $df = 1, 32$; $F = 0.1$; $p > 0.05$) but was significantly affected by nutrient additions (LME, $df = 3, 32$; $F = 10.5$; $p < 0.01$); HN-treatment reduced forb abundance by 49% compared to 0N being, the lowest, and P-treatment reduced forb abundance by 34% compared to 0N. However, there were no significant interactions between CO_2 and nutrients (LME, $df = 3, 32$; $F = 0.1$; $p > 0.05$) (Fig. 3.4A).

Grass abundance was nearly significantly affected by CO_2 (LME $df = 1, 28$; $F = 4.2$; $p = 0.0502$), where eCO_2 reduced grass abundance by 12% compared to aCO_2. Also, grass abundance was significantly affected by nutrient additions (LME, $df = 3, 32$; $F = 6.2$; $p < 0.01$), LN -treatment increased grass abundance by 11% compared to 0N, and P addition increased the abundance by 27% compared to 0N, and HN addition increased the abundance by 42% being significantly higher compared to 0N (Tukey, $p < 0.05$). However, there was no statistically significant interaction between CO_2 and nutrients (LME, $df = 3, 32$; $F = 0.4$; $p > 0.05$) (Fig. 3.4B).

Sedge abundance was not affected by CO_2 (LME, *df* = 1, 32; *F* = 0.4; $p > 0.05$) but was increased by nutrient treatments (LME, *df* = 3, 32; *F* = 42.9; $p < 0.01$). LN increased sedge abundance by 23% and HN by 35% compared to 0N. In contrast, P treatments reduced the abundance by 48% compared to 0N (Tukey, $p < 0.05$). However, there was no significant interaction between CO_2 and nutrients (LME, *df* = 3, 32; *F* = 0.5; $p > 0.05$) (Fig. 3.4C).

Legume abundance was significantly increased under eCO_2 by 57% compared to aCO_2 (LME, *df* = 1, 32; *F* = 5.3; $p < 0.05$) but not nutrient treatments (LME, *df* = 3, 32; *F* = 0.8; $p > 0.05$) and not their interaction (LME, *df* = 3, 32; *F* = 2.6; $p > 0.05$), generally significantly increasing in response to eCO_2, especially in combination with P addition (Fig. 3.4D).

Bryophyte abundance was significantly reduced under eCO_2 by 64% compared to aCO_2 (LME, *df* = 1, 32; *F* = 8.6; $p < 0.01$) but was not affected by nutrient treatments (LME, *df* = 3, 32; *F* = 0.5; $p > 0.05$) and there was no CO_2 and nutrient interaction (LME, *df* = 3, 32; *F* = 1.1; $p > 0.05$), (Fig. 3.4E).

Rush (a single species) was not affected by CO_2 (LME, *df* = 1, 32; *F* = 0.6; $p > 0.05$) but was affected by nutrient treatments (LME, *df* = 3, 32; *F* = 7.5; $p < 0.01$), generally being significantly higher in P treatments (Tukey, $p < 0.05$). However, there was no statistically significant interaction between CO_2 and nutrients (LME, *df* = 3, 32; *F* = 0.9; $p > 0.05$) (Fig. 3.4F).

A qualitative summary of the main effects of CO_2 and nutrient treatments on the abundance of grassland functional type is provided in Table 3.1. An additional graph combining all the PFT in each treatment and the percentage cover of each species individually has been provided in the appendix.

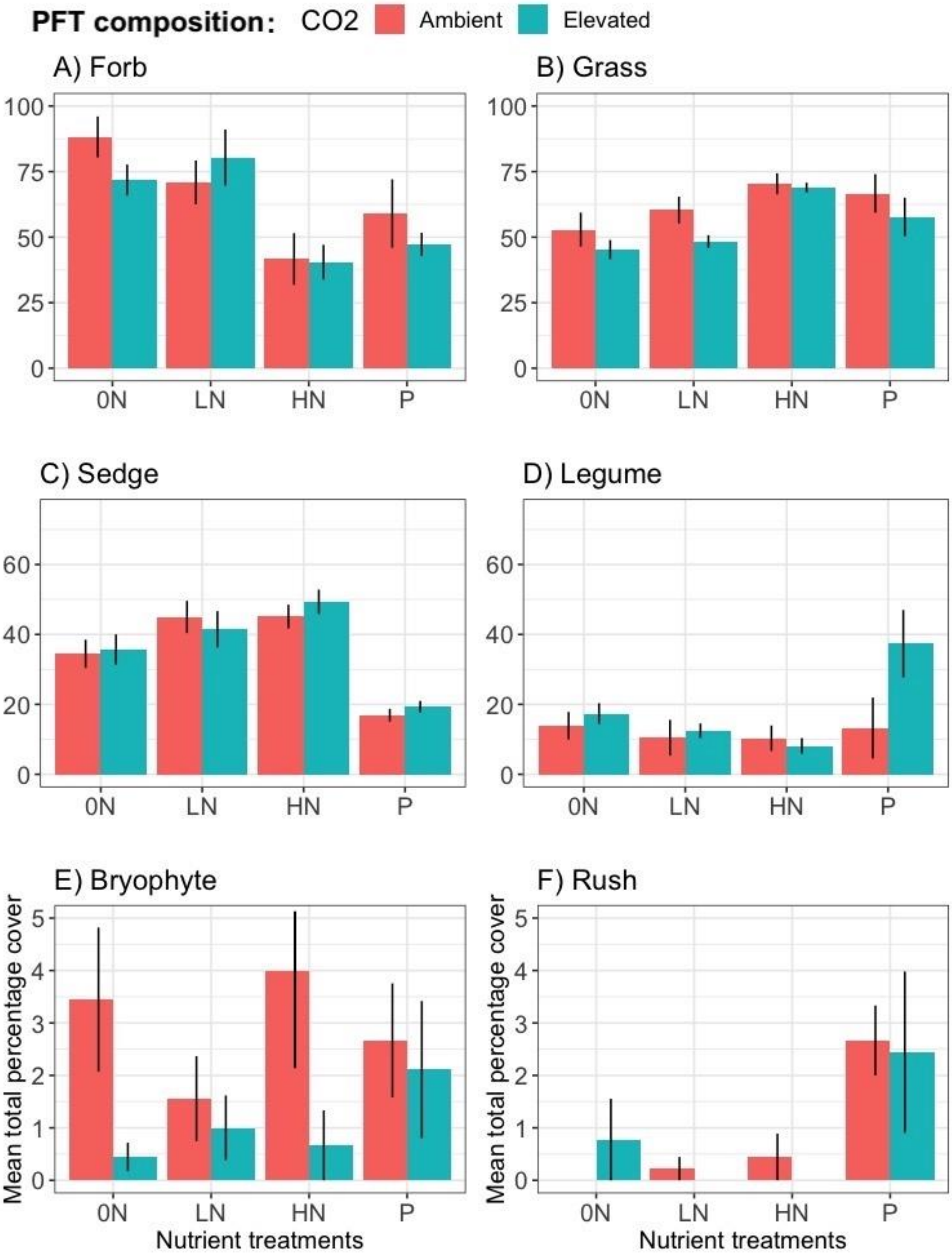

Figure 3.4: The mean total percentage cover of each plant functional type (PFT); A) Forb, B) Grass, C) Sedge, D) Legume, E) Bryophyte, F) Rush, across CO_2 treatments (ambient and elevated) and nutrient treatments (0N, LN, HN and P) and Error bars represent the standard errors of the means. Note the different Y-axis lengths.

Table 3.1: A summary of the effects of CO_2, nutrient treatments, and the interaction between CO_2 and nutrient treatments on grassland plant functional types (PFT) abundance.

Plant functional type	**CO_2 effect**	**Nutrient effect**	**CO_2* Nutrient**
Forb	NS	**	NS
Grass	*	**	NS
Sedge	NS	**	NS
Legume	*	NS	NS
Bryophyte	**	NS	NS
Rush	NS	**	NS

NS = P > 0.05, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

3.4 Discussion

3.4.1 Summary of the results

eCO_2 affected community composition, diversity, species richness, and functional composition of limestone grassland after five years of CO_2 fumigation. eCO_2 tended to increase species richness and diversity. eCO_2 also increased the abundance of legumes, especially in combination with P addition, but reduced the abundance of grass and bryophytes, causing a shift in overall community functional composition.

Nutrient treatments (N and P) strongly impacted community composition, diversity, species richness, and functional composition. N additions reduced species richness and diversity, especially under HN, compared to 0N, and P-treated communities were significantly less diverse. N and P additions altered functional composition by reducing forb abundance and increasing grass abundance. Also, N addition increased sedge abundance while P reduced sedge abundance but increased rush abundance.

3.4.2 eCO_2 effects

In this study, after five years of CO_2 fumigation, we observed that eCO_2 caused significant changes in the community composition and an increase in species richness under eCO_2 by 11% (+4 species on average compared to aCO_2); also, eCO_2 increased the diversity of species on Simpson's diversity index, where eCO_2 communities were more diverse compared to aCO_2. These changes in the community composition and the increase in species richness and diversity under eCO_2 are most likely due to the fact that plants are utilizing the extra carbon to acquire the most limited nutrient, which is P in this case (Jin et al. 2015; Phoenix et al. 2020), and this alleviates the P limitation which ultimately reduces the intensity of competition for the nutrients, and in turn, this should facilitate the coexistence of species (Phoenix et al. 2020). For instance, plants may use the extra C to capture more P (Jin et al. 2015; Phoenix et al. 2020) through trading metabolites with mycorrhizal partners such as most forbs and grasses (Smith and Read 2008), growing highly branching roots systems (Li et al. 2017), or secreting acidic exudates to mine P from soil (P-mining) (Shane et al. 2006), and growing specialist roots such as dauciform roots in sedges that release organic acids and chelators in spatially limited bursts (Shane et al. 2006),

It has been shown in the same experiment that there is an increase in productivity and photosynthesis rate in limestone grassland in response to eCO_2 (Keane et al. 2023; Chapter 2). In the context of the humped back curve theory (Al-Mufti et al. 1977), this suggests that limestone grassland is on the left side of the curve, which means eCO_2 pushes the limestone grassland to the right so that both productivity and species richness increase. Moreover, eCO_2 has been widely observed to enhance plant photosynthesis rate, including limestone grassland (Chapter 2) but decrease stomatal conductance (Ainsworth and Long 2005, 2021), including in limestone grassland (Chapter 2), as well modifying stomata morphology (Haworth et al., 2016). Consequently, plants can maintain a greater leaf water content (Tausz-Posch et al. 2015; Wullschleger et al. 2002), improve water use efficiency (WUE) (Li et al. 2017; Pazzagli et al. 2016; da Silva et al. 2017; Yan et al. 2017), including in limestone grassland (Chapter 2) and eventually, soil water increases (Dijkstra et al. 2008; Dijkstra et al. 2010). Given that the limestone grassland soil is shallow (~10cm deep), it’s possible it becomes periodically water limited, and the above improvements in water use efficiency could, therefore, also results in minimizing the decrease in growth associated with water stress under eCO_2.

eCO_2 significantly reduced grass abundance. This suggests other plant functional types may benefit more from the extra carbon under eCO_2 to obtain the limiting nutrient P; hence they out-compete grass, resulting in a reduction in the grass abundance. As mentioned above, sedges, for example, have dauciform roots that release organic acids and chelators in spatially limited bursts to overwhelm soil microbes and enhance plant P acquisition (Shane et al. 2006). In addition, some non-mycorrhizal forbs grow highly branching root systems (Li et al. 2017), trading metabolites with mycorrhizal partners such as most forbs (Smith and Read 2008). Each of these P-acquisition strategies has a 'carbon costs' (Lynch and Ho 2005), which can benefit from the additional carbon under eCO_2 and hence impact grassland community composition and biodiversity where some species or functional types benefit more than others (Lambers et al. 2008; Phoenix, Booth, et al. 2004).

eCO_2 significantly increased legume abundance, especially when eCO_2 was combined with P treatments. Our results support the findings of others who have hypothesized that the availability of both C and P may act as co-limiting factors for legume growth in calcareous grasslands (Niklaus et al. 1998). The process of N fixation is an energy-intensive one, both in terms of C resources (Minchin and Witty 2005) and P resources (Liu et al. 2018). Therefore, the provision of both C and P at the same time may constitute the optimum conditions for the expansion of legumes. This may mean that land managers in agricultural limestone grasslands may be able to reduce the requirement for N fertilizers and their related environmental impacts in a high CO_2 future through this eCO_2 promotion of biological N fixation even with very modest inputs of P (Fuchs et al. 2020).

Finally, eCO_2 significantly reduced bryophyte abundance. This may simply be because eCO_2 increased the biomass of vascular plants, thus shading out the bryophytes. A decline in bryophyte abundance has been previously observed in the same experiment (Taylor 2021).

3.4.3 Nutrient additions effects

N enrichment led to significant declines in species richness and diversity, which were primarily attributed to considerable reductions in forbs and a significant increase in grass and sedge abundance, resulting in communities even more dominated by graminoids. Comparable responses have been observed previously (Lu et al. 2021; You et al. 2017), including at the Wardlow field site where the limestone mesocosms were extracted from (Carroll et al. 2003; Lee and Caporn 1998; Stevens et al. 2004) and in this eCO_2 experiment (Taylor 2021). Numerous species that constitute limestone grasslands are calcicolous, meaning they are favourable to alkaline soils. This makes them vulnerable to the impacts of N-induced acidification (Tian et al. 2021). Acidification causes depletion of base cation availability, possibly contributing to forb declines (Horswill et al. 2008; Stevens et al. 2018), thereby making the environment conducive to the growth of stress-tolerant graminoids. HN and LN treatments enhanced the growth of slow-growing sedges such *Carex flacca* and *Carex panicea*, indicating that they may be capable of withstanding acidification and perhaps utilise N for extra P absorption, such as by stimulating the growth of their specialist P-acquiring dauciform roots. Treatments of N or P can cause a reduction of biodiversity because they promote the growth of fast-growing species, such as some grass species, which can result in the competitive exclusion of other species (Bobbink et al. 2010).

Carex species are particularly adapted to low-P conditions in this limestone grassland; therefore, sedge abundance increased with N additions, where we would anticipate P demand to be highest due to the need for maintaining a stoichiometric balance in plant tissues (Phoenix, Booth, et al. 2004; Taylor 2021), i.e. sedges increase not (only) because of the additional N supply, but because they are well adapted to cope with the extra P stress that arises with extra N. In P-limited soils, microbial immobilisation of soluble inorganic P can be rapid and important (Bünemann et al. 2012; Schneider et al. 2017), but the strong pulses of sedge exudation from their dauciform roots may overcome microbial competition for P (Phoenix et al. 2020), resulting in an efficient approach that becomes more so as P limitation increases (Lambers et al. 2008). The stimulation of this with N addition may enable sedges to potentially be stronger competitors against forbs for the limiting P and exploit 'niche-space' produced by forb reduction, especially under HN treatments.

In N-limited ecosystems, N loading can reduce biodiversity by facilitating the expansion of a competitive dominant species (Bobbink 1991) because aggressive grass species may take advantage of the additional nutrient resources available and outcompete other species (Bobbink et al. 2010; Lu et al. 2021; You et al. 2017). We, therefore, expected that relieving P limitation through the addition of P would similarly lead to grass dominance and decreased diversity in this P-limited grassland. This is exactly what has been observed in our results as P addition reduces the Simpson's diversity. In a comparable P-limited ecosystem, Stiles, Rowe, and Dennis (2017) also found losses in the diversity of up to 21% as a result of P enrichment. In addition, P enrichment has been found to reduce biodiversity in the same experiment at Bradfield (Taylor 2021). This emphasizes the crucial role that P limitation plays in sustaining the diversity of these grasslands (Ceulemans et al. 2013, 2014; Phoenix et al. 2020).

Since we surveyed the limestone community after 27 years of N additions, species richness reductions are also caused by long-term effects, such as alterations in plants' reproduction. As protective seed coats become increasingly decomposable under high N levels, N deposition has the potential to deplete seed banks and reduce the flowering of several Forb species, as found in Wardlow grasslands (Basto et al. 2015). These indirect impacts on plant fitness could become more prevalent during the long N deposition period, and if seedbanks are indeed depleted, could suggest that the declines in grassland biodiversity shown here are unlikely to be recovered rapidly where atmospheric N deposition declines.

3.5 Conclusion

After five years of CO_2 fumigation, eCO_2 had an effect on the community composition of limestone grassland, including an increase in species richness and diversity, causing a shift in functional composition. Since this is a P-limited ecosystem, the increased species richness and diversity under eCO_2 may be a consequence of plants utilizing additional C to acquire P, the most limited nutrient. This alleviates the P limitation, which consequently reduces the intensity of competition for the nutrients, facilitating the coexistence of species. Also, this may explain the changes in community composition and plant functional type.

The effects of long-term simulated N and P treatments on community composition, diversity, species richness, and functional composition were significant. N additions reduced species richness and diversity as a consequence of significant reductions in forbs and an increase in grass and sedge abundance, resulting in communities dominated by graminoids. In addition, communities treated with P were much less diverse.

Reductions in richness and diversity and a shift towards graminoids-dominated grassland under nutrient treatments (N and P) could potentially diminish the conservation and recreational value of these grasslands, as well as their usefulness as grazing lands. While the response of plant P uptake to eCO_2 is a plausible factor driving these alterations in this P-limited grassland, we highlight the necessity for deeper mechanistic understanding to comprehend why P-limited grassland evolves in response to eCO_2 and nutrient enhancement.

3.6 Appendix

This appendix provides extra figures related to the community composition dataset. Included is the mean cover percentage of each species recorded across all mesocosms. Individual species' percentage cover changes as a result of CO_2 and nutrition treatments are also shown.

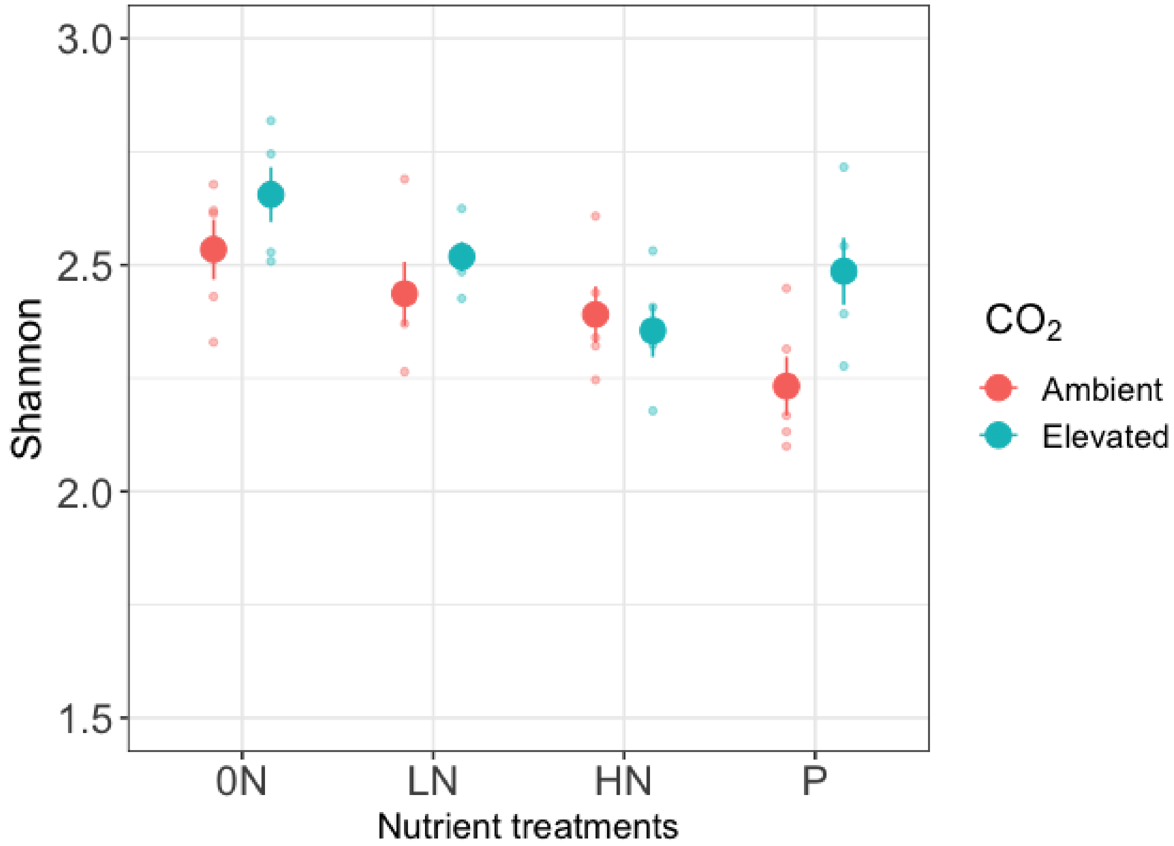

Figure S.3. 1: Shannon diversity index across CO_2 and nutrient treatments. The smaller transparent data points show the diversity respectively of individual mesocosms. The larger points represent the mean diversity across all mesocosms sharing the same treatment and are presented with their respective standard errors.

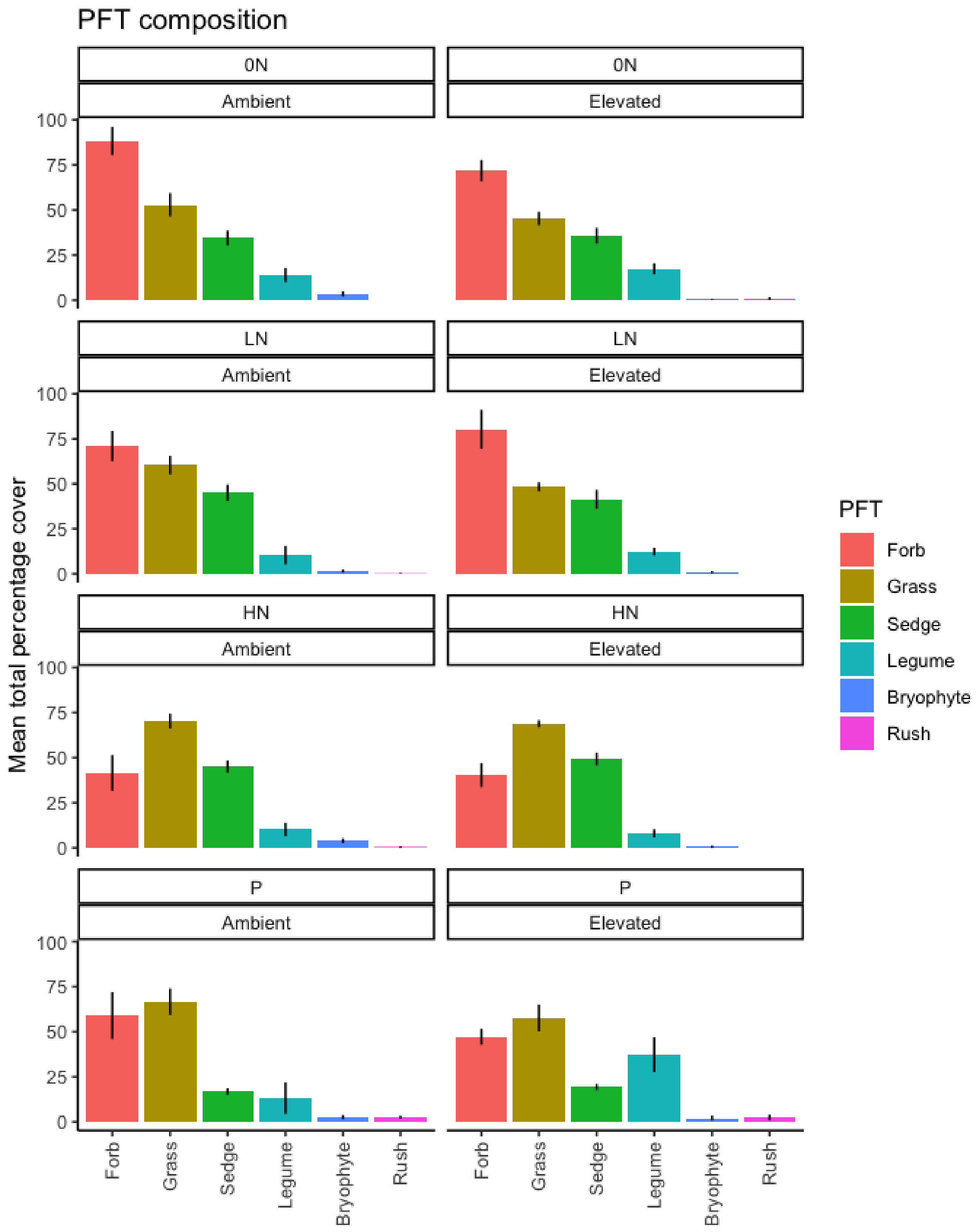

Figure S.3. 2: The mean total percentage cover of each plant functional type (PFT) across CO_2 treatments (ambient and elevated) and nutrient treatments (0N, LN, HN and P) and Error bars represent the standard errors of the means.

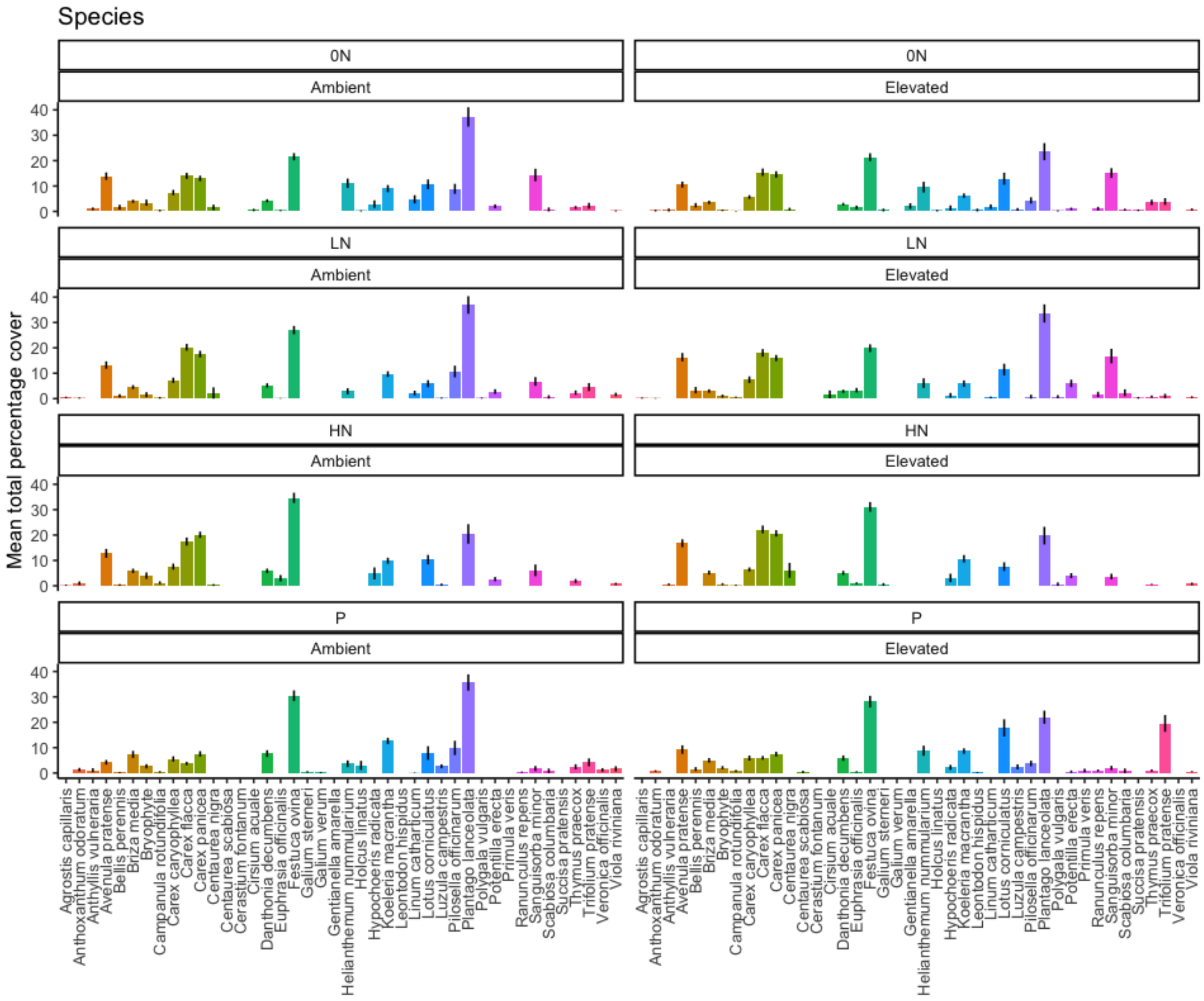

Figure S.3. 3: CO_2 and nutrient treatments effect on the mean percentage cover of each individual species with error bars representing the standard errors of the means.

