Trait-based methods in (plant) ecology: theory and practice

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Supervised by Professor Andrew Hector (University of Oxford)

This thesis is dedicated to my dear Nonna Anna who never stopped encouraging me to "get that piece of paper [degree]" and my great great-grandma Calie born into slavery who was never given the opportunity to flourish. They planted the seeds so I could blossom. I blossom with the hope young Black (plant) scientists can fully flourish.

Declaration

I declare that this thesis: *Trait-based methods in (plant) ecology: theory and practice* is my own work conducted under the supervision of Professor Andy Hector (University of Oxford). This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Oxford. None of the work included has been submitted, in whole or in part, in any previous application for a degree.

Sara Lil Middleton
October 2024

Abstract

From cells to canopies, plant responses to environmental stressors are not necessarily uniform across biological scales. Trait-based approaches are being increasingly used to help characterise, compare and contextualise key functions and processes of organisms and their responses to environmental change. Despite the popularity of trait-based research, there remain several challenges that are limiting our ability to synthesise trait data across taxa, spatial and temporal scales. This thesis uses a calcareous grassland climate change experiment at Wytham Woods, UK as a case study to assess individual to community scale responses to an imposed drought treatment over medium to short time scales.

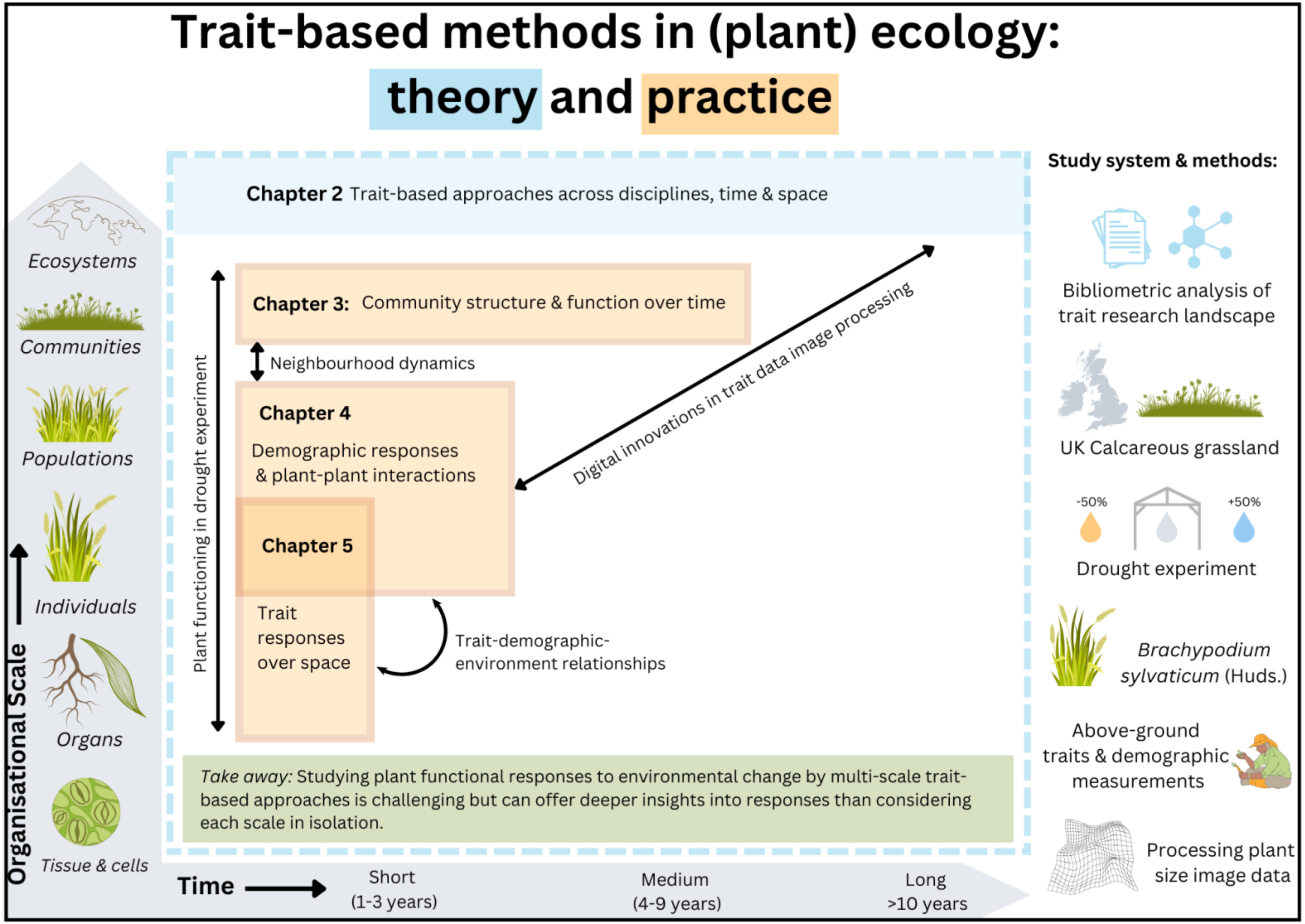
I conducted a bibliometric analysis to examine the trait research landscape across space, time and disciplines **(Chapter 2)**. This assessment revealed trait-based approaches have different conceptual origins and trajectories underlined by how space and time are accounted for, but with areas of overlap in their practical applications in environmental change research. I identified several conceptual and methodological challenges, and propose that by simplifying trait terminology, finding common trait currencies, contextualising trait data and leveraging field training courses, research synthesis and disciplinary links can be strengthened.

Experimental work examined the temporal dynamics of grassland community composition and functioning with altered growing season rainfall (**Chapter 3**). Results indicated diverging trends on community compositional and functional properties to drought treatment. Productivity and temporal stability decreased with drought, whilst species diversity and composition were resistant. There was a non-uniform response in plant functional groups driving reductions in productivity, with graminoid and legume species showing the greatest biomass losses compared to forbs. I discuss how calcareous grassland communities often display lagged reactions to

changes in rainfall patterns, which can lead to underestimating effects, emphasising the importance of long-term vegetation monitoring.

The trait-demographic studies (**Chapter 4** and **5)** focused on the dominant bunch grass false brome (*Brachypodium sylvaticum* Huds.). Results indicated false brome individuals adopt a conservative resource strategy and are drought tolerant. I observed high survival and low reproduction rates, stable above-ground size over three growing seasons, and only minor adjustments to leaf anatomy and leaf rolling behaviour in droughted individuals. There was evidence of delayed drought reactions in plant height and reproductive parameters, which showed signs of recovery. Accounting for neighbourhood plant interactions did not appear to help or hinder individuals across treatments. Examining trait distributions revealed most trait values converged towards the mean, suggesting false brome individuals had adequate trait-environment matching and that this species is more drought tolerant than other graminoids in the community. My experimental work showed that by assessing community-level functions (productivity) with trait-demographic responses of a dominant species, deeper insights into vegetation functional dynamics can be obtained than by considering responses at each organisational scale in isolation. Both at the individual species and community level, this calcareous grassland showed resistance and capacity to recover from drought, although it is unclear if dominant species will continue to compensate for the altered functioning of other community members. By combining a wide range of methodological approaches from bibliometric, experimental to analytical techniques, my thesis has demonstrated the need for interdisciplinary perspectives in trait-based environmental change research.

Graphical abstract



Trait-based methods in (plant) ecology: theory and practice

Chapter 2 Trait-based approaches across disciplines, time & space

Chapter 3: Community structure & function over time

Neighbourhood dynamics

Chapter 4

Demographic responses & plant-plant interactions

Chapter 5

Trait responses over space

Trait-demographic-environment relationships

Plant functioning in drought experiment

Digital innovations in trait data image processing

Take away: Studying plant functional responses to environmental change by multi-scale trait-based approaches is challenging but can offer deeper insights into responses than considering each scale in isolation.

Study system & methods:

Bibliometric analysis of trait research landscape

UK Calcareous grassland

Drought experiment

Brachypodium sylvaticum (Huds.)

Above-ground traits & demographic measurements

Processing plant size image data

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Formatting Statement

This thesis follows an integrated format, where each of the four research chapters have been prepared as manuscripts, with some chapters already published. Contributions from collaborators are listed below under “Author contributions'' for each chapter. The word count of this thesis (not including references, tables of contents, supplementary, acknowledgements, figures, tables and their captions) is 48,327.

Author Contributions

The contribution of other authors to any of the manuscripts is set out below.

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Contributions: Study was established by KM, CSL and AH. PI support and resources were provided by AH, RSG, KM, NH, and CSL. Data was collected primarily by CSL and AH, with contributions to data collection from all authors. JJ performed data analysis and exploration, with support from SLM, AH and RSG. JJ wrote the initial draft with support from SLM, AH and RSG. Critical manuscript feedback and approval was provided by all authors.

<u>Chapter 4</u>
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<u>Chapter 5</u>
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<u>Chapter 6</u>
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COVID-19 Impact and Mitigating Circumstances Statement

The Covid-19 pandemic has had a significant direct and indirect impact on the completion of this thesis. Three field seasons (2020, 2021 and 2022) were impacted by Covid-19, which influenced data collection and processing in **Chapters 4** and **5**. In data **Chapter 5**, a smaller trait dataset was obtained than planned partly due to the shortening of the field data collection window in June-July 2022, as I contracted Covid-19. Changes in the use of lab and greenhouse spaces I required to process demographic and trait data (**Chapters 4** and **5**) caused significant disruption. At each field season, I had to set up and calibrate equipment in a new space, which caused significant delays in digitising demographic data (**Chapter 4**). The extra adjustments to the digiting processes was not anticipated, however this resulted in a short publication which documented this process. Trait field data and lab processing was split across three sites/labs (instead of the two) which was more time and labour-intensive than anticipated contributing to the smaller sample size of the trait dataset (**Chapter 5**).

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This thesis has been the fruits of my labour made possible by the community of people rooting for me through sunshine and showers of this DPhil journey. Without their support and funding from the Natural Environment Research Council, this thesis would not have been possible.

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Chapter 1: General introduction

Sara L Middleton

(Image: Sara L Middleton)

1.1 Environmental change and the biosphere

Global environmental change is altering key processes and functioning of the biosphere, which has not been recorded in recent geological timescales (IPCC, 2013; Malhi *et al.*, 2020; Peñuelas and Nogué, 2023). The increasing levels of atmospheric carbon dioxide, rearrangement of the planet's land surface for human agriculture and settlement and the overharvesting of species are affecting the integrity of both marine and terrestrial biological systems (Malhi *et al.*, 2020). Future climate projections for the end of the 21st century indicate further destabilisation of the biosphere due to more frequent and intense heatwaves, wildfires, droughts and flooding (IPCC, 2013).

The disruption to the hydro-climatic system is one of the major environmental stressors for terrestrial vegetation (Seleiman *et al.*, 2021). In many areas globally, the amount, frequency and duration of precipitation events is predicted to change (Dai, 2013). Indeed, precipitation extremes are already being experienced, as evidenced by the 2003 European drought (Ciais *et al.*, 2005), and the excess monsoonal rainfall in Pakistan in 2022 (Otto *et al.*, 2023). These rainfall anomalies are manifested via increased variability of precipitation between years, more extreme wet and dry years and changes to amount of annual precipitation (IPCC, 2013; Knapp *et al.*, 2017). As water comprises 80-95% of fresh plant biomass, extreme changes to water availability can cause alterations to the functioning, structure, and productivity of vegetation from individual to ecosystem levels (Figure 1.1) (Seleiman *et al.*, 2021; Li *et al.*, 2023). Changes in vegetation functioning directly feed into the carbon and water cycles (e.g. via shifts in transpiration) that mediate the climate, which in turn plants respond to, creating a feedback loop (Li *et al.*, 2023).

1.2 Multidimensional effects of drought on vegetation

Drought, defined here from the ecological plant perspective, is a deficit in water input relative to normal levels or input relative to plant needs, increasing stress and mortality risk in natural plant, agricultural and silvicultural systems (Swann, 2018; Büntgen *et al.*, 2021; Carroll *et al.*, 2021). Characterising droughts is challenging, as defining them depends on which part of the Earth system (e.g., meteorological, hydrological, ecological, or agricultural) is considered and therefore the type of impacts (individual-to-ecosystem level) that are measured or observed (Wilhite *et al.,* 2007; Vicente-Serrano *et al.*, 2022). In addition to a reduction in the amount of precipitation, there are three other dimensions of drought that modulate plant responses: timing, duration, and preceding conditions (Figure 1.1) (Carroll *et al.*, 2021).

Climate change is predicted to widen the drought window, shifting away from drought events historically occurring during the peak growing period such as July in the US Great Plains (Felton and Goldsmith, 2023). The sensitivity of vegetation to this timing shift depends also on the plant water use efficiency strategy (Chandregowda *et al.*, 2022). For example, C_3 grasses gain the majority of biomass during the cooler months and therefore may be more vulnerable to droughts occurring earlier on in the growing season (Felton and Goldsmith, 2023). The length of a drought event influences the magnitude of ecosystem-level responses, as shown experimentally by Carroll *et al.* (2021) in their comparisons of pulse versus press drought disturbances on ecosystem productivity in a US mesic grassland. Whilst above-ground net primary productivity was reduced in both drought durations compared to ambient conditions, there was a significantly larger reduction in acute drought conditions (Carroll *et al.*, 2021). Plant community reactions to drought in a given year are also affected by previous precipitation conditions resulting in both lagged and cumulative responses (Zhao *et al.*, 2020).

The observed macro-level vegetation responses to drought are linked to reactions to the individual-level physiological and biochemical processes, which induce morphological changes. Typical drought symptoms include increased stomatal closure to reduce water loss at a cost of reduced gas exchange, leading to sub-optimal photosynthesis rates (Seleiman *et al.*, 2021). Persistent drought stress can result in a plant's inability to meet their metabolic needs due to the coupled mechanisms of hydraulic failure and carbon starvation, which can be lethal (Swann, 2018). Plant growth and development are dependent on cell division and differentiation which relies on optimally functioning plant apparatus which becomes hindered under drought stress. Morphological responses to drought include a reduction in size and number of leaves and in cases of severe drought, premature leaf senescence (Seleiman *et al.*, 2021). Individual plant species display a range of strategies in response to drought that have arisen due to natural selection allowing plants to cope in water-stressed environments (Laughlin, 2023).

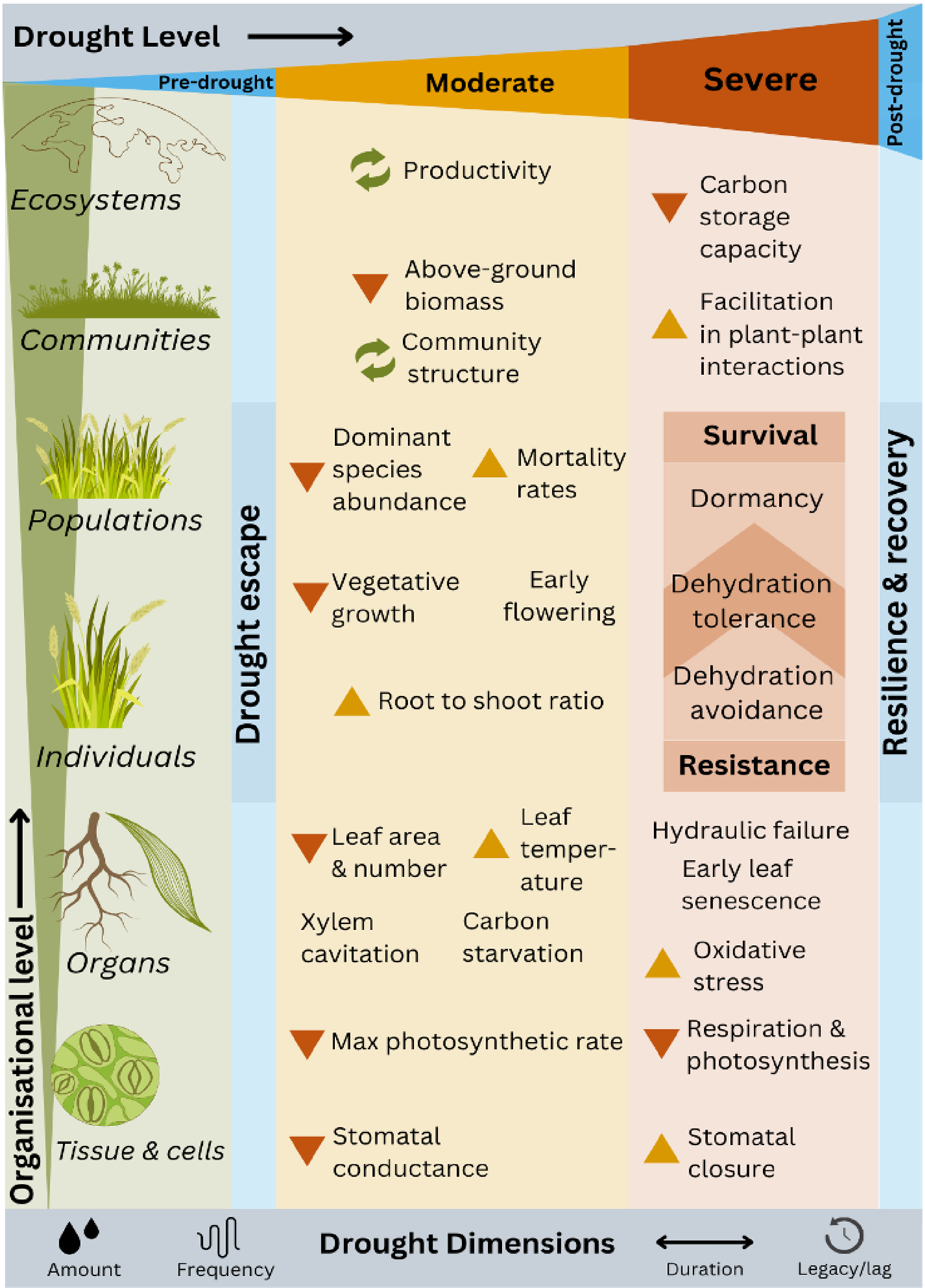


Figure 1.1 A Qualitative overview of the hierarchical plant responses to the different dimensions of drought (McDowell *et al.*, 2008; Seleiman *et al.*, 2021; Felton and Goldsmith, 2023). Key plant strategies are also listed according to drought levels: pre-drought: drought escape; severe drought: resistance, dehydration avoidance, dehydration tolerance, dormancy and survival and post drought: resilience and recovery (Lüscher *et al.,* 2020).

1.3 Plant strategies related to drought

Plants have three main drought response strategies: i) escape, ii) avoidance, and ii) tolerance (Figure 1.1) (Ilyas *et al.*, 2021). Drought escape is where plants (usually annuals) adjust their life cycle to avoid drought effects (e.g. grow, mature, flower and set seed during a wetter season) (Laughlin, 2023). Avoidance and tolerance strategies relate to the ability to regulate dehydration stress. Drought avoiders are plants that can continue physiological functioning either via minimising water loss or maximising water uptake during drought periods. With a tolerance strategy, plants are able to resist dehydration by making physiological adjustments (Ilyas *et al.*, 2021). Within the tolerance strategy, plants can show dormancy - the internally controlled process of severely reducing or stopping growth to enhance survival, particularly in extreme drought conditions (Volaire, 2018). These strategies are thought to be linked to the presence (or absence) of traits which determine the persistence, decline or even extinction of a population within a particular environment (Laughlin, 2023). As such, trait-based approaches have been increasingly used to assess plant species responses to environmental stressors (Shipley *et al.*, 2016).

1.4 Using trait-based methods in era of environmental change

1.4.1 Why use traits?

A current grand challenge is our ability to quantify and predict how biodiversity will respond to environmental change (Enquist *et al.*, 2015). In trying to address this grand challenge, ecologists are increasingly turning towards trait-based methods (Shipley *et al.,* 2016; Figure 1.2). In broad terms, traits are measurable properties of biological entities (typically individual organisms), which can be used to understand how they function and interact with the abiotic and biotic environment (Gallagher *et al.*, 2020; Volaire *et al.,* 2020). The benefits of trait-based approaches in environmental change studies are threefold: i) quantifying mechanisms, ii) generality and iii) prediction. Firstly, traits enable measurable characteristics of organisms to be mechanistically linked to their responses to abiotic and biotic components of their environment (Gallagher *et al.*, 2020). Secondly, linking the organism-environment relationship allows for generality, as these measurements can be compared over wider geographical contexts and independent from taxonomic or phylogenetic affiliation (Verbeeck *et al.*, 2014). Thirdly, with increased generality, traits can be used for their predictive capacity, such as predicting community assembly or nutrient cycling under a certain climate change scenario (Shipley *et al.*, 2016). These combined benefits have also translated to trait theory being used in applied contexts in conservation and restoration ecology (Gallagher *et al.*, 2021).

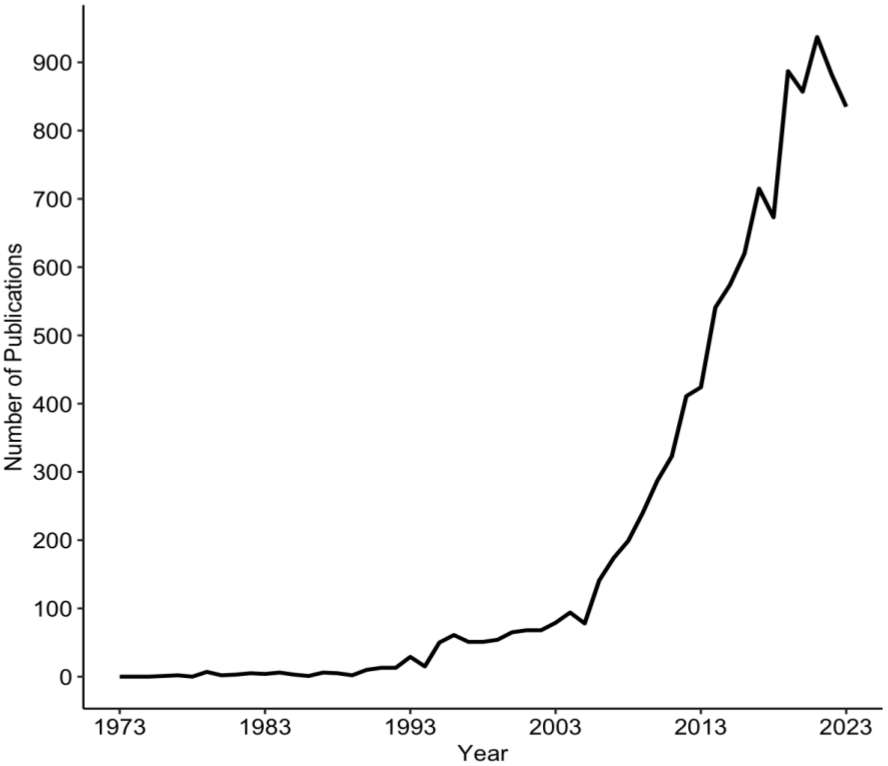


Figure 1.2 Trends in the number of publications within ecology adopting trait-based approaches in environmental change research between 1973 - 2023. Data obtained from Web of Science Core collection with the search terms: “traits” AND “climate change” OR “environmental change” accessed on 13/09/2024.

The concept of traits is not new, as through the scientific ages, natural philosophers such as Theophrastus and Darwin have used traits as a qualitative means to categorise the natural world (Nock *et al.,* 2016). A lot of our trait-based thinking has come from work in plants: In the 19th century, Humboldt examined physical plant characteristics to group plants into different forms such as lianas, ferns and gymnosperms and linked the diversity of plant forms to temperature (Heilmeier, 2019). This work on trait-environment linkages laid the foundations for functional classifications of plants such as Raunkiaer's life forms (Raunkiaer, 1934), which influenced the Box life form system and Grime's CSR (competitive, stress tolerant and ruderal) scheme (Grime, 1979; Box, 1981).

By focusing on basic plant units – meristems from which plants grow, Raunkiaer was able to classify vascular plants into five general life forms based on the location of buds during inactive periods (e.g. winter): phanerophytes, chamaephytes, hemicryptophytes, cryptophytes and therophytes. Phanerophytes (typically trees) have buds high above the ground, whilst chamaephytes (e.g. shrubs) have meristems closer to the ground. Buds are more protected in hemicryptophytes (e.g. herbaceous plants) and cryptophytes such as rhizomatous grasses or tuberous plants, as regrowth occurs in meristems at just above and at or below the soil/water surface respectively. Therophytes, instead are annual plants surviving as seeds during harsh environmental conditions (Raunkiaer, 1934). With additional data, Raunkiaer showed that the distribution of plant life-forms was non-random with respect to climate, as demonstrated by the large representation of hemicryptophytes over phanerophytes in artic environments (Keddy and Laughlin, 2021). His classification system, based on a single easy-to-measure trait - meristem location, has made it widely applicable across vegetation types. It has been used to study relationships between life forms and pace of life (Salguero-Gómez *et al.*, 2016), compare climate and land use as drivers in the distribution and abundance of life forms (McKeon *et al.*, 2023) and even as proxies for persistence strategies in island systems (Conti *et al.*, 2022).

1.4.2 The foundations for more integrative and comparative trait-based research

Building on work from Raunkiaer and MacArthur and Wilson's (1967) on r-K strategies, Grime's CSR classification scheme further integrated theory and practice in plant functional ecology, via the creation of an ordination framework that functionally grouped individual species in response to threats to plant existence (Grime, 1977). Species are classified based on how plants cope with competition, environmental stress and frequent disturbance. Competitors (C) are plants that occupy relatively undisturbed and resource-rich habitats by rapidly maximising resource uptake via acquisitive traits (high relative growth rate). Stress-tolerators (S) are found in undisturbed and resource-poor habitats where plants are characterised by conservative resource use, long longevity of organs and lifespans. Ruderals (R) reflect r-strategists, with short lifespans and high investment in reproduction found in low-stress disturbed environments (Grime, 1977). The three primary plant strategies can be mapped onto a ternary plot, where each axis represents one of the C, S and R strategies. In addition, species can take on intermediate forms such as stress-competitors (SC) (Grime *et al.,* 1988*)*.

The conceptual CSR framework was formalised quantitatively with the large-scale integrated trait screening programme on common British flora (Grime *et al.,* 1988). Traits such as germination and relative growth rate were measured in a systematic way on a large number of species under experimentally controlled conditions (Grime and Hunt, 1975; Grime *et al.*, 1981). It was revolutionary for its time. Collecting continuous trait measurements on tens of species in a standardised way enabled the assessment of fundamental trade-offs between traits, identification of plant strategies and inter-species comparisons (Keddy, 1992). This work by Grime and colleagues was hugely influential for the field of plant trait ecology and set a precedent for standardising trait measurements in field and laboratory settings beyond plants. A key outcome of the trait screening programme was the validation of resource investment trade-offs (resource acquisitive vs conservative strategy in productive vs unproductive habitats respectively) based on the measurement of 67 traits on 43 species (Grime *et al.*, 1997). Traits that assembled on this main axis of variation related to leaf morphology, longevity, growth rate and leaf litter decomposition rate. This particular work was a precursor to the widely used leaf economics spectrum (Wright *et al.,* 2004) and steered the ecological research agenda towards resource-use economics (Fridley and Pierce, 2021). Grime's plant strategy scheme and screening programme expanded the trait research landscape leaving a long legacy in community and functional ecology. Despite Grime's empirical work drawing on flora from Northen England, his methods have been tested and adapted to various ecological settings (e.g. the development of the StrateFy tool to calculate CSR strategies across different biomes (Pierce *et al.*, 2017)).

The 1990's and early 2000's saw a move towards more integrative and quantitative methods to quantify mechanisms governing plant form and function, with a hope to predict responses from environmental change (Diaz *et al.,* 1998). The last two decades have seen a rapid increase in adoption of trait-based approaches, as shown in Figure 1.2 with the steep rise in publications from around 2004. Since then, the field has seen developments in the release of measurement protocols (Pérez-Harguindeguy *et al.*, 2013), and the construction of trait databases such as TRY (Kattge *et al.*, 2011). These advancements have enabled leaf trait diversity to be condensed onto a single axis of variation - leaf economics spectrum (Wright *et al.*, 2004), to scaling organisational levels to link individual-level traits from genetic (Ramírez-Valiente and Robledo-Arnuncio, 2014), to ecosystem level processes (Sakschewski *et al.*, 2015) and use traits to predict community-ecosystem dynamics under environmental change (Díaz *et al.*, 2013; Gross *et al.*, 2017). There is increasing awareness in accounting for the dynamic nature of traits and trait-trait relationships (Messier *et al.,* 2010). Analysing trait variation across space and time can inform us of processes driving organ level trade-offs, phenotypic responses to environmental stressors, community assembly, and ecosystem functioning (Enquist *et al.*, 2015)

In the last decade, trait-based research has radiated to include a wider range of taxa such as terrestrial invertebrates (Moretti *et al.*, 2017; Wong *et al.,* 2019) and fungi and microbiota (Chagnon *et al.*, 2013; Rath *et al.,* 2019). Trait-based methods show great utility and promise in helping us characterise, compare and contextualise key functions and processes of organisms and their responses to environmental change. Despite significant conceptual and methodological advances in trait-based research, particularly with respect to plants, there remains fragmented progress and low conceptual and data integration across taxa, geography and disciplines (Schleuning *et al.,* 2023).

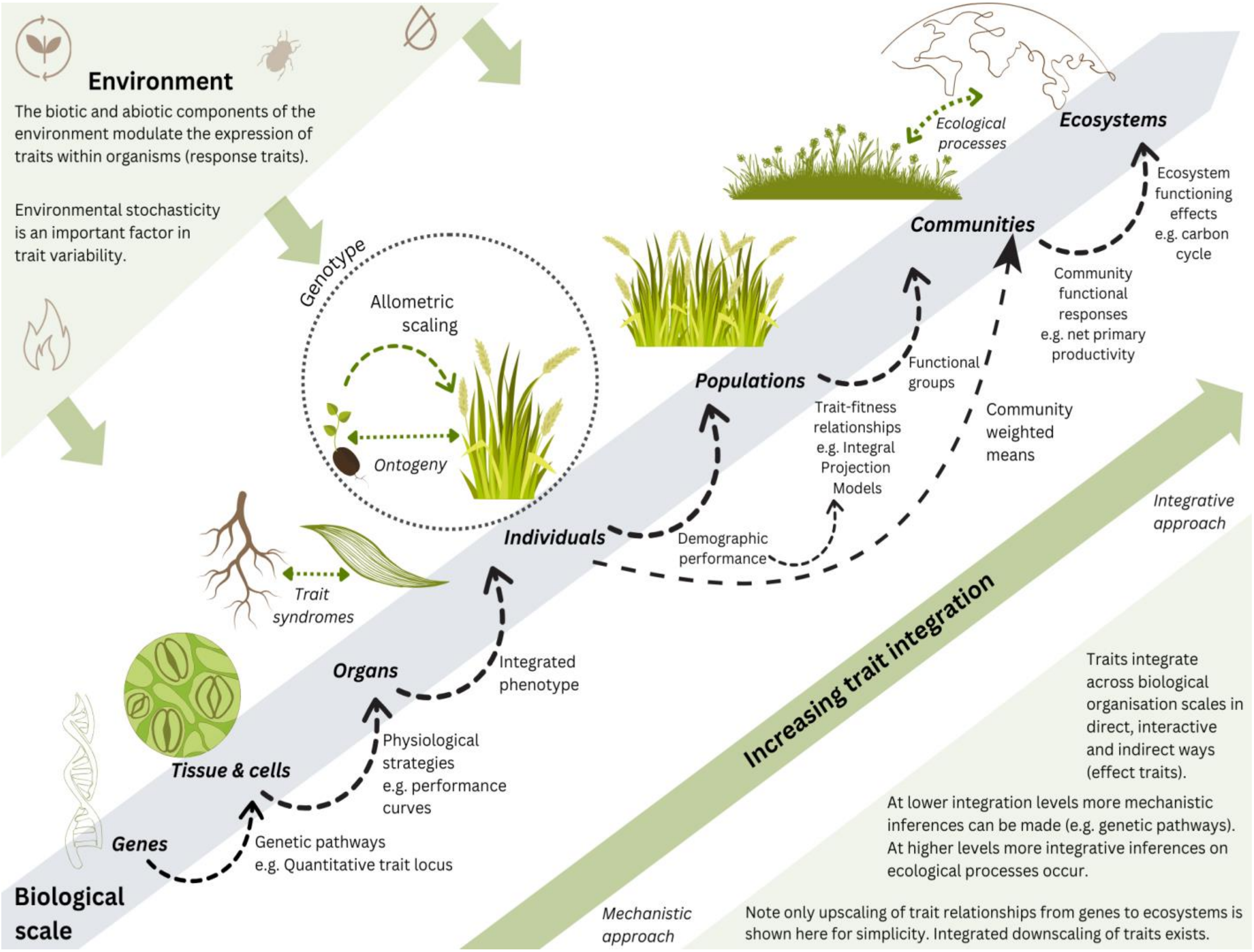
1.5 Challenges in trait-based research: from scales to semantics

Despite the widespread popularity of traits, there remains several challenges hindering the collective advancement of the field. As often occurs in rapidly expanding research areas, addressing assumptions (e.g. assumed harmonisation of terminology), acknowledging conceptual histories or moving beyond solving the “low hanging fruit” issues has been neglected (Shipley *et al.*, 2016). A reason why these issues are harder to solve is due to trait-based research adopting a broad mode of study defined by the way biological systems or processes are studied rather than by organisational or geographical scale (Shipley *et al.*, 2016; Volaire *et al.,* 2020). This broad scope has meant trait-based approaches have transcended the disciplinary divide, and are widely used in the fields of ecology, evolutionary biology and demography. Across these disciplines, a vast array of tools, concepts and datasets have been generated in recent decades, encompassing a range of trait types and biological scales. This wide scope has enhanced our understanding but also posed challenges in synthesising results across the trait research landscape.

Figure 1.3 illustrates the complex ways traits integrate along scales of biological organisation and how research approaches differ. From genes to ecosystems, traits interact in direct, interactive and indirect ways (Fontana *et al.,* 2021). Genes link to cells and tissues via metabolic pathways which then coordinate organ-level responses. At the level of the individual, the performance of phenotypes influences population demographic dynamics via a genotype and environment interaction. At the community level, organisms with similar responses can be grouped into functional groups or types which can then help to inform ecological processes at the community and ecosystem scales (e.g. community functioning and composition and nutrient cycling) (Violle *et al.*, 2007; Fontana *et al.*, 2021).

Referred to as “effect” traits, these individual trait signatures then propagate to higher levels of organisation influencing broader community and ecosystem processes such as trophic interactions, species composition, pollination, mutualisms and nutrient cycling (Lavorel and Garnier, 2002). In turn, biotic and abiotic components of the environment such as herbivory and water deficits modulate the expression and variability of traits (“response” traits) to create trait syndromes (Lavorel and Garnier, 2002). Although not depicted in Figure 1.3 for simplicity, it is important to note that the route from gene to ecosystem processes can be more direct and with high predictive power (Schweitzer *et al.*, 2004). Also noteworthy is the existence of “downscaling” of trait responses from higher integrated biological scales to lower levels (see commentary by Pelletier *et al.* (2009)). As such, each organisational scale requires different analytical approaches (see right corner of Figure 1.3).

Much of the focus on the scale of trait selection has been on the level of the individual multicellular organisms (Violle *et al.,* 2007). This has brought up some conceptual and practical challenges for integrating trait-based research on organisms where individuality is hard to define (e.g. eusocial insects, species in mutualistic relationships, unicellular organisms) (Dawson *et al.,* 2021). There are ongoing debates about what counts as an individual in biology, such as the discussion on corals as whether they are individuals or communities (Skillings, 2016).



The biotic and abiotic components of the environment modulate the expression of traits within organisms (response traits).

Environmental stochasticity is an important factor in trait variability.

Traits integrate across biological organisation scales in direct, interactive and indirect ways (effect traits).

At lower integration levels more mechanistic inferences can be made (e.g. genetic pathways). At higher levels more integrative inferences on ecological processes occur.

Note only upscaling of trait relationships from genes to ecosystems is shown here for simplicity. Integrated downscaling of traits exists.

Figure 1.3 A schematic showing how traits integrate across scales of biological organisation.

Even in plants, where much of the conceptual developments in traits have taken place, defining individuality is challenging given clonality (Violle *et al.,* 2007). Plants exhibit modularity, meaning their vegetative and reproductive units have a degree of autonomy. Clonal plants grow vegetatively by sending out extensions of themselves in the from multicellular runners (ramets). A collection of ramets that developed from a single seed are classed as genets (i.e. are genetically identical) (Harper, 1977; Clarke, 2012). Genets can become disconnected, appearing as separate individual plants with different lifespans, despite being genetically identical. Thus, analytical and practical difficulties can arise when trying to measure trait responses on individuals: what is the size of an individual? Ramet or genet - which is a better predictor of fitness when deciding which “type” of individual to measure? How feasible is it to account for both genets and ramets? These questions are particularly pertinent to field-based plant demographers interested in quantifying fitness but also how responses of these clonal traits integrate to higher levels of biological organisation (Bittebiere *et al.,* 2020). There is yet a consensus on ways to reconcile evolutionary view of individuality with practical field measurements (although see in depth review by Clarke (2012) on plant individuality from a demographer’s perspective).

When it comes to trait terminology, within and across disciplines, there exists a plethora of adjectives to describe traits such as “phenotypic”, “functional” and “life history” traits. These terms are often used interchangeably, implying not all traits have equal meaning. There have been attempts in plant ecology to reconcile differences in traits definitions such as Violle *et al.* (2007) who proposed that functional traits be defined as measurable characteristics of individuals which impact fitness. More recently, Volaire *et al.* (2020) argue for a broader definition where traits can be measured at any biological scale to widen applicability to other taxa such as microbes. Whilst their work has provided some clarity for plant trait ecologists, a broader exploration of trait terminology spanning ecology and evolutionary disciplines is lacking. Ambiguity around trait terminology has downstream effects along the research pipeline which presents additional empirical and analytical challenges. These challenges relate to which traits do we select in our study? How do we go about obtaining trait values? What scale of trait measurement is appropriate? By continuing to bypass these key issues it will likely result in trait-based research not yielding on the promises of versatility, scalability and prediction thereby limiting research synthesis and synergy across disciplines. In this thesis I adopt the trait definition by Dawson *et al.* (2021):

"*A trait is a measurable characteristic (morphological, phenological, physiological, behavioural, or cultural) of an individual organism that is measured at either the individual or other relevant level of organization*". This thesis aims to help stitch together some of the disparate pieces to add to the rich tapestry that is trait-based research by studying traits in a UK grassland climate change experiment as a case study (see Figure 1.4).

1.6 The study system

1.6.1 Grasslands, the unravelling of green carpets with climate change

Grasslands cover ~40% of the Earth's land surface and provide us with significant ecological, economic, and cultural value (Gibson, 2009). Grasslands exist across a wide climatic range including steppe, tropical savannahs, alpine and temperate grasslands (Lüscher *et al.*, 2022). In regions, with a woody climax community, such as temperate areas, conditions are maintained with grazing or mowing regimes (Lüscher *et al.*, 2022). Natural and managed grasslands play a key role in carbon budgets within the broad Earth-system processes (Chang *et al.*, 2021) and are estimated to hold 30% of the global below-ground carbon store (Scurlock and Hall, 1998). Grasslands are also sources of great biodiversity hosting many charismatic megafauna, endemic plants and elusive soil microbial communities (Wilsey, 2018; Middleton, 2022). In addition, grasslands provide important direct and indirect benefits (ecosystem services) to people such as pollination and food provisions (Wilsey, 2018). Given the pace of environmental change, there are concerns about the degradation of grassland ecosystems (Bardgett *et al.*, 2021). Despite their importance, assessing their impacts from environmental change, especially droughts are often overlooked (Parr *et al.*, 2014). Grasslands are particularly sensitive to drought compared to forested ecosystems due to lower water use efficiency and the reduced ability to exploit deeper pockets of soil water owing to shallower rooting depths (Felton and Goldsmith, 2023; Kowalski *et al.,* 2023). Drought tends to reduce primary productivity, measured through biomass (Craine *et al.*, 2012; Smith *et al.*, 2024). Grassland community responses can vary with geography (e.g. xeric grasslands generally being more drought sensitive than mesic ones (Knapp *et al.*, 2017; Griffin-Nolan *et al.*, 2019), successional stage (Grime *et al.*, 2000), or functional group (Bollig and Feller, 2014; Wellstein *et al.*, 2017). Grassland drought research has tended to focus on dryland areas, understandably so given they are most sensitive to changes in water availability (Knapp *et al.*, 2017; Yue *et al.*, 2019). Quantifying drought impacts on grasslands in regions such as northwest Europe have been vastly understudied, which is worrisome given their high agricultural, conservation and cultural value *(*Grime *et al.,* 2000; Pellaton *et al.,* 2022). In addition, in the last decade, Europe has experienced the most severe summer droughts of the last two millennia (Büntgen *et al.*, 2021).

Of particular concern are calcareous grasslands, which are host to some of the most species-rich plant communities in Europe with up to 70 plant species per m^2 (Bobbink and Willems, 1987). Calcareous grasslands are semi-natural habitats characterised by shallow, nutrient-poor, well-drained basic soils on a limestone or chalk bedrock which originated circa seven millennia ago with the widespread forest clearance (WallisDeVries *et al.,* 2002). Calcareous grasslands are endangered and in addition to climate change threats, have faced rapid declines due to land use change in the last century (e.g. agricultural expansion, changes in management, eutrophication and afforestation) (WallisDeVries *et al.,* 2002; Diekmann *et al.*, 2014). In Britain, the number of calcareous grassland sites decreased 39% between 1960 and 2013 (Ridding *et al.,* 2015). Studies on calcareous grassland response and resilience strategies to altered precipitation patterns have been limited due to the complexity of these systems across space and time (although see section 1.6.3). Generally, it is hypothesised that the high plant diversity found in calcareous grassland communities should be able to maintain community functioning under drought from their higher functional diversity (Craine *et al.*, 2012). But this is dependent on the proportion of grassland community members with drought tolerance strategies, where too few could lead to shifts in community structure and declines in functioning (e.g. lower productivity, Craine *et al.*, 2012). Studies have shown these calcareous grasslands to be drought resistant (Grime *et al.*, 2008), whilst others have shown these grasslands to be vulnerable, particularly with respect to long term seedbank dynamics (Basto *et al.*, 2018), or even as potentially benefitting by the suppression of dominant species (Bennie *et al.*, 2006). To unpack the mechanisms for these varied drought responses in calcareous grasslands to altered precipitation regimes, further long-term monitoring across organisational scales via experimental approaches are needed (Felton and Smith, 2017).

1.6.2 Climate change experiments

In recognising the rise in climatic extremes there has been a growth in experimental studies aimed at disentangling the complexity of responses, resistance, and resilience of plants to these extremes (Smith, 2011; Hoover *et al.,* 2014). Whilst these studies have generated useful data and insights, many of these experiments use unique approaches, with findings largely applicable at the local scale or taxonomic group (Borer *et al.*, 2014). This creates challenges in interpreting whether the locus of response is methodological or a study system property, especially given the pressing need to understand whole-earth processes (Smith, 2011; Knapp *et al.*, 2017). To address this context dependency, the value of coordinated multi-site and longer-term experiments such as the International Tundra Experiment (ITEX – www.gvsu.edu/itex/), Disturbance and Resources Across Global Grasslands (DRAGNet – www.dragnetglobal.weebly.com), Nutrient Network (NutNet - https://nutnet.org/), and Drought Network (DroughtNet – www.droughtnet.weebly.com) are increasingly being recognised (Knapp *et al.*, 2017). These distributed, co-ordinated network experiments share standardised set of protocols and baseline measurements enabling quantitative synthesis across sites (Smith *et al.*, 2024). These macro-scale insights into drought responses can then be used to integrate responses at lower scales of organisation. Ecosystem responses might be undetectable to environmental stressor(s), but manifest at individual or population levels which may propagate over longer time scales (Felton and Smith, 2017). Therefore, examining the interwoven drought responses with respect to organisational scale will help build a more coherent picture of the structure and functioning of grasslands.