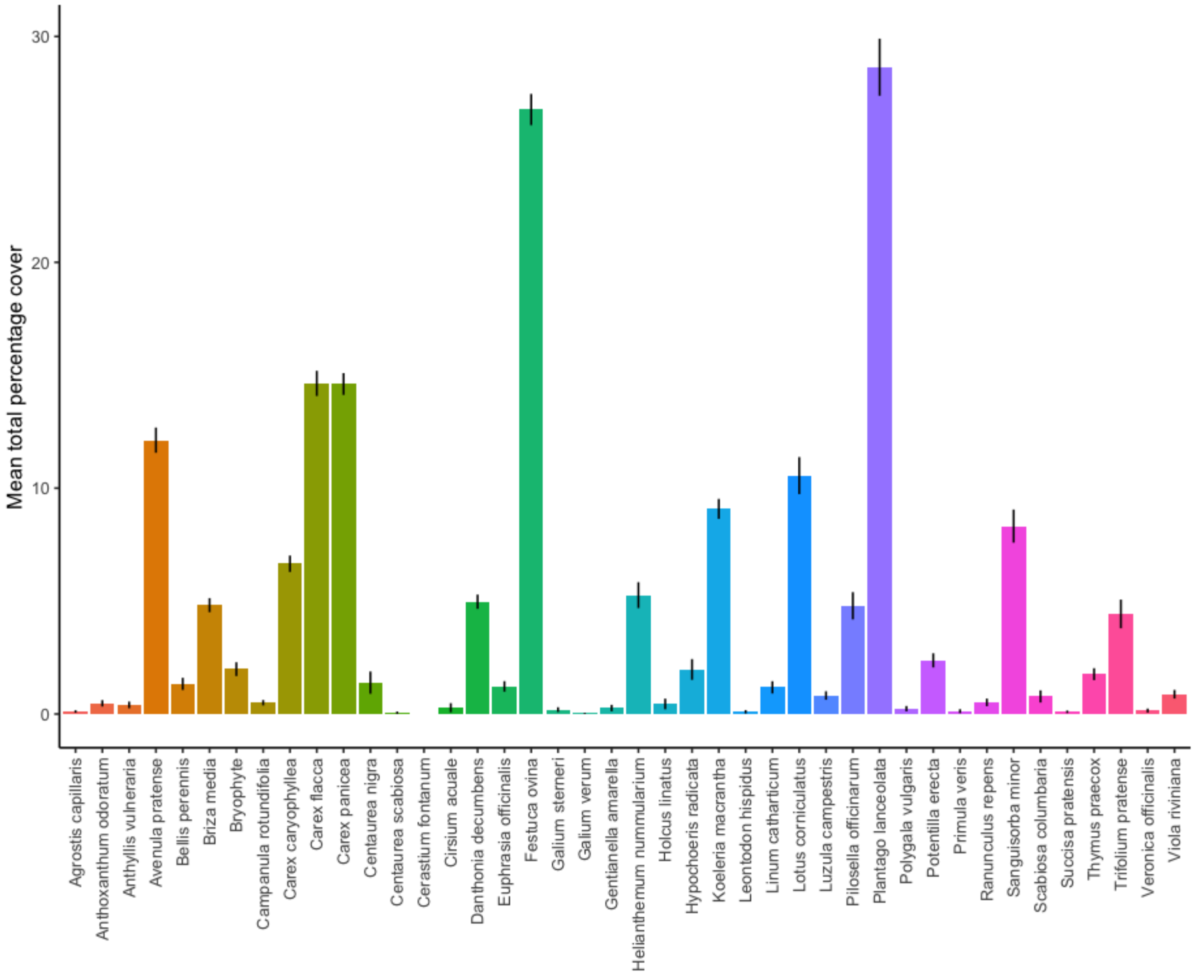

Figure S.3. 4: The mean percentage cover of each species was recorded across all mesocosms, with error bars representing the standard error of the means.

Chapter 4

Ecological and Physiological Comparisons and Contrasts between *Calluna vulgaris* (Heather) life stages

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4 Abstract

British moorlands are distinctive and important habitats, both biogeographically and in terms of carbon cycling. In addition, upland moorlands span 2 to 3 million hectares in the UK, accounting for 75% of the world's total. However, past and present management malpractices, including overgrazing, inadequate burning, and artificial drainage, are contributing to the degradation of this habitat and reducing its capacity to withstand climate change. It is anticipated that the peatlands of the United Kingdom (that include moorlands), which have historically served as a carbon sink, will begin to act as a carbon source in the latter years of the 20th century. Long-term, repeatable observation of moorland areas is required for assessing the amount and trajectory of ecological change. *Calluna vulgaris* (*C. vulgaris*) is a shrub dominant across all British moorland habitats and is known to be vulnerable to N deposition and climate change. Understanding the ecology, physiology, and biochemistry of *C. vulgaris* is crucial for both protecting and managing UK moorlands. The challenge is further amplified because *C. vulgaris* undergoes four distinct life phases, and there is a knowledge gap in understanding how the species' physiological and biochemical traits change across these stages. The aim of this study is, therefore, to i) investigate the physiological and biochemical variations among *C. vulgaris* growth stages, and ii) assess if distinct life stages can be detected using hyperspectral reflectance at both the leaf and canopy levels. This study was carried out at the University of Sheffield's Bradfield Environmental Laboratory site during the summer of 2020, using biochemical analysis of leaf chlorophyll content, hyperspectral reflectance, and gas-exchange measurements, along with ecological surveys. Our results showed that the average *C. vulgaris* plant height varied from 57 cm to 26.5 cm for the mature and pioneer phases, respectively. Flower positions on the stems differed through the life stage, with the pioneer phase having the highest flowering position (2 cm from the top of the stem) and mature and degenerate phases having the same flower position (0 cm). In addition, leaf biochemistry significantly differed through life stages, with the degenerate phase having the highest leaf chlorophyll and leaf nitrogen content, and the pioneer phase having the lowest leaf chlorophyll and leaf nitrogen content. The photosynthesis rate (*A*) was highest for the mature phase, with an average of 28.33 µmol m^{-2} s^{-1} and lowest for the pioneer phase, with an average of 21.77 µmol m^{-2} s^{-1}. Leaf-level reflectance, among all the tested indices, Normalized difference red edge index (NDRE) and MERIS terrestrial chlorophyll index (MTCI) showed a strong relationship with leaf chlorophyll and N among life stages. Canopy-level reflectance, among all the tested indices, Normalized difference vegetation index (NDVI), NDRE, MTCI, and Simple ratio (SR) showed a strong relationship with leaf chlorophyll. The results obtained indicated that each phase has traits that are unique and distinct to it and distinguish it from other stages, whether in terms of ecology, physiology, or biochemistry. Understanding and knowing these characteristics and differences may help facilitate moorland management.

4.1 Introduction

The moorlands of the UK are a distinctive and valuable habitat which plays an important role in biogeography and carbon cycling on a national and international scale (Allen et al. 2016; Chapman et al. 2010; Harris et al. 2011). However, ongoing and historical (mis)management practices refer to inappropriate human interventions or a lack of necessary interventions, such as overgrazing, poor burning, and artificial drainage (Allen et al. 2016; Chapman et al. 2010; Clarke et al. 1995; Gimingham 1960) are degrading this habitat and diminishing their environmental resilience to climate change. In the latter decades of the 21st century, British peatlands may be transformed from a carbon sink into a carbon source (Arthur and Malthus 2012; Bellamy et al. 2005). In order to evaluate the extent and trajectory of environmental change, it is necessary to conduct long-term, reproducible monitoring of moorland areas (Arthur and Malthus 2012; Chapman et al. 2010; Knoth et al. 2013). However, these areas are large (upland moorland covers 2 to 3 million hectares in the UK, 75% of the world's total) and so present challenges for monitoring (Aebischer et al. 2009; Holden et al. 2007; Thompson et al. 1995). Most British moorland environments are dominated by one plant species – the shrub *Calluna vulgaris* (*C. vulgaris*; commonly known as heather or ling), which is known to have sensitivity to climate change (Albert et al. 2011; Gordon et al. 1999). If we are to better protect and manage moorland from the threats of mismanagement and climate change, it is important to understand the ecology, physiology, and biochemistry of *C. vulgaris*. This is made more challenging by the fact that *C. vulgaris* has four distinct life stages and the fact that there is much less known about how the species' physiology, leaf biochemistry and hyperspectral reflectance varies throughout the life stages.

Calluna vulgaris is a low-growing, evergreen dwarf shrub (Gimingham 1960; Gordon et al. 1999; Sands and Gimingham 1975), producing densely packed shoots with purple flowers. Its competitive and hardy nature allows the maintenance of aggressive dominance. Gimingham (1975), based on the study of Watt (1955), identified four distinct phases in the growth of *C. vulgaris* (Fig. 4.2) that are associated with changes in biomass and shoot production (Barclay-Estrup 1970; Society 1971) and differences in the microenvironment (Barclay-Estrup and Gimingham 1969; Sands and Gimingham 1975):

I. **Pioneer phase:** Characterised by small individuals with limited lignification, resulting in a patchy distribution pattern with a lush green colour appearance. Flowering is rare, occasionally missing, and the patch is open, with a large number of bryophytes and vascular plants growing beneath.

II. **Building phase:** The dense, green canopy of larger individuals entirely covers the ground, with little light reaching the ground level. Flowering is vigorous, with a lack of other plants developing under *C. vulgaris*.

III. **Mature phase:** Plants keep expanding, although at a slower rate. A less thick canopy with more gaps is the result of this development type, with abundant flowering and the beginning of other plants growing beneath.

IV. **Degenerate phase:** Due to the collapse of the major branches, the canopy gaps produced during the mature phase get larger, and other plant species expand rapidly; the lichen *Parmelia physodes* grow on the stems during the degenerate phase (Gimingham et al. 1981; Sands and Gimingham 1975).

These phases are, therefore, each associated with characteristic physiology, morphology, and ecological composition (Gimingham 1989; Mead and Arthur 2020; Sands and Gimingham 1975).

Much of UK heather moorland is managed for grouse shooting. The gamekeepers burn patches of heather every 8 to 15 years to ensure there is a mix of young *C. vulgaris* and older *C. vulgaris* because each life stage has a different advantage for grouse (i.e. the young heather is used as food for grouse, and the old for nesting). In heather-dominated moorland, rotating controlled burning is the most commonly used management method (Velle et al. 2012). However, mismanagement, N-deposition, and grazing have reduced *C. vulgaris* habitats across Europe, including the UK (Damgaard 2019; Holden et al. 2007). High N deposition can change species composition by decreasing heather and increasing grass species, especially nitrogen-tolerant grasses, causing biodiversity reduction (Bobbink et al. 2010). In addition, mismanagement, such as inappropriate burning, can destroy habitats and peatlands and raise carbon emissions (Thompson et al. 1995). Another example of mismanagement which lacks to implement regeneration, can cause the *C. vulgaris* population to age, and diminish the resilience of the habitat (Thompson et al. 1995). Moreover, overgrazing by cattle or wild herbivores can seriously harm heather plants by creating soil compaction, erosion, and, eventually, a reduction in *C. vulgaris* abundance, leading to the dominance of less desirable plant species (Thompson et al. 1995).

Chlorophyll molecules are fundamental plant pigments that convert solar radiation into chemical energy through photosynthesis. The amount of solar radiation captured by a leaf is mostly a consequence of the foliar concentrations of photosynthetic pigments. Hence, reduced chlorophyll concentrations can significantly restrict photosynthetic capacity and, by extension, the primary production (Richardson et al. 2002). Chlorophyll content tends to change throughout a plant's lifetime in response to environmental stress and during senescence (Şevik et al. 2014; Swoczyna et al. 2022; Tamary et al. 2019). As a result, total chlorophyll measurements can provide important insights into plant-environment interactions (Richardson et al. 2002). In addition, leaf chlorophyll concentration is significantly associated with leaf nitrogen content (Daughtry et al. 2000), which provides a bio-indicator of plant physiological status, indicating areas of plant stress and perturbation (Anatoly et al. 2003; Sampson et al. 2003). Understanding variations in leaf pigments' (such as chlorophyll) in response to changing biotic and abiotic variables of species-specific and cross-specific are also essential for resource management (Richardson et al. 2002).

Monitoring changes in leaf biophysical or physiological traits across *C. vulgaris* life stages is therefore important for accurately modelling plant function over a range of spatial and temporal scales (Arthur and Malthus 2012; Nichol and Grace 2010; Varvia et al. 2018). While traditional ecological field-based observation methods of *C. vulgaris* growth, such as on-foot ecological surveys, can be time-consuming and spatially limited (Arthur and Malthus 2012; Mead and Arthur 2020), remote sensing methods for classifying *C. vulgaris* growth and canopy morphology at the leaf or canopy-scale may be of great interest. Hyperspectral remote sensing techniques offer the potential for precise, rapid, and scalable evaluation of the dynamic biophysical changes that may occur as a result of changes in *C. vulgaris'* life stages (Mead and Arthur 2020). Leaf reflectance is controlled by the occurrence of foliage pigments such as chlorophyll, N, and water content (Ustin et al. 2004) and by leaf structure (Croft et al. 2014). Various methods exist for quantifying these leaf variables from remotely sensed data, including spectral vegetation indices and radiative transfer models (Croft et al. 2017; Heenkenda et al. 2015; Main et al. 2011). Such approaches would therefore lend themselves to monitoring *C. vulgaris* at large spatial scales, across landscapes and (if distinguishable) for different life stages. However, the efficacy of hyperspectral techniques for detecting change throughout *C. vulgaris* life stages has yet to be investigated.

Our study aims to: i) revise and refine the ecological variation among *C. vulgaris* life stages (Gimingham 1975) and examine further new ecological traits such as stem length, length of the green leaf on the stems, flowers' position on the stems, the density of stems on the bush, percentage cover of *C. vulgaris*, and percentage cover of other species under *C. vulgaris*; and ii) investigate the physiological and biochemical differences among *C. vulgaris* life stages and investigate whether the different life stages can be detected through hyperspectral reflectance at both the leaf and canopy levels.

This study, therefore, addresses the following research questions: (I) What are the ecological and physiological variations among *C. vulgaris* (heather) life stages? (II) Can changes in leaf biochemical and physiological traits resulting from *C. vulgaris* (heather) life stages changes be detected using hyperspectral reflectance at both canopy and leaf levels? and (III) Are there vegetation indices that can distinguish between life stages?

4.2 Methods

4.2.1 Bradfield site description

Fieldwork was conducted between 26/08/2020 and 20/09/2020 at the University of Sheffield's Bradfield Environmental Laboratory (53°25N 1°35W) (Figure 4.1), 390 m above sea level, with mean annual air temperatures of 10.35 ^{o}C (Fick and Hijmans 2017). The mean annual precipitation is 893 mm (Fick and Hijmans 2017). Although the site is dominated by *C. vulgaris*, other plant species typical of acidic upland soils are common, including dwarf shrubs *Vaccinium myrtillus* and *Vaccinium vitis-idaea*, grasses species including *Deschampsia flexuosa, Festuca ovina,* and *Nardus stricta,* the rush s *Juncus squarrosus,* and forbs *Anthriscus sylvestris, Plantago major, Vicia sativa* and *Galium saxatile* (Figure 4.1).

Block selection

Across the Bradfield site, eight *C. vulgaris* blocks were selected, approximately 5x5 m^2 in size (Figure 4.1). Each block was dominated by *C. vulgaris* to the extent that at the canopy level, *C. vulgaris* was the only species (though other species were present under *C. vulgaris).*

Figure 4.1: Bradfield site, the yellow-border area. At the left is the main building. Orange circles *Calluna vulgaris* blocks, with a total of eight blocks, each block has four different life stages. Scale label and compass directions at the bottom right.

Within each block, four plots (50x50 cm^2) were selected, each representing a different life stage of *C. vulgaris* (Figure 4.2). The total number of plots across the eight blocks was, therefore, 32 50x50 cm plots, with each life stage represented eight times. The four different growth phases were selected based on the phenotypes of *C. vulgaris* as identified previously by Watt (1955) and Gimingham (1975) (Figure 4.2):

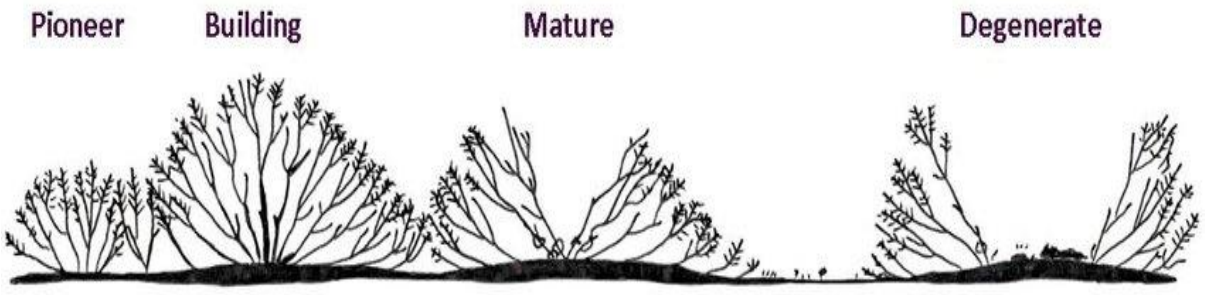

Figure 4.2: The four different growth phases of *Calluna vulgaris*. From left to right: 1. pioneer phase; 2. building phase; 3. mature phase; and 4. degenerate phase (source: Watt, 1955).

4.2.2 Ecological measurements: structural traits

Within each plot, *C. vulgaris* height was measured by measuring the height of five randomly selected points using a rigid tape measure. The stem length was measured by selecting five random stems and measuring the length of the stem from the soil. The length of the area on the stem covered in green leaves was also measured. The flowering position on the stems was measured as the distance from the top of the stem down to the first flower. The density of stems on the bush was measured using a quadrat by selecting five random spots 5x5 cm^2 in size and counting the stems within those.

The percentage of *C. vulgaris* ground cover was assessed by two methods; the first estimate was by visualising the ground cover of *C. vulgaris* for the whole plot (50x50 cm^2). The second was estimated using a quadrat by counting the presence or absence of *C. vulgaris* within each 5x5 cm^2 grid cell inside the quadrat. The percentage cover of other plant species growing under *C. vulgaris* was assessed by the same two methods.

Leaf area was measured using ImageJ (1.53a, Wayne Rosband, http://imagej.nih.gov/ij java 1.8.0.- 172). The leaves were captured via iPhone 7 sitting on a tripod and imported to ImageJ to measure the leaf area via polygon selections.

Soil moisture and temperature

Soil moisture was recorded on the same day on 10/09/2020, for all blocks using Hydrosense II handheld soil moister sensor HS2 (Campbell Scientific, Logan, Utah, USA), and soil temperature was recorded at the same time using a TP3001 digital thermometer (Metria).

4.2.3 Physiological measurements

Photosynthesis rate (*A*) was measured using a LiCor LI6400 portable photosynthesis system (LiCor, Germany) with a 6400-02B LED Light Source leaf chamber (6 cm^2) by recording three random replicates for each plot 50x50 cm^2. For all measurements, PAR was maintained at 1600 $\mu mol\ m^{-2}\ s^{-1}$ via red (680 nm) and blue (430 nm) LEDs integrated into the leaf chamber fluorometer. The CO_2 rate was set at 400 $\mu mol\ CO_2$ mol, and flow was standardised at 500 $\mu mol\ s^{-1}$. The temperature was standardised at 25 ^oC, and humidity was set between 50-70%. Before commencing measurements, the leaves were acclimated inside the leaf chamber for approximately 5–10 min until steady-state conditions were reached. Leaf gas exchange was measured between 11:00 to 14:00; this is for accurate measurement as the plants are at their maximum levels of activity. Because *C. vulgaris* leaves were relatively small in size, the leaves which had been measured were collected, and the leaf area was calculated using ImageJ (http://imagej.nih.gov/ij java 1.8.0.- 172).

4.2.4 Biochemistry measurements

Three leaves were sampled from each plot (the same leaves used for photosynthesis measurements) and kept at ~0 °C in dark conditions during transport back to the University of Sheffield (~10 km away) for laboratory analysis. From these samples, leaf disks were taken to extract chlorophyll using a hole puncher with a 6-mm diameter (28.3 mm^2). Each leaf sample was then immediately transferred into N, N-dimethylformamide (DMF) for foliar chlorophyll extraction following the methods of (Moran 1982), and the absorbance was subsequently measured at 663.8, 646.8 and 480 nm (Wellburn 1994) using a Spectrophotometer (Cecil CE1020 100 series spectrophotometer, Spectronic). Three fresh leaves with the remaining leaf material from chlorophyll punched leaves were dried for C/N concentrations and were oven dried at 80 ^{o}C prior to grinding by tissue-lyser (FastPrep-24TM 5G bead beating grinder and lysis system, MP Biomedical). To assess N and C tissue concentrations, approximately 2 – 4 mg of finely ground plant material was placed inside a tin capsule and sealed with tweezers for subsequent analysis by an isotope-ratio mass spectrometer (ANCA GSL 20-20 Mass Spectrometer, Sercon PDZ Europa). Using the same leaves, specific leaf area was calculated using the ImageJ leaf area and leaf dry weight, and leaf H_2O was calculated using leaf fresh wight and leaf dry weight.

4.2.5 Hyperspectral reflectance measurements

A Spectral Evolution PSR+ 3500 field spectrometer (Spectral Evolution Inc., MA, USA) was used to measure leaf-level hyperspectral reflectance from three leaves that were sampled from each plot (the same leaves used for photosynthesis measurement and leaf biochemistry). The PSR+ 3500 has a spectral range of 350-2500 nm and a spectral resolution of 2.8 nm at 700 nm. Leaf level reflectance was measured on individual leaves using a leaf clip attachment with an internal light source. To investigate if the adjustments in leaf physiological and biochemical traits among *C. vulgaris* life stages can be quantified remotely using hyperspectral reflectance at the leaf level, selected vegetation indices are regressed against measured leaf traits. A panel spectral was used as a calibration reference panel to convert reflected radiance to reflectance. For canopy measurements, a 4^{o} degree foreoptic was used, with the fiberoptic held at 1 m height above the ground, which gave an instantaneous field of view of 7 cm diameter. Reflectance data were collected on a sunny, blue-sky day on 12/09/2020, with three measurements taken in different locations within each 50 x 50 cm plot. To analyse the reflectance data, several spectral vegetation indices were selected according to their sensitivity to different structural, biochemical, or physiological traits (Table 4.1).

Table 4.1: Details of all published spectral vegetation indices tested in this study.

Index	Name	Equation	Reference
MTCI	MERIS terrestrial chlorophyll index	(R754 − R709)/ (R709 − R681)	(Dash and Curran 2004)
NDRE	Normalized Difference red edge index	(R790−R720)/(R790+R720)	(Fitzgerald et al. 2006)
NDVI	Normalized Difference Vegetation Index	(NIR – Red)/ (NIR + Red)	(Rouse et al. 1974)
mARI	Modified Anthocyanin Reflectance Index	((R760-R797) *(550) -(1/701))	(Anatoly et al. 2009)
MNDVI1	Modified NDVI	(R755-R745)/ (R755 + R745)	(Mutanga and Skidmore 2004)
MNDVI8	Modified NDVI	(R755-R730)/ (R755 + R730)	(Mutanga and Skidmore 2004)
RVII	Ratio Vegetation Index I	R810/R660	(Zhu et al. 2008)
RVIII	Ratio Vegetation Index II	R810/R560	(Xue et al. 2004)
CI$_{\textbf{red edge}}$	Red edge chlorophyll index	(NIR/Red Edge) − 1	(Gitelson et al. 2005; Gitelson et al. 2003)
RWC	Relative Water Content	(R1451/ R1263)	(Yu et al. 2000)
WBI	Water Band Index	(R970/ R900)	(Penuelas et al. 1993)
WCI	water content index	(R686- R955)/ (R955- R548)	(Mertens et al. 2021)
WPI	Water Poverty Index	(R665 - R715)/ (R715)	(Mertens et al. 2021)

4.2.6 Statistical analysis

Statistical analyses were carried out using R (R Studio, R Core (Team 2021)). One-way ANOVA models were used to assess the variation among *C. vulgaris* life stages for all ecological, photosynthesis and biochemical measurements. If there were significant overall differences among *C. vulgaris* life stages, Tukey multiple comparisons of means were used to determine between which *C. vulgaris* life stages differences occurred. The “userfriendlyscience” (Peters 2018) and “spectrolab” (Jose et al. 2023) packages were used for reflectance data analysis for calculating both the indices and also for determining whether the life stages had different reflectance features. (lm) function was used to calculate the linear regression among leaf physiology and biochemistry. All the plots were performed using the “ggplot2” function (Wilkinson 2011).

4.3 Results

4.3.1 Ecological variations among C. vulgaris life stages

Calluna vulgaris height was significantly different between life stages (Fig. 4.3A; *df* = 3, 28; *F* = 22.98; $P < 0.001$), where the mature phase showed the greatest height, with an average of 57 cm while the pioneer phase had the lowest height, with an average of 26.5 cm.

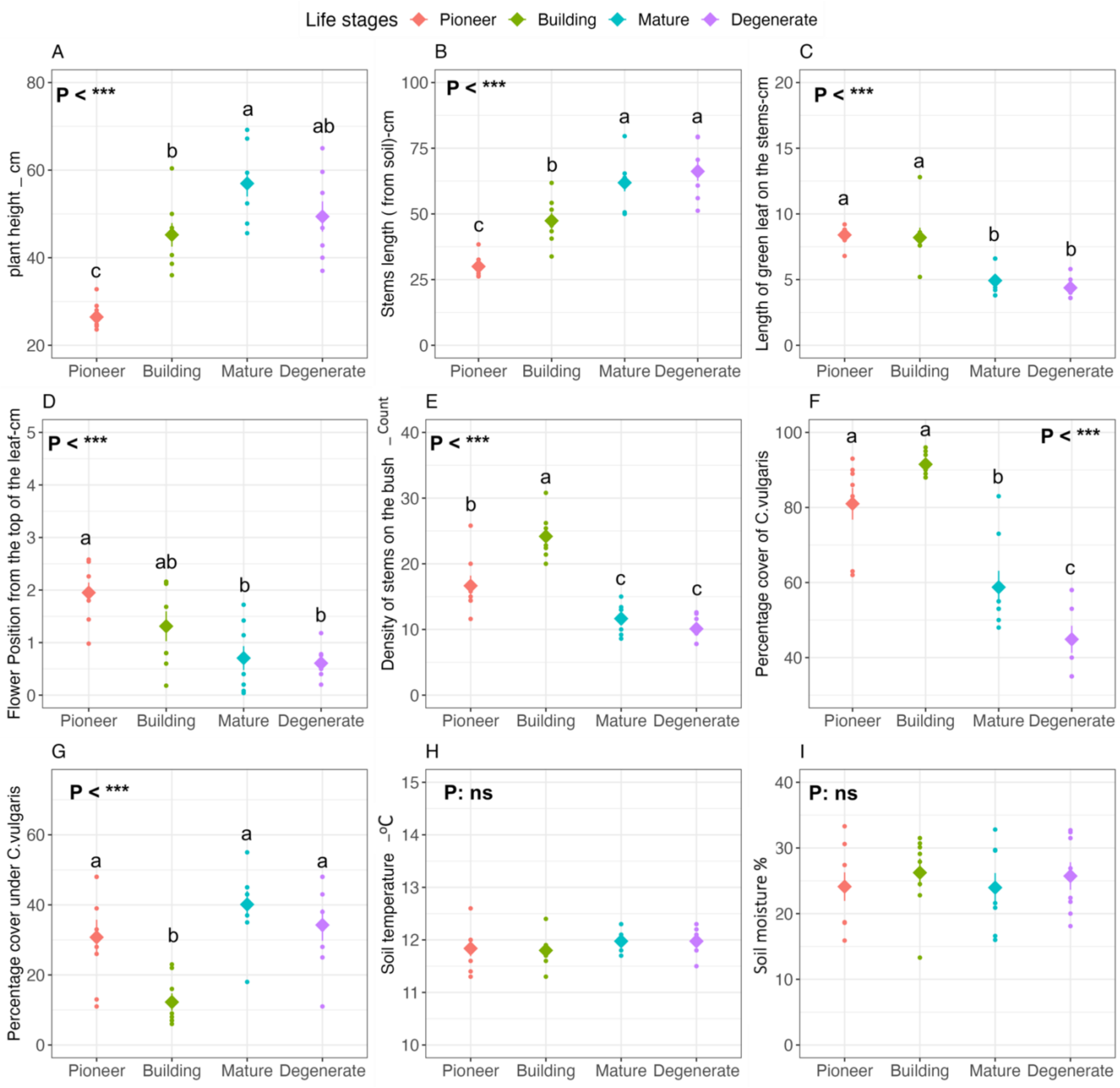

Figure 4.3: Variation in structural traits and soil conditions among *C. vulgaris* life stages; A) *C. vulgaris* height, B) stem length, C) length of the green leaf on the stems, D) flowers' position on the stems, E) density of stems on the bush, F) percentage cover of *C. vulgaris*, G) percentage cover of other species under *C. vulgaris*, H) soil temperature, I) soil moisture. Large diamond points with standard error bars indicate the mean, and the smaller points are individual data points. Life stages that do not share a letter are significantly different (Tukey, P < 0.05).

Calluna vulgaris stem length significantly differed between life stages (Fig. 4.3B; *df* = 3, 28; *F* = 30.54; P < 0.001). The degenerate phase showed the longest stems, with an average of 66.2 cm, and the pioneer phase had the shortest, with an average of 30 cm. *Calluna vulgaris* length of the green leaf area on stems also significantly differed among life stages (Fig. 4.3C; *df* = 3, 28; *F* = 20.91; P < 0.001). The pioneer and building phases had the longest green leaf on the stem, with an average of 8.4 cm for the pioneer and 8.2 cm for the building, while the degenerate and mature phases had the lowest green leaf on the stem, with an average of 4.37 cm for the degenerate and 4.9 cm for the mature. Also, following the same trend, *C. vulgaris* flowers' position on the stems was significantly different among life stages (Fig. 4.3D; *df* = 3, 28; *F* = 8.541; P < 0.001). The pioneer phase had the highest flowering position with flowers which means flowers started 2 cm from the top of the stem, while mature and degenerate phases had the same flower position on the stems, which means the flowers grew at the top of the stems (0 cm). *Calluna vulgaris* density of stems on the bush significantly differed among life stages (Fig. 4.3E; *df* = 3, 28; *F* = 32.62; P < 0.001). The building phase showed the highest density of stems, with an average of 24 stems, while the mature and degenerate phases were the lowest, with an average of 11 stems for mature and 10 stems for degenerate.

The percentage cover of *C. vulgaris* significantly differed among life stages (Fig. 4.3F; *df* = 3, 28; *F* = 34.94; P < 0.001). The building and pioneer phases had the highest percentage cover of *C. vulgaris*, with an average of 92% of cover for the building and 81% of cover for the pioneer, while the degenerate phase had the lowest, with an average of 44.8% of cover. The percentage cover of other species under *C. vulgaris* significantly differed among life stages (Fig. 4.3G; *df* = 3, 28; *F* = 8.871; P < 0.001). The degenerate, mature, and pioneer phases had a high percentage cover of other species under *C. vulgaris*, with an average of 34.5% for the degenerate, 40% for the mature and 30% for the pioneer, while the building phase had the lowest with an average of 12.2%.

Soil temperature and soil moisture were not significantly different between life stages (Fig. 4.3H and 4.3I; $df = 3, 28$; $F = 0.742$; $P > 0.05$; and $df = 3, 28$; $F = 0.264$; $P > 0.05$).

4.3.2 Leaf physiology and biochemistry variations among C. vulgaris life stages

Both leaf chlorophyll content and leaf nitrogen content were significantly different among life stages (Fig. 4.4A and 4.4D; leaf chlorophyll; $df = 3, 28$; $F = 12.35$; $P < 0.001$; and leaf nitrogen content; $df = 3, 28$; $F = 10.64$; $P < 0.001$), and both followed a similar trend with the degenerate phase having the highest leaf chlorophyll, with an average of 48.13 (μg cm^2), and leaf nitrogen content, with an average of 2.1 %, where pioneer phase had the lowest leaf chlorophyll, with an average of 40.69 μg cm^2, and leaf nitrogen content, with an average of 1.62 %.

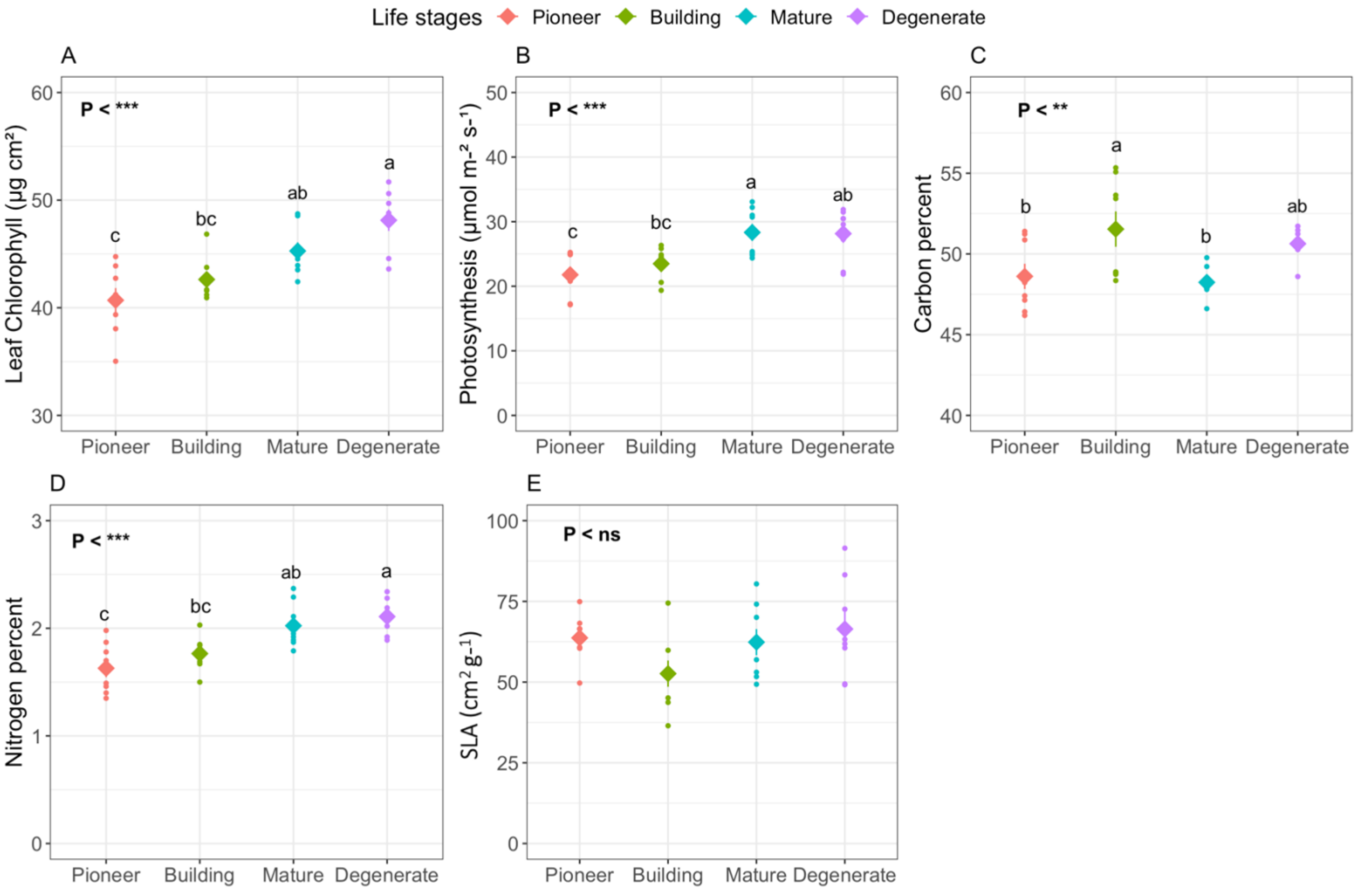

Figure 4.4: Leaf physiology and biochemistry variation among *C. vulgaris* life stages; A) leaf chlorophyll content, B) photosynthesis rate (A), C) leaf carbon content, D) leaf nitrogen content, E) Specific leaf area (SLA). Large diamond points with standard error bars indicate the mean, and the smaller points are individual data points. Life stages that do not share a letter are significantly different (Tukey, $P < 0.05$).

The photosynthesis rate (*A*) was also significantly different among life stages (Fig. 4.4B; *df* = 3, 28; *F* = 7.474; $P < 0.001$), again following a similar with the mature phase having the highest photosynthesis rate, with an average of 28.33 µmol m^{-2} s^{-1}, where the pioneer phase had the lowest photosynthesis rate, with an average of 21.77 µmol m^{-2} s^{-1}. Leaf carbon content significantly differed among life stages (Fig. 4.4C; *df* = 3, 28; *F* = 4.924; $P < 0.001$), and leaf carbon content dropped in the pioneer, with an average of 48.61 %, and mature phases, with an average of 48.24 %, and increased in the building phase, with an average of 51.53 %. However, specific leaf area (SLA) did not show differences among life stages (Fig. 4.4E; *df* = 3, 28; *F* = 2.117; $P > 0.05$).

4.3.3 Relationships between leaf gas exchange and leaf nutrients among C. vulgaris life stages

Leaf chlorophyll content showed a positive relationship with photosynthesis, with an overall regression coefficient of $R^2 = 0.779$; $p < 0.001$ (Fig. 4.5A). Mature and degenerate phases had the highest leaf chlorophyll with the highest photosynthesis. Leaf nitrogen content showed a positive relationship with photosynthesis, with an overall regression coefficient of $R^2 = 0.794$; $p < 0.001$ (Fig. 4.5B). Furthermore, leaf nitrogen content showed a positive relationship with leaf chlorophyll, with an overall regression coefficient of $R^2 = 0.913$; $p < 0.001$ (Fig. 4.5C). Mature and degenerate phases had the highest leaf chlorophyll with the highest leaf nitrogen.

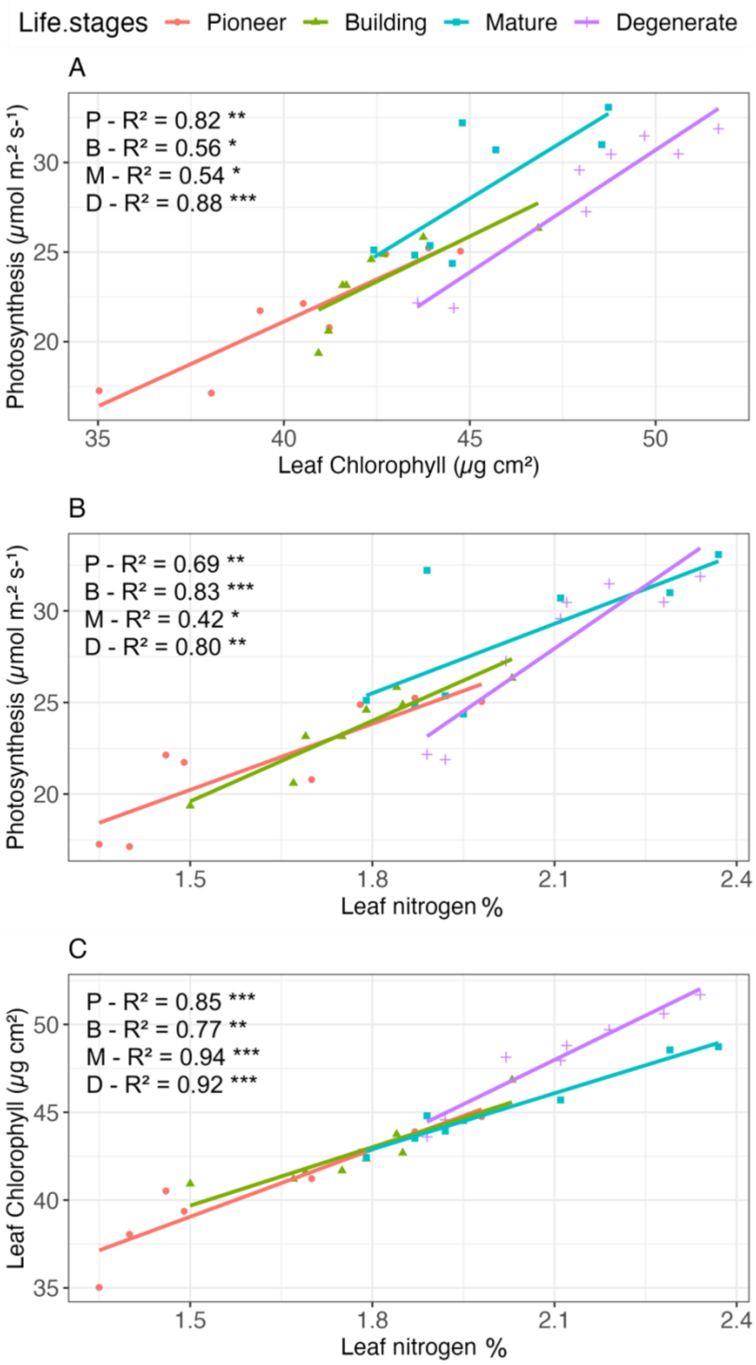

Figure 4.5: Regression analysis between *C. vulgaris* life stages, A) leaf chlorophyll and photosynthesis (overall regression coefficient of $R^2 = 0.779$; $p < 0.001$), B) leaf nitrogen and photosynthesis (overall regression coefficient of $R^2 = 0.794$; $p < 0.001$), C) leaf nitrogen and leaf chlorophyll (overall regression coefficient of $R^2 = 0.913$; $p < 0.001$). P, B, M, and D represent the life stage's first letter. *, ** and *** indicate significance at $p < 0.05$, $p < 0.01$, and $P < 0.001$, respectively.

4.3.4 Hyperspectral reflectance signatures for C. vulgaris different life stages

The ability of hyperspectral reflectance to discriminate among *C. vulgaris* different life stages was investigated at both the leaf and canopy levels (Figure 4.6).

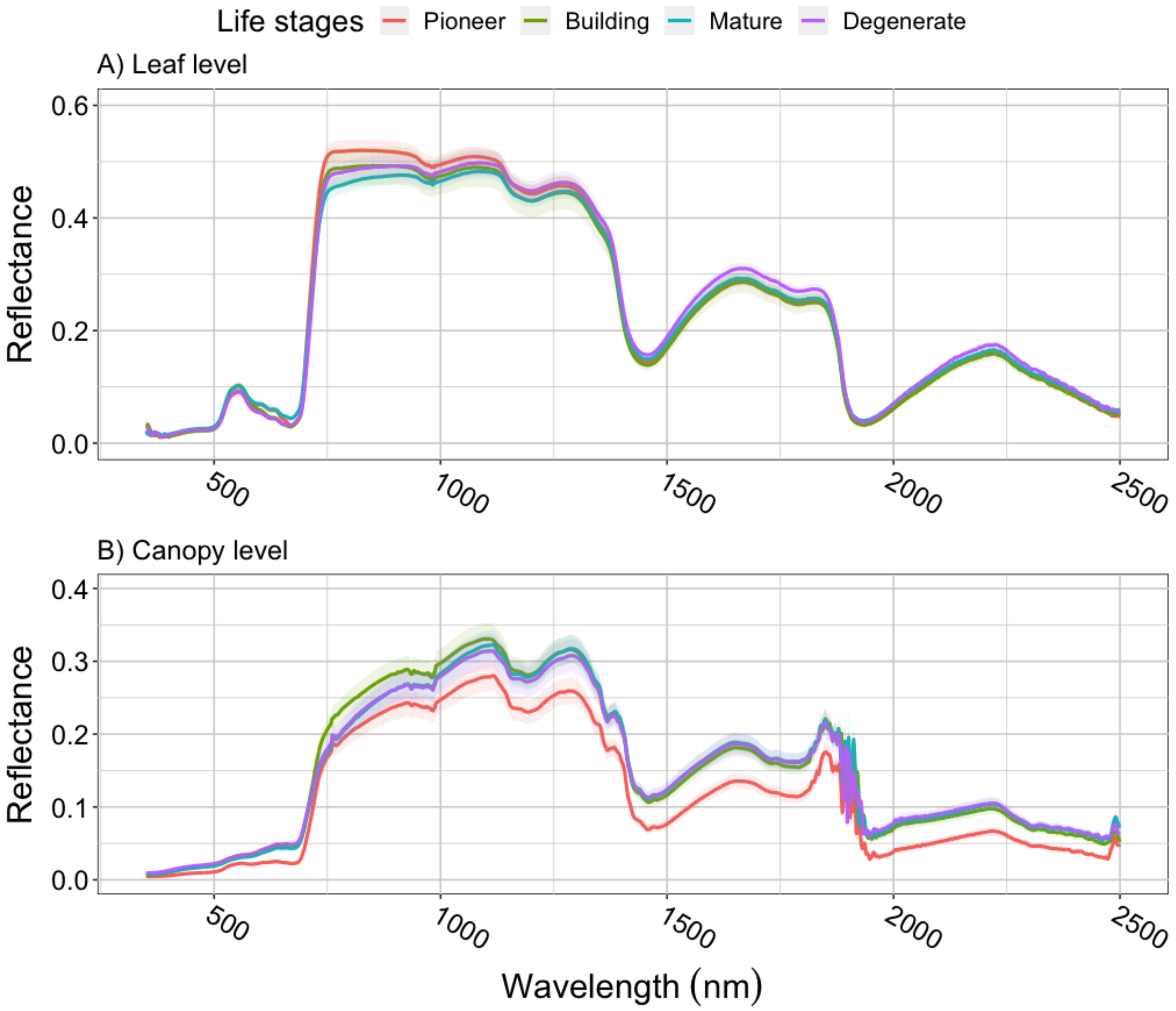

Figure 4.6: Spectral reflectance among *C. vulgaris* life stages, A) at the leaf level and B) at the canopy level; each colour represents a different life stage.

At the leaf level (Figure 4.6A), life stages did show differences in the visible light between 600 – 700 nm and in NIR, the building phase had a slight increase in the green peak. In addition, the degenerate phase showed a higher reflectance in the infrared regions and after 1500 nm compared to other life stages. There were no differences in reflectance in the water absorption bands at 1900 nm between life stages.

At the canopy level (Figure 4.6B), the pioneer phase did show lower reflectance overall and in the visible light and infrared regions, while the building phase showed a higher reflectance in the infrared regions.

4.3.5 Chlorophyll, nitrogen and water content and vegetation indices at the leaf level

To investigate if the adjustments in leaf physiological and biochemical traits among *C. vulgaris* life stages (Figure 4.4) can be detected remotely using hyperspectral reflectance at the leaf level, selected vegetation indices are tested (Figures 4.7 and 4.8).

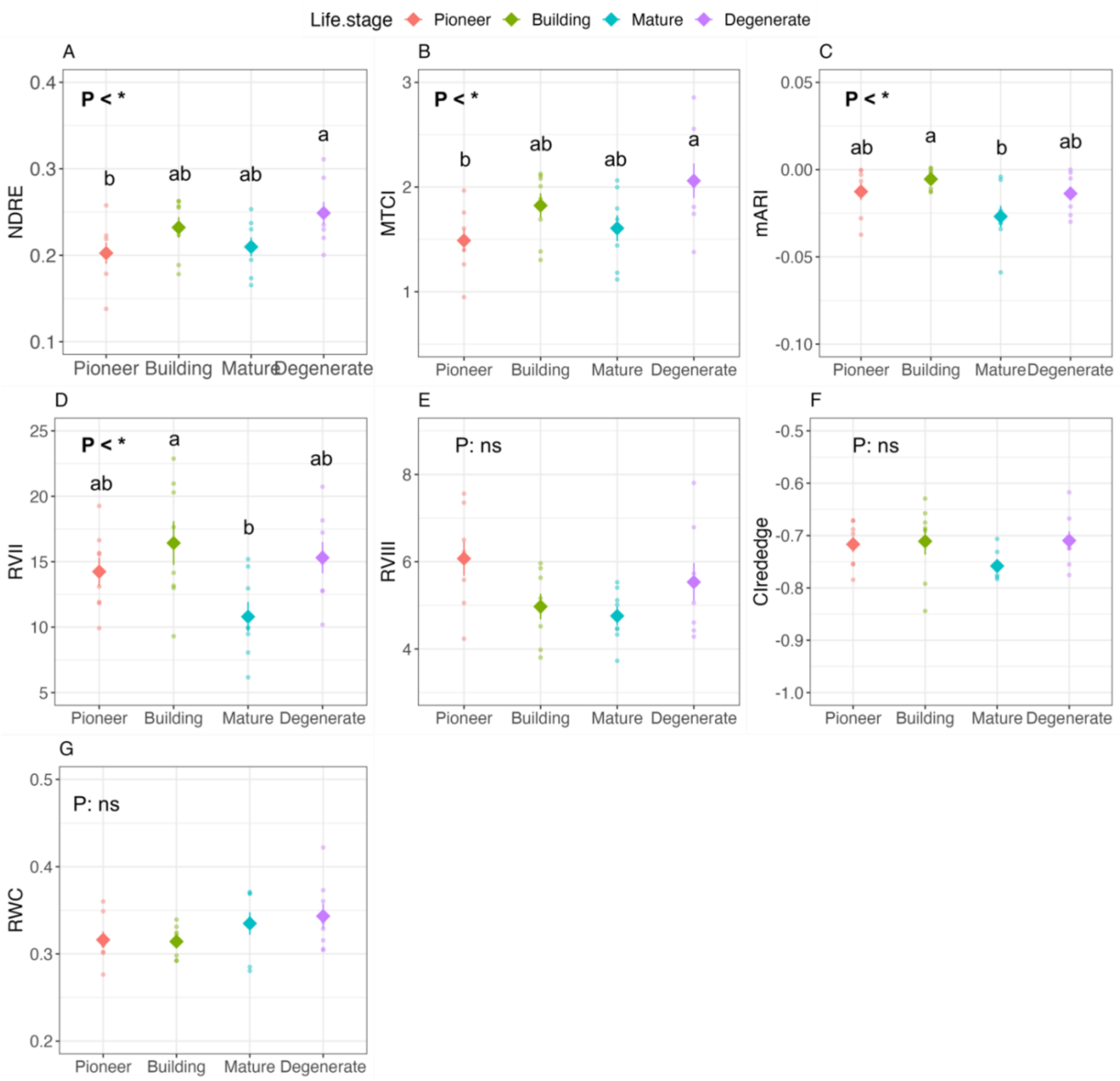

Figure 4.7: The leaf level hyperspectral reflectance variation among *C. vulgaris* life stages; A) NDRE - Normalized Difference red edge index, B) MERIS - terrestrial chlorophyll index, C) mARI - Modified Anthocyanin Reflectance Index, D) Ratio Vegetation Index I, E) Ratio Vegetation Index II, F) Red edge chlorophyll index, G) Relative Water Content. Large diamond points with standard error bars indicate the mean, and the smaller faint points are individual data points. Life stages that do not share a letter are significantly different (Tukey, $P < 0.05$). * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

Both normalized difference red edge index (NDRE) and MERIS - terrestrial chlorophyll index (MTCI) were significantly different among life stages (Fig. 4.7A and 4.7B; NDRE; *df* = 3, 28; *F* = 3.12; P < 0.05; and MTCI*; df* = 3, 28; *F* = 3.73; P < 0.05), and both followed a similar trend with the degenerate phase having the highest NDRE and MTCI reflectance where pioneer phase had the lowest. Interestingly, these results closely resembled the leaf chlorophyll and nitrogen contents that were observed in the laboratory (Fig. 4.4A and 4.4D). In addition, both modified anthocyanin reflectance index (mARI) and ratio vegetation index I (RVII) were significantly different among life stages (Fig. 4.7C and 4.7D; mARI; *df* = 3, 28; *F* = 3.96; P < 0.05; and RVII*; df* = 3, 28; *F* = 3.54; P < 0.05), and both followed a similar trend with the building phase having the highest mARI and RVII reflectance where mature phase had the lowest. However, ratio vegetation index II (RVII1), red edge chlorophyll index ($CI_{rededge}$), and relative water content (RWC) showed no significant difference among life stages (Fig. 4.7E, 4.7F, and 7G; RVII1; *df* = 3, 28; *F* = 2.91; P > 0.05; $CI_{rededge}$; *df* = 3, 28; *F* = 1.68; P > 0.05; and RWC; *df* = 3, 28; *F* = 1.63; P > 0.05).

For all life stages, leaf chlorophyll content had the strongest relationship with MERIS terrestrial chlorophyll index (MTCI), with a regression coefficient of $R^2 = 0.2$ ($p < 0.05$) (Fig. 4.8B). From all the indices tested, leaf nitrogen content also presented the strongest relationship with MTCI; $R^2 = 0.\ 17$, $p < 0.05$) (Fig. 4.8C). All the other indices tested in this study are also presented in (Figure 4.8), with their R^2 and P value.