1.6.3 UK grassland climate change experiments

The UK has a rich history in grassland ecological research, with the oldest ecological experiment in the world, Park Grass Experiment in Rothamsted in Hertfordshire running since 1856 (Silvertown *et al.*, 2006). The experiment was originally designed to address a key agricultural question of the time: namely what are the impacts of fertiliser addition on yields? In the decades since, research interests have shifted, and the experiment's longevity provided unique insights into plant population dynamics not observed over shorter time scales (Silvertown *et al.*, 2002) and demonstrated the scientific value of long-term experiments.

The 1980's saw increasing recognition of the scale and impact of climate change on ecological systems in academic and policy circles. Climate and dynamic vegetation models were emerging tools to study these impacts which required ground truthing with experimental field data (Cummins *et al.*, 1995). In response, the Terrestrial Initiative in Global Environmental Research (TIGER) programme was launched in the early 1990's which provided funding for a consortium of environmental projects (Cummins *et al.,* 1995). The Upper Seeds climate change experiment at the Wytham Estate (Oxfordshire, UK) was created as a "flagship" site to examine increasing winter temperatures and summer precipitation patterns and their interactions on belowground processes, vegetation dynamics and invertebrates (Morecroft and Taylor, 2011). Supplemental watering in summer had a positive effect on plant community response measured by vegetation cover and species richness, whilst a combination of winter warming and reduced summer rainfall led to significant reductions in cover and richness (Sternberg *et al.*, 1999). Plant litter instead showed apposing trends to vegetation cover with treatments. Key traits relating to root architecture (e.g. rooting depth) and bud position (sensu Raunkiaer (1934)), of dominant species influenced community responses to treatments. Overall, the experimental drought showed forbs, deep-rooted species and shorter-lived plants to be more tolerant of reduced summer rainfall (Sternberg *et al.*, 1999; Morecroft *et al.*, 2004). Vegetation responses to drought were partially compensated during wetter autumn and winter periods, highlighting the importance for accounting for weather conditions outside the peak growing season. This was especially the case for when belowground nutrient processes were considered (Jamieson *et al.*, 1998). Drought produced lag effects in soil N mineralisation rates, where summer drought periods of inhibited N mineralisation were followed by an increase during the autumn and winter. The seasonality of soil water availability impacted belowground microbial dynamics via the nutrient inputs of plant litter aboveground and in situ microbial lifecycles (Jamieson *et al.,* 1998). Drought impacts were not isolated to vegetation and soil microbial communities. Complex plant-invertebrate interactions and responses were found (e.g. Masters *et al.*, 1998) and these studies represented the bulk of research at the Upper Seeds climate change experiment (Morecroft and Taylor, 2011).

In parallel to the Upper Seeds climate change experiment, another summer drought and winter warming experiment was launched in Buxton (Derbyshire, UK) (Grime *et al.,* 2000). This experiment presented an interesting comparison of the two calcareous grassland sites with different management history, successional stage and topography, which will be revisited in the subsequent experimental chapters (Grime *et al.*, 2000; Askew *et al.*, 2011). The Buxton climate change experiment has entered its third decade of operation and is currently the longest running climate change experiment in the UK. Key findings to date show this grassland to have compositional stability at the community level, dominated by stress-tolerant species (Grime *et al.,* 2000, 2008). Community stability at the m^2 scale was masked by restructuring of species at fine-spatial scales (cm^2) in relation to variation in soil conditions. Changes in edaphic conditions like soil depth modulated treatment responses, whereby shallower microsites saw a decline in productive species (e.g. *Anthoxanthum odoratum*) with both warming and drought treatment compared to areas with deeper soil profiles (Fridley *et al.*, 2011). From a functional perspective, the treatments revealed gradual vegetation shifts. Winter warming promoted traits linked to faster resource acquisition and taller stature, whereas summer drought was associated with leaf-tissue investment traits (Fridley *et al.*, 2016). More recently, genetic studies at the site indicate some species may show potential to resist climate change effects (Ravenscroft *et al.,* 2014; Trinder *et al.,* 2020). Earlier climate change experiments at Upper Seeds, together with the sibling experiment at Buxton provide important historical perspectives from which the experimental chapters of this thesis draw on. They also highlight the need to examine responses of vegetation composition and functioning across spatial and temporal scales.

1.6.4 The RainDrop Drought Experiment and Study Species

The study system forming **Chapter's 4** and **5** of this thesis consists of the grass species false brome (*Brachypodium sylvaticum* Huds.) in an experimental drought treatment on a calcareous grassland at Upper Seeds, Wytham Woods, UK (Figure 1.4). Wytham Woods is an estate comprising about 400-hectare mosaic of mixed deciduous woodland and semi-natural grassland (Gibson and Kirby, 2011). Upper Seeds sits on a hill (160 m a.s.l) formed of Corallian Limestone, overlain with well-drained calcareous soils (Taylor *et al.,* 2011).

Information on the site usage and management prior to the 1940s is unknown, but between c. 1940-1980 it was ploughed and used for arable crops. Since the early 1980s the site was in recovery, naturally colonised with grassland and woodland-edge plant species maintained with sheep grazing. As outlined in section 1.6.2, during the site recovery phase the Upper Seeds climate change experiment, the precursor to RainDrop, was set up between 1993 and ran for 5 years (Morecroft *et al.,* 2004). In 2016, the RainDrop experiment was set up to examine how manipulating rainfall affects grassland community functioning and resilience. The experimental setup follows a replicated randomised block design where each of the five blocks contain eight 2.5 x 2.5 m plots with four different treatments: 1) irrigated with 50% more rainfall, 2) droughted with 50% less rainfall and two controls: 3) procedural and 4) ambient. Whilst two grass species *Brachypodium pinnatum* and *B. sylvaticum* dominate, the site is species rich, with over 100 graminoid, forb, leguminous forb and woody species. The site is managed with two cuts with above-ground biomass removal in mid-July and end of September. Biannual censuses in June and September since 2016 of above ground biomass and vascular plant species cover (in June) are carried out (Jackson *et al.*, 2024).

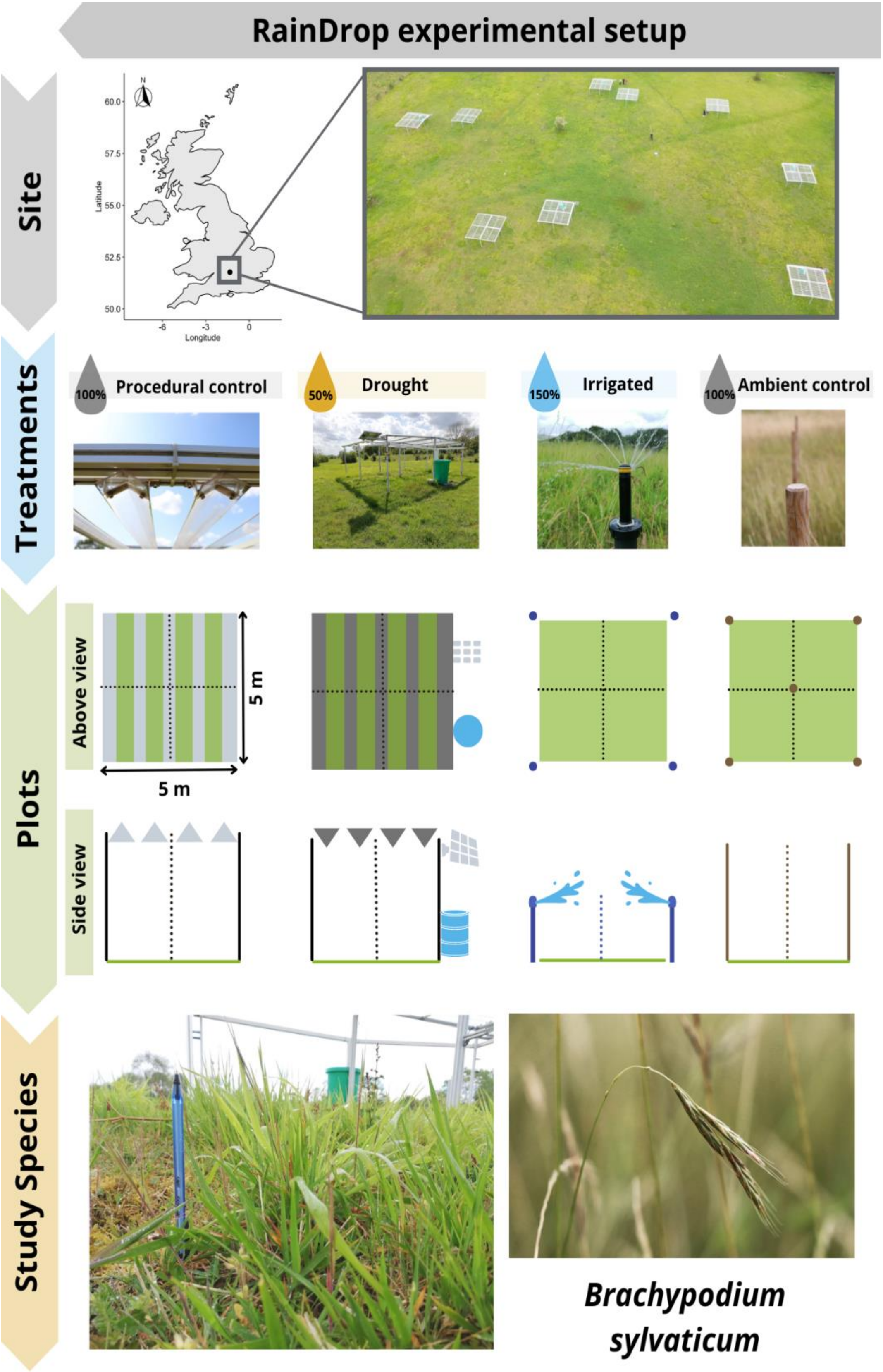


Figure 1.4 Overview of the study system at the RainDrop site at Upper Seeds, Wytham Woods, UK showing experimental set up of precipitation treatments and the study species *Brachypodium sylvaticum*.

Brachypodium sylvaticum is a bunch-forming perennial grass species native to Eurasia that is self-compatible, and wind pollinated (Stace, 2019; Monroe *et al.,* 2021). In the UK, it grows up to 1 m in height on woodland edges and scrub, favouring well-drained calcareous-neutral soils (Streeter *et al.,* 2009; Stace, 2019). This species is considered an “invasive” species in North America and is found in comparable habitats to those in its native range (Rosenthal *et al.,* 2008). The seedbank is short-lived, typically no more than one year (Davies and Waite, 1998) and individuals start out from seed usually near the parent plant, but short-distance seed dispersal can occur, typically with ungulates such as deer (Miller *et al.,* 2011; Roy *et al.,* 2011). Seedlings grow through the production of asexual tillers which usually flower after their second year (Roy *et al.*, 2011). *B. sylvaticum* flowers relatively late in the growing season, between July and August. Outside the growing season, particularly during cold winter months, plants will die back to ground level (Roy *et al.*, 2011). Given the complex nature of plant modularity and defining individuality (see section 1.5), for practical reasons in this study an individual was classed as a distinct bunch, which was unconnected aboveground to other bunches.

1.7 Thesis overview

A key motivation for my thesis title: *"Trait-based methods in (plant) ecology: theory and practice"* is to highlight my research philosophy of praxis: the cyclical process of reflection and action. The overall aim of my thesis is to:

"Examine how trait-based methods can be used to assess plant community to individual-scale impacts in the context of global environmental change."

This aim is addressed via **three thematic sections** that combine theory and practice in trait-based research to form **four chapters**, which is then contextualised in the general discussion **Chapter 6** (Figure 1.5):

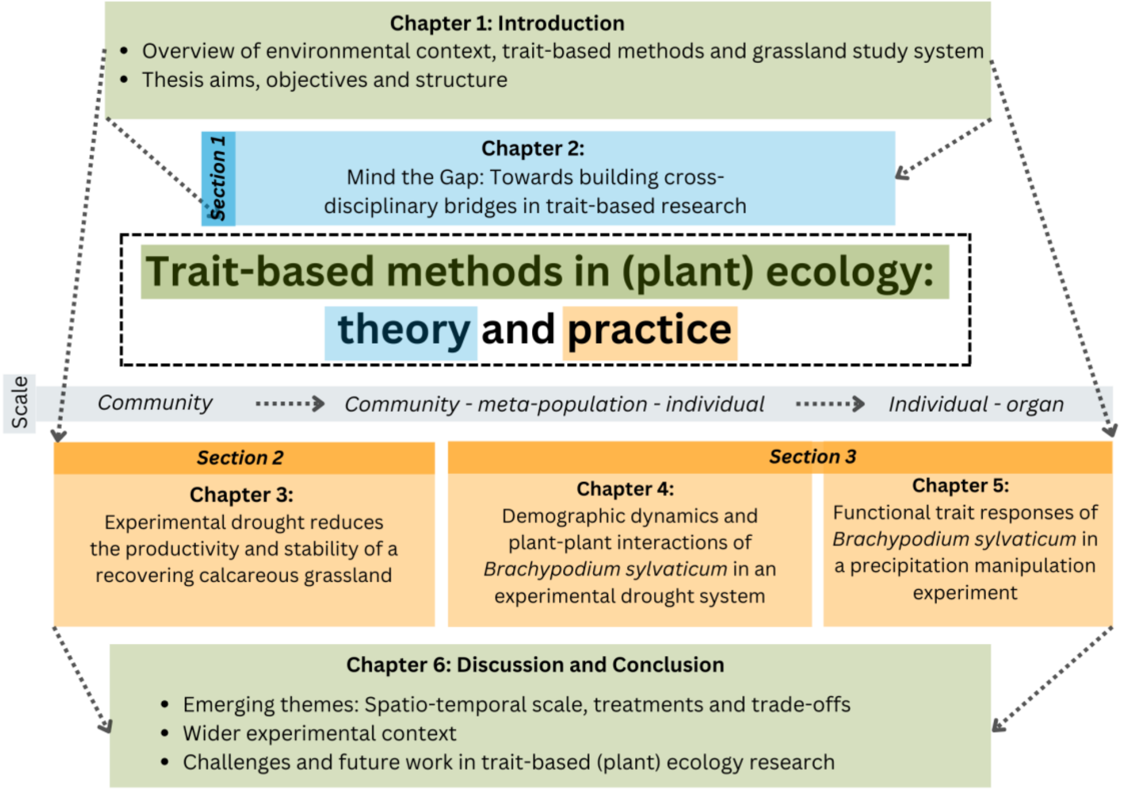


Figure 1.5 An overview of the thesis structure by thematic section and chapter. The thesis title (outlined in dashed box) is deconstructed to illustrate different approaches taken. The scale of interest differs amongst chapters and is outlined in the centre of the schematic. Chapter's 3-5 are based on investigations in the RainDrop drought experiment site in Oxfordshire.

Section 1: Key trait theories and concepts: **Chapter 2** is a literature-based assessment of the use of traits across space, time and disciplines. The research question for **Chapter 2** was:
What are the conceptual genealogies of "functional", "phenotypic" and "life history" trait terms, the main challenges and possible solutions to improve research synthesis and synergy between trait-based disciplines?

Section 2: Monitoring grassland communities and environmental change: **Chapter 3** examines grassland community structure and functioning in an experimental drought system, where the main research question was:
Under an imposed precipitation treatment, what are the temporal dynamics in the structure and functioning of a calcareous grassland community?

Section 3: Trait-demographic case study on *Brachypodium sylvaticum*: **Chapter's 4** and **5** explore the application of traits from organ to community level in an experimental drought system in a calcareous grassland. **Chapters 4** aimed to answer this research question:
How does drought influence the survival, above-ground vegetative and reproductive components of the bunch grass Brachypodium sylvaticum and in addition do biotic interactions mediate demographic responses?

Chapter 5 instead focused on addressing the question:
How do above-ground trait responses vary between and within Brachypodium sylvaticum individuals across precipitation treatments?

In the following section I will briefly provide an overview of the four chapters.

Trait-based methods have a long and complex history, evolving from ecology, evolutionary biology and demographic research streams. These varied conceptual origins and developments has led to differences in the understanding and usage of traits, which has raised several challenges in research synthesis and synergy amongst trait-based researchers/disciplines. Despite the promise of generality, prediction and scalability of trait-based approaches in addressing grand environmental challenges, an interdisciplinary review of the conceptual and empirical issues has been seldom qualified. Using bibliometric analyses, **Chapter 2** explores the "genealogy" of trait terms and concepts within these disciplines. I highlight key challenges in trait-based research and outline ways to better reconcile terminological differences and enhance interdisciplinary collaborations between trait-based researchers.

Grasslands are ecologically and socio-economically important and often overlooked biomes. **Section 2** sheds a light on these "green carpets" by taking a community perspective to assess the structure, functioning and plant life histories of a calcareous grassland community growing at the RainDrop drought experiment (Wytham Woods, Oxfordshire). **Chapter 3** uses a six-year dataset on plant species composition and above-ground biomass to quantify the stability and resistance of both productivity and community diversity at RainDrop. This chapter introduces the RainDrop experimental site and lays the foundations for **Section 3** and the final data **Chapter's 4** and **5**.

Empirical data on key demographic parameters and traits of perennial plants are limited on fine spatial scales (and long-time scales), particularly in grassland ecosystems hindering our ability to quantify and predict ecological processes. Often studies rely on mean trait values for a given species, which provides only a partial window into species responses across space and time, as intra-specific variation is not adequately characterised. **Chapter 4** combines three years of data on demographic dynamics (survival and reproduction) and biotic interactions to assess individual functioning of a bunch-forming grass species *Brachypodium sylvaticum* (false brome) under an imposed drought. Understanding the role of intra-specific trait variation both *amongst* and *within* individuals of the same species is critical for characterising plant performance and fitness. Within this context, **Chapter 5** uses a subset of the surviving *B. sylvaticum* individuals studied in **Chapter 4** to explore variation in trait-trait and trait-treatment relationships in a suite of above-ground morphological traits. The conclusion of this thesis is with **Chapter 6**, where I synthesise and discuss the emerging themes resulting from **Chapter's 2-5** and outline some of the outstanding questions and suggestions or future work in trait-based approaches in plant ecology.

1.7.1 Publications resulting from this thesis

The following articles pertaining to this thesis have been published and appear in the Appendix.

Jackson, J., **Middleton, S.L.,** Lawson, C.S., Jardine, E., Hawes, N., Maseyk, K., Salguero-Gómez, R. and Hector, A., (2024). Experimental drought reduces the productivity and stability of a calcareous grassland. *Journal of Ecology*, *112*(4), pp.917-931. DOI:10.1111/1365-2745.14282 (**Chapter 3**)

Middleton, S.L., (2023). Automating image segmentation for vegetation monitoring. *Nature Reviews Earth & Environment*, *4*(12), pp.807-807. DOI:10.1038/s43017-023-00466-1 (**Part of Chapter 4**) (Reproduced with permission from Springer Nature)

Middleton, S. Beyond a green carpet. *Wellcome Collection* (2022). https://wellcomecollection.org/articles/Ytf-3BEAACkA9nLA (**Part of Chapter 1**)

My work has also featured in two short documentaries alongside other members of the RainDrop network:

The Climate Time Machine (2021) available here:
https://youtu.be/a4J5Kd6YZTw?feature=shared

The RainDrop Experiment (2021) available here:
https://youtu.be/WwMzwqFD30g?feature=shared

1.7.2 Contributions to wider biological sciences

In addition, through my collaborations with the International Plant Functional Trait Course network (2019 - present) I have made contributions to manuscripts in the wider domain of plant trait-based ecology, which further support themes covered in the thesis:

Halbritter, A.H., Vandvik, V., Cotner, S.H., Farfan-Rios, W., Maitner, B.S., Michaletz, S.T., Oliveras Menor, I., Telford, R.J., Ccahuana, A., Cruz, R. et al., (2024). Plant trait and vegetation data along a 1314 m elevation gradient with fire history in Puna grasslands, Perú. *Scientific Data*, *11*(1), p.225. DOI:10.1038/s41597-024-02980-3

Geange, S.R., Von Oppen, J., Strydom, T., Boakye, M., Gauthier, T.L.J., Gya, R., Halbritter, A.H., Jessup, L.H., **Middleton, S.L**., Navarro, J. and Pierfederici, M.E., (2021). Next-generation field courses: Integrating Open Science and online learning. *Ecology and evolution*, *11*(8), pp.3577-3587. DOI: 10.1002/ece3.7009 (Case study presented in **Chapter 2**)

I have also co-authored a number of articles on the topics of Open Science, inclusion, equity and justice across biological science disciplines and wider academia. Exploring these topics helped inform my understanding of the importance of collaborations, ethics and integrity when conducting scientific research.

Middleton S., Iley, B., Sulik, J., Elsherif, M. E. and Azevedo, F. (2024). The Academic Wheel of Privilege: An equity-based tool for authorship order. https://forrt.org/publications/.