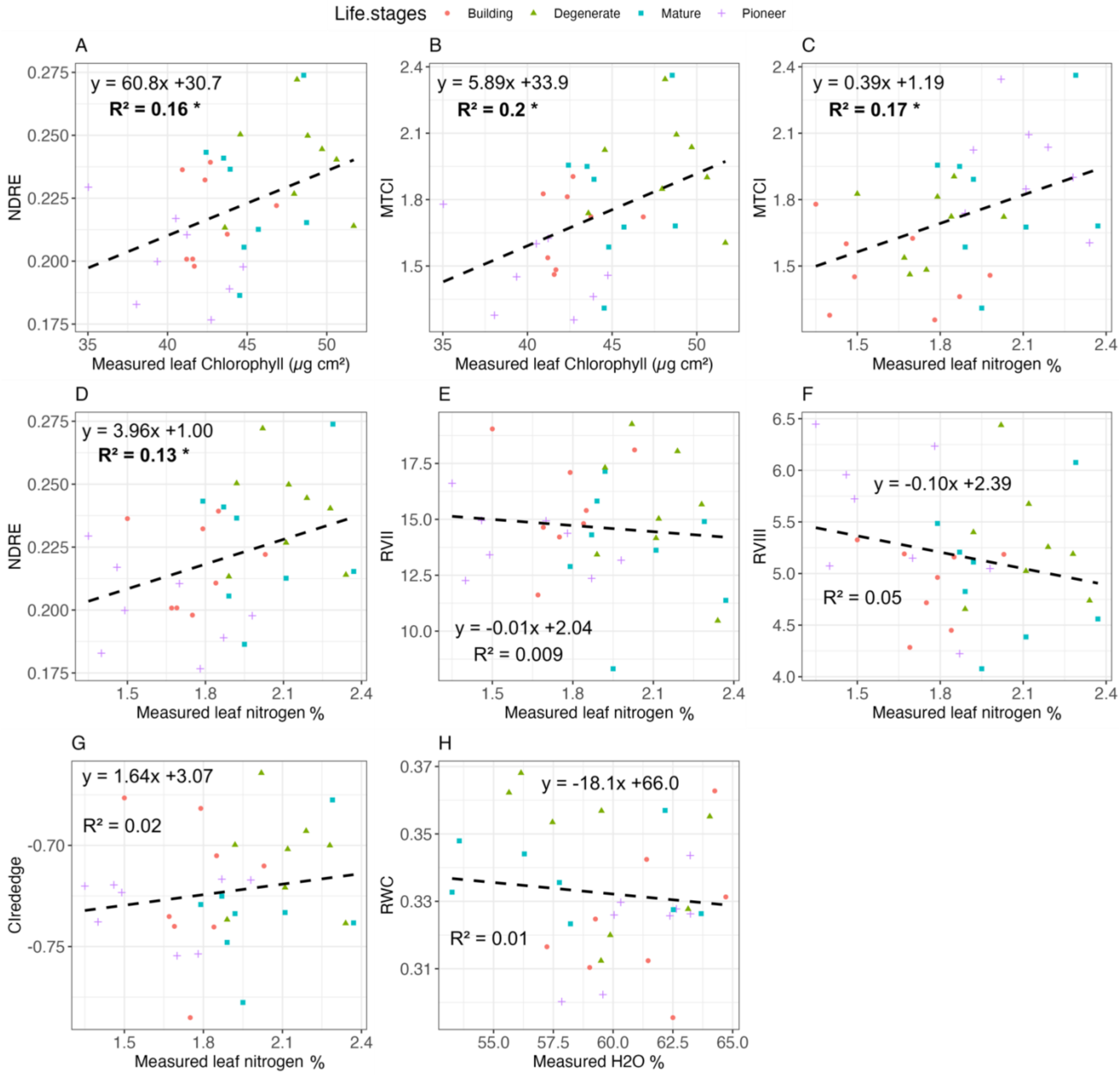

Figure 4.8: Leaf level Vegetation Indices (VIs) at the leaf level with regression against the most related measured variable for A) Normalized Difference red edge index, B) MERIS terrestrial chlorophyll index, C) MERIS terrestrial chlorophyll index, D) Normalized Difference red edge index, E) Ratio Vegetation Index I, F) Ratio Vegetation Index II, G) Red edge chlorophyll index, H) Relative Water Content. The overall R^2 values are shown on each plot. * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

4.3.6 Chlorophyll, nitrogen and water content and vegetation indices at the canopy level

To investigate if the adjustments in leaf physiological and biochemical traits among *C. vulgaris* life stages (Figure 4.4) can be quantified remotely using hyperspectral reflectance at the canopy level, selected vegetation indices are regressed against measured leaf traits (Figures 4.9 and 4.10).

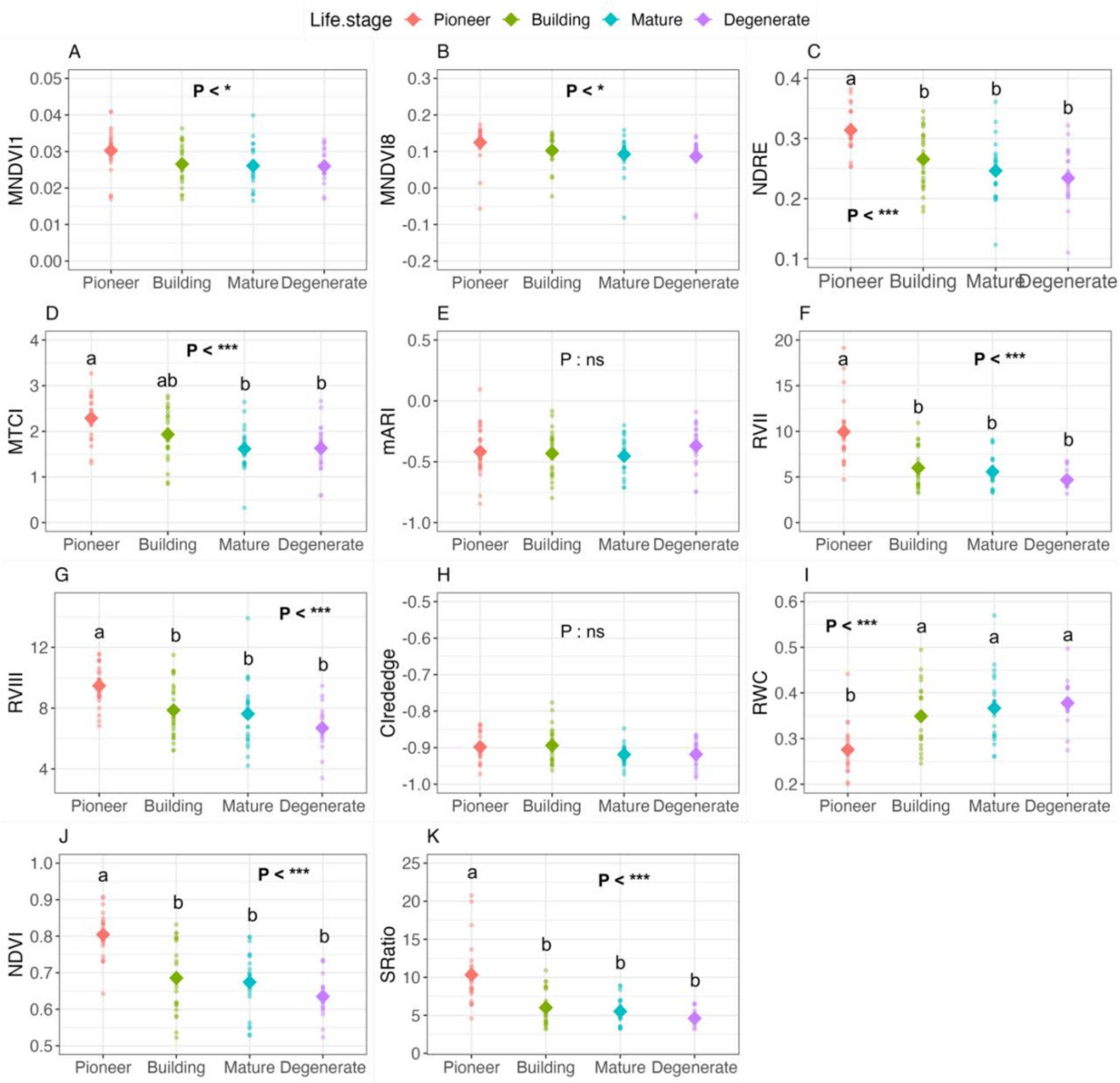

Figure 4.9: Canopy level hyperspectral reflectance variation among *C. vulgaris* life stages: A) Modified NDVI 1, B) Modified NDVI 8, C) Normalized Difference red edge index, D) MERIS terrestrial chlorophyll index, E) mARI – Modified Anthocyanin Reflectance Index, F) Ratio Vegetation Index I, G) Ratio Vegetation Index II, H) Red edge chlorophyll index, I) Relative Water Content, J) Normalized Difference Vegetation Index, K) Simple Ratio. Large diamond points with standard error bars indicate the mean, and the smaller faint points are individual data points. Life stages that do not share a letter are significantly different (Tukey, $P < 0.05$). *, ** and *** indicate significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

All these indices, normalized difference red edge index (NDRE), ratio vegetation index I (RVII), ratio vegetation index II (RVIII), normalized difference vegetation index (NDVI), Simple Ratio (SRatio), and MERIS terrestrial chlorophyll index (MTCI) were significantly different among life stages and followed a similar trend, with the pioneer phase having the highest reflectance where all other phases had the lowest with no significant differences among them (Fig. 4.9C, 4.9F, 4.9G, 4.9J, 4.9K, 4.9D; NDRE; *df* = 3, 89; *F* = 13.61; $P < 0.001$; RVII; *df* = 3, 89; *F* = 24.0; $P < 0.001$; RVIII; *df* = 3, 89; *F* = 10.35; $P < 0.001$; NDVI; *df* = 3, 89; *F* = 21.69; $P < 0.001$; SRatio; *df* = 3, 89; *F* = 23.04; $P < 0.001$; and MTCI; *df* = 3, 89; *F* = 9.17; $P < 0.001$). Relative water content (RWC) was also significantly different among life stages, with the pioneer phase being the lowest with no differences among the other stages (Fig. 10I; *df* = 3, 89; *F* = 13.86; $P < 0.001$). Modified NDVI 1 (MNDVI1) and Modified NDVI 8 (MNDVI8) were also significantly different among life stages (Fig. 4.9A and 4.9B; MNDVI1; *df* = 3, 89; *F* = 3.21; $P < 0.05$; and MNDVI8*; df* = 3, 89; *F* = 2.72; $P < 0.05$). However, modified anthocyanin reflectance index (mARI) and red edge chlorophyll index ($CI_{rededge}$) had no significant differences among life stages (Fig. 4.9E and 4.9H; mARI; *df* = 3, 89; *F* = 0.82; $P > 0.05$; and $CI_{rededge}$*; df* = 3, 89; *F* = 2.69; $P > 0.05$).

For all life stages, leaf chlorophyll content had the strongest relationship with NDRE, with a regression coefficient of $R^2 = 0.38$ ($p < 0.001$) (Fig. 4.10C). Also, leaf chlorophyll content had a strong relationship with NDVI, with a regression coefficient of $R^2 = 0.28$ ($p < 0.001$) (Fig. 4.10I). SR and MTCI had a strong relationship with leaf chlorophyll, with the same regression coefficient of $R^2 = 0.32$ ($p < 0.001$) (Fig. 4.10J and 4.10D). All the other indices tested in this study are also presented in (Figure 4.10), with their R^2 and P value.

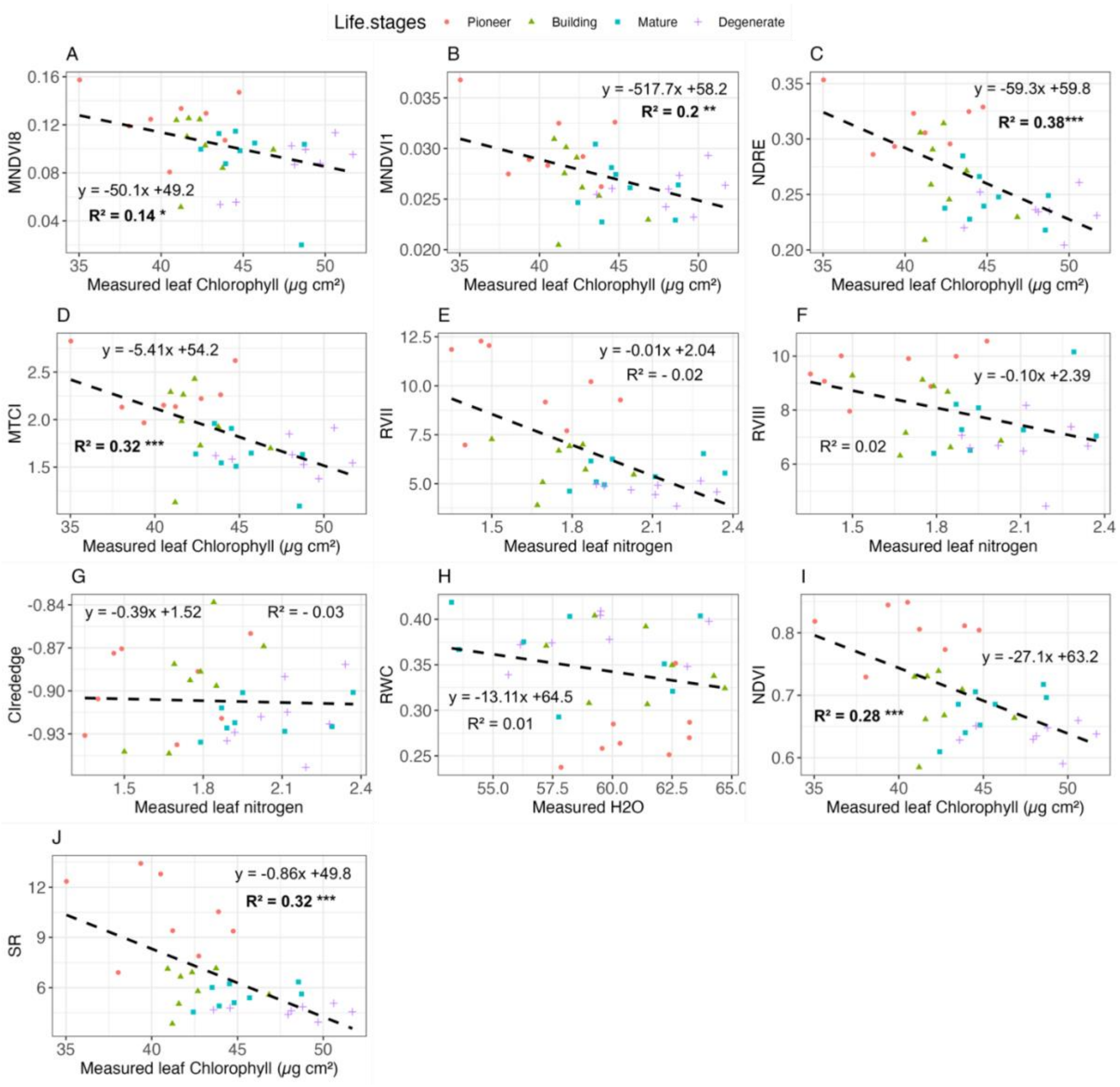

Figure 4.10: Canopy level Vegetation Indices (VIs) regressed against the most related measured variable: A) Modified NDVI 8, B) Modified NDVI 1, C) Normalized Difference red edge index, D) MERIS terrestrial chlorophyll index, E) Ratio Vegetation Index I, F) Ratio Vegetation Index II, G) Red edge chlorophyll index, H) Relative Water Content. The overall R^2 values are shown on each plot. * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

4.4 Discussion

4.4.1 Summary of the results

This study determined ecological variation in *C. vulgaris*. *Calluna vulgaris'* pioneer phase was the shortest, and the mature phase was the tallest. The stem length from the soil degenerate phase had the longest stem length. Degenerate and mature stems had the shortest green leaf area, while pioneer and building phases had the longest. The pioneer phase had the highest flowering position on *C. vulgaris* stems, while the mature and degenerate phases had the same flower position. The building phase had the most stems, and mature and degenerate had the least. In addition, physiological and leaf biochemistry features differed through *C. vulgaris* life stages, with leaf chlorophyll and N content significantly different across life stages and following a similar trend, with the degenerate phase having the highest and the pioneer phase having the lowest. Again, the mature phase had the maximum photosynthetic rate (*A*), whereas the pioneer phase had the lowest. The results show that each phase has distinct ecology, physiology, and biochemistry.

4.4.2 Ecological variations among C. vulgaris life stages

Calluna vulgaris height was maximum during the mature phase; this is attributed to the continued growth and the lack of collapse of main branches on the bush, which keeps the plant upright. Because the age of this stage ranges from 14 to 25 years, the plant reaches its peak activity at the mature phase, and this is reflected in the length of the plant (Sands and Gimingham 1975; Velle et al. 2012). In addition, the mature and the degenerate phases had the longest stems; since, in these two stages, the plant is still active and continues to grow (Gimingham 1989; Sands and Gimingham 1975). The degenerate phase was not the highest in plant height as the heather at this stage begins to collapse (Gimingham 1989; Sands and Gimingham 1975). Eventually, this led to decreased plant height while the stems continued to increase in length as they continued to grow (Gimingham 1989). The pioneer phase had the lowest height and shortest stems, as expected; this is due to being still in its early stages of growth and development (Gimingham 1989; Sands and Gimingham 1975).

These results regarding plant height are consistent with what has been observed in previous studies (Gimingham 1960; Gimingham 1989; Mead and Arthur 2020; Sands and Gimingham 1975; Thompson et al. 1995), which observed the morphological changes in the heather lifecycle, but this study has expanded understanding in terms of observing the length of the stem from the soil, which has shown the continuation of the growth of the stems despite the height reduction in degenerate phase due to the collapse.

The building phase had the highest percentage cover of *C. vulgaris*, with an average of 92% of cover due to the full growth and cover of the bush. As heather at this stage is fully grown, and there are no gaps in the bush (Barclay-Estrup 1970; Gimingham 1989). Also, as the heather in this stage has a robust root system that can support more extensive above-ground growth, the heather in the building stage invests energy in developing its stems and leaves, leading to increased length of green leaf on the stems and the density of the stems (Gimingham 1989; Sands and Gimingham 1975). In addition, because of the dense cover of the building stage, less light passes through to the ground level, and this affects the plant community under the building phase (Barclay-Estrup et al. 2020; Barclay-Estrup and Gimingham 1969; Gimingham 1989; Sands and Gimingham 1975); hence, the percentage cover of other species under *C. vulgaris* building phase was the lowest of all *C. vulgaris* life stages. This may help to explain one of the reasons for the dominance of *C. vulgaris* across large areas of upland because the building phase time is from 6 years up to 15 years which is long enough to have a significant influence on the ground cover and shade out other species (Barclay-Estrup and Gimingham 1969; Gimingham 1989; Sands and Gimingham 1975). However, the degenerate phase had the lowest percentage cover of *C. vulgaris* due to the collapse of the main branches (Gimingham, 1975; Gimingham et al.1981); this collapse and large gaps in the bush increased the cover of other species under this phase. As was also observed in this study that the degenerate phase had the highest cover of other species under the *C. vulgaris* and the lowest density of the stems on the bush due to the collapse and the death of the main branches. These findings are consistent with what was previously observed (Arthur and Malthus 2012; Barclay-Estrup 1970; Gimingham 1989; Mead and Arthur 2020; Sands and Gimingham 1975), and this study has expanded knowledge in terms of the percentage cover of other species under *C. vulgaris*, the length of green leaf on the stems, and the density of the stems.

Calluna vulgaris flowers' position on the stems was different among life stages. The pioneer and building phases had the lowest flowering position, which means the flowers were growing on the middle of the stems, not on the top of the stem. The flowering position has the potential to be used to determine the life stages and would be an important influence on the remote sensing canopy reflectance (Neumann et al. 2020). In the pioneer and building phases, because of the highest percentage cover and density of stems that reduce light passing through the bush, these two life stages have the green leaves growing at the top of the stems to better capture light for photosynthesis (Barclay-Estrup et al. 2020; Gimingham 1989). There is likely selection pressure to, therefore, not have flowers on top since there is a priority to present photosynthetic foliage to the sun rather than flowers. This may also explain why these two phases had the longest green leaf area on the stem. In contrast, the degenerate and mature phases had the flowers on top of the stem, which suggests a lower priority to have leaves there. This may be because lower leaves are not a disadvantage to photosynthesis in the degenerate and mature phases due to large gaps in the bush that allows light to pass through. This then means flowers can be presented higher up where they will be more visible to pollinators such as bees (Neumann et al. 2020). These findings emphasise the ecological variations among *C. vulgaris* life stages, and the impact of those variations throughout the life cycle on the plant community composition, and structural differences that likely impact productivity and pollination. In addition, these variations in heather ecological features across life stages, such as heather height, flowers' position on the stems, the length of green leaf on the stems and the density of the stems on the bush, are likely to also affect the plant's visibility and detectability in remote sensing data, which is an important consideration for ecological monitoring and management (Allen et al. 2016; Barclay-Estrup et al. 2020; Lefsky et al. 2002; Schlerf and Atzberger 2006).

4.4.3 Leaf physiology and biochemistry variations among C. vulgaris life stages

Leaf chlorophyll content was observed to increase with life stages, so as the plant gets older, the chlorophyll concentration in the leaf increases and is highest in the mature and degenerate phases. In addition, the degenerate phase increase in the leaf chlorophyll might be attributed to the branches beginning to die, which leads to a lack of green leaves, so the plant compensates for this deficiency by increasing the concentration of chlorophyll in the living leaves (Evans 1989; Gitelson et al. 2003; Nelson and Cox 2017; Taiz et al. 2015). Alternatively, chlorophyll concentrations in this phase may increase as the plant reallocates nutrients away from older, dying parts of the plant into new growing leaves (Evans 1989; Nelson and Cox 2017; Taiz et al. 2015). The pioneer phase had the lowest leaf chlorophyll concentration because leaves may still be developing and expanding in the early stages of a plant's life cycle, and energy and resources may be focused on the development of roots and basic structural elements of the plant. As a result, less N may be invested in the production of chlorophyll (Evans 1989; Taiz et al. 2015).

These changes in the leaf chlorophyll content are also reflected in the photosynthesis rate, as we found that the life stages of *C. vulgaris* that contain a higher percentage of chlorophyll (degenerate and mature phases) had a higher photosynthesis rate (Blankenship 2008; Evans 1989; Nelson and Cox 2017; Taiz et al. 2015).

Leaf N was found to change through life stages, being high in mature and degenerate phases and low in the pioneer phase, mirroring the leaf chlorophyll results. The strong relationship between N and chlorophyll is because N is a primary component of the chlorophyll (Evans 1989; Marschner 2011; Taiz et al. 2015). When plants have sufficient availability of nitrogen, they are normally able to produce chlorophyll efficiently, which in turn enhances the plants' capacity for healthy growth and development (Evans 1989; Taiz et al. 2015). In fact, the concentration of chlorophyll in plant tissue is often used as an indicator of the nitrogen status of the plant. Higher chlorophyll concentrations generally indicate sufficient or high nitrogen availability in the plant's environment (Evans 1989; Gitelson et al. 2003; Marschner 2011; Taiz et al. 2015).

4.4.4 Leaf and canopy level hyperspectral reflectance variations among C. vulgaris life stages and the challenges

In this study, NDRE and MTCI at the leaf level hyperspectral reflectance closely resembled the measured leaf chlorophyll content. Chlorophyll absorbs visible light strongly, particularly in the blue (430-450 nm) and red (640-680 nm) wavelengths, with maximum absorbance between 660 and 680 nm, while reflecting green light (500-570 nm), with maximum reflectance in green wavelengths at (560 nm) (Gitelson et al. 2003; Ustin et al. 2004; Zarco-Tejada et al. 2004). Both the MTCI and NDRE indices use the 'red edge' spectral region, which is the abrupt transition between the red and near-infrared regions of the electromagnetic spectrum, occurring between 680 and 750 nm (Dash and Curran 2004, 2007; Fitzgerald et al. 2006). As chlorophyll levels rise, the red edge shifts to longer wavelengths (Dash and Curran 2004, 2007; Fitzgerald et al. 2006; Gitelson et al. 2001). The NDRE index values range from -1 to 1, with higher values indicating more chlorophyll and, thus, more photosynthetic capacity (Clevers and Gitelson 2013; Gitelson and Merzlyak 1997; Haboudane et al. 2002). Therefore, NDRE is a powerful tool for monitoring plant growth. In addition, MTCI is a chlorophyll content index that uses a red-edge position to estimate chlorophyll content (Clevers and Gitelson 2013; Dash and Curran 2004, 2007). In addition, due to the strong relationship between leaf chlorophyll and leaf nitrogen, we can use the leaf chlorophyll index as an indicator of leaf nitrogen status (Clevers and Gitelson 2013).

However, the variable thickness and finite leaf width of *C. vulgaris* needle leaves complicate the relationship between reflectance from spectra and chlorophyll content, and nitrogen content (Zarco-Tejada et al. 2004; Zhang et al. 2008), making accurate remote sensing estimation difficult for *C. vulgaris*. At the moment, this work suggests that it may be difficult to use remote sensing methods to monitor *C. vulgaris* life stages across the landscape.

Several factors made it challenging to estimate reflectance at a canopy level: The spectrometer has an instantaneous field of view of 7 cm in diameter at 1 m height, which only captures part of the full plot of 50 cm. Secondly, the reflectance was significantly affected by the ecological changes between life phases, such as plant height, branches, flowers' positions, and the life stages' bush gaps (Gimingham 1989; Neumann et al. 2020; Sands and Gimingham 1975). The association between *C. vulgaris* needle leaf vegetation indices and leaf chlorophyll were found to be less strong at the leaf level, without the influence of canopy factors (Lewis and Disney 2007). It's possible that uncertainty in the leaf reflectance measurements contributed to the weaker correlations. Measuring the optical properties of needle leaves is complex, due to leaf structure and internal leaf structure, which influences the quantity of incoming light that is absorbed, scattered, and reflected via the top layer of the leaf, owing to changes in refraction between the air gaps within cells and the cell walls; needle leaves are characterized by a dense mesophyll that lacks differentiation and has thick cell walls (Blackburn 2007; Ollinger 2011). In addition, the finite width and varying thickness of needle leaves amplify the complexity of the relationship between spectral reflectance and chlorophyll content (Zarco-Tejada et al. 2004; Zhang et al. 2008). Thus, there have been comparatively fewer needle-leaf studies conducted in comparison to the broadleaf applications (Jacquemoud and Ustin 2001).

4.5 Conclusions

This study has revealed how structural, physiological, and leaf biochemical traits vary among *C. vulgaris* life stages, and indicates changing strategies with allocation of position to leaves vs flowers depending on canopy openness (related to collapse), and allocation of increasing chlorophyll and N to healthy leaves as other parts of the plant die with age. The work also provides insight into the extent that *C. vulgaris* performance and life stages might be monitored by remote sensing using reflectance data and finds that currently, this will be challenging due to insufficient separation of the life stages in canopy spectral data. None-the-less, more work could reveal a useful remote sensing approach for this. The combination of the trait and reflectance understanding may provide important tools in monitoring, managing and conserving *C. vulagris* and the heathlands it dominates.

4.6 Appendix

This appendix provides extra figures relating to the relationship between ecology and biochemistry. Also, the 15N, 13C and H_2O of the leaves among C. vulgaris life stages.

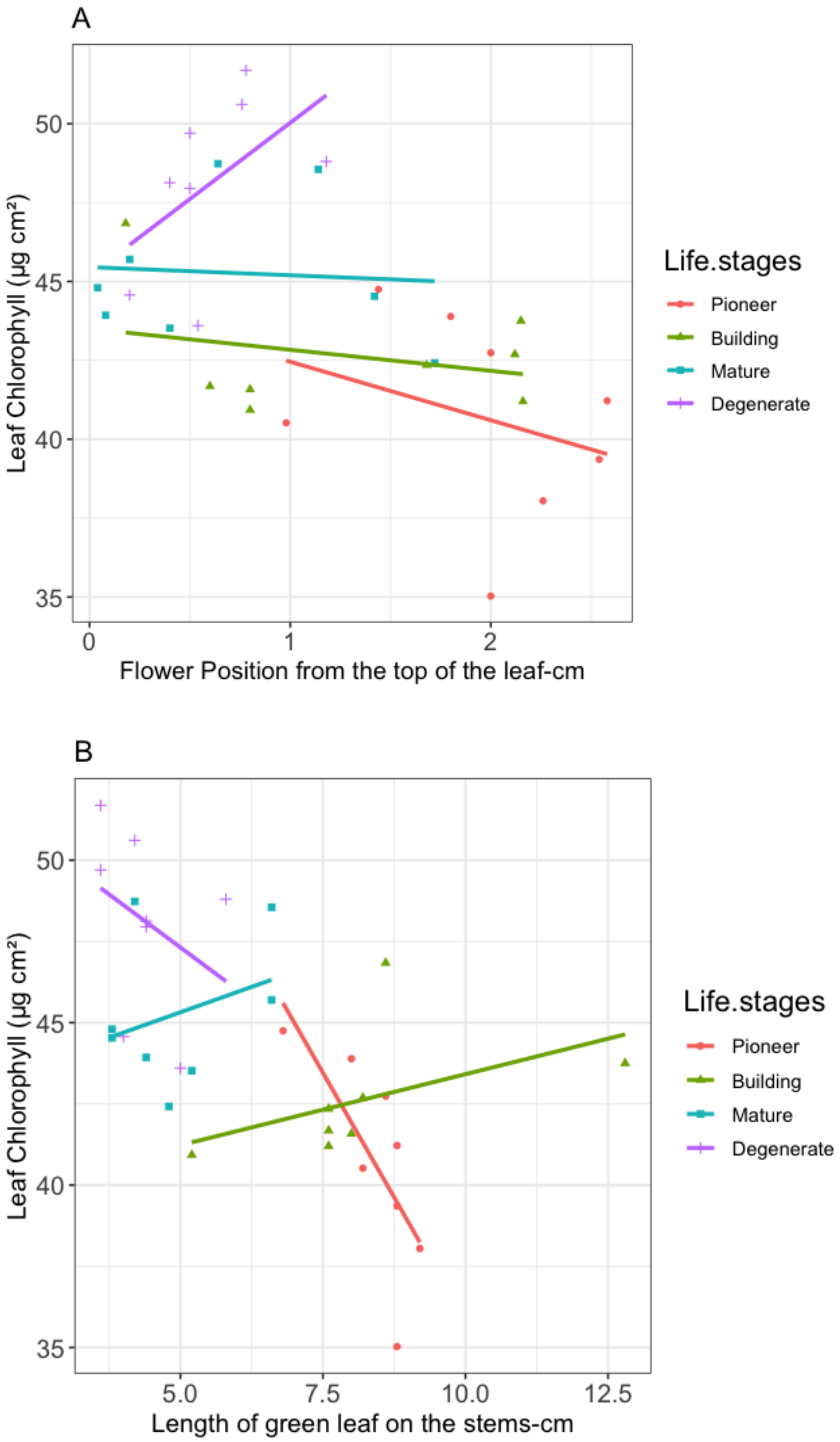

Figure S.4. 1: Regression analysis between C. vulgaris life stages, A) leaf chlorophyll and flower position, B) leaf chlorophyll and the length of green leaf on the stems.

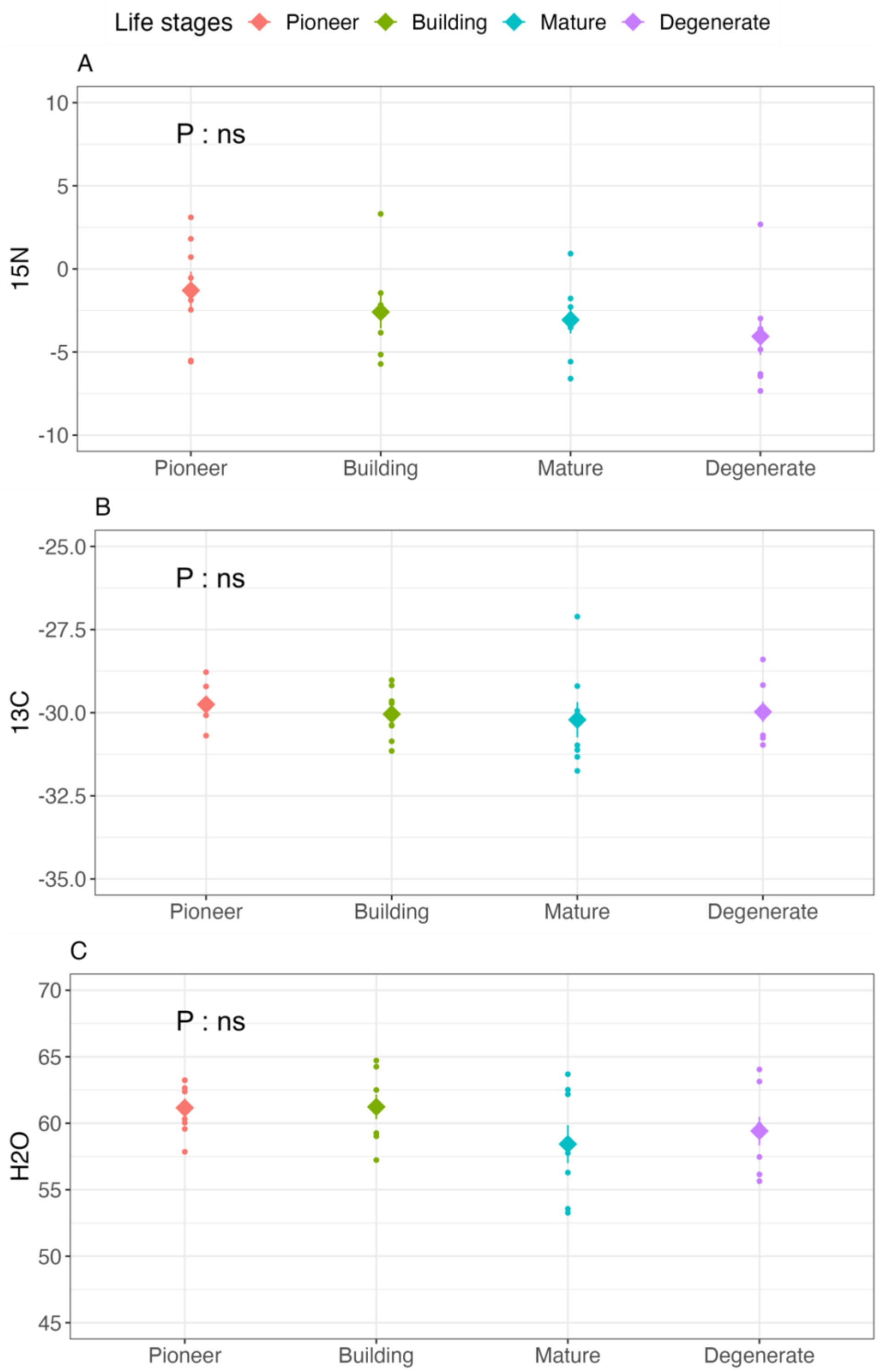

Figure S.4. 2: Leaf biochemistry variation among *C. vulgaris* life stages; A) leaf 15N, B) leaf 13C, C) leaf H_2O. Large diamond points with standard error bars indicate the mean, and the smaller points are individual data points.

Chapter 5

General Discussion

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5.1 Summary

This thesis has investigated how upland plant ecological, physiological, and biochemical traits change in response to environmental conditions and assesses if it is possible to measure these changes using proximal remote sensing techniques. The impacts of eCO_2 and nutrient enrichment (N and P) on both leaf physiology and shifts in plant community composition for a P-limited limestone grassland were examined. Leaf level hyperspectral reflectance, biochemistry and gas exchange measurements were used to investigate plant acclimation to CO_2 concentration and nutrient content in *Plantago lanceolata*. Finally, the ecological and physiological changes among *C. vulgaris* life stages and the potential for detecting these life stages through remote sensing techniques was explored.

These findings are in the chapters as follows:

1. *Plantago lanceolata* physiology, leaf biochemistry and leaf-level hyperspectral reflectance (Chapter 2)

a) The impacts of 3 years of CO_2 fumigation and long-term (N and P) enrichment on leaf gas exchange and leaf biochemistry.

b) The ability of hyperspectral reflectance and vegetation indices to quantify the adjustments in leaf physiological and biochemical traits in response to CO_2 and nutrient treatment.

2. **Plant community composition (Chapter 3)**

   a) The impacts of eCO_2 after five years of CO_2 fumigation and long-term nutrient enrichment (N and P) on plant community, species richness, and diversity in P-limited limestone grassland.

3. ***Calluna vulgaris* life stage variations ecologically, physiologically, and biochemically (Chapter 4)**

   a. The ecological variations among *C. vulgaris* life stages.
   b. The physiological and biochemical differences among *C. vulgaris* life stages and whether the different life stages can be detected through hyperspectral reflectance at both the leaf and canopy levels

The following sections of the general discussion provide a summary of the findings of this research as well as the consequences and implications of those findings. In addition, recommendations and areas for further future research are proposed.

5.2 Effects of eCO_2 and nutrient enrichment (P and N) on leaf physiology, biochemistry, and leaf-level hyperspectral reflectance (Chapter 2)

Chapter 2 has investigated the physiological and biochemical response of the common grassland forb, *Plantago lanceolata*, to eCO_2 (600 ppm) after 3 years of eCO_2 fumigation and long-term (>25 years) N and P enrichment, in P-limited limestone grassland. In this study, an increase in photosynthesis rate (A) under eCO_2 by 14% was observed. This photosynthesis increase is attributed to a higher rate of carboxylation and a reduction of the rate of the competing oxygenation reactions in Rubisco due to the increased CO_2 concentration (Long et al. 2004). However, results revealed a weaker photosynthesis response to eCO_2 compared to previous research and model predictions, likely due to photosynthesis acclimation after three years of exposure. Indeed, photosynthetic acclimation to elevated CO_2 concentrations was evident, with a significant decrease in Rubisco activity (V_{cmax}) of 15% under eCO_2, which is attributed to Rubisco amount reduction (Pastore et al. 2019; Ainsworth & Long, 2005; Ainsworth & Rogers, 2007; Ainsworth and Rogers 2007a; Maier et al. 2008; Rogers and Humphries 2000; Tissue et al. 1999). Photosynthesis acclimation occurs as a result of the suppression of Rubisco gene expression due to nonstructural carbohydrate accumulation, which is accelerated under eCO_2 (Byeon et al. 2021; Jacob et al. 1995). Interestingly, despite being a P-limited grassland, N addition had a greater effect on photosynthesis than P addition under eCO_2, such that N addition under eCO_2 alleviated the acclimation response. Previous studies have also suggested that photosynthesis acclimation to eCO_2 is alleviated at high N concentrations (Ainsworth and Long 2005; Ruiz-Vera et al. 2017). This is due to the fact that under eCO_2, Rubisco suppression is associated with N reallocation from a non-limiting source to other limiting photosynthetic components (Evans 1989; Kanno et al. 2017). In our study, eCO_2 reduced g_s by 26 % on average, attributable to increased intercellular CO_2 concentration under eCO_2, because under eCO_2, guard cells close to maintain intercellular CO_2 between 20 to 30% lower than the CO_2 concentration at the leaf surface (Ainsworth & Rogers, 2007). In addition, g_s reduction is one of the primary findings which indicate photosynthesis acclimation to eCO_2 because lower g_s reduces the photosynthetic rate due to limiting supply of CO_2, which may be in response to photosynthetic needs being met with the lower g_s and higher external CO_2 concentration. This is consistent with other studies (Pastore et al. 2019); the reduction in g_s increased WUE by 34% under eCO_2. Leaf N content was found to decrease under eCO_2 by 16 % on average compared to aCO_2, whereas leaf chlorophyll content decreased under eCO_2

by 13%; the chlorophyll reduction is due to the reduction in leaf N and the reduction in Rubisco (Loladze 2002; Mcgrath and Lobell 2013; Taub and Wang 2008). Lastly, N additions increased leaf chlorophyll and leaf N contents, whereas P addition increased leaf P concentration.

In the visible and infrared regions, eCO_2_HN had a higher reflectance than aCO_2_HN, and the HN treatment had the lowest reflectance under aCO_2 compared to aCO_2 0N and P. Interestingly, the reflectance of HN treatments varied depending on the CO_2 treatment. The P treatment remained the same under aCO_2 and eCO_2; additionally, 0N reflectance nearly remained the same under aCO_2 and eCO_2 with a slightly greater reflectance in the visible light peak under eCO_2. Leaf reflectance from 0N and P treatments separated in the green peak and after 1400 nm for aCO_2 but not for eCO_2. Changes in leaf-level hyperspectral reflectance between eCO_2 and aCO_2, caused by changes in *Plantago lanceolate* physiological status and leaf biochemistry, respectively, are affected by CO_2 and nutrient treatments and have the potential to influence the plant's spectral signature (Cha et al. 2017; Zheng et al. 2020).

In this study, among all the indices tested, MTCI at the leaf-level hyperspectral reflectance showed the strongest relationship with the measured leaf chlorophyll in the lab, with $R^2 = 0.92$, $p < 0.001$ (Clevers and Gitelson 2013; Dash and Curran 2004, 2007). In addition, among all the indices tested, MTCI at the leaf-level hyperspectral reflectance showed a strong relationship with the measured leaf N in the lab with $R^2 = 0.\ 86$, $p < 0.001$.

After three years of eCO_2 exposure, strong evidence of clear photosynthesis acclimation to eCO_2 was found. Our work, therefore, suggests that increased productivity of P-limited grasslands may be limited due to the acclimation of photosynthesis. Furthermore, our results also reveal that P-limited grasslands exhibit eCO_2 responses in leaf photosynthesis and biochemistry remarkably consistent with those observed in N-limited ecosystems. Hence, despite the lack of research on eCO_2 effects in P-limited ecosystems, this thesis suggests that knowledge derived from N-limited ecosystems could be utilized in P-limited ecosystems when formulating models for leaf photosynthesis. Consistent with this, the study also demonstrates that, even in a P-limited grassland ecosystem, it was N that had the most significant influence on photosynthesis rather than P. This finding broadens the understanding of N's dominant role over P in controlling photosynthesis, extending it to P-limited ecosystems. These results are significant for comprehending how plants in P-limited ecosystems respond physiologically to eCO_2, and how their ability to sequester C in a world with high CO_2 levels might be affected by this eCO_2 and the availability of N and P nutrients.

5.3 Plant community responses to five years of CO_2 fumigation and long-term nutrient enrichment (P and N) in P-limited limestone grassland (Chapter 3)

In this study, after five years of CO_2 fumigation, eCO_2 significantly shifted the community composition, increasing species richness under eCO_2 by 11% (+4 species on average compared to aCO_2) and increasing species diversity as determined by Simpson's diversity index, with eCO_2 communities being more diverse than aCO_2. Plants under eCO_2 are likely utilizing the extra C to acquire the most limited nutrient, in this instance, P (Jin, Tang, and Sale 2015; Phoenix et al. 2020). This reduces P limitation and, consequently, reduces the competition for nutrients, and hence facilitating species coexistence (Phoenix et al. 2020). In addition, in the same experiment, it was observed that there was an increase in productivity and photosynthesis rate in limestone grassland (Keane et al. in press; Chapter 2). eCO_2 significantly reduced grass and bryophyte abundance but increased legume abundance, especially when eCO_2 was combined with P enrichment. This implies that other plant functional types might be better equipped to leverage the additional C under eCO_2 to secure the limited resource P, consequently out-competing grasses, leading to a decrease in grass abundance.

Communities became even more dominated by graminoids as a result of N enrichment, which caused a substantial decrease in species richness and diversity due to a significant increase in grasses and sedges abundance at the expense of forbs. Previously, comparable responses were found (Lu et al. 2021; You et al. 2017), including at the Wardlow field site where the limestone mesocosms were taken from (Carroll et al. 2003; Lee and Caporn 1998; Stevens et al. 2004) and in this eCO_2 experiment (Taylor 2021). HN and LN treatments increased the growth of slow-growing sedges like *Carex flacca* and *Carex panicea*, showing that they may be able to withstand acidification and use N for more P absorption by stimulating their dauciform roots. N and P additions can benefit fast-growing species, such as some grass species, which can compete with other species and reduce biodiversity (Bobbink et al. 2010).

eCO_2 increased species richness and diversity in limestone grassland after five years of CO_2 fumigation, shifting its functional composition possibly because some plants may benefit from the additional C in using it to obtain P. In addition, Long-term simulated N and P treatments significantly affected community composition, diversity, species richness, and functional composition. N enrichment caused forbs to decline and grass and sedge to flourish, resulting in graminoids dominating communities. P-treated communities were less diverse. Under fertilizer treatments (N and P), grasslands may lose richness and diversity and become graminoids-dominated, decreasing their conservation, recreational, and grazing value. While the response of plant P absorption to eCO_2 is a likely mechanism driving these modifications in this P-limited grassland, there remains much need for a deeper mechanistic understanding as to why P-limited grassland changes in response to eCO_2 and nutrient addition.

5.4 Differences among *C. vulgaris* life stages, ecologically and physiologically, and biochemistry (Chapter 4)

This study determined ecological variation in *C. vulgaris*. *Calluna vulgaris* was tallest in the mature phase, and its stems were longest in the mature and degenerate phases since the plant remains active and growing (Gimingham 1989; Sands and Gimingham 1975). Due to its early development, the pioneer phase had the lowest height and shortest stem length (Gimingham 1989; Sands and Gimingham 1975). Due to the bush's full growth and lack of gaps, *C. vulgaris* cover was highest during the building phase. Consequently, the building phase had the lowest cover of other species under *C. vulgaris*. On the other hand, the degenerate phase had the lowest percentage cover of *C. vulgaris* and the lowest stem density on the bush due to the collapse and death of the main branches (Gimingham, 1975; Gimingham et al.1981). Consequently, the degenerate phase had the highest cover of other ground flora under *C. vulgaris*, likely because of greater light penetration through the collapsed bush. The pioneer and building phases of *C. vulgaris* had the lowest flowering position, meaning the flowers were located in the middle of the stems. In contrast, the degenerate and mature phases had flowers on the top of the stems. These changes suggest a priority for presenting green leaf cover to the light in the earlier life stages, and then a priority to presenting flowers at the top of the plant in the later life stages. In the later life stages (especially the degenerate stage), the opening of the canopy may also reduce the need to have leaves at the tips of stems because more light penetrates deeper into the canopy.

In the mature and degenerate phases, leaf chlorophyll and leaf N concentrations were highest, with the greatest photosynthetic rates, while the pioneer phase had the lowest leaf chlorophyll, leaf N concentrations and photosynthetic rates. At the leaf level, visible light variations occurred between 600 and 700 nm, and in NIR, the building phase had a slight rise in the green peak, while the degenerate phase had increased reflectance in the infrared and after 1500 nm. At the canopy level, the pioneer phase had lowered reflectance overall and in the visible light and infrared regions, whereas the building phase had increased infrared reflectance. Compared with the other indices, NDRE and MTCI at the leaf and canopy levels, respectively, had the highest R^2 values.

The research shows how structural, physiological, and leaf biochemical traits vary among *C. vulgaris* life stages, including changing strategies with leaf flower position according to canopy openness (related to collapse) and allocation of increasing chlorophyll and N to healthy leaves as other parts of the plant die with age. The work also provides insight into the extent to which the performance and life stages of *C. vulgaris* could be monitored by remote sensing using reflectance data and concludes that, due to the insufficient separation of life stages in canopy spectral data, this will be challenging. However, additional research may uncover a practical remote sensing technique for this. The combination of the trait and reflectance understanding may provide important tools in monitoring, managing and conserving *C. vulagris* and the heathlands it dominates.

5.5 Future research proposals

5.5.1 P-limited grassland

Chapter 2 investigated the leaf gas exchange, biochemistry, and hyperspectral reflectance in P-limited limestone grassland in *Plantago lanceolata*. However, due to the lack of studies on P-limited grassland and the consequently large knowledge gap, a range of unanswered questions remain. An area requiring study is understanding how the leaf and stomatal morphology of *Plantago lanceolata* responds to eCO_2 indeed, provides valuable insights into how plants adapt to changing environmental conditions. The stomata play a vital role in controlling gas exchange, and their size and density can significantly affect a plant's ability to absorb CO_2 and lose water through transpiration.

Research in some ecosystems has shown that eCO_2 can lead to changes in stomatal density (the number of stomata per unit leaf area) and stomatal size (Haworth et al. 2016). These changes can have significant impacts on photosynthesis, transpiration, and water-use efficiency (Ainsworth and Rogers 2007a; Pastore et al. 2019). For instance, some studies have found that plants grown under eCO_2 conditions often have lower stomatal densities, which can help improve water-use efficiency by reducing water loss without significantly impacting CO_2 intake (Ainsworth and Rogers 2007a; Haworth et al. 2016; Pastore et al. 2019). However, the impact of eCO_2 on stomatal characteristics can be species-specific and may also depend on other environmental factors. Therefore, it's not yet clear whether these findings can be generalized to all ecosystems or to all species within an ecosystem.

In P-limited ecosystems, which are characterized by low P availability, plants may show different responses to eCO_2 compared to those in other ecosystems.

Studying the leaf and stomatal morphology of *Plantago lanceolata* in response to eCO_2 in P-limited ecosystems could help to fill this knowledge gap. However, this will require detailed morphological studies, which can be challenging and time-consuming. Advanced microscopy techniques, possibly in combination with physiological measurements, would likely be needed to accurately measure changes in leaf and stomatal characteristics under different CO_2 concentrations. This is an exciting area of research with many open questions, and the results could have important implications for our understanding of plant responses to climate change.

Another area requiring study is understanding the responses of different species from P-limited grassland to eCO_2 is crucial for predicting ecosystem-level responses to environmental change. A focus on a single species, while valuable for depth of understanding, will not capture the diversity of responses that could occur within a community of multiple species. Furthermore, some species might respond differently due to differences in their physiology, growth form, or life history traits, and this variation in responses can have significant implications for community dynamics and ecosystem functions (Taylor 2021).

However, measuring leaf gas exchange in different species can indeed be challenging. As has been noted, issues can arise due to the small size of the leaves, or the positioning of the plants close to the ground. This makes it difficult to attach tools such as the LI-6800 leaf chamber, which is commonly used to measure photosynthesis and other leaf gas exchange parameters. Possible solutions could involve the use of custom-designed chambers or other equipment better suited to these particular challenges. Additionally, non-invasive methods such as stable isotope analysis or remote sensing could be explored as potential ways to infer leaf gas exchange without direct measurement.

Another area requiring further study is, expanding the study of hyperspectral reflectance beyond leaf-level measurements in a single species could provide valuable insights into ecosystem-level changes in community composition, plant physiology, and biochemistry under eCO_2 conditions. Hyperspectral reflectance can provide a wealth of information about these variables, as different plant species and physiological or biochemical states can be associated with distinct spectral signatures (Blackburn 1998, 2007; Croft et al. 2017).

Studying canopy-level reflectance would offer a more comprehensive view of the ecosystem, capturing the combined effects of all the plant species within the mesocosms. It could also reveal how changes in the structure of the plant community, such as species composition or canopy architecture, influence overall reflectance patterns.

However, interpreting canopy reflectance data can be challenging due to the complexity of the canopy structure and the many factors that can influence reflectance, including leaf angle, the proportion of sunlit and shaded leaves, and the presence of non-photosynthetic elements such as stems and soil (Chou et al. 2017; Serbin and Townsend 2020). Therefore, it will be important to pair remote sensing measurements with ground-based observations and experiments to validate and interpret the remote sensing data.

In terms of developing a phosphorus (P) index based on leaf P concentration, it may be that the indices tested in this study were not sensitive enough to detect variations in leaf P concentration, or that other factors were confounding the relationship between leaf P and reflectance. Further research could explore different methods for deriving a P index, potentially by combining multiple spectral bands or by using machine learning techniques to identify complex patterns in the hyperspectral data. Moreover, it may also be worthwhile to explore whether other spectral indices could serve as proxies for leaf P concentration. For example, indices based on the red edge position, or the chlorophyll absorption regions could potentially be sensitive to changes in leaf P concentration, as P is an essential nutrient that influences many aspects of plant physiology and biochemistry, including chlorophyll synthesis and photosynthesis.

In conclusion, while there are certainly challenges to overcome, these research directions could greatly enhance our understanding of plant and ecosystem responses to eCO_2, and could ultimately help to improve our predictions of how ecosystems will change in a future, higher-CO_2 world.

5.5.2 The C. vulgaris life stages

In Chapter 4, we investigated new ecological aspects, such as leaf biochemistry, photosynthesis, and hyperspectral reflectance. This was done in relation to *C. vulgaris* life stages. Even though *C. vulgaris* is a species that has been studied extensively; there are some remaining unanswered questions, especially from the remote sensing perspective. Exploring remote sensing perspectives in the study of *C. vulgaris* does indeed offer interesting research directions. Specifically, investigating the influence of flower density on canopy-level reflectance during the flowering phase is a novel approach. The spectral signature of plants in flower is often different from that of plants not in flower or vegetative stages due to the unique optical properties of flower structures and pigments. If flower density shows a consistent relationship with reflectance, it could serve as a remotely sensed indicator of plant reproductive effort or health status.

In terms of studying leaf-level reflectance in needle leaves presents its own set of challenges due to the unique structure of these leaves, and may require specialized instruments or methodologies. Needle leaves are often much smaller and narrower than broadleaf species, and their shape can create complex shading and reflectance patterns. Specialized instruments or attachment accessories designed for needle leaf measurement, such as needle leaf clips, can be used to ensure accurate and consistent measurements.

In addition, drone-based remote sensing provides a promising avenue for canopy-level reflectance studies. This approach can effectively cover large areas in a relatively short amount of time, making it ideal for monitoring changes in the *C. vulgaris* life stages (Duffy et al. 2018; Neumann et al. 2020). Drones equipped with hyperspectral sensors can capture detailed spectral data, potentially revealing subtle changes in vegetation health or composition. However, implementing drone technology also has its challenges. These include the need for expertise in drone operation, data analysis, and potential legal restrictions on drone usage. Additionally, high-resolution hyperspectral data comes with substantial data storage and processing requirements. Despite these challenges, the unique insights that can be gained make this a valuable area of future research.

Technological improvements and advancements in data processing techniques, such as machine learning and artificial intelligence, are continually expanding the possibilities for remote sensing research. These advanced techniques can help in the classification and identification of *C. vulgaris* life stages, also detection of plant health and disease, and the prediction of various plant functional traits.

Furthermore, combining ground-based field measurements with drone-based remote sensing could offer a powerful tool for validating and scaling up findings. Ground truthing is a critical step in ensuring the accuracy of remote sensing analysis, and when paired with larger-scale remote sensing, it can provide a more comprehensive picture of the ecosystem being studied.

Overall, while there is much, we already know about *C. vulgaris* and similar upland ecosystems, the application of remote sensing offers new opportunities to deepen our understanding of these important and complex environments. These future research directions can provide valuable insights to inform conservation and management strategies, particularly in the face of environmental change.

5.6 Conclusion

Anthropogenic impacts on the Earth's biogeochemistry have resulted in unprecedented alterations to the cycles of C, N, and P. This thesis has investigated some of the potential consequences of the responses of P-limited grasslands to eCO_2, N deposition and P enrichment with a particular focus on their effects on leaf physiology, leaf biochemistry, community composition, and hyperspectral reflectance. In addition, this thesis has also investigated some of the ecological, physiological, and biochemistry variations during *C. vulgaris* life stages, with the possibility of detecting the *C. vulgaris* life stages using remote sensing applications.

Considering that grasslands around the world are predicted to experience increases in both C (by eCO_2) and N (via deposition), chapters 2 and 3 of this thesis explored the potential implications on grassland plants. eCO_2 increased photosynthesis but with strong evidence of acclimation, and N addition, alleviated the magnitude of acclimation. Also, eCO_2 altered leaf biochemistry, such as Chlorophyll and N concentration, and the effects of eCO_2 and nutrient additions created unique plant communities. In addition, Chapter 4 of this thesis explored the variation among *C. vulgaris* life stages. Consequently, the ecological, physiological, and biochemical characteristics of *C. vulgaris* at different life stages changed substantially.

Understanding from this thesis contributes to our knowledge of limestone grassland and heather moorland ecology and physiology, and can help with predicting the consequences of global change, and how this might be monitored remotely.

References

Ač, Alexander et al. 2009. “Near-Distance Imaging Spectroscopy Investigating Chlorophyll Fluorescence and Photosynthetic Activity of Grassland in the Daily Course.” *Functional Plant Biology*.

Aebischer, Nicholas J., Julie A. Ewald, and Stephen C. Tapper. 2009. “Driven Grouse Shooting In Britain: A Form Of Upland Management With Wider Conservation Benefits.” In *Symposium Proceedings on the Ecologic and Economic Benefits of Hunting,*.