Parsons, S., Azevedo, F., Elsherif, M. M. M., Guay, S., Shahim, O. N., Govaart, G. H., Norris, E.,O'Mahony, A., Parker, A. J., Todorovic, A., Pennington, C. R., Garcia-Pelegrin, E., Lazí c, A., Robert-son, O. M., **Middleton, S. L**., Valentini, B., McCuaig, J., Baker, B. J., Collins, E., … and Aczel, B. (2022). A Community-Sourced Glossary of Open Scholarship Terms. *Nat Hum Behav. 6*(3), pp.312-318. DOI: 10.1038/s41562-021-01269-4

Elsherif, M. M., **Middleton, S. L**., Phan, J. M., Azevedo, F., Iley, B. J., Grose-Hodge, M., Tyler, S., Kapp, S., Gourdon-Kanhukamwe, A., Grafton-Clarke, D., Kit Yeung, S., Shaw, J. J., Hartmann, H., and Dokovova, M. (2022). Bridging Neurodiversity and Open Scholarship: How Shared Values Can Guide Best Practices for Research Integrity, Social Justice, and Principled Education. Retrieved from osf.io/preprints/metaarxiv/k7a9p

Chacón Labella, J., Boakye., M., Enquist, B. J., Farfan-Rios, W., Gya, R., Halbritter, A. H., **Middleton, S. L.,** von Oppen J., Pastor-Ploskonka, S., Strydom, T., Vandvik, V. and Geange, S. R. (2021). From a crisis to an opportunity: Eight insights for doing science in the Covid-19 era and beyond. *Ecology and Evolution*. *11*(8), pp.3588-3596. DOI: 10.1002/ece3.7026

Rudd L. F., Allred S., Bright Ross J. G., Hare D., Nkomo M. N., Shanker K., Allen T., Biggs D., Dickman A., Dunaway M., Ghosh R., González N. T., Kepe T., Mbizah M. M., **Middleton S. L.**, Oommen M. A., Paudel K., Sillero-Zubiri C. and Dávalos A. (2021). Overcoming racism in the twin spheres of conservation science and practice. *Proc. R. Soc. B. 288*(1962), p.20211871. DOI: 10.1098/rspb.2021.1871

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Chapter 2: Mind the gap: towards building cross-disciplinary bridges in trait-based research

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(Image: Sara L Middleton)

Abstract

Trait-based methods are widely used in ecological, evolutionary and demographic research, as traits bypass taxonomic identity, allowing for generalisations and predictions of patterns across spatial and organisational scales.

Despite a wealth of studies, differences in the understanding and usage of traits together with five other interrelated challenges (trait *selection*, *sourcing*, *scale*, data *structure* and *standardisation*) are hindering progress through ambiguity, redundancy and research inefficiency, while creating barriers for research synthesis.

Using bibliometric analyses, I examine the origins, evolution and current scope of three commonly used terms “functional”, “phenotypic” and “life history” trait across biological disciplines.

I outline suggestions for building stronger links between functional ecologists and evolutionary biologists and demographers who engage in trait-based research and insights into future directions in trait-based research.

Keywords: Bibliometrics, Comparative ecology, Functional trait, Interdisciplinary, Life history trait, Phenotypic trait, Terminology, Trait databases

2.1 Introduction

Trait-based approaches have emerged as a key method to classify, compare and contextualise organismal functioning and processes through space and time. In simplified terms, a trait is a measurable property of a biological entity (typically an individual organism, although not always, as will be discussed throughout this chapter). Utilising the trait record offers a window into the environmental context of an individual's trait expression and variation beyond their taxonomic identity, making trait-based approaches highly useful and versatile across disciplines (Keller *et al.*, 2023). Traits can inform us of the patterns and processes of how organisms perform along environmental gradients, respond to species interactions, change community composition or networks and influence ecosystem dynamics (e.g. response and effect traits; (Volaire *et al.,* 2020; Schleuning *et al.,* 2023). The inherent flexibility across organisational scale and wide applicability of traits has resulted in an exponential increase in studies adopting them (Shipley *et al.*, 2016). The popularity of traits is partly fuelled by the promise of gaining mechanistic understanding and generalised predictions ideal for addressing environmental change challenges in the biosphere: biodiversity conservation (Kindsvater *et al.*, 2018), biological invasions (Van Kleunen *et al.,* 2010) and ecosystem services (Carlucci *et al.*, 2020).

Trait-based research has been most prominent in plant sciences, which has developed from qualitative descriptions or grouping of plants to a more quantitative and predictive science (Nock *et al.,* 2016; Laliberté, 2017). The field has seen developments in the release of measurement protocols (Pérez-Harguindeguy *et al.*, 2013), and the construction of (open access) trait data repositories from: regional, LEDA (Kleyer *et al.*, 2008) to global coverage, TRY (Kattge *et al.*, 2011), BIEN (Enquist *et al.*, 2016), GLOPNET (Wright *et al.*, 2004). These advancements have enabled leaf trait diversity to be condensed onto a single axis of variation - leaf economics spectrum (Wright *et al.*, 2004), to scaling organisational levels to link individual-level traits from genetic (Alberto Ramirez-Valiente and Jose Robledo-Arnuncio, 2014), to ecosystem level processes (Sakschewski *et al.*, 2015) and use traits to predict community-ecosystem dynamics under environmental change (Díaz *et al.*, 2013; Gross *et al.*, 2017). In the last decade, trait-based research has radiated to include a wider range of taxa across the Tree of Life: Animals, including vertebrates (Luck *et al.*, 2012; Pigot *et al.*, 2020), aquatic vertebrates (Cano-Barbacil *et al.,* 2020), corals (McWilliam *et al.*, 2020), terrestrial invertebrates (Pey *et al.*, 2014; Moretti *et al.*, 2017; Wong *et al.,* 2019; Ferrín *et al.*, 2023), fungi, lichens and other microbiota (Chagnon *et al.*, 2013; Rath *et al.,* 2019; Ellis *et al.*, 2021). Despite significant conceptual and methodological advances in trait-based research, particularly with respect to plants, there remains fragmented progress and low conceptual and data integration across taxa, geography and disciplines (Schleuning *et al.,* 2023).

A major challenge to the collective advancement of trait-based science is the lack of consensus on unambiguous trait definitions (Schneider *et al.*, 2019). The absence of standardised trait terminology impacts elements across the whole trait data lifecycle, from the types of research questions asked to the methods used to collect, store, synthesise and disseminate trait data (Keller *et al.*, 2023). Within different disciplines, the trait term is often preceded by words such as “effect”, “super”, “functional”, “phenotypic” or “life history”, implying that not all traits are equal in meaning across the disciplines that adopt them (Figure 2.1). Are these differences in terminology solely an academic issue of semantics? To the best of my knowledge there has been no broad overview examining trait terminology across disciplines.

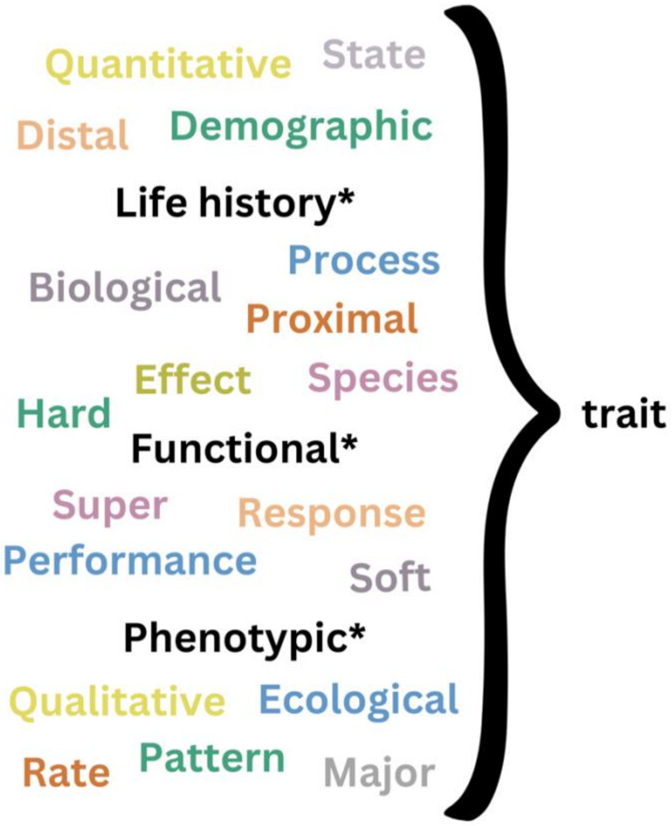

Figure 2.1. A word cloud illustrating some of the different adjectives used to describe traits found in the literature. Asterisked (*) trait terms denote focus of this study. Note that synonyms for “trait” such as characters, features or attributes are also found in the literature.

This review draws on bibliometric approaches to outline the conceptual origins, development and application of traits, highlighting challenges and setting out ways of drawing greater synergies within trait-based biological research. Trait-based science contains a broad spectrum of applications and requires interdisciplinary approaches to better characterise organisms’ responses to and effects on the environment across space and time. The word cloud in Figure 2.1 shows the many trait terms in circulation in the literature, and providing a comprehensive review of all terms here is unfeasible. Instead, I focus attention to three trait terms: “functional”, “phenotypic” and “life history”. These three terms were chosen firstly because they are frequently used and straddle over multiple sub-fields of biological sciences (e.g. ecology, evolution, demography), thus allowing for interdisciplinary comparison of these terms. Secondly, there is ample contemporary and older literature on each of these trait terms individually (e.g. Nock *et al.*, 2016) from which to draw on and assess changes in their usage over time. Lastly, and more generally, the conceptualisation of this review came from informal conversations between three trait-based researchers: myself, an ecologist who uses plant “functional” traits, an evolutionary biologist who studies “phenotypic” traits in birds and a demographer who uses “life history” traits across the tree of life. Each of us examined traits to answer similar questions (e.g. how does an environmental stressor affect the variation in trait responses?), albeit on diverse study systems, whilst adopting different terminology and perspectives based on our training and research backgrounds.

This review aims to unpack possible reasons for these apparent disciplinary divides between the three trait terms: “functional”, “phenotypic” and “life history”. I first outline six key challenges facing trait-based biological research and argue a broader perspective is needed to address issues. Next, I present the development of trait terms from their conceptual origins to their contemporary usage and identify commonalities between trait terms in terms of scope. The influence of rapidly advancing big data and technology on measuring and analysing traits are then discussed. Lastly, I provide some recommendations to increase synergies within trait-based biological research. Strengthening links across taxa, organisational scales and disciplines would benefit both basic and applied trait-based researchers in ecology, evolution, biomonitoring, natural history collections, crop science, conservation and beyond.

2.2 Six key challenges with trait-based research

There are six key challenges in trait-based research which hinder trait data synthesis and knowledge exchange amongst researchers and disciplines (Figure 2.2). These challenges are interrelated and occur along various points in the research project pipeline: i) *Semantic* issues in trait definitions and concepts within and across disciplines, ii) Appropriate trait *selection*, iii) acquisition of trait data whether from primary or secondary *sources*, iv) the organisation and temporal *scale* of trait measurement, and the v) trait data *structure*, and vi) *standardisation* and skills required to appropriately sample traits and manage, analyse and share data.

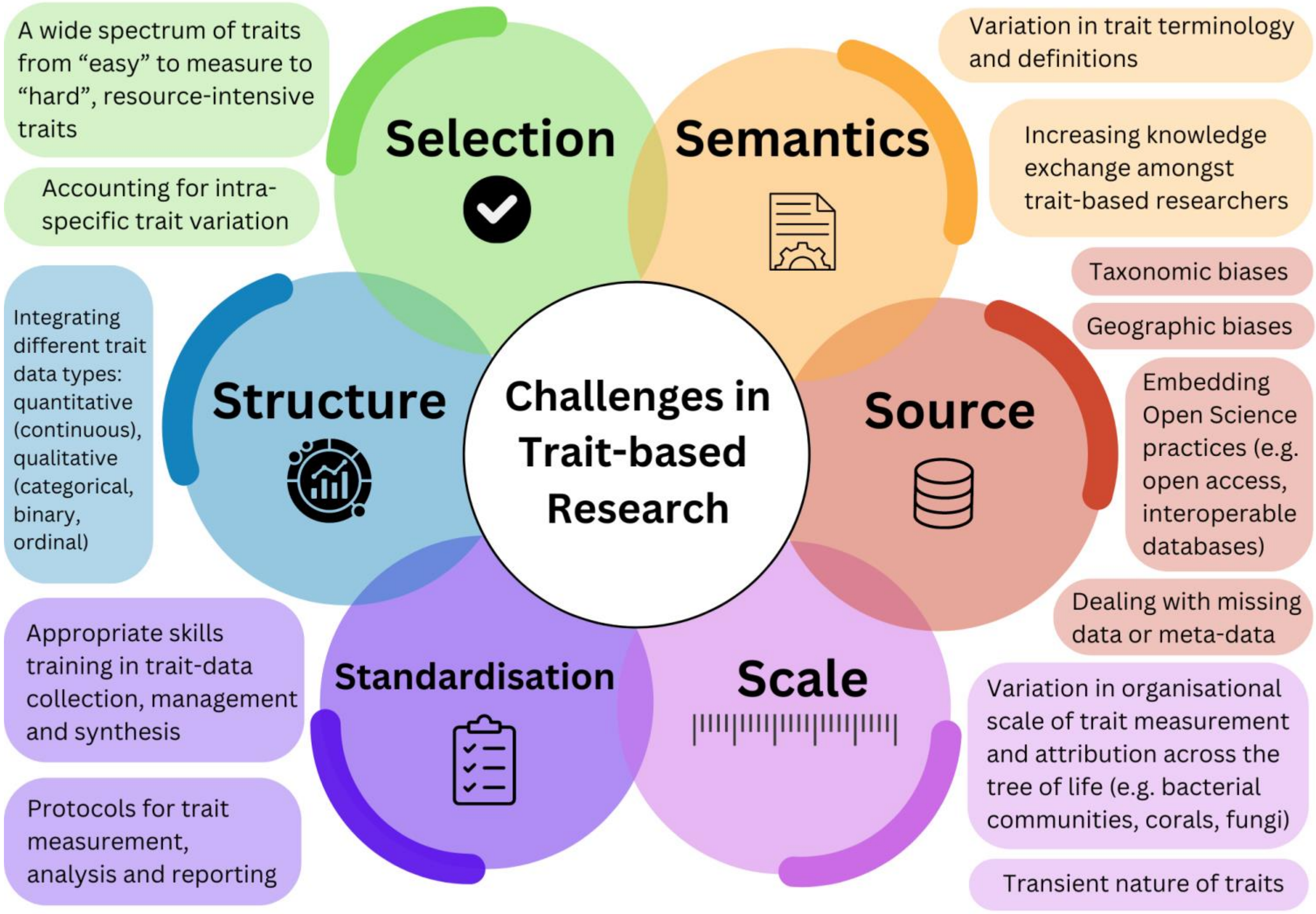

Figure 2.2 An overview of the six key challenges in trait-based research.

2.2.1 Semantic issues

Much research focus and effort has been concentrated on utilising traits for their mechanistic and predictive capacity, especially when applied to urgent environmental change challenges (Malik *et al.*, 2020; de Bello *et al.*, 2021). As often occurs in rapidly expanding research areas, addressing assumptions (e.g. assumed harmonisation of terminology) or acknowledging conceptual histories has been neglected (Shipley *et al.*, 2016). By continuing to bypass these foundational issues it will likely result in trait-based research not yielding on the promises of versatility, scalability and prediction of traits. A reason why these issues are harder to solve is due to trait-based research adopting a broad mode of study defined by the way biological systems or processes are studied rather than by organisational or geographical scale (Shipley *et al.*, 2016; Volaire *et al.,* 2020). The flexibility and versatility of traits has allowed for their integration into different biological disciplines (e.g. genetics, population ecology, community ecology, palaeoecology, ecosystem science, conservation, agriculture and evolutionary biology) (Violle *et al.*, 2007). Each of these disciplines adopting traits exist on different conceptual timelines and trajectories. Each field has a distinct foundational understanding of traits which has evolved into a new body of ideas, adapting concepts to contexts pertinent in each of the semi-isolated disciplines. This situation mirrors demic structure in evolutionary biology, whereby individuals of a given species in a population are more likely to have ecological or reproductive interactions between individuals from the same population than outside populations (Grantham, 2000; Nettle and Frankenhuis, 2019). Similarly in trait-based research, exchange of ideas (analogous to biological interactions) occurs more frequently within than across disciplines (akin to populations). Demic structure of scientific ideas is a means to generate a wide diversity of concepts and terminology. Issues arise when disciplines remain isolated for a significant time. Reduced cross-fertilisation of ideas between disciplines makes classification of terminology complex, leading to miscommunications overtime (Nettle and Frankenhuis, 2019; Peacor *et al.*, 2020).

The lack of common understanding and consensus of trait terms hinders progress through redundancy and research inefficiency (Peacor *et al.*, 2020; Dawson *et al.*, 2021), while creating barriers for comparative analyses (Garnier *et al.*, 2017; Weiss and Ray, 2019; Sobral, 2021), and cross-disciplinary knowledge transfer and collaborations. These ontological disparities arise from terminological differences (different words being used to mean the same thing e.g. characters, traits, forms), syntactic differences (different representations of the same term; e.g., due to differences in spelling, life history vs life-history), and semantic differences (the same words being used to mean different things in various sources, as highlighted by Violle *et al.* (2007) (Hughes *et al.*, 2008). These disparities pose a challenge for researchers conducting literature searches, as different trait keywords can yield different results and papers could be inadvertently missed. For example, life history traits tend to be associated with animal (demographic) studies and only searching within this field could result in missing papers that are conceptually similar but from different study systems. Indeed, a primary motivation for this chapter has come about from difficulties navigating the trait literature.

There have been attempts, primarily within (plant) ecology, to address the terminological issues or promote more synergies amongst trait-based researchers (see also commentary by Mahner and Kary (1997) in trait-based evolutionary biology). Violle *et al.*’s (2007) *Let the concept of a trait be functional* article is a widely recognised and cited definition of a functional trait. Focusing on plants, Violle *et al.* (2007) put forward the case for a strict usage of traits, centering functionality with two main criteria: i) traits are measured at the level of the individual organism, ii) traits must link to fitness through an organism's vital rates (survival, growth and reproduction). Violle *et al.*'s (2007) trait definition has provided a step towards a harmonised trait definition, through providing context for the organisational scale traits should be measured and applied at. More recently, the utility of ascribing functionality to traits has been questioned (Volaire *et al.,* 2020; Sobral, 2021; Streit and Bellwood, 2023). Volaire *et al.* (2020) and Streit and Bellwood (2022) offer broader definitions of (functional) traits that aim to disentangle the spatial and temporal environmental components of traits into pattern (state) and process (rate) traits. Pattern traits are analogous to a photograph representing a snapshot in time (e.g. beak shape), whereas process traits are a series of assembled snapshots (e.g. relative growth rate) producing a film. Sobral (2021) challenges the notion of a hierarchy of functional traits implied by Violle *et al.*'s (2007) trait definition by suggesting that, through an evolutionary lens all traits are functional. As there is currently no accepted definition of a trait, researchers have taken to adding new adjectives to trait terms or adapting definitions to suit their study systems or discipline, which is limiting comparability between studies.

2.2.2 Trait selection

There remains an implementation gap between theory and practice in trait-based research when it comes to selecting suitable traits for a given study. The task of deciding which and how many traits and where to source trait data remains a non-trivial exercise with strong implications for study outcomes (Gallagher *et al.*, 2021; Weigelt *et al.*, 2023). Researchers often chose traits (e.g. body size) that are abundant in the literature that can be obtained easily via databases or quick to measure in the field. Thus, trait selection is not always grounded in a solid theoretical bases used to guide hypotheses (de Bello *et al.,* 2021). This has likely resulted in part to numerous cases of mismatch in trait applications to demographic rates relationships (e.g. Yang *et al.,* 2018) or to conservation outcomes (e.g. Gallagher *et al.,* 2021). There are instances where exploratory trait analysis is more suitable. It can be difficult to decide *a priori* which traits to measure, as linking traits to a particular ecological mechanism or process is not always known from the outset, particularly in understudied taxa (e.g. bryophytes) or ecological settings (Coe *et al.*, 2024).

The question of how many traits to measure is also challenging. Traits coordinate responses and the sum of these trait-trait interactions influence higher levels of biological organisation (e.g. populations and communities). The mechanisms underlying trait-trait and trait-environment processes might only be apparent when multiple traits are considered (Armbruster *et al.*, 2014; Yang *et al.,* 2018). In plants, the leaf economics spectrum describes broad-scale patterns in leaf trait coordination, which relate to resource acquisitive or conservative plant strategies (Wright *et al.*, 2004; Reich, 2014). Thus, the focus on a single trait, especially if using species' mean trait values, might only provide a partial window into the underlying mechanisms (Yang *et al.,* 2018). It is important to note that including multiple traits into a study just because they are easy to access, or measure will not necessarily improve analysis outcomes and risks generating misleading outcomes. This is especially the case if traits highly coordinate, as using uncorrelated vs correlated traits provides richer information for characterising community structure (Laughlin, 2014).

A challenge related to the multidimensional nature of traits of an organism is intra-specific trait variation (ITV). Within-species trait variation whether from genotypic variation or phenotypic plasticity is an important consideration for assessing which and how many traits and the number of replicate individuals to include in a study. ITV can increase the difficulty in interpreting outcomes in both single and comparative studies, particularly when species mean traits are used (Shipley *et al.,* 2016). Some traits are more plastic than others (e.g. wingspan of a butterfly (Gentile *et al.,* 2021) or specific leaf area) and the degree of plasticity depends on the trait, an organism's developmental stage (ontogeny) and the environmental gradient in question (Pérez-Harguindeguy *et al.*, 2013). Questions remain as to when it is appropriate to disregard ITV or how best to balance trait data quality with limited sampling resources. To help guide researchers in the complex decision-making process in trait selection, a hypothesis-driven framework has been outlined, which differentiates between response and effect traits (de Bello *et al.,* 2021). This together with standardised trait sampling guides across taxa such as plants (Pérez-Harguindeguy *et al.*, 2013), terrestrial invertebrates (Moretti *et al.*, 2017) and fungi (Dawson *et al.*, 2019) is a positive step towards addressing this key empirical challenge.