Ainsworth, E. A. et al. 2003a. “Is Stimulation of Leaf Photosynthesis by Elevated Carbon Dioxide Concentration Maintained in the Long Term? A Test with Lolium Perenne Grown for 10 Years at Two Nitrogen Fertilization Levels under Free Air CO2 Enrichment (FACE).” *Plant, Cell and Environment* 26(5): 705–14.

———. 2003b. “Is Stimulation of Leaf Photosynthesis by Elevated Carbon Dioxide Concentration Maintained in the Long Term? A Test with Lolium Perenne Grown for 10 Years at Two Nitrogen Fertilization Levels under Free Air CO2 Enrichment (FACE).” *Plant, Cell and Environment* 26(5).

Ainsworth, Elizabeth A., and Stephen P. Long. 2005. “What Have We Learned from 15 Years of Free-Air CO2 Enrichment (FACE)? A Meta-Analytic Review of the Responses of Photosynthesis, Canopy Properties and Plant Production to Rising CO2.” *New Phytologist* 165(2).

———. 2021. “30 Years of Free-Air Carbon Dioxide Enrichment (FACE): What Have We Learned about Future Crop Productivity and Its Potential for Adaptation?” *Global Change Biology* 27(1).

Ainsworth, Elizabeth A., and Alistair Rogers. 2007a. “The Response of Photosynthesis and Stomatal Conductance to Rising [CO 2]: Mechanisms and Environmental Interactions.” *Plant, Cell and Environment* 30(3): 258–70.

———. 2007b. “The Response of Photosynthesis and Stomatal Conductance to Rising [CO 2]: Mechanisms and Environmental Interactions.” *Plant, Cell and Environment* 30(3).

Al-Mufti, M. M. et al. 1977. “A Quantitative Analysis of Shoot Phenology and Dominance in Herbaceous Vegetation.” *The Journal of Ecology* 65(3).

Albert, Kristian Rost et al. 2011. “Interactive Effects of Drought, Elevated CO2 and Warming on Photosynthetic Capacity and Photosystem Performance in Temperate

Heath Plants.” *Journal of Plant Physiology* 168(13).

Albertson, Kevin, Jonathan Aylen, Gina Cavan, and Julia McMorrow. 2010. “Climate Change and the Future Occurrence of Moorland Wildfires in the Peak District of the UK.” *Climate Research* 45(1).

Aleklett, Kristin, Pelle Ohlsson, Martin Bengtsson, and Edith C. Hammer. 2021. “Fungal Foraging Behaviour and Hyphal Space Exploration in Micro-Structured Soil Chips.” *ISME Journal* 15(6).

Ali, Iftikhar et al. 2016. “Satellite Remote Sensing of Grasslands: From Observation to Management.” *Journal of Plant Ecology* 9(6).

Alipanah, Leila et al. 2018. “Molecular Adaptations to Phosphorus Deprivation and Comparison with Nitrogen Deprivation Responses in the Diatom Phaeodactylum Tricornutum.” *PLoS ONE* 13(2).

Allen, Katherine A. et al. 2016. “Prescribed Moorland Burning Meets Good Practice Guidelines: A Monitoring Case Study Using Aerial Photography in the Peak District, UK.” *Ecological Indicators* 62.

Allen, Katherine A., Michael P.K. Harris, and Rob H. Marrs. 2013. “Matrix Modelling of Prescribed Burning in Calluna Vulgaris-Dominated Moorland: Short Burning Rotations Minimize Carbon Loss at Increased Wildfire Frequencies.” *Journal of Applied Ecology* 50(3): 614–24.

Anderson, A. J. B. 1971. “Ordination Methods in Ecology.” *The Journal of Ecology* 59(3).

Anderson, Karen et al. 2009. “Progress in Field Spectroscopy Progress in Field Spectroscopy.” *Remote Sensing of Environment* 00(August 2017): 7–9. http://dx.doi.org/10.1016/j.rse.2007.08.001.

Anderson, Marti J. 2017. “ Permutational Multivariate Analysis of Variance (PERMANOVA) .” In *Wiley StatsRef: Statistics Reference Online,.*

Arróniz-Crespo, María, Jonathan R. Leake, Peter Horton, and Gareth K. Phoenix. 2008. “Bryophyte Physiological Responses to, and Recovery from, Long-Term Nitrogen Deposition and Phosphorus Fertilisation in Acidic Grassland.” *New Phytologist* 180(4).

Mac Arthur, Alasdair, and Tim Malthus. 2012. “Calluna Vulgaris Foliar Pigments and Spectral Reflectance Modelling.” *International Journal of Remote Sensing* 33(16): 5214–39.

Ashraf, M., and P. J.C. Harris. 2013. “Photosynthesis under Stressful Environments: An Overview.” *Photosynthetica* 51(2).

Aspinall, R. et al. 2011. “Status and Changes in the UK Ecosystems and Their Services to

Society: Scotland (Chapter 19).” *The UK National Ecosystem Assessment Technical Report*.

Assmann, Jakob J., Jeffrey T. Kerby, Andrew M. Cunliffe, and Isla H. Myers-Smith. 2019. “Vegetation Monitoring Using Multispectral Sensors — Best Practices and Lessons Learned from High Latitudes.” *Journal of Unmanned Vehicle Systems* 7(1): 54–75.

Baggaley, N et al. 2021. “Understanding Carbon Sequestration in Upland Habitats 1 Executive Summary.” (January): 1–32. http://dx.doi.org/10.7488/era/1021.

Bai, Yongfei et al. 2010. “Tradeoffs and Thresholds in the Effects of Nitrogen Addition on Biodiversity and Ecosystem Functioning: Evidence from Inner Mongolia Grasslands.” *Global Change Biology* 16(1).

Bansod, Babankumar, Rangoli Singh, Ritula Thakur, and Gaurav Singhal. 2017. “A Comparision between Satellite Based and Drone Based Remote Sensing Technology to Achieve Sustainable Development: A Review.” *Journal of Agriculture and Environment for International Development* 111(2).

Barber, S. A., J. M. Walker, and E. H. Vasey. 1963. “Mechanisms for the Movement of Plant Nutrients from the Soil and Fertilizer to the Plant Root.” *Journal of Agricultural and Food Chemistry* 11(3).

Barclay-Estrup, P. 1970. “The Description and Interpretation of Cyclical Processes in a Heath Community: II. Changes in Biomass and Shoot Production during the Calluna Cycle.” *The Journal of Ecology* 58(1): 243.

———. 2020. “ASD FieldSpec FR.” *The Journal of Ecology* 57(1): 5214–39. https://www.facebook.com/groups/563872007130301/members/.

Barclay-Estrup, P., and C. H. Gimingham. 1969. “The Description and Interpretation of Cyclical Processes in a Heath Community: I. Vegetational Change in Relation to the Calluna Cycle.” *The Journal of Ecology* 57(3): 737.

Barker, D. H., G. G.R. Seaton, and S. A. Robinson. 1997. “Internal and External Photoprotection in Developing Leaves of the CAM Plant Cotyledon Orbiculata.” *Plant, Cell and Environment*.

Basto, Sofía et al. 2015. “Long-Term Nitrogen Deposition Depletes Grassland Seed Banks.” *Nature Communications* 6.

Bates, Douglas, Martin Mächler, Benjamin M. Bolker, and Steven C. Walker. 2015. “Fitting Linear Mixed-Effects Models Using Lme4.” *Journal of Statistical Software* 67(1).

Bellamy, Pat H. et al. 2005. “Carbon Losses from All Soils across England and Wales 1978-2003.” *Nature* 437(7056).

Blackburn, G. A. 1998. “Spectral Indices for Estimating Photosynthetic Pigment Concentrations: A Test Using Senescent Tree Leaves.” *International Journal of Remote Sensing*.

Blackburn, George Alan. 1998. “Quantifying Chlorophylls and Carotenoids at Leaf and Canopy Scales: An Evaluation of Some Hyperspectral Approaches.” *Remote Sensing of Environment*.

———. 2007. “Hyperspectral Remote Sensing of Plant Pigments.” *Journal of Experimental Botany* 58(4): 855–67.

Blankenship, Robert E. 2008. Molecular Mechanisms of Photosynthesis *Molecular Mechanisms of Photosynthesis*.

Bobbink, R. 1991. “Effects of Nutrient Enrichment in Dutch Chalk Grassland.” *The Journal of Applied Ecology* 28(1).

———. 2010. “Global Assessment of Nitrogen Deposition Effects on Terrestrial Plant Diversity: A Synthesis.” *Ecological Applications* 20(1).

Bonn, Aletta et al. 2011. “The UK National Ecosystem Assessment Technical Report. Chapter 5. Mountains, Moorlands and Heaths.” *Broad habitats*: 1–105. http://uknea.unep-wcmc.org/LinkClick.aspx?fileticket=CZHaB2%2FJKlo%3D&tabid=82%0Ahttp://uknea.unep-wcmc.org/LinkClick.aspx?fileticket=CZHaB2%2FJKlo%3D&tabid=82http://uknea.unep-wcmc.org/LinkClick.aspx?fileticket=CZHaB2%2FJKlo%3D&tabid=82.

Bontti, Eliana E. et al. 2009. “Litter Decomposition in Grasslands of Central North America (US Great Plains).” *Global Change Biology* 15(5).

Brodribb, Timothy J., Jennifer Powers, Hervé Cochard, and Brendan Choat. 2020. “Hanging by a Thread? Forests and Drought.” *Science* 368(6488).

Broge, N. H., and E. Leblanc. 2001. “Comparing Prediction Power and Stability of Broadband and Hyperspectral Vegetation Indices for Estimation of Green Leaf Area Index and Canopy Chlorophyll Density.” *Remote Sensing of Environment* 76(2).

Brundrett, Mark C. 2009. “Mycorrhizal Associations and Other Means of Nutrition of Vascular Plants: Understanding the Global Diversity of Host Plants by Resolving Conflicting Information and Developing Reliable Means of Diagnosis.” *Plant and Soil* 320(1–2).

Bünemann, E. K. et al. 2012. “Rapid Microbial Phosphorus Immobilization Dominates Gross Phosphorus Fluxes in a Grassland Soil with Low Inorganic Phosphorus Availability.”

Soil Biology and Biochemistry 51.

Byeon, Siyeon et al. 2021. “Down-Regulation of Photosynthesis to Elevated CO2 and N Fertilization in Understory Fraxinus Rhynchophylla Seedlings.” *Forests* 12(9).

Callaghan, Terry V. et al. 2011. “Multi-Decadal Changes in Tundra Environments and Ecosystems: Synthesis of the International Polar Year-Back to the Future Project (IPY-BTF).” *Ambio*.

Carbutt, Clinton, William D. Henwood, and Louise A. Gilfedder. 2017. “Global Plight of Native Temperate Grasslands: Going, Going, Gone?” *Biodiversity and Conservation* 26(12).

Carroll, J. A. et al. 2003. “The Interactions between Plant Growth, Vegetation Structure and Soil Processes in Semi-Natural Acidic and Calcareous Grasslands Receiving Long-Term Inputs of Simulated Pollutant Nitrogen Deposition.” *Environmental Pollution* 121(3).

Carstensen, Andreas et al. 2018. “The Impacts of Phosphorus Deficiency on the Photosynthetic Electron Transport Chain1[OPEN].” *Plant Physiology* 177(1).

Ceulemans, Tobias et al. 2014. “Soil Phosphorus Constrains Biodiversity across European Grasslands.” *Global Change Biology* 20(12).

———. 2017. “Phosphorus Resource Partitioning Shapes Phosphorus Acquisition and Plant Species Abundance in Grasslands.” *Nature Plants* 3.

Ceulemans, Tobias, Roel Merckx, Maarten Hens, and Olivier Honnay. 2013. “Plant Species Loss from European Semi-Natural Grasslands Following Nutrient Enrichment - Is It Nitrogen or Is It Phosphorus?” *Global Ecology and Biogeography* 22(1).

Cha, Sangsub, Hee Myung Chae, Sang Hoon Lee, and Jae Kuk Shim. 2017. “Effect of Elevated Atmospheric CO2 Concentration on Growth and Leaf Litter Decomposition of Quercus Acutissima and Fraxinus Rhynchophylla.” *PLoS ONE* 12(2).

Chapin, F. S., Matson, P. A., and Vitousek, P. M. 2011. “Nutrient Cycling, in: Principles of Terrestrial Ecosystem Ecology.” *Springer, New York*: 259–96.

Chapman, Daniel S., Aletta Bonn, William E. Kunin, and Stephen J. Cornell. 2010. “Random Forest Characterization of Upland Vegetation and Management Burning from Aerial Imagery.” *Journal of Biogeography* 37(1).

Chater, Caspar et al. 2015. “Elevated CO2-Induced Responses in Stomata Require ABA and ABA Signaling.” *Current Biology* 25(20).

Chen, Ji et al. 2020. “Long-Term Nitrogen Loading Alleviates Phosphorus Limitation in Terrestrial Ecosystems.” *Global Change Biology* 26(9).

Chen, Ruirui et al. 2014. “Soil C and N Availability Determine the Priming Effect: Microbial

N Mining and Stoichiometric Decomposition Theories." *Global Change Biology* 20(7).

Cheng, Yen Ben et al. 2006. "Estimating Vegetation Water Content with Hyperspectral Data for Different Canopy Scenarios: Relationships between AVIRIS and MODIS Indexes." *Remote Sensing of Environment*.

Chou, Shuren et al. 2017. "Canopy-Level Photochemical Reflectance Index from Hyperspectral Remote Sensing and Leaf-Level Non-Photochemical Quenching as Early Indicators of Water Stress in Maize." *Remote Sensing* 9(8).

Chu, Shanshan et al. 2018. "Physiological and Proteomics Analyses Reveal Low-Phosphorus Stress Affected the Regulation of Photosynthesis in Soybean." *International Journal of Molecular Sciences* 19(6).

Clarke, J. L., D. Welch, and I. J. Gordon. 1995. "The Influence of Vegetation Pattern on the Grazing of Heather Moorland by Red Deer and Sheep. II. The Impact on Heather." *The Journal of Applied Ecology* 32(1).

Clevers, J. G.P.W., and A. A. Gitelson. 2013. "Remote Estimation of Crop and Grass Chlorophyll and Nitrogen Content Using Red-Edge Bands on Sentinel-2 and-3." *International Journal of Applied Earth Observation and Geoinformation* 23(1).

Collins, Matthew et al. 2013. "Long-Term Climate Change: Projections, Commitments and Irreversibility. In: Climate Change 2013: The Physical Science." *Climate Change 2013 the Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (January 2014).

Cotton, D. E., and W. H.G. Hale. 1994. "Effectiveness of Cutting as an Alternative to Burning in the Management of Calluna Vulgaris Moorland: Results of an Experimental Field Trial." *Journal of Environmental Management* 40(2).

Croft, H., J. M. Chen, and Y. Zhang. 2014. "The Applicability of Empirical Vegetation Indices for Determining Leaf Chlorophyll Content over Different Leaf and Canopy Structures." *Ecological Complexity* 17(1).

Croft, H., J. M. Chen, Y. Zhang, and A. Simic. 2013. "Modelling Leaf Chlorophyll Content in Broadleaf and Needle Leaf Canopies from Ground, CASI, Landsat TM 5 and MERIS Reflectance Data." *Remote Sensing of Environment* 133.

Croft, Holly et al. 2017. "Leaf Chlorophyll Content as a Proxy for Leaf Photosynthetic Capacity." *Global Change Biology* 23(9).

Crous, Kristine Y. et al. 2019. "Nitrogen and Phosphorus Retranslocation of Leaves and Stemwood in a Mature Eucalyptus Forest Exposed to 5 Years of Elevated CO2." *Frontiers in Plant Science* 10.

Damgaard, Christian. 2019. “Is the Degree of Spatial Aggregation of Calluna Vulgaris Regulated by Nitrogen Deposition or Precipitation?” *Acta Oecologica* 97.

Damm, A. et al. 2018. “Remote Sensing of Plant-Water Relations: An Overview and Future Perspectives.” *Journal of Plant Physiology* 227.

Dash, J., and P. J. Curran. 2004. “The MERIS Terrestrial Chlorophyll Index.” *International Journal of Remote Sensing*.

———. 2007. “Evaluation of the MERIS Terrestrial Chlorophyll Index (MTCI).” *Advances in Space Research* 39(1).

Daughtry, C. S.T. et al. 2000. “Estimating Corn Leaf Chlorophyll Concentration from Leaf and Canopy Reflectance.” *Remote Sensing of Environment* 74(2).

Davey, P. A. et al. 2006. “Can Fast-Growing Plantation Trees Escape Biochemical down-Regulation of Photosynthesis When Grown throughout Their Complete Production Cycle in the Open Air under Elevated Carbon Dioxide?” *Plant, Cell and Environment* 29(7).

David, Fowler et al. 2013. “The Global Nitrogen Cycle in the Twenty-First Century.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 368(1621).

Davies, J. A.C. et al. 2016. “Long-Term P Weathering and Recent N Deposition Control Contemporary Plant-Soil C, N, and P.” *Global Biogeochemical Cycles* 30(2).

Demarez, V., and J. P. Gastellu-Etchegorry. 2000. “A Modeling Approach for Studying Forest Chlorophyll Content.” *Remote Sensing of Environment* 71(2).

Deng, Qi et al. 2015. “Down-Regulation of Tissue N:P Ratios in Terrestrial Plants by Elevated CO2.” *Ecology* 96(12).

Devau, Nicolas et al. 2009. “Soil PH Controls the Environmental Availability of Phosphorus: Experimental and Mechanistic Modelling Approaches.” *Applied Geochemistry* 24(11).

Dijkstra, F. A. et al. 2008. “Long-Term Enhancement of N Availability and Plant Growth under Elevated CO2 in a Semi-Arid Grassland.” *Functional Ecology* 22(6).

Dijkstra, Feike A. et al. 2010. “Elevated CO2 Effects on Semi-Arid Grassland Plants in Relation to Water Availability and Competition.” *Functional Ecology* 24(5): 1152–61.

Dijkstra, Feike A., Yolima Carrillo, Elise Pendall, and Jack A. Morgan. 2013. “Rhizosphere Priming: A Nutrient Perspective.” *Frontiers in Microbiology* 4(JUL).

Dixon, Philip. 2003. “VEGAN, a Package of R Functions for Community Ecology.” *Journal of Vegetation Science* 14(6).

Drake, Bert G., Miquel A. Gonzàlez-Meler, and Steve P. Long. 1997. “More Efficient Plants: A Consequence of Rising Atmospheric CO2?” *Annual Review of Plant Biology* 48.

Du, Chenjun et al. 2019. “Effects of Elevated CO 2 on Plant C-N-P Stoichiometry in Terrestrial Ecosystems: A Meta-Analysis.” *Science of the Total Environment* 650: 697–708. https://doi.org/10.1016/j.scitotenv.2018.09.051.

Du, Enzai et al. 2020. “Global Patterns of Terrestrial Nitrogen and Phosphorus Limitation.” *Nature Geoscience* 13(3).

Duffy, James P. et al. 2018. “Location, Location, Location: Considerations When Using Lightweight Drones in Challenging Environments.” *Remote Sensing in Ecology and Conservation* 4(1): 7–19.

Duursma, Remko A. 2015. “Plantecophys - An R Package for Analysing and Modelling Leaf Gas Exchange Data.” *PLoS ONE* 10(11).

Edwards, Everard J., Stephanie McCaffery, and John R. Evans. 2005. “Phosphorus Status Determines Biomass Response to Elevated CO2 in a Legume: C4 Grass Community.” *Global Change Biology* 11(11).

Eitel, Jan U.H., Troy S. Magney, Lee A. Vierling, and Günter Dittmar. 2014. “Assessment of Crop Foliar Nitrogen Using a Novel Dual-Wavelength Laser System and Implications for Conducting Laser-Based Plant Physiology.” *ISPRS Journal of Photogrammetry and Remote Sensing* 97.

Elser, James J. et al. 2007. “Global Analysis of Nitrogen and Phosphorus Limitation of Primary Producers in Freshwater, Marine and Terrestrial Ecosystems.” *Ecology Letters* 10(12).

Esmeijer-Liu, A. J. et al. 2009. “Nitrogen Enrichment Lowers Betula Pendula Green and Yellow Leaf Stoichiometry Irrespective of Effects of Elevated Carbon Dioxide.” *Plant and Soil* 316(1–2).

Evans, John R. 1989. “Photosynthesis and Nitrogen Relationships in Leaves of C3 Plants.” *Oecologia* 78(1).

Evans, John R. 2016. “International Association for Ecology Photosynthesis and Nitrogen Relationships in Leaves of C_3 Plants Author (s): John R . Evans Published by : Springer in Cooperation with International Association for Ecology Stable URL : Http://Www.Jstor.Org/Stable/.” 78(1): 9–19.

FAO, and ITPS. 2020. Global Soil Organic Carbon Map (GSOCmap) Version 1.5 *Global Soil Organic Carbon Map Version 1.5: Technical Report*. https://doi.org/10.4060/ca7597en.

Fay, Philip A. et al. 2015. “Grassland Productivity Limited by Multiple Nutrients.” *Nature Plants* 1.

Fick, Stephen E., and Robert J. Hijmans. 2017. “WorldClim 2: New 1-Km Spatial Resolution Climate Surfaces for Global Land Areas.” *International Journal of Climatology* 37(12).

Filella, I., L. Serrano, J. Serra, and J. Penuelas. 1995. “Evaluating Wheat Nitrogen Status with Canopy Reflectance Indices and Discriminant Analysis.” *Crop Science* 35(5).

Fitzgerald, G. J. et al. 2006. “Spectral and Thermal Sensing for Nitrogen and Water Status in Rainfed and Irrigated Wheat Environments.” *Precision Agriculture* 7(4).

Fleischer, Katrin et al. 2019. “Amazon Forest Response to CO2 Fertilization Dependent on Plant Phosphorus Acquisition.” *Nature Geoscience* 12(9).

Fowler, David, Jennifer B.A. Muller, and Lucy J. Sheppard. 2004. “The GaNE Programme in a Global Perspective.” *Water, Air, and Soil Pollution: Focus* 4(6).

Friedlingstein, Pierre et al. 2019. “Global Carbon Budget 2019.” *Earth System Science Data* 11(4).

———. 2022. “Global Carbon Budget 2022.” *Earth System Science Data* 14(11).

Fuchs, Kathrin et al. 2020. “Evaluating the Potential of Legumes to Mitigate N2O Emissions From Permanent Grassland Using Process-Based Models.” *Global Biogeochemical Cycles* 34(12).

Fuller, R. M. 1987. “The Changing Extent and Conservation Interest of Lowland Grasslands in England and Wales: A Review of Grassland Surveys 1930-1984.” *Biological Conservation* 40(4).

Gamon, J. A. et al. 2019. “Assessing Vegetation Function with Imaging Spectroscopy.” *Surveys in Geophysics* 40(3): 489–513. https://doi.org/10.1007/s10712-019-09511-5.

Gamon, J. A., and J. S. Surfus. 1999. “Assessing Leaf Pigment Content and Activity with a Reflectometer.” *New Phytologist* 143(1).

Gao, Feng, and Xiaoyang Zhang. 2021. “Mapping Crop Phenology in Near Real-Time Using Satellite Remote Sensing: Challenges and Opportunities.” *Journal of Remote Sensing* 2021.

Garnett, M. H., P. Ineson, and A. C. Stevenson. 2000. “Effects of Burning and Grazing on Carbon Sequestration in a Pennine Blanket Bog, UK.” *Holocene* 10(6).

Gerhards, Max, Martin Schlerf, Kaniska Mallick, and Thomas Udelhoven. 2019. “Challenges and Future Perspectives of Multi-/Hyperspectral Thermal Infrared Remote Sensing for Crop Water-Stress Detection: A Review.” *Remote Sensing* 11(10).

Gerke, Jörg. 2015. “The Acquisition of Phosphate by Higher Plants: Effect of Carboxylate Release by the Roots. A Critical Review.” *Journal of Plant Nutrition and Soil Science* 178(3).

Ghannoum, Oula et al. 2010. “Photosynthetic Responses of Two Eucalypts to Industrial-Age Changes in Atmospheric [CO2] and Temperature.” *Plant, Cell and Environment* 33(10): 1671–81.

Gifford, Roger M., Damian J. Barrett, and Jason L. Lutze. 2000. “The Effects of Elevated [CO2] on the C:N and C:P Mass Ratios of Plant Tissues.” *Plant and Soil* 224(1).

Gimingham, C. H. 1960. “Calluna Salisb.” *The Journal of Ecology* 48(2).

GIMINGHAM, C. H. 1989. “Heather and Heathlands.” *Botanical Journal of the Linnean Society* 101(3).

Gimingham, C. H., R. J. Hobbs, and A. U. Mallik. 1981. “Community Dynamics in Relation to Management of Heathland Vegetation in Scotland.” *Vegetatio* 46–47(1).

Gimingham, C. H., F. J. Kruger, D. T. Mitchell, and J. U. M. Jarvis. 1985. “Mediterranean-Type Ecosystems: The Role of Nutrients.” *The Journal of Ecology* 73(1).

Gitelson, A. A., and M. N. Merzlyak. 1997. “Remote Estimation of Chlorophyll Content in Higher Plant Leaves.” *International Journal of Remote Sensing* 18(12).

Gitelson, Anatoly A. et al. 2005. “Remote Estimation of Canopy Chlorophyll Content in Crops.” *Geophysical Research Letters* 32(8).

Gitelson, Anatoly A., Olga B. Chivkunova, and Mark N. Merzlyak. 2009. “Nondestructive Estimation of Anthocyanins and Chlorophylls in Anthocyanic Leaves.” *American Journal of Botany* 96(10).

Gitelson, Anatoly A., Yuri Gritz, and Mark N. Merzlyak. 2003. “Relationships between Leaf Chlorophyll Content and Spectral Reflectance and Algorithms for Non-Destructive Chlorophyll Assessment in Higher Plant Leaves.” *Journal of Plant Physiology* 160(3).

Gitelson, Anatoly A., Yoram J. Kaufman, Robert Stark, and Don Rundquist. 2002. “Novel Algorithms for Remote Estimation of Vegetation Fraction.” *Remote Sensing of Environment* 80(1): 76–87.

Gitelson, Anatoly A., Mark N. Merzlyak, and Olga B. Chivkunova. 2001. “Optical Properties and Nondestructive Estimation of Anthocyanin Content in Plant Leaves¶.” *Photochemistry and Photobiology* 74(1).

Goll, D. S. et al. 2012. “Nutrient Limitation Reduces Land Carbon Uptake in Simulations with a Model of Combined Carbon, Nitrogen and Phosphorus Cycling.” *Biogeosciences* 9(9).

Gordon, C., S. J. Woodin, I. J. Alexander, and C. E. Mullins. 1999. “Effects of Increased Temperature, Drought and Nitrogen Supply on Two Upland Perennials of Contrasting Functional Type: Calluna Vulgaris and Pteridium Aquilinum.” *New Phytologist* 142(2).

Gray, Julie E. et al. 2000. "The HIC Signalling Pathway Links CO2 Perception to Stomatal Development." *Nature* 408(6813).

Griffin, K. L., D. T. Tissue, M. H. Turnbull, and D. Whitehead. 2000. "The Onset of Photosynthetic Acclimation to Elevated CO2 Partial Pressure in Field-Grown Pinus Radiata D. Don. after 4 Years." *Plant, Cell and Environment* 23(10).

Grulke, N. E., J. L. Hom, and S. W. Roberts. 1993. "Physiological Adjustment of Two Full-Sib Families of Ponderosa Pine to Elevated CO2." *Tree Physiology* 12(4).

Grünzweig, José M., and Christian Körner. 2001. "Biodiversity Effects of Elevated CO2 in Species-Rich Model Communities from the Semi-Arid Negev of Israel." *Oikos* 95(1).

———. 2003. "Differential Phosphorus and Nitrogen Effects Drive Species and Community Responses to Elevated CO2 in Semi-Arid Grassland." *Functional Ecology* 17(6).

Habel, Jan Christian et al. 2013. "European Grassland Ecosystems: Threatened Hotspots of Biodiversity." *Biodiversity and Conservation* 22(10).

Haboudane, Driss et al. 2002. "Integrated Narrow-Band Vegetation Indices for Prediction of Crop Chlorophyll Content for Application to Precision Agriculture." *Remote Sensing of Environment* 81(2–3).

Hammond, John P., and Philip J. White. 2008. "Sucrose Transport in the Phloem: Integrating Root Responses to Phosphorus Starvation." In *Journal of Experimental Botany*,.

Hanley, M. E., S. Trofimov, and G. Taylor. 2004. "Species-Level Effects More Important than Functional Group-Level Responses to Elevated CO2: Evidence from Simulated Turves." *Functional Ecology* 18(3): 304–13.

Harris, Mike P.K. et al. 2011. "Factors Affecting Moorland Plant Communities and Component Species in Relation to Prescribed Burning." *Journal of Applied Ecology* 48(6).

Hassler, Samuel C., and Fulya Baysal-Gurel. 2019. "Unmanned Aircraft System (UAS) Technology and Applications in Agriculture." *Agronomy* 9(10).

Hatfield, Jerry L., and Christian Dold. 2019. "Water-Use Efficiency: Advances and Challenges in a Changing Climate." *Frontiers in Plant Science* 10.

Hautier, Yann, Pascal A. Niklaus, and Andy Hector. 2009. "Competition for Light Causes Plant Biodiversity Loss after Eutrophication." *Science* 324(5927).

Haworth, Matthew et al. 2016. "Carbon Dioxide Fertilisation and Supressed Respiration Induce Enhanced Spring Biomass Production in a Mixed Species Temperate Meadow Exposed to Moderate Carbon Dioxide Enrichment." *Functional Plant Biology* 43(1).

Heenkenda, Muditha K., Karen E. Joyce, Stefan W. Maier, and Sytze de Bruin. 2015.

"Quantifying Mangrove Chlorophyll from High Spatial Resolution Imagery." *ISPRS Journal of Photogrammetry and Remote Sensing* 108.

Herrmann, I. et al. 2010. "SWIR-Based Spectral Indices for Assessing Nitrogen Content in Potato Fields." *International Journal of Remote Sensing* 31(19).

Hodge, Angela. 2014. "Interactions between Arbuscular Mycorrhizal Fungi and Organic Material Substrates." In *Advances in Applied Microbiology*,.

Hoekstra, Jonathan M., Timothy M. Boucher, Taylor H. Ricketts, and Carter Roberts. 2005. "Confronting a Biome Crisis: Global Disparities of Habitat Loss and Protection." *Ecology Letters* 8(1).

Holden, J. et al. 2007. "Environmental Change in Moorland Landscapes." *Earth-Science Reviews* 82(1–2).

Holford, I. C.R. 1997. "Soil Phosphorus: Its Measurement, and Its Uptake by Plants." *Australian Journal of Soil Research* 35(2).

Homolová, Lucie et al. 2013. "Review of Optical-Based Remote Sensing for Plant Trait Mapping." *Ecological Complexity* 15: 1–16.

Hooftman, D. A.P., and J. M. Bullock. 2012. "Mapping to Inform Conservation: A Case Study of Changes in Semi-Natural Habitats and Their Connectivity over 70years." *Biological Conservation* 145(1).

Horswill, Paul et al. 2008. "Base Cation Depletion, Eutrophication and Acidification of Species-Rich Grasslands in Response to Long-Term Simulated Nitrogen Deposition." *Environmental Pollution* 155(2).

Huang, Wenjuan et al. 2015. "Plant Stoichiometric Responses to Elevated CO 2 Vary with Nitrogen and Phosphorus Inputs: Evidence from a Global-Scale Meta-Analysis." *Scientific Reports* 5(June): 1–8.

Huemmrich, K. Fred et al. 2022. "Leaf-Level Chlorophyll Fluorescence and Reflectance Spectra of High Latitude Plants." *Environmental Research Communications* 4(3).

IPCC Working Group 1, I. et al. 2013. "IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change." *IPCC* AR5.

Isbell, Forest et al. 2011. "High Plant Diversity Is Needed to Maintain Ecosystem Services." *Nature* 477(7363).

JACOB, J., C. GREITNER, and B. G. DRAKE. 1995. "Acclimation of Photosynthesis in Relation to Rubisco and Non-structural Carbohydrate Contents and in Situ Carboxylase Activity in Scirpus Olneyi Grown at Elevated CO2 in the Field." *Plant, Cell &*

Environment 18(8).

Jacquemoud, S. et al. 1996. “Estimating Leaf Biochemistry Using the PROSPECT Leaf Optical Properties Model.” *Remote Sensing of Environment* 56(3): 194–202.

Jacquemoud, S, and Susan L Ustin. 2001. “Leaf Optical Properties: A State of the Art.” *Proc. 8th International Symposium Physical Measurements & Signatures in Remote Sensing* (January).

Jacquemoud, Stéphane, and Susan Ustin. 2019. “Variation Due to Leaf Structural, Chemical, and Physiological Traits.” In *Leaf Optical Properties*,.

Jakobsen, Iver et al. 2016. “Plant Growth Responses to Elevated Atmospheric CO2 Are Increased by Phosphorus Sufficiency but Not by Arbuscular Mycorrhizas.” *Journal of Experimental Botany* 67(21).

Janes-Bassett, Victoria, Jessica Davies, Ed C. Rowe, and Edward Tipping. 2020. “Simulating Long-Term Carbon Nitrogen and Phosphorus Biogeochemical Cycling in Agricultural Environments.” *Science of the Total Environment* 714.

Jin, Jian, Caixian Tang, and Peter Sale. 2015. “The Impact of Elevated Carbon Dioxide on the Phosphorus Nutrition of Plants: A Review.” *Annals of Botany* 116(6).

JNCC, Defra. JNCC, Peterborough, UK (2012). 2010. “‘UK Post-2010 Biodiversity Framework.’”

Johnson, D., J. R. Leake, and J. A. Lee. 1999. “The Effects of Quantity and Duration of Simulated Pollutant Nitrogen Deposition on Root-Surface Phosphatase Activities in Calcareous and Acid Grasslands: A Bioassay Approach.” *New Phytologist* 141(3).

Jones, M. B., and Alison Donnelly. 2004. “Carbon Sequestration in Temperate Grassland Ecosystems and the Influence of Management, Climate and Elevated CO2.” *New Phytologist* 164(3).

Jose, Author et al. 2023. “Package ‘ Spectrolab .’”

Junttila, S. et al. 2022. “Close-Range Hyperspectral Spectroscopy Reveals Leaf Water Content Dynamics.” *Remote Sensing of Environment* 277.

Kanno, Keiichi, Yuji Suzuki, and Amane Makino. 2017. “A Small Decrease in Rubisco Content by Individual Suppression of RBCS Genes Leads to Improvement of Photosynthesis and Greater Biomass Production in Rice under Conditions of Elevated CO2.” *Plant and Cell Physiology* 58(3).

Kattge, Jens, Wolfgang Knorr, Thomas Raddatz, and Christian Wirth. 2009. “Quantifying Photosynthetic Capacity and Its Relationship to Leaf Nitrogen Content for Global-Scale Terrestrial Biosphere Models.” *Global Change Biology* 15(4).

Kayoumu, Mirezhatijiang et al. 2023. “Phosphorus Availability Affects the Photosynthesis and Antioxidant System of Contrasting Low-P-Tolerant Cotton Genotypes.” *Antioxidants* 12(2).

Keane, J. Ben et al. 2020. “Soil C, N and P Cycling Enzyme Responses to Nutrient Limitation under Elevated CO2.” *Biogeochemistry* 151(2–3).

Keane, J Ben et al. 2023. “Grassland Responses to Elevated CO 2 Determined by Plant – Microbe Competition for Phosphorus.”

Kleynhans, Elizabeth J., Sarah P. Otto, Peter B. Reich, and Mark Vellend. 2016. “Adaptation to Elevated CO2 in Different Biodiversity Contexts.” *Nature Communications* 7.

Klupar, Ian, Adrian V. Rocha, and Edward B. Rastetter. 2021. “Alleviation of Nutrient Co-Limitation Induces Regime Shifts in Post-Fire Community Composition and Productivity in Arctic Tundra.” *Global Change Biology* 27(14).

Knoth, Christian, Birte Klein, Torsten Prinz, and Till Kleinebecker. 2013. “Unmanned Aerial Vehicles as Innovative Remote Sensing Platforms for High-Resolution Infrared Imagery to Support Restoration Monitoring in Cut-over Bogs.” *Applied Vegetation Science* 16(3): 509–17.

Kovar, Marek et al. 2019. “Evaluation of Hyperspectral Reflectance Parameters to Assess the Leafwater Content in Soybean.” *Water (Switzerland)* 11(3).

Kume, Atsushi, Tomoko Akitsu, and Kenlo Nishida Nasahara. 2019. “Correction to: Why Is Chlorophyll b Only Used in Light-Harvesting Systems? (Journal of Plant Research, (2018), 131, 6, (961-972), 10.1007/S10265-018-1052-7).” *Journal of Plant Research* 132(3).

Kuzyakov, Yakov. 2002. “Review: Factors Affecting Rhizosphere Priming Effects.” *Journal of Plant Nutrition and Soil Science* 165(4): 382–96.

Laliberté, Etienne et al. 2013. “How Does Pedogenesis Drive Plant Diversity?” *Trends in Ecology and Evolution* 28(6).

Lambers, Hans et al. 2013. “Phosphorus Nutrition of Phosphorus-Sensitive Australian Native Plants: Threats to Plant Communities in a Global Biodiversity Hotspot.” *Conservation Physiology* 1(1).

Lambers, Hans, and William C. Plaxton. 2015. “Phosphorus: Back to The Roots.” In *Phosphorus Metabolism in Plants*,.

Lambers, Hans, John A. Raven, Gaius R. Shaver, and Sally E. Smith. 2008. “Plant Nutrient-Acquisition Strategies Change with Soil Age.” *Trends in Ecology and Evolution* 23(2).

Lê, Sébastien, Julie Josse, and François Husson. 2008. “FactoMineR: An R Package for

Multivariate Analysis." *Journal of Statistical Software* 25(1).

Leakey, Andrew D.B. et al. 2009. "Elevated CO2 Effects on Plant Carbon, Nitrogen, and Water Relations: Six Important Lessons from FACE." In *Journal of Experimental Botany*,.

LeBauer, David S., and Kathleen K. Treseder. 2008. "Nitrogen Limitation of Net Primary Productivity in Terrestrial Ecosystems Is Globally Distributed." *Ecology* 89(2).

Lee, J. A., and S. J.M. Caporn. 1998. "Ecological Effects of Atmospheric Reactive Nitrogen Deposition on Semi-Natural Terrestrial Ecosystems." *New Phytologist* 139(1).

Lee, Tali D., Mark G. Tjoelker, Peter B. Reich, and Michael P. Russelle. 2003. "Contrasting Growth Response of an N2-Fixing and Non-Fixing Forb to Elevated CO2: Dependence on Soil N Supply." *Plant and Soil* 255(2).

Lefsky, Michael A., Warren B. Cohen, Geoffrey G. Parker, and David J. Harding. 2002. "Lidar Remote Sensing for Ecosystem Studies." *BioScience* 52(1).

Lewis, P., and M. Disney. 2007. "Spectral Invariants and Scattering across Multiple Scales from Within-Leaf to Canopy." *Remote Sensing of Environment* 109(2).

Li, Hongbo et al. 2017. "Diverse Belowground Resource Strategies Underlie Plant Species Coexistence and Spatial Distribution in Three Grasslands along a Precipitation Gradient." *New Phytologist* 216(4).

Li, Leilei et al. 2022. "Ameliorative Effects of Silicon against Salt Stress in Gossypium Hirsutum L." *Antioxidants* 11(8).

Li, Xiangnan, Dong Jiang, and Fulai Liu. 2016. "Dynamics of Amino Acid Carbon and Nitrogen and Relationship with Grain Protein in Wheat under Elevated CO2 and Soil Warming." *Environmental and Experimental Botany* 132.

Li, Yafei, Xiangnan Li, Jingjie Yu, and Fulai Liu. 2017. "Effect of the Transgenerational Exposure to Elevated CO2 on the Drought Response of Winter Wheat: Stomatal Control and Water Use Efficiency." *Environmental and Experimental Botany* 136.

Liu, Ailin, Carolina A. Contador, Kejing Fan, and Hon Ming Lam. 2018. "Interaction and Regulation of Carbon, Nitrogen, and Phosphorus Metabolisms in Root Nodules of Legumes." *Frontiers in Plant Science* 871.

Liu, Chao et al. 2021. "Hyperspectral Characteristics and Inversion Model Estimation of Winter Wheat under Different Elevated CO2 Concentrations." *International Journal of Remote Sensing* 42(3).

Liu, Jingran et al. 2021. "High Soil Available Phosphorus Favors Carbon Metabolism in Cotton Leaves in Pot Trials." *Journal of Plant Growth Regulation* 40(3).

Loladze, Irakli. 2002. “Rising Atmospheric CO2 and Human Nutrition: Toward Globally Imbalanced Plant Stoichiometry?” *Trends in Ecology and Evolution* 17(10).

Long, Stephen P., Elizabeth A. Ainsworth, Alistair Rogers, and Donald R. Ort. 2004. “Rising Atmospheric Carbon Dioxide: Plants FACE the Future.” *Annual Review of Plant Biology* 55.

Lu, Hong et al. 2023. “Molecular Mechanisms and Genetic Improvement of Low-Phosphorus Tolerance in Rice.” *Plant Cell and Environment* 46(4).

Lu, Peng et al. 2021. “Ambient Nitrogen Deposition Drives Plant-Diversity Decline by Nitrogen Accumulation in a Closed Grassland Ecosystem.” *Journal of Applied Ecology* 58(9).

Luo, Xiangzhong et al. 2021. “Global Variation in the Fraction of Leaf Nitrogen Allocated to Photosynthesis.” *Nature Communications* 12(1).

Lynch, Jonathan P., and Melissa D. Ho. 2005. “Rhizoeconomics: Carbon Costs of Phosphorus Acquisition.” In *Plant and Soil*,.

Ma, Wenming et al. 2022. “Root Exudates Contribute to Belowground Ecosystem Hotspots: A Review.” *Frontiers in Microbiology* 13.

MacDonald, Graham K., Elena M. Bennett, Philip A. Potter, and Navin Ramankutty. 2011. “Agronomic Phosphorus Imbalances across the World’s Croplands.” *Proceedings of the National Academy of Sciences of the United States of America* 108(7).

Maier, Chris A., Sari Palmroth, and Eric Ward. 2008. “Short-Term Effects of Fertilization on Photosynthesis and Leaf Morphology of Field-Grown Loblolly Pine Following Long-Term Exposure to Elevated CO 2 Concentration.” In *Tree Physiology*,.

Main, Russell et al. 2011. “An Investigation into Robust Spectral Indices for Leaf Chlorophyll Estimation.” *ISPRS Journal of Photogrammetry and Remote Sensing* 66(6).

Le Maire, G., C. François, and E. Dufrêne. 2004. “Towards Universal Broad Leaf Chlorophyll Indices Using PROSPECT Simulated Database and Hyperspectral Reflectance Measurements.” *Remote Sensing of Environment* 89(1).

Malenovský, Zbynk et al. 2009. “Scientific and Technical Challenges in Remote Sensing of Plant Canopy Reflectance and Fluorescence.” *Journal of Experimental Botany* 60(11).

Måren, Inger E. et al. 2010. “Prescribed Burning of Northern Heathlands: Calluna Vulgaris Germination Cues and Seed-Bank Dynamics.” *Plant Ecology* 207(2).

Marschner, Petra. 2011. Marschner’s Mineral Nutrition of Higher Plants: Third Edition *Marschner’s Mineral Nutrition of Higher Plants: Third Edition*.

[Masson-Delmotte, V. et al. 2021. Cambridge University Press. In Press. *IPCC, 2021:*

Climate Change 2021: The Physical Science Basis.

Mcgrath, Justin M., and David B. Lobell. 2013. “Reduction of Transpiration and Altered Nutrient Allocation Contribute to Nutrient Decline of Crops Grown in Elevated CO2 Concentrations.” *Plant, Cell and Environment* 36(3).

Mead, L., and Mac Arthur. 2020. “Environmental Condition in British Moorlands: Quantifying the Life Cycle of Calluna Vulgaris Using UAV Aerial Imagery.” *International Journal of Remote Sensing* 41(2): 573–83. https://doi.org/10.1080/2150704X.2019.1646931.

Mertens, Stien et al. 2021. “Proximal Hyperspectral Imaging Detects Diurnal and Drought-Induced Changes in Maize Physiology.” *Frontiers in Plant Science* 12.

Michelet, Laure et al. 2013. “Redox Regulation of the Calvin-Benson Cycle: Something Old, Something New.” *Frontiers in Plant Science* 4(NOV).

Miglietta, F. et al. 2001. “Spatial and Temporal Performance of the Miniface (Free Air CO2 Enrichment) System on Bog Ecosystems in Northern and Central Europe.” *Environmental Monitoring and Assessment* 66(2).

Milton, E. J. 1987. “Review Article: Principles of Field Spectroscopy.” *International Journal of Remote Sensing* 8(12).

Minchin, Frank R., and John F Witty. 2005. “Respiratory/Carbon Costs of Symbiotic Nitrogen Fixation in Legumes.” In *Plant Respiration*,.

Mo, Qifeng et al. 2020. “Leaf Nonstructural Carbohydrate Concentrations of Understory Woody Species Regulated by Soil Phosphorus Availability in a Tropical Forest.” *Ecology and Evolution* 10(15).

Moran, Rami. 1982. “ Formulae for Determination of Chlorophyllous Pigments Extracted with N,N -Dimethylformamide .” *Plant Physiology* 69(6).

Morecroft, M. D., E. K. Sellers, and J. A. Lee. 1994. “An Experimental Investigation into the Effects of Atmospheric Nitrogen Deposition on Two Semi-Natural Grasslands.” *The Journal of Ecology* 82(3).

Mu, Xiaohuan, and Yanling Chen. 2021. “The Physiological Response of Photosynthesis to Nitrogen Deficiency.” *Plant Physiology and Biochemistry* 158.

Mutanga, O., and L. Kumar. 2007. “Estimating and Mapping Grass Phosphorus Concentration in an African Savanna Using Hyperspectral Image Data.” *International Journal of Remote Sensing* 28(21).

Mutanga, O., and A. K. Skidmore. 2004. “Narrow Band Vegetation Indices Overcome the Saturation Problem in Biomass Estimation.” *International Journal of Remote Sensing*

25(19).

Mutanga, O., A. K. Skidmore, and H. H.T. Prins. 2004. “Predicting in Situ Pasture Quality in the Kruger National Park, South Africa, Using Continuum-Removed Absorption Features.” *Remote Sensing of Environment* 89(3).

Myneni, R. B. et al. 1997. “Increased Plant Growth in the Northern High Latitudes from 1981 to 1991.” *Nature*.

Näsholm, Torgny, Knut Kielland, and Ulrika Ganeteg. 2009. “Uptake of Organic Nitrogen by Plants.” *New Phytologist* 182(1).

Nelson, David L., and Michael M. Cox. 2017. “Lehninger Principles of Biochemistry 7th.” *W.H. Freeman and Company* 2.

Nelson, Jim A. et al. 2004. “Elevated CO2 Increases Soil Moisture and Enhances Plant Water Relations in a Long-Term Field Study in Semi-Arid Shortgrass Steppe of Colorado.” *Plant and Soil* 259(1–2).

Neocleous, Damianos, and Dimitrios Savvas. 2019. “The Effects of Phosphorus Supply Limitation on Photosynthesis, Biomass Production, Nutritional Quality, and Mineral Nutrition in Lettuce Grown in a Recirculating Nutrient Solution.” *Scientia Horticulturae* 252.

Neumann, Carsten et al. 2020. “The Colors of Heath Flowering – Quantifying Spatial Patterns of Phenology in Calluna Life-Cycle Phases Using High-Resolution Drone Imagery.” *Remote Sensing in Ecology and Conservation* 6(1): 35–51.

Nguyen, Van Lam, Lachlan Palmer, and James Stangoulis. 2022. “Higher Photochemical Quenching and Better Maintenance of Carbon Dioxide Fixation Are Key Traits for Phosphorus Use Efficiency in the Wheat Breeding Line, RAC875.” *Frontiers in Plant Science* 12.

Nichol, C. J., and J. Grace. 2010. “Determination of Leaf Pigment Content in Calluna Vulgaris Shoots from Spectral Reflectance.” *International Journal of Remote Sensing* 31(20): 5409–22.

Niklaus, Pascal A., Paul W. Leadley, Jürg Stöcklin, and Christian Körner. 1998. “Nutrient Relations in Calcareous Grassland under Elevated CO2.” *Oecologia* 116(1–2).

Niu, Junpeng et al. 2022. “Exogenous Melatonin Promotes the Growth of Alfalfa (Medicago Sativa L.) under NaCl Stress through Multiple Pathways.” *Ecotoxicology and Environmental Safety* 242(August): 113938. https://doi.org/10.1016/j.ecoenv.2022.113938.

Novotny, Amy M. et al. 2007. “Stoichiometric Response of Nitrogen-Fixing and Non-Fixing

Dicots to Manipulations of CO2, Nitrogen, and Diversity." *Oecologia* 151(4).

Nowak, Robert S., David S. Ellsworth, and Stanley D. Smith. 2004. "Functional Responses of Plants to Elevated Atmospheric CO2 - Do Photosynthetic and Productivity Data from FACE Experiments Support Early Predictions?" *New Phytologist* 162(2).

Ollinger, S. V. 2011. "Sources of Variability in Canopy Reflectance and the Convergent Properties of Plants." *New Phytologist* 189(2).

Osborne, S. L., J. S. Schepers, D. D. Francis, and M. R. Schlemmer. 2002. "Detection of Phosphorus and Nitrogen Deficiencies in Corn Using Spectral Radiance Measurements." *Agronomy Journal* 94(6).

Pandey, Renu et al. 2015. "Physiological and Molecular Alterations in Plants Exposed to High [CO2] under Phosphorus Stress." *Biotechnology Advances* 33(3–4).

Pang, Jiayin et al. 2018. "Leaf Transpiration Plays a Role in Phosphorus Acquisition among a Large Set of Chickpea Genotypes." *Plant Cell and Environment* 41(9).

Pasquini, S. C., and L. S. Santiago. 2012. "Nutrients Limit Photosynthesis in Seedlings of a Lowland Tropical Forest Tree Species." *Oecologia* 168(2).

Pastore, Melissa A., Tali D. Lee, Sarah E. Hobbie, and Peter B. Reich. 2019. "Strong Photosynthetic Acclimation and Enhanced Water-Use Efficiency in Grassland Functional Groups Persist over 21 Years of CO2 Enrichment, Independent of Nitrogen Supply." *Global Change Biology* 25(9): 3031–44.

Pauli, Duke et al. 2017. "Investigation of the Influence of Leaf Thickness on Canopy Reflectance and Physiological Traits in Upland and Pima Cotton Populations." *Frontiers in Plant Science* 8.

Payne, Richard J. et al. 2017. "Nitrogen Deposition and Plant Biodiversity: Past, Present, and Future." *Frontiers in Ecology and the Environment* 15(8).

Pazzagli, Pietro T., Jacob Weiner, and Fulai Liu. 2016. "Effects of CO2 Elevation and Irrigation Regimes on Leaf Gas Exchange, Plant Water Relations, and Water Use Efficiency of Two Tomato Cultivars." *Agricultural Water Management* 169.

Penuelas, J. et al. 1993. "The Reflectance at the 950-970 Nm Region as an Indicator of Plant Water Status." *International Journal of Remote Sensing* 14(10).

Péret, Benjamin et al. 2014. "Root Architecture Responses: In Search of Phosphate." *Plant Physiology* 166(4).

Peters, Gjalt -. Jorn. 2018. "Userfriendlyscience: Quantitative Analysis Made Accessible." *https://userfriendlyscience.com*.

Phoenix, Gareth K. et al. 2003. "Effects of Enhanced Nitrogen Deposition and Phosphorus

Limitation on Nitrogen Budgets of Semi-Natural Grasslands.” *Global Change Biology* 9(9): 1309–21.

Phoenix, Gareth K., Jonathan R. Leake, et al. 2004. “Accumulation of Pollutant Nitrogen in Calcareous and Ccidic Grasslands: Evidence from N Flux and 15N Tracer Studies.” *Water, Air, and Soil Pollution: Focus* 4(6).

Phoenix, Gareth K., Rosemary E. Booth, et al. 2004. “Simulated Pollutant Nitrogen Deposition Increases P Demand and Enhances Root-Surface Phosphatase Activities of Three Plant Functional Types in a Calcareous Grassland.” *New Phytologist* 161(1): 279–90.

Phoenix, Gareth K. et al. 2012. “Impacts of Atmospheric Nitrogen Deposition: Responses of Multiple Plant and Soil Parameters across Contrasting Ecosystems in Long-Term Field Experiments.” *Global Change Biology* 18(4).

———. 2020. “Niche Differentiation and Plasticity in Soil Phosphorus Acquisition among Co-Occurring Plants.” *Nature Plants* 6(4): 349–54. http://dx.doi.org/10.1038/s41477-020-0624-4.

Pimstein, Agustin, Arnon Karnieli, Surinder K. Bansal, and David J. Bonfil. 2011. “Exploring Remotely Sensed Technologies for Monitoring Wheat Potassium and Phosphorus Using Field Spectroscopy.” *Field Crops Research* 121(1).

Plante, Alain F. 2006. “Soil Biogeochemical Cycling of Inorganic Nutrients and Metals.” In *Soil Microbiology, Ecology and Biochemistry: Third Edition*,.

Polley, H. Wayne, Virginia L. Jin, and Philip A. Fay. 2012. “Feedback from Plant Species Change Amplifies CO 2 Enhancement of Grassland Productivity.” *Global Change Biology* 18(9): 2813–23.

Poorter, Hendrik et al. 2012. “Biomass Allocation to Leaves, Stems and Roots: Meta-Analyses of Interspecific Variation and Environmental Control.” *New Phytologist* 193(1).

Poorter, Hendrik, and Oscar Nagel. 2000. “The Role of Biomass Allocation in the Growth Response of Plants to Different Levels of Light, CO2, Nutrients and Water: A Quantitative Review.” In *Australian Journal of Plant Physiology*,.

Poorter, Hendrik, and Marie Laure Navas. 2003. “Plant Growth and Competition at Elevated Co2: On Winners, Losers and Functional Groups.” *New Phytologist* 157(2).

Prananto, Jeremy Aditya, Budiman Minasny, and Timothy Weaver. 2020. “Near Infrared (NIR) Spectroscopy as a Rapid and Cost-Effective Method for Nutrient Analysis of Plant Leaf Tissues.” In *Advances in Agronomy*,.

Prior, Stephen A., Seth G. Pritchard, and G. Brett Runion. 2004. “Leaves and the Effects of Elevated Carbon Dioxide Levels.” In *Encyclopedia of Plant and Crop Science*,.

Quemada, Carlos et al. 2021. “Remote Sensing for Plant Water Content Monitoring: A Review.” *Remote Sensing* 13(11).

Quirk, Joe et al. 2012. “Evolution of Trees and Mycorrhizal Fungi Intensifies Silicate Mineral Weathering.” *Biology Letters* 8(6).

R Core Team. 2022. “R Core Team 2021 R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Https://Www.R-Project.Org/.” *R Foundation for Statistical Computing* 2.

Ramchunder, S. J., L. E. Brown, and J. Holden. 2009. “Environmental Effects of Drainage, Drain-Blocking and Prescribed Vegetation Burning in UK Upland Peatlands.” *Progress in Physical Geography* 33(1).