2.2.3 Trait sources

Trait values can be obtained from primary or secondary sources. Primary trait data collection (i.e. in situ observational and experimental approaches) are regarded as the preferable option to secondary sources (literature and databases) for studies at small spatial scales or focusing on local adaptation (Wong *et al.,* 2019; de Bello *et al.*, 2021). Obtaining trait values from primary data collection typically results in higher quality data, however, some practical challenges remain: i) ensuring traits measurements are comparable with other studies ii) navigating practical or resource constraints at field sites (e.g. sampling endangered taxa or at remote field sites) iii) ensuring adequate knowledge and training in trait sampling techniques for a study species. In cases where direct in situ trait measurements are not feasible, extracting data from the literature or databases is warranted.

Recent decades have seen a marked rise in the volume and velocity of trait data collection and storage, with more than 45 trait databases currently in operation (Gallagher *et al.*, 2020, Open Traits Network 2024, https://opentraits.org/datasets.html). These repositories cover a wide range of taxonomic groups, mostly within the plant and animal kingdoms, with some emerging databases in the fungi and microbial domain (Table S2.1). Currently, large, centralised repositories are the dominant way of compiling and storing large volumes of trait data (Schneider *et al.*, 2019). These databases have advanced from the first generation of databases which were born out of the needs of individual researchers or research groups, often based in a small region, studying a limited set of traits using independent protocols (Fraser, 2020). This disconnected and decentralised approach affords some advantages, like increased autonomy and flexibility of use (Gallagher *et al.,* 2020). However, this results in issues with data veracity, limiting interoperability, whilst also increasing risk of duplication. Centralised repositories such as TRY (Kattge *et al.*, 2011), have aided in the harmonising and standardisation of disparate trait data (Gallagher *et al.,* 2020), leading to our advanced understanding of traits, particularly at a macroecological level (e.g. defining a global trait space of plant form and function, Díaz *et al.*, 2016). Nonetheless, centralised databases still remain narrow in scope and taxon or region specific (Schneider *et al.,* 2019).

Missing trait values, metadata or changes in species taxonomy can limit the utility and interoperability of trait databases. Missing trait data can arise for similar reasons outlined with primary data collection. Removing incomplete trait observations reduces the sample size and thereby the statistical power (Penone *et al.*, 2014). Some techniques such as phylogenetic imputation – the substitution of trait values of the focal species with a congeneric species are being used to overcome missing data (see Johnson *et al.* (2021) for imputation method comparisons). The availability of auxiliary information accompanying trait values such as measurement protocols, geographic location, local environmental conditions and trait variability hampers the utility of trait data repositories. For taxa with wide geographical extents this can be especially problematic. Relying on a single mean trait value averaged over a wide climatic gradient of a species can distort inferences for studies operating at macro ecological scales (de Bello *et al.,* 2021). There is also a need to reconcile data gaps across the spectrum of trait measurements from the easy to measure (e.g. many morphological traits) to the harder and (more resource intensive) to measure such as eco-physiological or behaviour traits (Gallagher *et al.*, 2021).

2.2.4 Trait data structure

Trait data can take on many forms from continuous (e.g. invertebrate leg length in cm), circular continuous (e.g. flowering time) or qualitative: categorical (e.g. diet type), binary (e.g. presence/absence of a behavioural trait), ordinal (e.g. plant size classes). The structure of the data affects the outcomes and interpretations when modelling community metrics. For example, functional diversity indices rely on distance-based calculations (e.g. Gower distance, Gower, 1971) to quantify trait dissimilarity between species standardised onto a common scale. Analysing a trait dataset with both quantitative and qualitative variables can introduce complications, as categorical variables contribute disproportionately higher weighting in multi-dimensional trait space (Davis, 2021; de Bello *et al.,* 2021). Within computational ecology there have been some developments in addressing this issue with software packages such as ‘gawdis’ (de Bello *et al.*, 2021) or evaluating different methodological approaches (Davis, 2021; Palacio *et al.,* 2024) to help guide researchers. Given these computational challenges remain, it is recommended, where possible continuous trait data are used. Continuous variables are better able to capture strength of trait-trait and trait-environment relationships, including characterising intra-specific trait variation (Wong *et al.,* 2019).

2.2.5 Traits across organisational and temporal scales

Traits can provide a window into the patterns and processes governing an organism’s co-ordination in physiological functioning, phylogenetic relationships, and environmental tolerances across space and time (He *et al.*, 2019). As such, reconciling these two scales in trait-based research both conceptually and in practice across the diversity of life forms on Earth under global change remains tricky. Well-structured research questions should guide the suitable scale traits are sampled at for the context of the study. There is a conceptual rigidity in identifying the “correct” sampling scale across trait-based studies (Keller *et al.,* 2022), driven in part from trait data providers and users operating in different research spheres. There are numerous situations where adopting a plant-centric definition of traits fails to fully capture complexity across the Tree of Life. Plants are generally easy to observe, immobile, largely restricted to a single trophic level and relatively easy to distinguish between organisms (but note asexual reproduction can complicate matters). The widely used trait definitions and concepts do not translate easily in practice to single-celled organisms (Rath *et al.,* 2019). Whilst some organisms have clearly defined boundaries of individuality (the typical measurement level of (functional) traits, Violle *et al.* 2007) such as a bison or a sunflower, defining the appropriate scale of measurement in practice for other taxa is more complex. For example, microbial taxa exist as communities, which can be hard to cultivate. These microbial communities are based on complex interactions integrated via food webs (Rath *et al.,* 2019, see also Schleuning *et al.* (2022) for incorporating traits across trophic levels). What is more, defining and isolating microbial entities such as bacteria is challenging given horizontal gene transfer (Westoby *et al.*, 2021). Thus, it is more appropriate and biologically meaningful to measure microbial traits as an aggregated community trait than as individual taxa.

Complexities also lie in integrating trait data across spatial scales. Translation issues exist between the spatial scale traits are sampled at (e.g. individual level) and their applications to other levels of organisation (e.g. their use in community and ecosystem modelling) (He *et al.*, 2019). Increasingly, with global change, trait-based studies “upscale” traits, that is using organ or individual level measurements to answer research questions at macro-ecological scales (Rissanen *et al.*, 2023). Extrapolating trait measurements to larger spatial extents can involve collapsing trait information into functional groupings, such as plant functional types (Wullschleger *et al.*, 2014), herbivore functional types (Berzaghi *et al.*, 2024) or planktonic functional types (Anderson, 2005), which groups species with broadly similar traits and roles within an ecosystem. When global climate models rely on a limited set of traits for a functional type it can obscure trait variation within and across spatial scales. Analysis has shown large ranges in specific leaf area values at global ($\bar{x}$ = 13.9, range = 2.7 – 65.2 m^2 kg^{-1}) and local ($\bar{x}$ = 5.7, range = 4.7 – 37.7 m^2 kg^{-1}) extents for the broadleaf evergreen tropical tree plant functional type (Butler *et al.*, 2017). There is often a mismatch in units when trying to translate organ-level traits into ecosystem-level scale where functions relate to units of land area. The issue of integrating scales has been resolved for some traits like for leaf area mm^2 (organ level) to ecological-scale equivalent of leaf area index, but for more resource-intensive traits (e.g. leaf reflectance) additional work is needed (He *et al.*, 2019).

Combining multi-source datasets with diverging sampling protocols with uncharacterised trait variation has led to generally weak global trait-environment relationships in plant ecology (Anderegg, 2023, see Berzaghi *et al.*'s (2024) commentary for mammal macro-trait ecology). The mechanisms driving trait-environment relationships vary with spatial scale (Messier *et al.,* 2010; Moran *et al.,* 2016). For example, at the regional scale (tens of km), climate conditions are expected to primarily shape trait responses, genetic influences are likely more significant at site level (m to km scale), whilst at the level of organs (mm to cm scale) within organism resource allocation is expected to have a larger influence on traits (Messier *et al.,* 2010). Synthesising trait data sampled across scales makes disentangling the multiple ecological and evolutionary processes challenging, leading to ambiguities and misattribution of trait-environment linkages both within and across studies (He *et al.*, 2019; de Bello *et al.*, 2021). Integrating spatial scales requires adequate sampling of taxa and replicates of traits to get a representative sample of a community structure and functioning. As previously mentioned (trait sources section), trait-databases contain taxonomic and geographic biases and often focus on dominant species. Multi-scale trait approaches are an emerging and promising avenue which will help elucidate the stability of trait-environment relationships across organisational scales (Rissanen *et al.,* 2023).

Traits are not static across time and conceptual differences in characterising the temporal component of traits across fields exist (Messier *et al.,* 2010). Assessing trait-time relationships is inherent to evolutionary and demographic research as these approaches broadly study the origins, variation of life forms and population dynamics respectively over time periods from single lifespans to multiple generations (Metcalf and Pavard, 2007; Raja *et al.*, 2021). Time is a central currency for traits pertaining to an organism’s life history such as survival probability for a given year, relative growth rate or average age at reproductive maturity, which can be mapped onto the “fast-slow continuum” of life history strategies (Stott *et al.*, 2024). In ecology, the temporal aspects of traits have been more implicit, with trait values often representing a single time point for a given organisational scale (Cope *et al.*, 2022). Time-based trait ecology is an emerging area, with recent advances in intraspecific trait temporal variation (Chen *et al.*, 2019) and phenology (Nordt *et al.*, 2021), accelerated by the need to assess and predict global change impacts. Additional conceptual work is needed to find common trait currencies across disciplines, to aid knowledge exchange and global synthesis efforts.

2.2.6 Trait data standardisation

Lastly the issue of standardisation of trait data collection, management, analysis and reporting touches on all the other five key challenges (semantics, source, scale and structure) previously outlined. There has been a concerted effort in the last decade or so to standardise data collection protocols which has been discussed at length elsewhere (e.g. Schneider *et al.,* 2019)**.** With the rise and increasing acceptance of Open Science practices such as open access, open data and code the standardising of management, analysis and reporting of trait data is improving (Gallagher *et al.*, 2020; Geange *et al.*, 2021). However, an outstanding issue is the availability of training and guidelines for researchers to adopt standard practices along the whole trait data cycle (although see Keller *et al.,* 2022 and de

Bello *et al.,* 2021). A communication gap between trait data collectors, managers and users often means a lack of integrated standardisation practices. This stems from a lack of awareness, training and resources to understand the requirements of each of the researchers/practioners involved (Keller *et al.,* 2022). The breadth of the trait-based literature can be overwhelming to navigate, especially for researchers newly entering the field. Whilst protocols exist for an aspect of the trait data cycle such as sampling protocols (Pérez-Harguindeguy *et al.*, 2013) and data management and sharing guidelines (Wieczorek *et al.*, 2012), there is a need for accessible guidelines that integrate theoretical concepts, empirical and analytical methods in trait-based research. As outlined throughout this section on challenges, conceptual ambiguities or biases in foundational aspects of the field such as terminology or sourcing trait data has downstream consequences in the research pipeline.

In the pursuit for unifying concepts or standardisation in trait-based research, it is important to take stock of the variety of methodological approaches used, reflecting the vast diversity of life forms we study. There is a need for pragmatism, openness and cross-disciplinary communication to address the conceptual and empirical challenges in this promising field. Next, I contextualise the challenges by delving into the conceptual “genealogy” through an inter-disciplinary perspective to examine how some of the trait-based approaches in biological sciences came to be.

2.3 What's in a name?: a genealogy of trait terms and concepts

The concept of a trait is not new. There has been a long history of classifying living things according to their characteristics, which has influenced contemporary approaches in trait-based science. This mode of inquiry focuses on the function or role of living things rather than what they are made of. Natural philosophers such as Aristotle (ca. 350 BCE) classified animals based on their life strategies where 'birds' were living things that flew, and 'fish' were any aquatic animal (Figura, 2024). Theophrastus (ca. 300 BCE) grouped plants into trees, shrubs, herbs and grasses, according to their height and degree of woodiness (Weiher *et al.*, 1999).

In the 19th century, traits were examined beyond simply cataloguing them. Traits were studied in relation to demographic performance or environmental variables to help explain patterns of species assembly and diversity. Darwin used traits, referred to as "features" or "characters" to assess an organism's performance (e.g. beak size linked to survivorship) (Darwin, 1859). Humboldt examined physical plant characteristics to further group plants into 18 different forms such as lianas, ferns and gymnosperms (Heilmeier, 2019). Humboldt hypothesised that the diversity of plant forms was linked to temperature (Heilmeier, 2019). This work on trait-environment linkages laid the foundations for functional classifications of plants such as Raunkiaer's life forms and Grime's CSR (competitive, stress tolerant and ruderal) scheme (Raunkiaer, 1934; Grime, 1979). Similar functional classification for animals have been developed such as guild – the grouping of animals that share similar environmental resources and have overlapping niches (Root, 1967). Functional traits have strong ties to ecology, as the term evolved from the birth of the new sub-discipline of functional ecology (Calow, 1987). Functional trait approaches are typically defined at the individual scale and act as surrogates for quantifying individual performance (i.e. on growth, reproduction and survival) and ultimately fitness (Violle *et al.,* 2007, Table 2.1). The fitness component of functional traits is often implied and not frequently empirically tested, as sampling is typically conducted at one time point and on few individuals (Shipley *et al.*, 2016; Pistón *et al.*, 2019). Central to functional trait approaches in ecology is the idea of environmental filtering. The persistence of a species in a given location is governed by a suite of traits, which if they do not adequately match the environmental conditions, species are not able to persist (i.e. are filtered out) (Keddy, 1992). This concept of environmental filtering has given rise to the study of finding patterns and mechanisms for trait-environment linkages along various environmental gradients (Bruelheide *et al.*, 2018; Kemppinen *et al.*, 2021). Trait-environment relationships in animal studies are often referred to in the literature as "ecometrics" (Eronen *et al.*, 2010), and traits are often analysed at higher levels of biological organisation (e.g. via community weighted means). Examining coordination and trade-offs between traits (i.e. trait-trait links) are also a key functional trait application and have been used to generate quantitative schemes (e.g. leaf-height-seed scheme (Westoby, 1998); leaf economics spectrum (Wright *et al.*, 2004).

Life history trait approaches have more quantitative roots in demography, population ecology and evolutionary biology research streams, underpinned by life history theory (Stearns, 1992). Fitness is central to the definition of life history traits, based on conceptual work from natural historians like Darwin and Wallace combined with modelling frameworks (e.g. Euler-Lotka equation) (Laughlin, 2023b). Life history traits differ from functional traits in how they are obtained (Table 2.1). Life history traits represent quantitative properties directly relating to the three fitness components (survival, growth and reproduction) of an individual, which are derived from demographic population models (Griffith *et al.*, 2016; Laughlin, 2023b). As such, life history trait approaches seek to examine how natural selection shapes the life cycle of organisms and resulting population dynamics in particular environmental contexts. These methods require large volumes of longitudinal data on individuals to adequately parametrise an organism's life table (Salguero-Gomez *et al.*, 2018). Life history traits of animals and plants have been mapped onto a "fast – slow" continuum which captures an organism's life history strategy resulting from trait covariation or trade-offs in reproduction and growth versus survival (Salguero-Gómez *et al.*, 2016; Stott *et al.*, 2024). Life history trait empirical approaches typically do not incorporate genetic components, a key pillar in phenotypic trait approaches (although this is changing with advances in genetic sequencing (Flatt and Heyland, 2011)).

Table 2.1 Examples of how "functional", "phenotypic" and "life history" trait terms are used in the literature, with comparisons in definitions, frameworks and spatio-temporal scales. The spatio-temporal scales traits are defined at determine the main trait relationships (trait links) studied. Examples shown here are non-exhaustive and are adapted from Violle *et al.* (2007) and Klausmeier *et al.* (2020).

Trait term	Definition	Example frameworks	Trait links	Temporal scale of definition	Spatial scale of definition	References
Functional	Morphological, biochemical, physiological, structural, phenological, or behavioural characteristics of individuals that impact fitness indirectly via their effects on growth, reproduction and survival, the three components of individual performance. *Examples:* Plant height, fur density, feeding strategy	CSR scheme Response-effect framework Economics Spectrum	Trait-environment, trait-trait	Usually fixed	Individual (frequently scaled to higher levels of biological organisation)	Grime, 1979 Violle *et al.,* 2007 Wright *et al.,* 2004
Phenotypic	Observable morphological, behavioural or physiological properties of an organism or cell resulting from the interaction of the genes and the environment. *Examples:* Metabolic rate, foraging efficiency, seed size	Quantitative genetics Morphology-performance-fitness pathway	Trait-genes, trait-trait, trait-environment	Usually dynamic	Individual (frequent reference to sub individual levels, cells and genes)	Levins, 1968; Lande and Arnold 1983; Arnold 1983; Charlesworth and Charlesworth, 1987.
Life history	Quantitative properties of organisms relating to key moments along their life cycle describing survival, development, and/or reproduction components of fitness usually derived from population growth models. *Examples:* Age at maturity, life span, size at birth	Euler–Lotka Life history theory Fast-slow continuum	Trait-demographic, trait-trait, trait-environment	Dynamic	Individual and population	Stearns, 1983, 1992; Kingsolver *et al.,* 2001

Phenotypic trait approaches have branched out of eco-evolutionary and agronomic research spheres. The introduction of a new multivariate analytical method to examine selection on trait-trait relationships by Arnold (1983) and Lande and Arnold (1983) provided more empirical frameworks to a traditionally theoretical discipline (Svensson, 2023). The morphology-performance-fitness pathway provided mechanistic links to quantify variation in phenotypic “characters” (traits) first illustrated in snakes (Arnold, 1983). The Lande-Arnold analytical toolbox integrated statistics from animal and plant breeding studies, concepts from palaeontology (e.g. adaptive landscape) and field methods which provided conceptual bridges between ecology and evolution fields (Lande and Arnold, 1983; Svensson, 2023). Phenotypic trait approaches frequently “down scale”, especially in agronomic research, where traits are primarily observable features of organisms measured to develop genotype-phenotype maps (e.g. quantitative trait loci analysis, (Yarnes *et al.*, 2013)). When phenotypic traits are related to the environment, they can be regarded as life history traits, but only if they contribute positively to fitness (i.e. they are functional) (Laughlin, 2023a).

The functional, phenotypic and life history trait terms have emerged from different research streams and thus adopted different conceptual approaches to answer broadly similar research questions (e.g. the trait links shown in Table 2.1). There are instances where the three terms share fuzzy boundaries (e.g. phenotypic traits are functional traits when they impact fitness, (Laughlin, 2023a)). These areas of overlap indicate potential pathways towards interdisciplinary communication and harmonisation. In the next section I explore the bibliometric trait landscape through time and disciplinary space.

2.3.1 Methods for literature review

To examine the connection between the “functional”, “phenotypic” and “life history” trait terms across time and disciplines, a bibliometric analysis was carried out 9-11th June 2021. A literature search in the ISI Web of Science database (Core Collection) inputting the terms “functional trai*” OR “phenotypic trai*” OR “life*history trai*”. To remain within the study scope, only articles pertaining to biological, ecological, agricultural, environmental, molecular sciences and microbiology categories were included (ensuring any references to medical sciences were excluded). To examine how trait term usage and level of integration between fields evolved, results were subset into three 10-year time spans (1991-2000, 2001-2010 and 2011-2020). A 30-year time span was chosen as few articles containing the three trait terms were found before 1990’s (n = 62, 1960-1990), where their inclusion as a separate time period would not have allowed for meaningful patterns to be drawn. The potential limitations in the exclusion of other trait terms and time periods are discussed briefly in section 2.3.4*.* Titles, abstracts and keywords from articles with original data, reviews and conference papers were included and saved as text files. To build the network visualisation maps for each decade, VOSViewer (v1.6.16) was used by extracting terms from text files (van Eck and Waltman, 2017). Network visualisation maps contain three key attributes: i) bubbles representing a key term or phrase, with their size proportional to the frequency of occurrence, ii) clusters forming from closely associated bubbles, and iii) links which denote how closely associated bubbles are to each other. Full details of selection criteria, thresholds, counting and clustering analyses used are available in Supplementary Note 2.1.

2.3.2 Trait evolution across disciplines

A total of 12,880 articles were published between 1991 and 2020 containing one or more of the phenotypic, life history and functional trait terms, with ~85% articles published in the 2011-2020 period (n = 10,884) (Figure 2.3). Aside from the increase in volume of publications, the clustering analysis revealed three key patterns: i) earlier decades are characterised by long-term evolutionary and demographic trait approaches whilst proceeding decades more clusters classify macro-level approaches over shorter time scales; ii) a taxonomic split between plant and animal research remains broadly stable over the three decades; iii) the 2011-2020 period saw the emergence of new sub-fields or taxa adopting trait-based approaches like mycology and bacterial ecology.

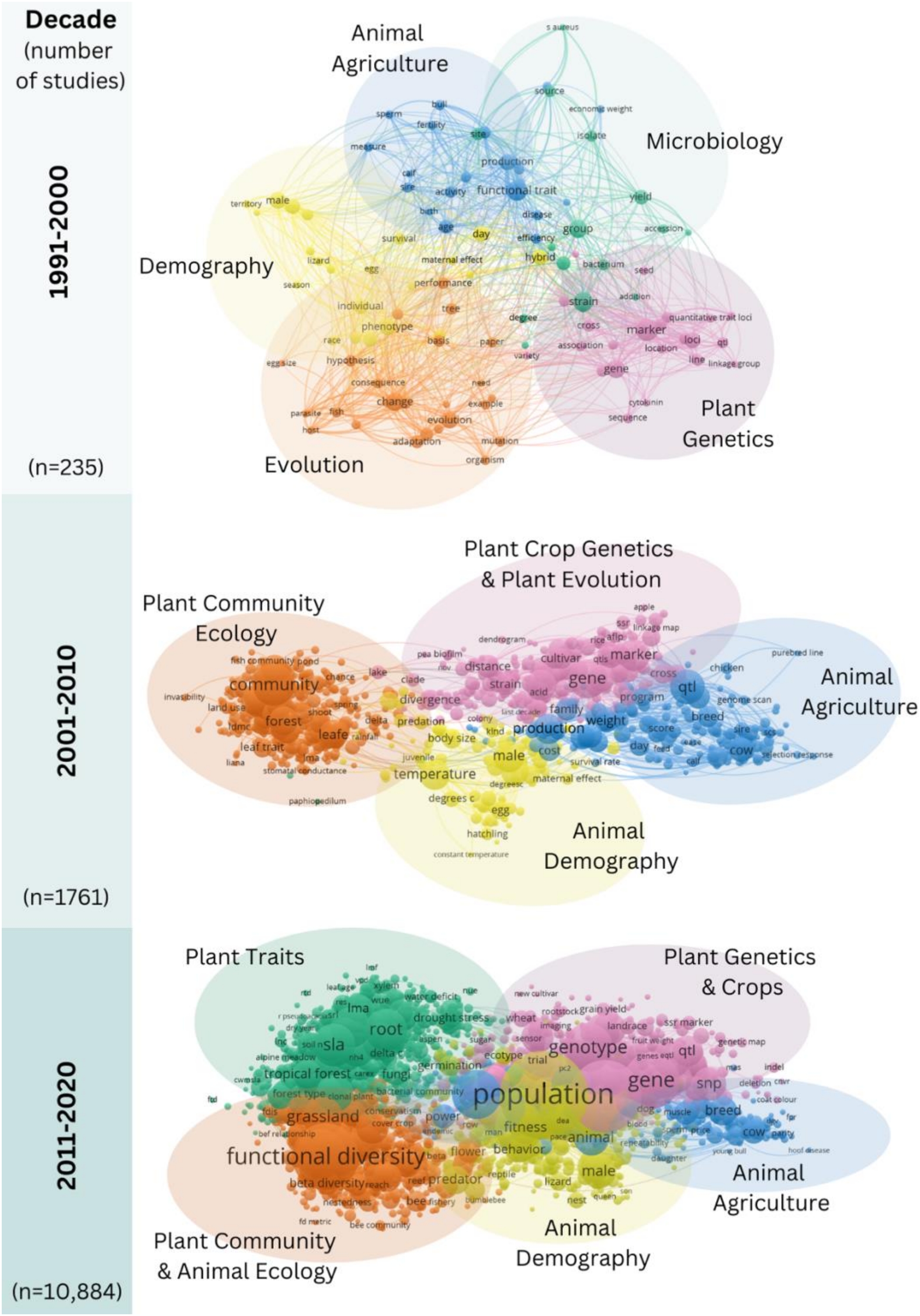

Figure 2.3 Network visualisation map for the trait term associations over three decades, 1991-2020 constructed using VOSViewer (v1.6.16). Maps are based on studies (n) containing one or a combination of the three key trait terms: “functional trai*”, “phenotypic trai*” and “life*history trai*” extracted from ISI Web of Science Core Collection, where the words from titles, abstracts and keywords were analysed. Full details on search criteria and construction of visualisation maps can be found in SI. Each bubble represents a key term or phrase, and their size indicates the frequency of occurrence. Colours denote key clusters according to close associations of key terms or phrases. Major cluster themes are labelled for each decade.

2.3.3 Space-time splits in trait approaches

In the earliest decade (1991-2000), the five distinct clusters were characterised by smaller organisational scales (individuals to genes) as unit of study within evolutionary, genetic and demographic fields (Table 2.2). Research priorities in trait-based studies in this decade were shaped by enhancing agricultural productivity (e.g. genetic engineering of crops for abiotic tolerance or increased yield) to meet the needs of growing human population (Carroll *et al.*, 2014). The rise of genome sequencing and genetic marker technologies likely fuelled the applied trait analysis in the agricultural domain (Miflin, 2000).

In the first two decades of the millennium, ecology emerged as a prominent cluster. The ecology clusters were associated with trait studies researching at population and community organisational scales (Table 2.2) with strong taxonomic split. This aligns with the rise and establishment of ecology as a discipline concerned with addressing grand challenges of biodiversity loss and environmental change (Santangelo and Bramanti, 2006). The space-time split is particularly evident in the weaker association (shown qualitatively by the longer inter-cluster links) between the plant community ecology cluster and evolution and demography clusters in 2002-2010 (Figure 2.3). This weak disciplinary link echoes issues raised in the challenges section and review by Swenson *et al.* (2019). Evolutionary biologists and demographers differ in the biological unit of study to ecologists hindering pathways for communication and empirical analyses. With the former, researchers speak the language of individuals as dynamic entities, whilst the latter primarily operate with aggregate static traits at population or community levels (Swenson *et al.*, 2019).

Table 2.2 A list of top ten word or phrase bubbles found in the network analysis of functional, phenotypic and life history trait studies between 1991 and 2020. Numbers in brackets indicate frequency of occurrence. The threshold of occurrence for a word or phrase was 10 for 1991-2000 and 2001-2010 time periods and 15 for 2011-2020. Text data extracted from titles, abstracts and key word of articles. See Supplementary Note 2.1 for details of methods.

Time period	Top ten terms (frequency of occurrence)
1991-2000	Marker (98)
	Functional trait (85)
	Strain (80)
	Change (72)
	Group (71)
	Gene (69)
	Evolution (61)
	Phenotype (59)
	Male (58)
	Production (55)
2001-2010	Community (527)
	Gene (464)
	QTL (quantitative trait loci) (356)
	Marker (304)
	Genotype (291)
	Male (286)
	Temperature (276)
	Production (275)
	Forest (245)
	Leaf (245)
2011-2020	Population (4350)
	Phenotypic trait (2904)
	Functional diversity (2475)
	Selection (1811)
	Gene (1764)
	Species richness (1394)
	Evolution (1363)
	Production (1354)
	Genotype (1300)
	Phenotype (1087)

2.3.4 Taxonomic divide linked to discipline

The plant and animal kingdoms are the dominant studied taxa using traits. The network analysis showed low integration across taxa over the three decades examined (Figure 2.3). Animal traits were more closely associated with demographic and agricultural clusters, whilst plant traits were more linked to community ecology and genetics clusters. Interestingly, the plant trait formed its own cluster in the 2011-2020 period and was the largest of the five clusters containing 35% (n = 1086) of the total word bubbles (n = 3071) (Supplementary Note 1). These taxonomic splits are the remnants of the historical divide between demographic concepts developed in animal study systems, particularly mammals and birds (Salguero-Gomez, 2017, but see Harper and White (1974)) and trait-based ecology where theories and empirical approaches have been plant-centric (Dawson *et al.,* 2021).

The last decade showed two notable findings: i) signs of taxonomic linkages via the community ecology cluster, and ii) the emergence of new taxa studied such as fungi and bacterial communities. These results likely reflect the rise in macro-scale frameworks used to assess trait-environment links across multiple taxa for a given environmental stressor or ecosystem (Dray and Legendre, 2008; Aubin *et al.*, 2013; Díaz *et al.*, 2016; Simons *et al.,* 2016). In addition, the launch of databases that integrate multiple taxa, traits and ecosystems has aided in this research endeavour (Kattge *et al.*, 2011). The increase in taxonomic diversity adopting trait-based approaches shown here suggests a shift towards more holistic view of ecosystem functioning (e.g. role of fungi in decomposition, (Treseder and Lennon, 2015)).

There is an important caveat to the contextualise the results of this network analysis. The network maps were constructed only from articles mentioning one or more of the three trait terms (functional, phenotypic and life history traits). As mentioned previously with terminological issues, the number of word bubbles shown here are likely an undercount, particularly for 1991-2000 time period. Trait-based research from earlier decades often referred to traits as “characters” or “components” (e.g. Sparnaaij and Bos, 1993), meaning these studies were not picked up in this bibliometric analysis. Work on harmonising trait terminology has been more prevalent in recent decades (Violle *et al.,* 2007). The exclusion of some trait synonyms here reiterates the importance of building cross taxonomic and disciplinary bridges to gain new insights and advance trait-based bioscience.

2.4 Recommendations for bridging the gaps in trait-based research

To realise the full potential of trait-based research, one of mechanistic understanding, generality and prediction, several conceptual, methodological and empirical hurdles need to be acknowledged and addressed. There is no quick fix. Embracing the complexity of the task at hand, here I offer some actionable suggestions for the research community.

2.4.1 Go back to basics

Firstly, striving for an all-encompassing definition of a trait that agrees across taxa and disciplines is a tall order. It is an admirable goal, however in practice it is likely a futile exercise and perhaps reductive. The vast array of life forms on Earth means traits capture diverse features, functions and processes which is reflected in the range of sampling and analytical approaches reported in the literature. A strict definition that works in one system might not translate to another, thus a simplified definition of a trait that suits a wider range of trait-based researchers is needed. I agree with Dawson *et al.*'s (2021) pragmatic approach to return to basics with their more inclusive trait definition: "A trait is a measurable characteristic (morphological, phenological, physiological, behavioural, or cultural) of an individual organism that is measured at either the individual or other relevant level of organization."

This definition is more inclusive of trait-based researchers studying taxa where the measurement unit is harder to determine at the individual level such as fungi with undefined individuality, symbiotic taxa (e.g. corals) or individuals acting as a super organism (e.g. eusocial insects) (Dawson *et al.*'s 2021). At a minimum, studies should clarify which trait definition their study is adopting, to reduce ambiguity and allow for comparability between studies. A further suggestion would be for researchers to drop additional adjectives like "functional", "phenotypic", and "life history" trait terms unless explicitly linking a function to a process or vital rate where both aspects are measured. This is particularly the case of the former two traits, as their definitions in the literature have been more fluid and frequently used interchangeably. Going forward, adopting more simplified trait terminology should increase discoverability of articles in databases, thus improving cross-disciplinary communication. Retrospectively harmonising trait terms is not possible and so researchers should keep in mind synonyms (e.g. terms in Figure 2.1) when searching for studies in databases especially for older trait-based literature.

2.4.2 Common ground and context for comparisons

The genealogy of the terms functional, phenotypic and life history traits have evolved from different disciplines and study systems. Yet, despite different origins, their contemporary applications often align in quantifying and predicting global environmental change impacts. Finding a common language to communicate conceptually and empirically between these three trait spheres will likely come from adopting traits that represent a precise function, or so called “hard” traits (Belluau and Shipley, 2018). Adopting a thermodynamic view of organisms, as energy exchange systems, captured by energy and mass-based traits has the potential to strengthen our mechanistic understanding, with emerging theoretical work already underway (Kearney *et al.*, 2021).

Reviewing the literature revealed body size as a key trait commonly associated with the three terms. Indeed, the bibliometric trait network analysis showed body size centrally located between the four clusters in 2001-2010 network map (Figure 2.3). Body/cell size is often regarded as a “universal trait”, providing insights into organismal functioning (Woodward *et al.*, 2005; Enquist *et al.*, 2015; Fritschie and Olden, 2016; Nock *et al.,* 2016). Body size is an inherit property of organisms that can be expressed as body mass (g) (e.g. above-ground plant biomass), body length, width or height (cm) (e.g. plant height, shell length) or area (cm^2) (coral or bacterial colony size). At the nexus of ecological, evolutionary and demographic approaches, body size governs many trait-trait (e.g. allometric scaling (Lande, 1979), trait-environment (e.g. extinctions (Parker *et al.,* 2023), trophic interactions (Martini *et al.*, 2021)) and trait-demographic (e.g. fecundity (Harper and White, 1974)) relationships. Body size is fairly easy trait to measure and with advances in image capturing technologies and processing techniques can further speed up data collection (Christie *et al.,* 2021; Middleton, 2023). What is more body size can be applied to trait-environment modelling of fossil data to help inform contemporary trait-environment relationships (i.e. hindcasting) impacted by global change (Parker *et al.*, 2023).

Where possible, trait data should be collected on the same individual or relevant biological unit and environmental context to minimise “Frankenstein” trait-environment datasets (a sentiment echoed by Salguero-Gomez *et al.* (2018)). In situ trait measurements will allow for improved characterisation of traits across space and time. The following thesis **Chapters** (**3**, **4** and **5**) aim to fill this empirical gap.

2.4.3 Increasing knowledge exchange between researchers

Journal editors and academic societies can be active facilitators in knowledge exchange amongst trait-based researchers. Introducing more special journal features (e.g. (Salguero-Gomez *et al.*, 2018; Schleuning *et al.,* 2023) that expose researchers to a wider range of trait concepts and approaches would be a valuable effort. Journals can also support the publishing of trait-data sets or databases (e.g. MOSAIC) to improve availability and synthesis of trait data (Bernard *et al.*, 2023; Keller *et al.*, 2023). Conferences are apt arenas to create more opportunities for interdisciplinary communication and collaboration. Creating workshops and thematic sessions that include researchers from traditional trait-based disciplines (ecology, evolutionary and population biology) and also platforming researchers from emerging and allied fields such as mycology, computer science and bioinformatics would greatly improve synergies amongst researchers and help address the taxonomic divide. Future journal features, editorials and conference events could examine themes such as: i) how can trait-based researchers integrate machine learning approaches to assess large volumes of trait data? ii) Which trait-based empirical and analytical methods can be adopted from the microbiome to the macrobiome and vice versa? iii) How can we better integrate the needs between trait data collectors, data managers and users?

2.4.4 Training courses in trait-based research

Training courses are an under-utilised opportunity to address issues with standardisation in trait-based research. Training courses, especially field courses provide an opportunity for participants to acquire and develop practical skills by applying learned theoretical concepts (Geange *et al.,* 2021). Field trait courses can act as a bridge between theory and practice as they incorporate existing guidelines (e.g. Halbritter *et al.*, 2020) and measurement protocols (Pérez-Harguindeguy *et al.,* 2013), data management and analysis (e.g. Götzenberger *et al.*, 2020) into the course structure. Box 2.1 outlines a case study of the International Plant Functional Trait Course (PFTC, https://plantfunctionaltraitscourses.w.uib.no/) which provides an example of how training researchers can help with further harmonising trait practices from study design to reporting of research outcomes. PFTC provides hands-on training on trait-based methods in plant ecology from designing a research project, field and lab data collection, documentation, analysis and reporting (Geange *et al.,* 2021). Open Science principles are embedded within course structure, which helps address some of the previously outlined challenges in trait-based research (Figure 2.2). The principles of the trait course are relevant across taxonomic groups and can be adapted accordingly.

Box 2.1 The International Plant Functional Trait Course: A case study into addressing the challenge in trait standardisation in trait-based ecology

The International Plant Functional Trait Course has had seven iterations in locations including Peru, China and Norway which have focused on trait-based research within climate change and ecosystem science. There are three phases of the course: **phase 1:** pre-field course, learning key theory and trait-environment concepts, designing group research projects, which occurs via regular virtual classes. **Phase 2:** Hands-on training in field and lab trait measurements and data documentation. **Phase 3:** Post field course data analysis, reporting and project write up (Geange *et al.,* 2021).

Trait sampling along an elevational gradient during the fifth iteration of the course in Peru in 2020.

Standardisation of trait data collection and documentation

In advance of data collection, course participants are trained in the standardised steps involved from sampling to documentation of leaf traits, summarised by the ten step “Trait Wheel” (PFTC and Halbritter, 2020):

1. **Collect leaves**

Select individual plants and sample leaves from the field according to established measurement protocols

2. **Prepare leaves**

Correctly assign taxonomic and sample ID and store leaves ready for measurement

3. **Wet weight**

Weigh and record the leaf wet mass, according to established measurement protocols

4. **Leaf area**

Carefully scan leaf to obtain leaf area using ImageJ and LeafArea R package (Schneider *et al.,* 2012; Katabuchi, 2015)

5. **Leaf thickness**

Measure the thickness of the leaf according to established measurement protocols

6. **Dry weight**

Dry the leaf samples according to established measurement protocols

7. **Data entry**

Enter leaf trait data carefully into spreadsheets in .csv format

8. **Data checking**

Visually inspect data for errors, typos or missing data

9. **Data documentation**

Systematically record each step of methods, auxiliary data, note down cases of deviation from established protocols and create a data dictionary (e.g. units, data type) of trait variables

10. **Chemical leaf traits.**

Measure chemical traits such as Δ13C using established measurement protocols and follow steps 7-9

Participants then share protocols and datasets between the different research groups and are encouraged to disseminate these to the wider research community via open access publications (see Vandvik *et al.* (2020) and Halbritter *et al.* (2024) for examples).

2.5 Big data in trait-based eco-evo-demo research

Trait-based research is at an exciting time with rapid digital innovations offering novel tools to address pressing eco-evolutionary and demographic questions. Digital innovations (big data) have greatly expanded the capacity of data collection and analysis in trait-based research through large trait databases and more recently with emerging tools in machine learning (Farley *et al.*, 2018; Gallagher *et al.*, 2020; Xia *et al.,* 2020). Big data can be summarised by five dimensions, known as the five Vs (Nativi *et al.,* 2015), which includes high quantity of data (Volume), speed of gathering data (Velocity), diversity in origins and structure of data (Variety), accuracy and reliability of data (Veracity), effective graphical or pictorial representation of complex data (Visualisation). For big data to be of value to research, all the five Vs need to be satisfied (Xia *et al.,* 2020).

Advances in image capturing and processing techniques have the potential to address several of the five Vs of big data. Utilising remote sensing and other autonomous methods to measure traits has the potential to improve the spatial and temporal resolution of trait data (Jackson *et al.*, 2022). They can be used to fill data gaps in understudied regions or taxa (e.g. tropical forests) and help resolve trait-environment context dependency issues present in many trait databases (Aguirre-Gutiérrez *et al.*, 2021). On smaller spatial scales, there is also emerging work to apply automatic data collection and monitoring techniques used by agronomists in controlled environments to more natural settings via field robots (Gadd *et al.*, 2024). These robots could aid in collecting “hard” trait data (e.g. ecophysiological traits) more rapidly, which would be particularly helpful in data hungry demographic trait-based research. Processing meaningful trait data from images is often time and labour-intensive. Open-source automation tools (e.g. Segment Anything Model) for image segmentation are expanding and could transform how large volumes of trait data are visualised (Kirillov *et al.*, 2023). The applications of automating image segmentation in trait-based research and practice are numerous, transcending disciplines (Middleton, 2023).

Increases in digitisation of museum specimens and herbaria present novel sources of trait data (Heberling, 2022; Brown *et al.*, 2023). Although traditionally in the domain of taxonomy and systematics, utilising collections as repositories for traits could help fill the taxonomic gaps in established trait databases. Collection-derived traits could be used to assess temporal intra and inter-specific trait variation in the more recent past (e.g. comparing field and herbarium traits in alpine grassland species, Jaroszynska *et al.*, 2023) or even deep time (e.g. fossil coral traits, Raja *et al.,* 2022). Advances in image and natural language processing tools (e.g. FloraTraiter) could compliment this effort further by automating and synthesising trait information from text in specimen records, monographs and floras (Folk *et al.,* 2024). Many of these new tools that integrate Big Data to trait-based methods are still in their infancy. They have the potential to generate new insights helping to fulfil the promise of trait-based approaches in addressing grand environmental challenges.

2.6 Concluding remarks

Traits encode rich and diverse information on biological entities such as individual organisms. Trait data are multidimensional, occupying a broad-spectrum of types (e.g. morphological, behavioural, phenological, physiological), sampling methods and sources (e.g. field measurements, remote sensing, databases) and structures (e.g. continuous, categorical, binary with diverse units). Just as trait data in themselves are diverse, so too are the disciplines that adopt trait-based approaches. From Aristotle (ca. 350 BCE) to Arnold (1983), traits have been used to classify, compare and contextualise the functioning and processes of biological entities through space and time. Trait-based approaches adopt a broad mode of study defined by the way biological entities or processes are studied rather than by organisational or geographical scale, making traits highly versatile and scalable. Increasingly, traits are being used mechanistically link and predict trait-trait and trait-environment relationships in the era of global change. Despite a rapid increase in trait-based studies, there remains a number of challenges that is hindering progress (Shipley *et al.,* 2016).

One of the six challenges I highlighted in this review was semantic issues around trait definitions and concepts within and across disciplines. Terminological issues influence the other five challenges (trait *selection*, *sourcing*, *scale*, data *structure* and *standardisation*). Focusing on three commonly used trait terms: functional, phenotypic and life history, I outlined the conceptual origins and developments of each term. Despite different conceptual genealogies, the traits terms share fuzzy boundaries in how they are used and applied. Functional traits approaches fall primarily in the ecology research stream, whilst evolution/genetics and population ecology/demography characterise phenotypic and life history trait approaches respectively. Common to all three trait approaches was individual organisms as the primary measurement unit, where life history traits are derived from population growth models. The bibliometric analysis showed some integration of disciplines in the latest decade of the 30-year (1991 and 2020) period examined, although taxonomic divides remained prevalent.

To improve synthesis and synergy within and between taxa and researchers/disciplines that adopt trait-based methods, the definition of a trait should be simplified to i) include broader definition of biological unit of measurement (to be inclusive of taxa where individuality is hard to define e.g. corals, eusocial insects or clonal organisms), ii) dropping reference to trait adjectives “functional”, “phenotypic” and “life history” unless specifically measuring a function or process (Dawson *et al.,* 2021). Finding common trait currencies (e.g. energy-mass based or body size traits) and contextualising trait data (e.g. sampling both traits and environmental variables in the same location) are two other ways to build closer empirical links between disciplines. Increasing knowledge exchange between researchers can be facilitated by increasing the number of journals issues and conference workshops examining themes pertinent to a wide range of researchers. Lastly, training courses (e.g. International Plant Functional Trait Course) can strengthen existing individual researcher knowledge of trait-based methods by considering the whole trait-data life cycle from project design to dissemination by adopting Open Science practices (Geange *et al.,* 2021). The incorporation of Big Data into trait-based research through automation of trait data collection, processing and analysis is likely to generate new insights and presents an opportunity for collaborations beyond the biological sciences (e.g. software developers, engineers and bioinformaticians).

In the same vein as the call that evolutionary biologists and functional ecologists should become demographers (Metcalf and Pavard, 2007; Laughlin, 2023a), I advocate that all researchers adopt an interdisciplinary lens while conducting trait-based research and be curious about conceptual genealogies. Innovative work by Lande and Arnold (1983), is an example of how building interdisciplinary bridges can help the collective advancement of trait-based theory and practice.