Raza, Sajjad et al. 2020. “Dramatic Loss of Inorganic Carbon by Nitrogen-Induced Soil Acidification in Chinese Croplands.” *Global Change Biology* 26(6).

Reddy, Attipalli R., Girish K. Rasineni, and Agepati S. Raghavendra. 2010. “The Impact of Global Elevated CO2 Concentration on Photosynthesis and Plant Productivity.” *Current Science* 99(1).

Reed, M. S. et al. 2009. “The Future of the Uplands.” *Land Use Policy* 26(SUPPL. 1).

Reich, Peter B. et al. 2001. “Do Species and Functional Groups Differ in Acquisition and Use of C, N and Water under Varying Atmospheric CO2 and N Availability Regimes? A Field Test with 16 Grassland Species.” *New Phytologist* 150(2).

———. 2009. “Elevated CO2 Reduces Losses of Plant Diversity Caused by Nitrogen Deposition.” *Science* 326(5958).

Reich, Peter B., Jacek Oleksyn, and Ian J. Wright. 2009. “Leaf Phosphorus Influences the Photosynthesis-Nitrogen Relation: A Cross-Biome Analysis of 314 Species.” *Oecologia* 160(2).

Reich, Peter B., Michael B. Walters, and David S. Ellsworth. 1997. “From Tropics to Tundra: Global Convergence in Plant Functioning.” *Proceedings of the National Academy of Sciences of the United States of America* 94(25).

Richardson, Andrew D., Shane P. Duigan, and Graeme P. Berlyn. 2002. “An Evaluation of Noninvasive Methods to Estimate Foliar Chlorophyll Content.” *New Phytologist*.

Rodwell, J. S. 1992. “British Plant Communities. Volume 3: Grasslands and Montane Communities.” *British plant communities. Volume 3: grasslands and montane communities*.

Rogers, A., and S. W. Humphries. 2000. “A Mechanistic Evaluation of Photosynthetic Acclimation at Elevated CO2.” *Global Change Biology* 6(8).

Roslim, Muhammad Huzaifah Mohd et al. 2021. “Using Remote Sensing and an Unmanned Aerial System for Weed Management in Agricultural Crops: A Review.” *Agronomy* 11(9).

Rotbart, Nativ et al. 2013. “Estimating Olive Leaf Nitrogen Concentration Using Visible and Near-Infrared Spectral Reflectance.” *Biosystems Engineering* 114(4).

Rouse, J. W, R. H Haas, J. A Schell, and D. W Deering. 1974. “Monitoring Vegetation Systems in the Great Plains with ERTS. NASA Special Publication.” *NASA special publication* 24(1).

Royimani, Lwando et al. 2019. “Advancements in Satellite Remote Sensing for Mapping and Monitoring of Alien Invasive Plant Species (AIPs).” *Physics and Chemistry of the Earth* 112.

Ruiz-Vera, Ursula M., Amanda P. De Souza, Stephen P. Long, and Donald R. Ort. 2017. “The Role of Sink Strength and Nitrogen Availability in the Down-Regulation of Photosynthetic Capacity in Field-Grown Nicotiana Tabacum L. at Elevated CO2 Concentration.” *Frontiers in Plant Science* 8.

Russell V. Lenth et al. 2022. “Estimated Marginal Means, Aka Least-Squares Means [R Package Emmeans Version 1.8.3].” *American Statistician* 34(4).

Sage, Rowan F. 1994. “Acclimation of Photosynthesis to Increasing Atmospheric CO2: The Gas Exchange Perspective.” *Photosynthesis Research* 39(3).

Sage, Rowan F., Danielle A. Way, and David S. Kubien. 2008. “Rubisco, Rubisco Activase, and Global Climate Change.” In *Journal of Experimental Botany*,.

Sala, Osvaldo E. et al. 2000. “Global Biodiversity Scenarios for the Year 2100.” *Science* 287(5459).

Sampson, Paul H. et al. 2003. “Hyperspectral Remote Sensing of Forest Condition: Estimating Chlorophyll Content in Tolerant Hardwoods.” *Forest Science* 49(3).

Sanderson, Roy, Samuel Newton, and Jen Selvidge. 2020. “Effects of Vegetation Cutting on Invertebrate Communities of High Conservation Value Calluna Upland Peatlands.” *Insect Conservation and Diversity* 13(3).

Sands, Martin J. S., and C. H. Gimingham. 1975. “Ecology of Heathlands.” *Kew Bulletin* 30(3).

Sanz-Sáez, Álvaro et al. 2010. “Photosynthetic Down-Regulation under Elevated CO2 Exposure Can Be Prevented by Nitrogen Supply in Nodulated Alfalfa.” *Journal of Plant*

Physiology 167(18).

Schachtman, Daniel P., Robert J. Reid, and S. M. Ayling. 1998. “Phosphorus Uptake by Plants: From Soil to Cell.” *Plant Physiology* 116(2).

Schielzeth, Holger et al. 2020. “Robustness of Linear Mixed-Effects Models to Violations of Distributional Assumptions.” *Methods in Ecology and Evolution* 11(9).

Schimel, Joshua P. 2018. “Life in Dry Soils: Effects of Drought on Soil Microbial Communities and Processes.” *Annual Review of Ecology, Evolution, and Systematics* 49.

Schlerf, Martin, and Clement Atzberger. 2006. “Inversion of a Forest Reflectance Model to Estimate Structural Canopy Variables from Hyperspectral Remote Sensing Data.” *Remote Sensing of Environment* 100(3).

Schlesinger, William H. 2009. “On the Fate of Anthropogenic Nitrogen.” *Proceedings of the National Academy of Sciences of the United States of America* 106(1).

Schlüter, Urte et al. 2013. “Adaptation of Maize Source Leaf Metabolism to Stress Related Disturbances in Carbon, Nitrogen and Phosphorus Balance.” *BMC Genomics* 14(1).

Schneider, Kimberley D. et al. 2017. “Microbially-Mediated P Fluxes in Calcareous Soils as a Function of Water-Extractable Phosphate.” *Soil Biology and Biochemistry* 106.

de Schrijver, An et al. 2011. “Cumulative Nitrogen Input Drives Species Loss in Terrestrial Ecosystems.” *Global Ecology and Biogeography* 20(6).

Serbin, Shawn P., and Philip A. Townsend. 2020. “Scaling Functional Traits from Leaves to Canopies.” *Remote Sensing of Plant Biodiversity* (May): 43–82.

Serrano, Lydia. 2008a. “Effects of Leaf Structure on Reflectance Estimates of Chlorophyll Content.” *International Journal of Remote Sensing* 29(17–18).

———. 2008b. “Effects of Leaf Structure on Reflectance Estimates of Chlorophyll Content.” *International Journal of Remote Sensing* 29(17–18): 5265–74.

Şevik, Hakan, Nur Belkayalı, and Gülnur Aktar. 2014. “Change of Chlorophyll Amount in Some Landscape Plants.” *Journal of Biotechnological Sciences (J. Biotechnol. Sci.)* 2(1).

Shane, Michael W. et al. 2006. “Specialized ‘dauciform’ Roots of Cyperaceae Are Structurally Distinct, but Functionally Analogous with ‘Cluster’ Roots.” *Plant, Cell and Environment* 29(10).

Shannon, C. E. 1948. “A Mathematical Theory of Communication.” *Bell System Technical Journal* 27(3).

Shen, Hao et al. 2019. “Effects of Simulated N Deposition on Photosynthesis and Productivity of Key Plants from Different Functional Groups of Alpine Meadow on

Qinghai-Tibetan Plateau.” *Environmental Pollution* 251.

Shen, Hao, Shikui Dong, Jiannan Xiao, and Yangliu Zhi. 2022. “Effects of N and P Enrichment on Plant Photosynthetic Traits in Alpine Steppe of the Qinghai-Tibetan Plateau.” *BMC Plant Biology* 22(1).

Shen, Jianbo et al. 2011. “Phosphorus Dynamics: From Soil to Plant.” *Plant Physiology* 156(3).

da Silva, Jefferson Rangel et al. 2017. “Photosynthetic Acclimation to Elevated CO2 Combined with Partial Rootzone Drying Results in Improved Water Use Efficiency, Drought Tolerance and Leaf Carbon Balance of Grapevines (Vitis Labrusca).” *Environmental and Experimental Botany* 134.

Simic, Anita, Jing M. Chen, and Thomas L. Noland. 2011. “Retrieval of Forest Chlorophyll Content Using Canopy Structure Parameters Derived from Multi-Angle Data: The Measurement Concept of Combining Nadir Hyperspectral and off-Nadir Multispectral Data.” *International Journal of Remote Sensing* 32(20).

Simpson, E. H. 1949. “Measurement of Diversity [16].” *Nature* 163(4148).

Sims, Daniel A., and John A. Gamon. 2002. “Relationships between Leaf Pigment Content and Spectral Reflectance across a Wide Range of Species, Leaf Structures and Developmental Stages.” *Remote Sensing of Environment* 81(2–3): 337–54.

Smith, Pete et al. 2007. “ECOSSE: Estimating Carbon in Organic Soils - Sequestration and Emissions: Final Report.” *Environmental Research*.

Smith, S E, and D J Read. 2008. “Mycorrhizal Symbiosis 3rd Edn Academic Press: San Diego.” *Academic Press: San Diego,and London*.

Smith, Sally E., and David Read. 2008. “The Symbionts Forming Arbuscular Mycorrhizas.” In *Mycorrhizal Symbiosis*,.

Society, British Ecological. 1971. “The Description and Interpretation of Cyclical Processes in a Heath Community : III . Microclimate in Relation to the Calluna Cycle Author (s): P . Barclay-Estrup Published by : British Ecological Society Stable URL : Https://Www.Jstor.Org/Stable/225845.” 59(1): 143–66.

Southon, Georgina E. et al. 2013. “Nitrogen Deposition Reduces Plant Diversity and Alters Ecosystem Functioning: Field-Scale Evidence from a Nationwide Survey of UK Heathlands.” *PLoS ONE* 8(4).

Spellerberg, Ian F., and Peter J. Fedor. 2003. “A Tribute to Claude-Shannon (1916-2001) and a Plea for More Rigorous Use of Species Richness, Species Diversity and the ‘Shannon-Wiener’ Index.” *Global Ecology and Biogeography* 12(3).

Steffen, Will et al. 2015. “Planetary Boundaries: Guiding Human Development on a Changing Planet.” *Science* 347(6223).

Stevens, Carly J., Ken Thompson, et al. 2010. “Contribution of Acidification and Eutrophication to Declines in Species Richness of Calcifuge Grasslands along a Gradient of Atmospheric Nitrogen Deposition.” *Functional Ecology* 24(2).

Stevens, Carly J., Cecilia Dupr, et al. 2010. “Nitrogen Deposition Threatens Species Richness of Grasslands across Europe.” *Environmental Pollution* 158(9).

Stevens, Carly J., Thomas I. David, and Jonathan Storkey. 2018. “Atmospheric Nitrogen Deposition in Terrestrial Ecosystems: Its Impact on Plant Communities and Consequences across Trophic Levels.” *Functional Ecology* 32(7).

Stevens, Carly J., Nancy B. Dise, J. Owen Mountford, and David J. Gowing. 2004. “Impact of Nitrogen Deposition on the Species Richness of Grasslands.” *Science* 303(5665).

Stigter, Kyla A., and William C. Plaxton. 2015. “Molecular Mechanisms of Phosphorus Metabolism and Transport during Leaf Senescence.” *Plants* 4(4).

Stiles, William A.V., Edwin C. Rowe, and Peter Dennis. 2017. “Long-Term Nitrogen and Phosphorus Enrichment Alters Vegetation Species Composition and Reduces Carbon Storage in Upland Soil.” *Science of the Total Environment* 593–594.

Stitt, Mark, and A. Krapp. 1999. “The Interaction between Elevated Carbon Dioxide and Nitrogen Nutrition: The Physiological and Molecular Background.” *Plant, Cell and Environment* 22(6).

Stöcklin, Jürg, Kathrin Schweizer, and Christian Körner. 1998. “Effects of Elevated CO2 and Phosphorus Addition on Productivity and Community Composition of Intact Monoliths from Calcareous Grassland.” *Oecologia* 116(1–2): 50–56.

Suding, Katharine N. et al. 2005. “Functional- and Abundance-Based Mechanisms Explain Diversity Loss Due to N Fertilization.” *Proceedings of the National Academy of Sciences of the United States of America* 102(12).

SUN, Jing Qi et al. 2023. “The Monitoring of Plant Physiology and Ecology: From Materials to Flexible Devices.” *Chinese Journal of Analytical Chemistry* 51(2).

Sun, Zhongyu et al. 2021. “UAVs as Remote Sensing Platforms in Plant Ecology: Review of Applications and Challenges.” *Journal of Plant Ecology* 14(6).

Swoczyna, Tatiana et al. 2022. “Environmental Stress - What Can We Learn from Chlorophyll a Fluorescence Analysis in Woody Plants? A Review.” *Frontiers in Plant Science* 13.

Taiz, Lincoln, Eduardo Zeiger, Ian Max Møller, and Angus Murphy. 2015. 6 Sinauer

Associates *Plant Physiology and Development,Sixth Edition*.

Tamary, Eyal et al. 2019. “Chlorophyll Catabolism Precedes Changes in Chloroplast Structure and Proteome during Leaf Senescence.” *Plant Direct* 3(3).

Taub, Daniel R., and Xianzhong Wang. 2008. “Why Are Nitrogen Concentrations in Plant Tissues Lower under Elevated CO2? A Critical Examination of the Hypotheses.” *Journal of Integrative Plant Biology* 50(11): 1365–74.

Tausz-Posch, Sabine et al. 2015. “Does a Freely Tillering Wheat Cultivar Benefit More from Elevated CO2 than a Restricted Tillering Cultivar in a Water-Limited Environment?” *European Journal of Agronomy* 64.

Taylor, Christopher R. et al. 2021. “Organic Phosphorus Cycling May Control Grassland Responses to Nitrogen Deposition: A Long-Term Field Manipulation and Modelling Study.” *Biogeosciences* 18(13): 4021–37.

Taylor, Christopher Rhys. 2021. “A Tale of Two Grasslands: The Interactions of Nutrient Limitation, Nitrogen Deposition and Elevated CO 2 on the Biogeochemical Cycling and Biodiversity of Two Contrasting UK Grasslands Christopher Rhys Taylor.” (October).

Team, R Core. 2021. “R: A Language and Environment for Statistical Computing.” *R Foundation for Statistical Computing*.

Teng, Nianjun et al. 2006. “Elevated CO2 Induces Physiological, Biochemical and Structural Changes in Leaves of Arabidopsis Thaliana.” *New Phytologist* 172(1).

Teste, Francois P., Erik J. Veneklaas, Kingsley W. Dixon, and Hans Lambers. 2014. “Complementary Plant Nutrient-Acquisition Strategies Promote Growth of Neighbour Species.” *Functional Ecology* 28(4).

Thomas, Richard B., and Boyd R. Strain. 1991. “Root Restriction as a Factor in Photosynthetic Acclimation of Cotton Seedlings Grown in Elevated Carbon Dioxide.” *Plant Physiology* 96(2).

Thompson, D. B.A., A. J. MacDonald, J. H. Marsden, and C. A. Galbraith. 1995. “Upland Heather Moorland in Great Britain: A Review of International Importance, Vegetation Change and Some Objectives for Nature Conservation.” *Biological Conservation* 71(2).

Tian, Qiuying et al. 2021. “Processes at the Soil–Root Interface Determine the Different Responses of Nutrient Limitation and Metal Toxicity in Forbs and Grasses to Nitrogen Enrichment.” *Journal of Ecology* 109(2).

Tilman, David, Forest Isbell, and Jane M. Cowles. 2014. “Biodiversity and Ecosystem Functioning.” *Annual Review of Ecology, Evolution, and Systematics* 45.

Tipping, E. et al. 2017. “Long-Term Increases in Soil Carbon Due to Ecosystem Fertilization by Atmospheric Nitrogen Deposition Demonstrated by Regional-Scale Modelling and Observations." *Scientific Reports* 7(1).

Tipping, Edward et al. 2021. "Long-Term Effects of Atmospheric Deposition on British Plant Species Richness." *Environmental Pollution* 281.

Tissue, David T., Kevin L. Griffin, and J. Timothy Ball. 1999. "Photosynthetic Adjustment in Field-Grown Ponderosa Pine Trees after Six Years of Exposure to Elevated CO2." *Tree Physiology* 19(4–5).

Tjoelker, M. G., J. Oleksyn, and P. B. Reich. 1998. "Seedlings of Five Boreal Tree Species Differ in Acclimation of Net Photosynthesis to Elevated CO2 and Temperature." *Tree Physiology* 18(11).

Tomlinson, Samuel J., Edward J. Carnell, Anthony J. Dore, and Ulrike Dragosits. 2021. "Nitrogen Deposition in the UK at 1 Km Resolution from 1990 to 2017." *Earth System Science Data* 13(10).

Tyler, Germund, and Lena Ström. 1995. "Differing Organic Acid Exudation Pattern Explains Calcifuge and Acidifuge Behaviour of Plants." *Annals of Botany* 75(1).

Urban, O. 2003. "Physiological Impacts of Elevated CO2 Concentration Ranging from Molecular to Whole Plant Responses." *Photosynthetica* 41(1).

Ustin, Susan L. et al. 2004. "Using Imaging Spectroscopy to Study Ecosystem Processes and Properties." *BioScience* 54(6).

Vance, Carroll P., Claudia Uhde-Stone, and Deborah L. Allan. 2003. "Phosphorus Acquisition and Use: Critical Adaptations by Plants for Securing a Nonrenewable Resource." *New Phytologist* 157(3).

Varvia, Petri, Miina Rautiainen, and Aku Seppänen. 2018. "Bayesian Estimation of Seasonal Course of Canopy Leaf Area Index from Hyperspectral Satellite Data." *Journal of Quantitative Spectroscopy and Radiative Transfer* 208.

Velle, Liv G., Liv S. Nilsen, and Vigdis Vandvik. 2012. "The Age of Calluna Stands Moderates Post-Fire Regeneration Rate and Trends in Northern Calluna Heathlands." *Applied Vegetation Science* 15(1).

Victor Roch, Gurusunathan, Theivanayagam Maharajan, Stanislaus Antony Ceasar, and Savarimuthu Ignacimuthu. 2019. "The Role of PHT1 Family Transporters in the Acquisition and Redistribution of Phosphorus in Plants." *Critical Reviews in Plant Sciences* 38(3).

Vile, Denis et al. 2005. "Specific Leaf Area and Dry Matter Content Estimate Thickness in Laminar Leaves." *Annals of Botany* 96(6).

Vitousek, Peter M., and Heraldo Farrington. 1997. “Nutrient Limitation and Soil Development: Experimental Test of a Biogeochemical Theory.” *Biogeochemistry* 37(1).

Walker, Anthony P. et al. 2014. “The Relationship of Leaf Photosynthetic Traits - Vcmax and Jmax - to Leaf Nitrogen, Leaf Phosphorus, and Specific Leaf Area: A Meta-Analysis and Modeling Study.” *Ecology and Evolution* 4(16): 3218–35.

Walker, T. W., and J. K. Syers. 1976. “The Fate of Phosphorus during Pedogenesis.” *Geoderma* 15(1).

Wang, Cuiting, Yuan Sun, Han Y.H. Chen, and Honghua Ruan. 2021. “Effects of Elevated CO2 on the C:N Stoichiometry of Plants, Soils, and Microorganisms in Terrestrial Ecosystems.” *Catena* 201.

Wang, Hehua, Haoxin Fan, and Huaiying Yao. 2020. “Effects of Elevated CO2 on Tomato (Lycopersicon Esculentum Mill.) Growth and Rhizosphere Soil Microbial Community Structure and Functionality.” *Agronomy* 10(11).

Wang, Ruzhen et al. 2021. “Nitrogen Enrichment Buffers Phosphorus Limitation by Mobilizing Mineral-bound Soil Phosphorus in Grasslands.” *Ecology*.

Wang, Y. H. et al. 2019. “Elevated CO2 Alters the Structure of the Bacterial Community Assimilating Plant-Derived Carbon in the Rhizosphere of Soya Bean.” *European Journal of Soil Science* 70(6).

Wang, Ying Ping, B. Z. Houlton, and C. B. Field. 2007. “A Model of Biogeochemical Cycles of Carbon, Nitrogen, and Phosphorus Including Symbiotic Nitrogen Fixation and Phosphatase Production.” *Global Biogeochemical Cycles* 21(1).

Warren, Jeffrey M. et al. 2015. “Carbon Dioxide Stimulation of Photosynthesis in Liquidambar Styraciflua Is Not Sustained during a 12-Year Field Experiment.” *AoB PLANTS* 7(1).

Waters, Colin N. et al. 2016. “The Anthropocene Is Functionally and Stratigraphically Distinct from the Holocene.” *Science* 351(6269).

Watson R, Albon S, Aspinall R, Austen M, Bardgett B, Bateman I, Berry P, Bird W, Bradbury R, Brown C. 2011. *UK National Ecosystem Assessment: Technical Report*.

Watt, Alex S. 1955. “Bracken Versus Heather, A Study in Plant Sociology.” *The Journal of Ecology* 43(2).

Weiss, M., F. Jacob, and G. Duveiller. 2020. “Remote Sensing for Agricultural Applications: A Meta-Review.” *Remote Sensing of Environment* 236.

Wellburn, Alan R. 1994. “The Spectral Determination of Chlorophylls a and b, as Well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different

Resolution." *Journal of Plant Physiology* 144(3).

Wen, Zhihui et al. 2022. "Differences in Foliar Phosphorus Fractions, Rather than in Cell-Specific Phosphorus Allocation, Underlie Contrasting Photosynthetic Phosphorus Use Efficiency among Chickpea Genotypes." *Journal of Experimental Botany*.

Westoby, Mark et al. 2004. "The Worldwide Leaf Economics Spectrum." *Nature* 428: 821–827. www.nature.com/nature%0Ahttps://idp.nature.com/authorize/casa?redirect_uri=https://www.nature.com/articles/nature02403&casa_token=CtYdn6u63BcAAAAA:cNAkoHi669d547DoKaM3keUixX15dhKLnmyUD_oX2GBEB1VTe9mtWcMdFoXauJPFpb4iQItw5D0oAvb3.

Wickham, Hadley et al. 2019. "Welcome to the Tidyverse." *Journal of Open Source Software* 4(43).

Wilkinson, Leland. 2011. "Ggplot2: Elegant Graphics for Data Analysis by WICKHAM, H." *Biometrics* 67(2).

Willeit, M., A. Ganopolski, R. Calov, and V. Brovkin. 2019. "Mid-Pleistocene Transition in Glacial Cycles Explained by Declining CO2 and Regolith Removal." *Science Advances* 5(4).

Wu, Jin et al. 2017. "Convergence in Relationships between Leaf Traits, Spectra and Age across Diverse Canopy Environments and Two Contrasting Tropical Forests." *New Phytologist* 214(3).

Wujeska-Klause, Agnieszka, Kristine Y. Crous, Oula Ghannoum, and David S. Ellsworth. 2019. "Lower Photorespiration in Elevated CO 2 Reduces Leaf N Concentrations in Mature Eucalyptus Trees in the Field." *Global Change Biology* 25(4): 1282–95.

Wullschleger, Stan D., T. J. Tschaplinski, and R. J. Norby. 2002. "Plant Water Relations at Elevated CO2 - Implications for Water-Limited Environments." *Plant, Cell and Environment* 25(2).

Xu, Shan, Jordi Sardans, Jinlong Zhang, and Josep Peñuelas. 2020. "Variations in Foliar Carbon:Nitrogen and Nitrogen:Phosphorus Ratios under Global Change: A Meta-Analysis of Experimental Field Studies." *Scientific Reports* 10(1).

Xue, Jinru, and Baofeng Su. 2017. "Significant Remote Sensing Vegetation Indices: A Review of Developments and Applications." *Journal of Sensors* 2017.

Xue, Lihong et al. 2004. "Monitoring Leaf Nitrogen Status in Rice with Canopy Spectral Reflectance." *Agronomy Journal* 96(1).

Yan, Fei, Xiangnan Li, and Fulai Liu. 2017. "ABA Signaling and Stomatal Control in

Tomato Plants Exposure to Progressive Soil Drying under Ambient and Elevated Atmospheric CO2 Concentration.” *Environmental and Experimental Botany* 139.

Yan, Zheng Bing, Shu Wen Liu, and Jin Wu. 2022. “Hyperspectral Remote Sensing of Plant Functional Traits: Monitoring Techniques and Future Advances.” *Chinese Journal of Plant Ecology* 46(10).

Yang, X., P. E. Thornton, D. M. Ricciuto, and W. M. Post. 2014. “The Role of Phosphorus Dynamics in Tropical Forests - A Modeling Study Using CLM-CNP.” *Biogeosciences* 11(6).

Yang, Xi et al. 2016. “Seasonal Variability of Multiple Leaf Traits Captured by Leaf Spectroscopy at Two Temperate Deciduous Forests.” *Remote Sensing of Environment* 179.

You, Chengming et al. 2017. “Grass and Forbs Respond Differently to Nitrogen Addition: A Meta-Analysis of Global Grassland Ecosystems.” *Scientific Reports* 7(1).

Yu, Gui Rui et al. 2000. “A Proposal for Universal Formulas for Estimating Leaf Water Status of Herbaceous and Woody Plants Based on Spectral Reflectance Properties.” *Plant and Soil* 227(1–2).

Zaehle, Sönke et al. 2015. “Nitrogen Availability Reduces CMIP5 Projections of Twenty-First-Century Land Carbon Uptake.” *Journal of Climate* 28(6).

Zarco-Tejada, Pablo J. et al. 2004. “Needle Chlorophyll Content Estimation through Model Inversion Using Hyperspectral Data from Boreal Conifer Forest Canopies.” *Remote Sensing of Environment* 89(2).

Zavaleta, Erika S. et al. 2003. “Additive Effects of Simulated Climate Changes, Elevated CO2, and Nitrogen Deposition on Grassland Diversity.” *Proceedings of the National Academy of Sciences of the United States of America* 100(13).

Zhang, Dan et al. 2009. “Detection of Quantitative Trait Loci for Phosphorus Deficiency Tolerance at Soybean Seedling Stage.” *Euphytica* 167(3).

Zhang, Jingcheng et al. 2019. “Monitoring Plant Diseases and Pests through Remote Sensing Technology: A Review.” *Computers and Electronics in Agriculture* 165.

Zhang, Lin et al. 2011. “Effects of Elevated CO 2 and N Addition on Growth and N 2 Fixation of a Legume Subshrub (Caragana Microphylla Lam.) in Temperate Grassland in China.” *PLoS ONE* 6(10).

Zhang, Yongguang, Mirco Migliavacca, Josep Penuelas, and Weimin Ju. 2021. “Advances in Hyperspectral Remote Sensing of Vegetation Traits and Functions.” *Remote Sensing of Environment* 252.

Zhang, Yongqin, Jing M. Chen, John R. Miller, and Thomas L. Noland. 2008. “Retrieving Chlorophyll Content in Conifer Needles from Hyperspectral Measurements.” *Canadian Journal of Remote Sensing* 34(3).

Zheng, Guowei, Jia Chen, and Weiqi Li. 2020. “Impacts of CO2 Elevation on the Physiology and Seed Quality of Soybean.” *Plant Diversity* 42(1).

Zheng, Zemei et al. 2020. “Differential Effects of N and P Additions on Foliar Stoichiometry between Species and Community Levels in a Subtropical Forest in Eastern China.” *Ecological Indicators* 117.

Zhu, Yan et al. 2008. “Analysis of Common Canopy Vegetation Indices for Indicating Leaf Nitrogen Accumulations in Wheat and Rice.” *International Journal of Applied Earth Observation and Geoinformation* 10(1).