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Supplementary

Table S2.1. A selection of existing trait databases across the tree of life. Table adapted from de Bello *et al.* (2021). See also Open Traits Network (https://opentraits.org/datasets.html) for a more comprehensive repository of open access trait databases. Note source links may change.

Taxa	**Database**	**Geographic coverage**	**Main ecosystem(s)**	**Source (reference)**
Diverse taxa				
Plants and animals	MOSAIC	Global (mostly North America and Europe)	Terrestrial, marine	mosaicdatabase.web.ox.ac.uk (Bernard *et al.,* 2023)
Plants and animals	CESTES	Global (mostly North America and Europe)	Terrestrial	https://icestes.github.io/database (Jeliazkov *et al.,* 2020)
Plants and animals	COM(P)ADRE	Global	Terrestrial, marine, freshwater	https://compadre-db.org/ (Salguero-Gómez *et al.,* 2015)
Plants				
Vascular plants	TRY	Global	Terrestrial	https://www.try-db.org/TryWeb/Home.php (Kattge *et al.,* 2011)
Vascular plants	EcoFlora	Britain and Ireland	Terrestrial	http://ecoflora.org.uk/ (Fitter and Peat 1994)
Vascular plants	LEDA	Northwest Europe	Terrestrial	https://uol.de/en/landeco/research/leda (Kleyer *et al.,* 2008)
Vascular plants	AusTraits	Australia	Terrestrial	https://austraits.org/ (Falster *et al.,* 2021)
Vascular plants	FunAndes	South America	Terrestrial	https://doi.org/10.6084/m9.figshare.19665471 (Báez *et al.,* 2022)

Vascular plants	FLAMmability plant traiTS (FLAMITS)	Global	Terrestrial	https://doi.org/10.5061/dryad.h18931zr3 (Ocampo-Zuleta *et al.*, 2023)
Palms	PalmTraits	Global	Terrestrial	https://doi.org/10.6084/m9.figshare.9766919 (Kissling *et al.*, 2019)
Bryophytes	Bryophytes of Europe Traits (BET)	Europe	Terrestrial	https://envidat.ch/#/metadata/bet (van Zuijlen *et al.*, 2023)
Fine roots	Fine-Root Ecology Database (FRED)	Global	Terrestrial	https://roots.ornl.gov/ (Inversen *et al.*, 2017)
Clonal growth	CLO-PLA	Central Europe	Terrestrial	https://clopla.butbn.cas.cz/ (Klimešová *et al.*, 2017)
Algae	AlgaeTraits	Europe	Marine	https://algaetraits.org/ (Vranken *et al.*, 2023)
Fungi and Lichens				
Fungi	FungalTraits aka fun^{fun}	Global	Terrestrial	https://github.com/traitecoevo/fungaltraits (Flores-Moreno *et al.*, 2019)
Lichen	LIAS Light	Global	Terrestrial	https://liasgtm.lias.net/gtm.php (Rambold *et al.*, 2016)
Micro-organisms				
Bacteria	BactoTraits	Global	Terrestrial, freshwater	https://doi.org/10.24396/ORDAR-53 (Cébron *et al.*, 2021)
Bacteria and archaea	Bacterial and archaeal phenotypic trait database	Global	Terrestrial, freshwater, Marine	https://github.com/bacteria-archaea-traits/bacteria-archaea-traits (Madin *et al.*, 2020)

Invertebrates				
Ants	GlobalAnts	Global	Terrestrial	https://globalants.org/ (Parr *et al.*, 2017)
Spiders	The World Spider Trait database	Global	Terrestrial	https://spidertraits.sci.muni.cz/ (Pekár *et al.*, 2021)
Saproxylic beetles	Morphological trait database of saproxylic beetles	Europe	Terrestrial	https://doi.org/10.5061/dryad.2fqz612p3 (Hegge *et al.*, 2021)
Copepods	A trait database for marine copepods	Global	Marine	doi:10.1594/PANGAEA.862968 (Brun *et al.*, 2017)
Benthic invertebrates	The Arctic Traits Database	pan-Arctic	Marine	https://arctictraits.univie.ac.at/ (Degen and Faulwetter, 2019)
Freshwater invertebrates (Arthropoda, Mollusca, Annelida)	DISPERSE	Europe	Freshwater	https://doi.org/10.6084/m9.figshare.13148333 (Sarremejane *et al.*, 2020)
Freshwater invertebrates	Trait database for southern African freshwater invertebrates	Southern Africa	Freshwater	https://doi.org/10.6084/m9.figshare.22109269.v1 (Odume *et al.*, 2023)
Corals	The Coral Trait Database	Global	Marine	https://coraltraits.org (Madin *et al.*, 2016)
Corals	Ancient Reef Traits Database (ARTD)	Global	Marine	https://art.nat.fau.de/ (Raja *et al.*, 2022)
Diverse soil fauna	BETSI	Europe	Terrestrial	https://portail.betsi.cnrs.fr/ (Pey *et al.*, 2014)

Vertebrates				
Mammals	PanTHERIA	Global	Terrestrial	https://doi.org/10.6084/m9.figshare.c.3301274.v1 (Jones *et al.*, 2009)
Bats	AfroBaT	Africa	Terrestrial	https://doi.org/10.6084/m9.figshare.23599740.v1 (Cosentino *et al.*, 2023)
Birds	AVONET	Global	Terrestrial, aquatic	https://figshare.com/s/b990722d72a26b5bfead (Tobias *et al.*, 2022)
Fishes	The Pelagic Species Trait Database	Global	Marine	https://doi.org/10.5683/SP3/0YFJED (Gleiber *et al.*, 2024)
Amphibians	AmphiBIO	Global	Terrestrial	https://doi.org/10.6084/m9.figshare.4644424 (Oliveira *et al.*, 2017)
Reptiles	ReptTraits	Global	Terrestrial	https://doi.org/10.6084/m9.figshare.24572683.v4 (Oskyrko *et al.*, 2024)

Supplementary Note 2.1

Method used for constructing network visualisation map for the trait term associations over three decades shown in (Figure 2.3)

Studies were extracted from Web of Science (WoS) and then analysed in VOSViewer (V1.6.16). The sections below outline the process of searching, extracting, and decision-making process for constructing the visualisation maps for each decade.

1. 1991-2000

Search date:
9/6/2021

Search terms:
(from Web of Science Core Collection)
You searched for: **TOPIC:** ("functional trai*") *OR* **TOPIC:** ("life*history trai*") *OR* **TOPIC:** ("phenotypic trai*")

Results:
235

Refinements:
Refined by: WEB OF SCIENCE CATEGORIES: (ECOLOGY OR MATHEMATICAL COMPUTATIONAL BIOLOGY OR ENTOMOLOGY OR PLANT SCIENCES OR ENVIRONMENTAL SCIENCES OR SOIL SCIENCE OR MYCOLOGY OR EVOLUTIONARY BIOLOGY OR AGRICULTURE DAIRY ANIMAL SCIENCE OR AGRONOMY OR ZOOLOGY OR FORESTRY OR BIOLOGY OR HORTICULTURE OR MARINE FRESHWATER BIOLOGY OR FISHERIES OR VETERINARY SCIENCES OR AGRICULTURE MULTIDISCIPLINARY OR OCEANOGRAPHY)
Timespan: 1991-2000. Indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC.

- Categories that did not pertain to biological, ecological, agricultural or environmental sciences were excluded. Molecular and microbiology categories contained references to medical sciences so were excluded.

Document types:
All (e.g. articles, review, conference papers)

Selection criteria to create network visualisation in VOSViewer for 1991-2000 data extracted from Web of Science (WoS)

Step	**Action/Result**	**Comment**
Choose type of data	Create a map based on text data	Interest is to create network map of trait term associations from text data in studies
Choose data source	Read data from bibliographic datafiles	WoS
Select files	Select text files (.txt) from folder in computer	
Choose fields	Selecting Title and abstract from which terms will be extracted	Ignore structured abstract labels and copyright statements
Choose counting method	Full counting	All occurrences of the term are counted
Choose threshold	10	Of 7326 terms, 177 occurred more than 10 times
Choose number of terms	106	60% relevancy score of the 177 terms that occurred more than 10 times
Verify terms		Check to see if terms are ok
Number of clusters	5	Cluster 1 “evolution” = 25 items Cluster 2 “demography” = 22 items Cluster 3 “plant genetics” = 22 items Cluster 4 “animal agriculture” = 21 items Cluster 5 “microbiology” = 16 items
Top 10 terms (occurrences)	1= Marker (98) 2= Functional trait (85) 3= Strain (80) 4= Change (72) 5= Group (71) 6= Gene (69) 7= Evolution (61) 8= Phenotype (59) 9= Male (58) 10= Production (55)	Terms that occur with the highest frequency
Number of links	2456	
Total link strength	22482	
Weights	Based on occurrences	
Clustering	Minimum clustering size = 5	Terms that form a cluster of less than 5 terms are merged with larger clusters

2. 2001-2010

Search date:
11/6/2021

Search terms:
(from Web of Science Core Collection)
You searched for: **TOPIC:** ("functional trai*") *OR* **TOPIC:** ("life*history trai*") *OR* **TOPIC:** ("phenotypic trai*")
Results:
1,761

Refinements:
Refined by: WEB OF SCIENCE CATEGORIES: (ECOLOGY OR ENTOMOLOGY OR PLANT SCIENCES OR OCEANOGRAPHY OR EVOLUTIONARY BIOLOGY OR FISHERIES OR AGRICULTURE DAIRY ANIMAL SCIENCE OR AGRONOMY OR FORESTRY OR MYCOLOGY OR ZOOLOGY OR PARASITOLOGY OR BIOLOGY OR ENVIRONMENTAL SCIENCES OR HORTICULTURE OR MARINE FRESHWATER BIOLOGY OR LIMNOLOGY OR BIODIVERSITY CONSERVATION OR VETERINARY SCIENCES OR AGRICULTURE MULTIDISCIPLINARY OR MATHEMATICAL COMPUTATIONAL BIOLOGY OR SOIL SCIENCE)
Timespan: 2001-2010. Indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC.

- Categories that did not pertain to biological, ecological, agricultural or environmental sciences were excluded. Molecular and microbiology categories contained references to medical sciences so were excluded.

Document types:
All (e.g. articles, review, conference papers)

Selection criteria to create network visualisation in VOSViewer for 2001-2010 data extracted from Web of Science (WoS)

Step	**Action/Result**	**Comment**
Choose type of data	Create a map based on text data	Interest is to create network map of trait term associations from text data in studies
Choose data source	Read data from bibliographic datafiles	WoS
Select files	Select text files (.txt) from folder in computer	
Choose fields	Selecting Title and abstract from which terms will be extracted	Ignore structured abstract labels and copyright statements
Choose counting method	Full counting	All occurrences of the term are counted
Choose threshold	10	Of 42450 terms, 1357 occurred more than 10 times

Choose number of terms	814	60% relevancy score of then 814 terms that occurred more than 10 times
Verify terms		Check to see if terms are ok
Number of clusters	4	Cluster 1 “Plant community ecology” = 344 items Cluster 2 “plant crop genetics and plant evolution” = 211 items Cluster 3 “animal agriculture” = 161 items Cluster 4 “animal demography” = 97 items
Top 10 terms (occurrences)	1= Community (527) 2= Gene (464) 3= QTL (356) 4= Marker (304) 5= Genotype (291) 6= Male (286) 7= Temperature (276) 8= Production (275) 9= Forest (245) 10= Leafe (245)	Terms that occur with the highest frequency
Number of links	58332	
Total link strength	362173	
Weights	Based on occurrences	
Clustering	Minimum clustering size = 5	Terms that form a cluster of less than 5 terms are merged with larger clusters

3. 2011-2020

Search date:
11/6/2021

Search terms:
(from Web of Science Core Collection)
You searched for: **TOPIC:** ("functional trai*") *OR* **TOPIC:** ("life*history trai*") *OR* **TOPIC:** ("phenotypic trai*")

Results:
10,884

Refinements:
Refined by: WEB OF SCIENCE CATEGORIES: (ECOLOGY OR PLANT SCIENCES OR ENVIRONMENTAL SCIENCES OR REMOTE SENSING OR FORESTRY OR MYCOLOGY OR EVOLUTIONARY BIOLOGY OR ENVIRONMENTAL STUDIES OR MULTIDISCIPLINARY SCIENCES OR BIODIVERSITY CONSERVATION OR MARINE FRESHWATER BIOLOGY OR AGRONOMY OR ORNITHOLOGY OR PARASITOLOGY OR BIOLOGY OR ZOOLOGY OR AGRICULTURE DAIRY ANIMAL SCIENCE OR SOIL SCIENCE OR HORTICULTURE OR

AGRICULTURE MULTIDISCIPLINARY OR VETERINARY SCIENCES OR OCEANOGRAPHY OR ENTOMOLOGY OR MATHEMATICAL COMPUTATIONAL BIOLOGY OR FISHERIES OR LIMNOLOGY) Timespan: 2011-2020. Indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC.

- Categories that did not pertain to biological, ecological, agricultural or environmental sciences were excluded. Molecular and microbiology categories contained references to medical sciences so were excluded.

Document types:
All (e.g. articles, review, conference papers)

Selection criteria to create network visualisation in VOS Viewer for 2011-2020 data extracted from Web of Science (WoS)

Step	**Action/Result**	**Comment**
Choose type of data	Create a map based on text data	Links to aim
Choose data source	Read data from bibliographic datafiles	WoS
Select files	Select text files (.txt) from folder in computer	
Choose fields	Selecting Title and abstract from which terms will be extracted	Ignore structured abstract labels and copyright statements
Choose counting method	Full counting	All occurrences of the term are counted
Choose threshold	15	Of 197330 terms, 5119 occurred more than 15 times. Due to large volume of terms, the threshold was increased from 10 to 15
Choose number of terms	3071	60% relevancy score of then 3071 terms that occurred more than 15 times
Verify terms	Check to see if terms are ok	
Number of clusters	5	Cluster 1 “plant traits” = 1086 items Cluster 2 “plant community ecology + animal ecology” = 852 items Cluster 3 “plant genetics/crops” = 485 items Cluster 4 “animal demography” = 418 items Cluster 5 “animal agriculture” = 230 items
Top 10 terms (occurrences)	1= Population (4350) 2= Phenotypic trait (2904) 3= Functional diversity (2475) 4= Selection (1811)	Terms that occur with the highest frequency

	5= Gene (1764) 6= Species richness (1394) 7= Evolution (1363) 8= Production (1354) 9= Genotype (1300) 10= Phenotype (1087)	
Number of links	463664	
Total link strength	2369673	
Weights	Based on occurrences	

Chapter 3: Experimental drought reduces the productivity and stability of a calcareous grassland

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Abstract

1. Grasslands, which encompass 40% of terrestrial ecosystems, hold global significance for food production, carbon storage, and other ecosystem services. However, grasslands in many areas are becoming increasingly exposed to extreme wet and dry periods resulting from climate change.

2. Therefore, understanding how grasslands will respond to precipitation extremes is a pressing issue for managing changes to biodiversity and ecosystem service provision.

3. Here, we use experimental manipulations of precipitation (50% increase and 50% decrease of growing-season precipitation) over six years to investigate the stability and resistance of both productivity and community diversity in a calcareous grassland community.

4. We found that decreased growing season precipitation led to reductions of mean productivity (25 % decrease in peak above-ground biomass) and its temporal stability (54 % increase in the coefficient of variation of biomass across years). Productivity losses were more pronounced for graminoids and legumes, but this was not reflected in species percent cover. Community composition was resistant to the precipitation manipulations, with no clear differences in overall community compositional turnover, dissimilarity, or biodiversity indices. There was no detectible effect of increased precipitation on productivity or community composition, which may indicate a lack of efficacy of the irrigation treatment in increasing soil moisture. Finally, the precipitation manipulations had no effect on temporal trends of

community change, with an overall increase in richness and shift in grassland community composition across the study period independent of treatment.

5. While the diversity and composition of this calcareous grassland was resistant to precipitation extremes (at least in the short term), sustained reductions in growing-season precipitation reduced productivity and its temporal stability, particularly for graminoids and legumes. Therefore, we highlight that different properties of grasslands can vary in their responses to changes in precipitation.

Keywords: Biodiversity change, climate change, community resilience, DroughtNet, field experiment, precipitation.

3.1. Introduction

As a dominant terrestrial biome important for ecosystem services and food security, understanding the response of grasslands to climate change, and in particular changing precipitation patterns, is critical (Fay *et al.,* 2008; Franklin *et al.,* 2016; Hopkins and Prado, 2007). Despite the threat of climate change, its effects on plant communities are not uniform, and complex responses highlight the need for targeted community-level experiments (Gupta *et al.,* 2020; Harrison *et al.,* 2015; Kardol *et al.,* 2010; Parmesan and Hanley, 2015). Key components of climate change that are expected to influence plant communities are more changeable, and more extreme, precipitation patterns (Hopkins and Prado, 2007; Knapp *et al.*, 2015). For grasslands, which occupy 40% of terrestrial ecosystems and provide many ecosystems services (Abberton *et al.,* 2010; O'Mara, 2012; Petermann and Buzhdygan, 2021), declines in use and extent after agricultural intensification, conversion, and grazing may be exacerbated by precipitation shifts (Meng *et al.,* 2021; Peeters, 2009). Thus, studying how grassland communities will respond to precipitation change will aid in in safeguarding these globally important habitats.

In grasslands, one way that community responses to precipitation change have been explored is through productivity. Productivity in grasslands is typically measured through biomass (Fahey and Knapp, 2007). Indeed, there have been wide efforts to understand how grassland biomass changes with precipitation (Grime *et al.,* 2008; Knapp *et al.,* 2017; Kröel-Dulay *et al.,* 2022; Smith *et al.,* 2024). Generally, drought conditions reduce primary productivity in grasslands (Herben *et al.,* 1995; Kardol et al., 2010; Smith et al., 2024; Wang *et al.,* 2007), which can impact ecosystem services such as pollination (Phillips *et al.,* 2018). However, despite overall reductions in productivity, grassland biomes with different baseline climatic conditions react differently, where arid or xeric biomes are more susceptible to losses in productivity with drought than mesic biomes (Cleland *et al.,* 2013; Huxman *et al.,* 2004; Moran *et al.,* 2014). Productivity may also recover quickly when drought conditions subside, even in semi-arid grasslands (Luo *et al.,* 2023). Conversely, there are relatively fewer studies investigating increases to precipitation (or irrigation), but a global meta-analysis (although largely in North America) revealed that ecological experiments with supplemented watering alone did not change biomass (DeMalach *et al.,* 2017). Overall, there are grassland productivity declines in drought conditions, but understanding temporal dynamics in productivity with respect to precipitation is crucial in a wider range of grassland habitat types.

Community composition differences driven by changing precipitation patterns are less clear in grasslands. Harrison *et al.* (2015) presented findings that reductions in midwinter precipitation over 14 years reduced biodiversity in Californian grasslands. Furthermore, a recent study found that after 11 drought years, grassland communities in a German semi-arid grassland shifted to favour dry-grassland specialists and ruderal species, and composition change was mediated by microclimatic conditions of site topology (Mazalla *et al.,* 2022). However, another long-term study of experimental precipitation manipulation in an infertile UK grassland found that although communities in drought (but not irrigated) conditions differed from control plots, interannual variation in natural precipitation and broad shifts in the community through time were better explanations for community change (Grime *et al.,* 2008). Several studies have found that grassland communities are resistant to both experimentally-induced drought and natural variation in precipitation (Cleland *et al.,* 2013; Craine *et al.*, 2013; Grime *et al.,* 2000; Sternberg *et al.,* 1999). A key mechanism proposed for community resistance to precipitation extremes is buffering via increased species diversity, whereby more species create redundancy in functional composition that is better adapted to withstanding precipitation extremes (Craine *et al.,* 2013; Craven *et al.,* 2018; Griffin-Nolan *et al.*, 2019; Wagg *et al.,* 2017). Furthermore, perceived resistance may be observed due to the lifespan of perennial plant species in a community, with much greater community differences in the seed-bank resulting in a delayed response (Basto *et al.,* 2018). To fully elucidate the temporal dynamics of community composition as precipitation patterns change, we need further long-term monitoring of grassland systems.

A notable case-study of a grassland that is vulnerable to climate change are the calcareous grasslands, which we focus on here. Calcareous grasslands are typified by alkaline soils, often occurring on limestone or chalk bedrock, supporting up to 700 vascular plant species in Europe, and providing a wide range of ecosystem services such as pollination, carbon sequestration, and recreation *(*Gibson and Brown, 1991; Grêt-Regamey *et al.,* 2014; Klaus *et al.,* 2021; Willems, 1990). There have been declines in the extent of many European calcareous grasslands, following agricultural intensification, scrub encroachment and grazer management (Grêt-Regamey *et al.,* 2014; Ridding *et al.,* 2020), which may accentuate effects of climate change. To preserve high levels of species diversity, calcareous grasslands are listed as recovering ecosystems and part of wider conservation management schemes in countries such as the United Kingdom (Gibson, 1986; Gibson and Brown, 1991; Maddock, 2008; Poschlod *et al.,* 1998).

Here, we use six-years (2016-2021) of biodiversity data from an experimental manipulation (50% increase and 50% decrease) of precipitation in a calcareous grassland to examine productivity and community resistance to precipitation change, an important component of climate change. We extend previous work on calcareous grasslands (Grime *et al.,* 2000, 2008) through an analysis of detailed temporal dynamics in community composition and productivity, using annually replicated data. Specifically, we answered three key questions: i) whether changes in precipitation affect above-ground annual net primary productivity (above-ground ANPP) and its temporal stability across years, ii) whether calcareous grassland communities have a high resistance to drought conditions, and which species are important for driving community differences, and iii) whether the effect of precipitation manipulation change over time, and whether these temporal dynamics affected by natural precipitation patterns. We address these questions by quantifying stability in productivity and resistance in community composition, which are useful conceptual terms to integrate community responses to climate change and tools to compare communities from different ecosystems (Allen *et al.,* 2019; Donohue *et al.,* 2013; Pimm, 1984; Van Meerbeek *et al.,* 2021). For stability, we monitored shifts in the mean and variability of productivity through time with respect to precipitation treatments. Shifts in abundance or productivity serve as early warning signals of community shifts, and indicate decreased stability in ecological communities (Clements and Ozgul, 2016; Pimm, 1984). Then, we use the definition of resistance for community composition from Van Meerbeek *et al.* (2021), where community resistance is the ability of community composition to resist changes in system variables in response to a perturbation, which here is drought or increase to precipitation through time.

3.2. Methods

3.2.1 Study site

The RainDrop (rainfall and drought platform) experiment is situated in a ~2 ha area (‘five-acre field’) in the Upper Seeds grasslands (51°46’16.8”N 1°19’59.1”W, 166 m a.s.l) of the University of Oxford’s Wytham Woods estate, Oxfordshire, UK (Figure 3.1). Upper Seeds is a recovering calcareous grassland, which was intermittently used for arable agriculture from the second world war until the late 1970s, before the site was managed as a grassland beginning in 1978 (Gibson, 1986; Grime *et al.,* 2000). In the 1990’s the site was also home to a climate change experiment with winter warming and summer drought treatments (Cummins *et al.,* 1995). Grazing by sheep has been excluded throughout the experiment but did occur before the experiment. Upper Seeds, as with other calcareous grasslands, has a high level of floral biodiversity, in which graminoids constitute ~60% of species by biomass. Management consists of mowing all above-ground vegetation in mid-July at the peak of the growing season, and again in early October, coinciding with the end of the growing season. Biomass is removed following mowing. The site has a shallow soil depth (300-500 mm), alkaline soils (Gibson and Brown, 1991), a daily average temperature range of -5 °C to 26 °C (2016-2020), and a daily total precipitation range of 0-40 mm (2016-2020) (Rennie *et al.,* 2017).

3.2.2 Experimental design

We explored grassland biodiversity responses to precipitation in the context of the global drought network (DroughtNet) international drought experiment. DroughtNet’s international drought experiment is a coordinated distributed experiment with over 100 sites globally (https://droughtnet.weebly.com/) (Smith *et al.,* 2024). The goal of the

DroughtNet experimental network is to explore ecosystem sensitivity to precipitation extremes through experimental manipulations of precipitation (Knapp *et al.,* 2017). Precipitation manipulation is carried out by modifying natural precipitation patterns in each plot with rainout shelters, acting as a press disturbance (continuous change in environment) maintained across several years. The manipulation was implemented in RainDrop as a randomised, replicated block design in which four treatments were repeated across five blocks (n = 20 experimental plots; Figure 3.1a blocks A-E), in order to account for spatial variation and site topography, which varies by 5 m within the experimental area. Plots were therefore spatially nested within each block, which we accounted for with nested hierarchical random effects of plots within blocks in analyses.

Each replicated unit of 5 m × 5 m plots had one of four experimental treatments: ambient control plots (Ambient control), -50% precipitation rainout shelters to simulate drought (Drought), +50% irrigated plots with sprinklers to simulate increased precipitation (Irrigated), and procedural controls (Procedural control; precipitation shelter with no change to precipitation) (Figure 3.1a). Biodiversity data collection occurs in the central 1 m × 1 m quadrat in one quarter of each 5 m × 5 m plot, where the data collection quarter was randomised at the beginning of the experiment (Figure 3.1c). Therefore, each experimental quadrat has a buffer of 75 cm, which prevents wind-driven rain from entering the experimental quadrat (Figure 3.1c).

Rainout shelters consist of metal structures 1.5-2 m above the ground with transparent Perspex guttering. In the drought treatment, the rainout shelter gutters are approximately 10.4 cm wide, and spaced such that 50% of the surface area of the plot is blocked by guttering. Irrigated treatment plots are supplied by water containers from the drought treatment, which collects precipitation with the transparent Perspex gutters, such that the 50% additional precipitation is comprised of precipitation lost to the shelter (Gherardi and Sala, 2013). Procedural control plots were crucial to test the potential confounding effect of the drought treatment, in which similar structures and guttering are in place to simulate the micro-climatic conditions of the shelter, but with guttering inverted to allow natural precipitation levels (Figure 3.1b). For example, although Perspex guttering was transparent to allow light to reach the plot, it will reduce the light available to the grassland community to some degree. Thus, the procedural control enabled us to account for any such micro-climatic effects. Precipitation manipulation percentages were selected through an assessment of long-term precipitation records, which found that extremes of annual precipitation differed from average years by ~40% (Knapp *et al.,* 2015). At RainDrop, all drought treatments are removed between October and March of each year, when the Perspex gutters are inverted to restore natural precipitation levels, such that the experimental treatments are active during the growing season. However, rainout shelters and guttering remain throughout the year. To further test the validity of the experimental treatments at this site, we used preliminary data on soil moisture recorded from 13 of the 20 plots between August-September 2022. We found that simulated drought reduced median raw soil moisture by 37%, while there was only a modest soil moisture increase of 7% in the Irrigated treatment (Figure S3.1).

3.2.3 Data collection

The core experimental protocol consists of biodiversity and productivity monitoring within experimental plots, namely species diversity and abundance, and aboveground net biomass production. To explore how precipitation manipulation influences grassland dynamics and composition, we monitored three main features of biodiversity: total community above-ground annual net primary productivity (ANPP), functional group level above-ground biomass, and species-level percentage cover of vascular plants, for each 1 m × 1 m quadrat in each year between 2016-2021. We define ANPP as the total above-ground biomass of vascular plants across the growing season in each year, which estimates the investment of energy into growth of plant structures. We collected biomass at the peak of the growing season, 20th June – 14th July, and at the end of September, such that ANPP was estimated for the entire growing season. We estimated ANPP using a 'clip strip' of all vascular plant material in a 1 m × 0.25 m strip in the centre of each quadrat, collected after percentage cover data. The size of clip strips was chosen to efficiently measure biomass across all plots in sufficient time, and representativeness of the biomass samples was ensured through spatial replication in blocks. Clip strips were gathered using hand trimmers ~ 1 cm above the soil surface. Within one day of collection, we sorted clip strips in to five functional groups: graminoids, legumes, non-leguminous forbs, woody species, and bryophytes and dried them at 70°C for 48 hrs, before weighing the dry biomass with an accuracy of ± 0.1g. Forbs are defined as any herbaceous flowering plant other than a graminoid. We used both functional group-level estimates of biomass and summed values of ANPP in analyses, which were scaled by a factor of four to the standardised measure of g m^{-2}. Due to smaller biomasses estimated for woody (1.65 % of total biomass) and bryophyte groups (1.20 % of total biomass), we only included graminoids, legumes and forbs in subsequent ANPP analyses.

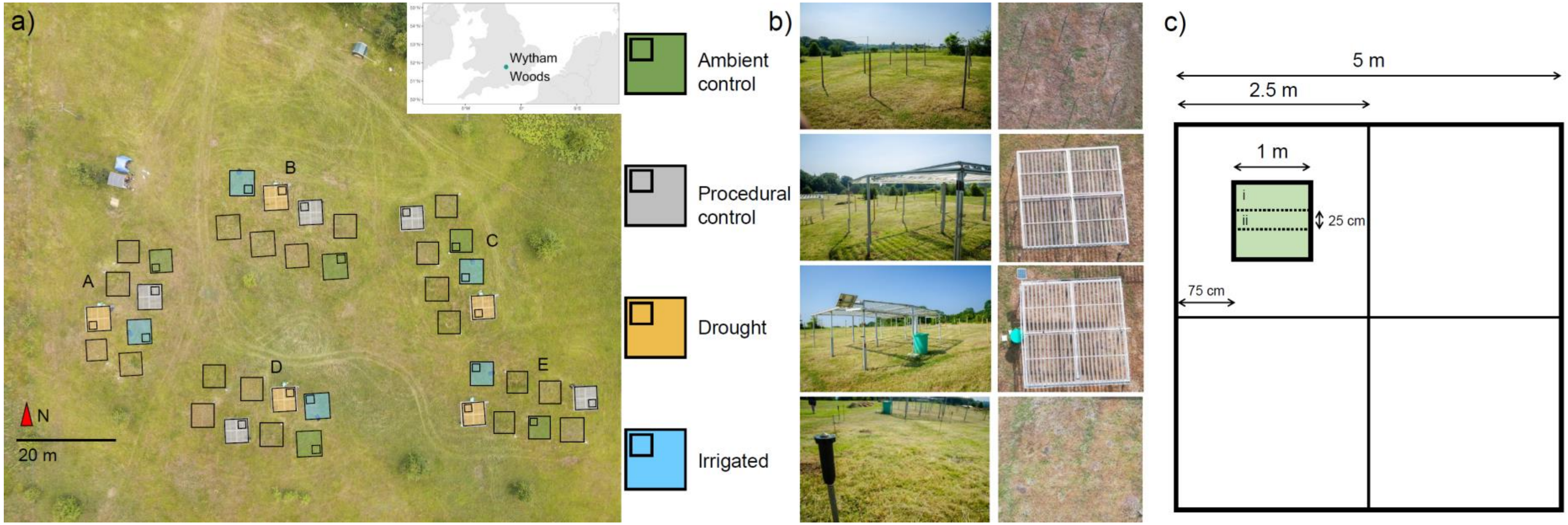

Figure 3.1 Experimental schematic of RainDrop on Upper Seeds, Wytham Woods with DroughtNet coordinated distributed experiment plots. a) DroughtNet treatments are denoted by the colour of each 5 m × 5 m plot, and include: Ambient control (green; no manipulation), Procedural control (grey; rainfall shelter but no change in rainfall), Drought (orange; -50% rainfall shelter), and Irrigated (blue; +50% rainfall with sprinklers). Letters indicate the five replicated and randomised experimental blocks A-E. b) Photos including ground-level (left) and aerial (right) views of each treatment type. Smaller squares indicate the biodiversity data collection area in each plot, the positions of which were randomised. c) Experimental design schematic for each plot. Full 5 m × 5 m plot area, subdivided into quarters (2.5 m × 2.5 m), one of which contained the observation quadrat (green square - 1 m × 1 m) used in this experiment. Species percentage cover (i) and biomass (ii) was collected from the observation quadrat. Each quadrat had a buffer of 75 cm.

Percentage cover data collection occurred before the peak of the growing season in each year, in mid to late June. We estimated the percentage cover of all vascular plant species in each quadrat. Because species overlapped spatially, percentage cover estimates exceed 100%. Species names follow the International Plant Names Index (IPNI, 2022).

We added environmental context and explored how biodiversity changes are influenced by local weather patterns using weather data from the National Environment Research Council (NERC) Environmental Change Network (Rennie *et al.*, 2017). A meteorological station was present in five-acre field within 100 m of all experimental quadrats. Raw meteorological data consisted of 16 weather variables, which were gathered at hourly intervals between 2016-2020, but data were not available in 2021. We used the mean hourly precipitation and temperature in the spring (21st March – 20th June) and summer (21st June – 22nd September) (the growing season) for each year of study as weather variables of interest.

3.2.4 General analysis

We analysed the experimental data with hierarchical Bayesian regression models using the *brms* package (Bürkner, 2017) in R version 4.1.3 (R Core Team, 2022). To perform model selection, we estimated the out-of-sample predictive performance of candidate models relative to base models that excluded predictor variables of interest. For each candidate model, we performed leave-one-out cross validation with the *loo* criterion and the expected log-wise predictive density ($elpd$, where $\Delta elpd$ gives the change in $elpd$ relative to another explanatory model) (Vehtari *et al.,* 2017). Therefore, $elpd$ gives an estimate of predictive performance that is analogous to an information criterion. Where two candidate models were comparable in $elpd$ ($\Delta elpd < 2$), we reported the model with fewer explanatory variables and explored the posterior coefficients of the model to make inference. Models were run across four Markov Chain Monte Carlo chains for 4,000 iterations with 2,000 warm-up iterations, and the convergence of the model across chains was assessed by inspecting $\hat{R}$ values, which assesses the degree of mixing between chains (Bürkner, 2017). Model priors were selected using prior predictive simulations, where ranges of coefficient values were assessed for plausibility relative to prior distribution parameters. For the full set of model priors, please refer to model code (10.5281/zenodo.8135588). Following model selection, we performed a set of Bayesian hypothesis tests (Bürkner, 2017) to investigate whether there were consistent differences in biodiversity and biomass measures between procedural control and ambient control treatments, and the proportion of variance explained by the random effect of experimental block. Differences between control treatments were evaluated by comparing posterior distributions between ambient control and procedural control groups. We used the intraclass correlation coefficients (ICC) (Nakagawa and Schielzeth, 2010) to assess the proportion of variance explained by the random effect of block relative to the total population-level variance.

3.2.5 Testing the effect of precipitation on grassland productivity and its temporal stability

To answer our first key question, we analysed how precipitation manipulation influences ANPP and its temporal stability. We examined responses at both the whole community level and at the functional group level, as well as the inter-annual temporal stability of above-ground ANPP. We define the temporal stability of productivity as the inverse of the interannual coefficient of variation (CV) of above-ground ANPP (He *et al.,* 2022) for each quadrat. Where the response variable was the annual total and group-level ANPP, raw ANPP values were transformed using the natural logarithm, which were then modelled using a Gaussian distribution (although similar results were obtained using a Gamma distribution; Figure S3.3). In the ANPP models, the key predictors of interest were precipitation treatment and observation year. We used model selection to test the predictive performance for a set of candidate models including precipitation treatment (categorical variable, four levels), a linear effect of the observation year (continuous variable, z-scored), an autoregressive term for the observation year (order = 1), and two-way interactions between precipitation treatment and observation year (linear). We investigated linear effects of observation year to capture broad changes in the community through time, but with insufficient temporal sampling to ascertain non-linear dynamics. Additional annual fluctuations were tested with effects of local environmental variables, and random effects of observation year. We compared candidate models to base models that excluded predictor variables of precipitation treatment and observation year. ANPP models estimated at the level of functional group also included terms for functional group (categorical variable, three levels). The full set of candidate models for each ANPP response variable is detailed in supplementary tables S1-3. We also included a categorical predictor term for the month of harvest (middle or later part of growing season).

In all models, we included a nested (hierarchical), intercept-only random effect of the precipitation treatment (four levels) within block (five levels) to account for the experimental structure of RainDrop, and an intercept-only random effect of observation year (six levels) to capture additional interannual variability. Nested intercept-only random effects were appropriate for this study due to the static spatial organisation of plots (each with one replicate of each treatment) within blocks in the current experiment (Figure 3.1a), for which we aimed to account for additional variance from the treatments x block interaction. Models of the stability of ANPP do not include temporal effects, because the metric captured interannual variability in biomass for each quadrat resulting in a single value per plot. Thus, for temporal stability models we tested a candidate model with the precipitation treatment to the base model with no predictor variables (Table S3.3). When analysing temporal stability of ANPP, we validated the patterns in stability by testing the log-linear relationship between variance in ANPP and mean ANPP for each plot using Pearson's correlation, to test the role of Taylor's Power Law in driving these effects (Reckling et al., 2021) (Figure S3.5). In ANPP models, we used weakly informed priors of $N(3.5, 0.5)$ for the global intercept term and $N(0, 1)$ for predictor variables. The intercept-only random effects were fitted using exponential priors with rates between 4-8. Exponential priors aligned with the variance terms of random effects, which are constrained to be positive (unlike a gaussian distribution).

3.2.6 Testing the resistance of grassland communities to precipitation treatments

We answered our second key question by investigating whether the grassland community was resistant to precipitation treatments in three ways: with broad diversity indices, using community composition and turnover, and exploring individual species contributions to community change. First, we explored how broad diversity indices at the quadrat level were influenced by the precipitation treatments using linear hierarchical mixed-effects model selection, in an identical procedure as described in section 3.2.4. We calculated biodiversity indices using the relative proportions, p, of each species from percentage cover estimates. The three biodiversity indices included were vascular plant species richness, the Shannon-

Weiner diversity index, $H = -\sum p \ \ln p$ (Shannon and Weaver, 1963), and the Simpson's diversity index, $D = \sum p^2$ (Simpson, 1949). For the Shannon-Weiner and Simpson's indices, response variables were z-scored (mean and variance centered on 0) for analyses and models were fitted using a Gaussian distribution. Models with species richness counts were fitted using a Poisson distribution and a log link. Shannon-Weiner and Simpson's models were fitted with regularising priors of $N(0, 0.5)$ for both intercept and predictor terms, and Richness models were fitted with a prior of $N(3, 0.25)$ for the intercept.

To assess how the grassland community composition varied between precipitation treatments we tested community dissimilarity using non-metric multi-dimensional scaling (NMDS) implemented in the *vegan* package (Clarke, 1993; Oskanen *et al.,* 2022). Community-level data consisted of species percentage cover data for each quadrat, which is a single treatment in a given plot each year. We fitted the NMDS using the Bray-Curtis dissimilarity index with a dimension of three, and up to 1000 random starts to reach convergence in stress values. Then, we compared the first two dominant NMDS axes between precipitation treatments. We used hierarchical linear mixed-effects models with a response variable of the NMDS axis scores, with model selection as described in section 3.2.4. We fitted these models with regularising priors of $N(0,0.1)$ for both the global intercept and predictor variables. Then, in addition to the linear modelling framework, we explored statistical differences in Bray-Curtis similarities between precipitation treatments using analysis of similarities. Analysis of similarities tests differences in dissimilarity within sampling units compared to between sampling units (Oksanen *et al.,* 2022). We also explored block-level community effects by testing the dissimilarity between experimental blocks by pooling data from the replicates within each block.

To further explore the drivers of community composition differences between sampling groups of precipitation treatments and observation years, we investigated which species were most important for community differences using Similarity Percentage analysis. Similarity Percentage is an extension of the Bray-Curtis dissimilarity measure for individual species within a community, for which the dissimilarity metric per species is averaged across pairs of sampling groups to give an overall contribution (standardised to sum to 1) of each species to differences for a set of sampling groups of interest (Clarke, 1993; Oskanen *et al.,* 2022). We tested Similarity Percentage for the sampling groups of precipitation treatment, observation year, and blocks. Then, following Similarity Percentage analysis, we explored variation in the relative abundance, *p*, of influential species between communities.

3.2.7 Testing the temporal dynamics of the community and its drivers

We answered our third key question by exploring the temporal dynamics in the stability and resistance of the grassland community, and its potential climatic drivers. We estimated temporal trends in productivity, diversity indices and NMDS axes, as well as the similarities in floral communities between observation years. We extracted temporal trends from linear models of biodiversity specified in section 3.2.5, which included linear or autoregressive effects of observation year, as well as two-way interactions between precipitation treatments and observation year. In addition to model selection, we also performed analysis of similarity tests for the NMDS communities between observation years (Oskanen *et al.,* 2022). Therefore, by assessing the temporal change in diversity indices and NMDS scores, we tested the temporal dynamics in this calcareous grassland. Furthermore, we explored which species had the largest changes in abundance over the study period, and thus which may have contributed most to temporal dynamics. For each species, we estimated the linear association between observation year and relative abundance, and explored which species had significant changes in relative abundance across plots. Finally, to explore whether environmental drivers mediated temporal dynamics in the community, we tested how local weather variables influenced ANPP and biodiversity indices. Following the model selection framework in 3.2.4, we implemented linear models to investigate the impact of mean temperature and precipitation across the spring and summer influenced biodiversity indices. In these models, linear terms for observation year were replaced with annual mean weather variables. We included both weather data for the current year and the previous year relative to biodiversity data collection, to test for current and lagged impacts of local weather on biodiversity differences.

3.3 Results

3.3.1 Simulated drought reduces biomass production and its temporal stability

We found that simulated drought substantially reduced both above-ground ANPP and productivity's temporal stability (Figure 3.2). Across all observation years and experimental quadrats, there was a mean total ANPP of 303 ± 138 (S.D.) g m^{-2}. Graminoids were the dominant functional group by biomass, with 63.6% of all biomass measured, compared to 20.4% for legumes and 16.0% for forbs. For total annual above-ground ANPP between 2016-2021, the model with the highest predictive performance, and fewest parameters, was the model including only the categorical effect precipitation treatment ($\Delta elpd$ = 5.03 relative to the base model; Table S3.1). Total above-ground ANPP was substantially reduced in the drought treatment (β = -0.77 [-1.04; -0.49]; β coefficients give the posterior mean difference compared to the ambient control treatment on the log-scale, with the 95%

credible intervals), with a mean total above-ground ANPP of 137 ± 156 (S.D.) g m^{-2} compared to 182 ± 137 (S.D.) g m^{-2} for the ambient control treatment (Figure 3.2a). Thus, compared to ambient conditions, the mean ANPP was reduced by 24.7% in the drought treatment. In contrast, irrigated plots did not have any substantial change in ANPP compared to the ambient control (β = -0.10 [-0.37; 0.18]; Figure 3.2a). We did not find evidence for either an overall linear temporal trend in ANPP, or for an interaction between observation year and treatment (Table S3.1; Figure S3.2). The model with the highest predictive performance included the linear effect of observation year, but the $elpd$ was not substantially increased relative to the simpler model including only the effect of treatment (Table S3.1), and the raw data showed no clear temporal pattern in ANPP (Figure S3.2). There was weak negative skew in raw ANPP values, but skew did not impact model convergence (Figure S3.3). Furthermore, we re-ran model selection using a gamma distribution, which did not change the qualitative results (Figure S3.3b). We observed a similar pattern for temporal stability in productivity, with a $\Delta elpd$ of 3.15 for the model including precipitation treatment compared to the base model (Table S3.3). Temporal stability in productivity was substantially reduced in the drought treatment (β = -0.19 [-0.32; -0.06]), equating to a 53.5% increase in the coefficient of variation of biomass (CV = 0.56) relative to the ambient control treatment (CV = 0.37) (Figure 3.2b). There were no discernible differences in temporal stability in productivity for both ambient control and procedural control treatments or the irrigation treatment (Figure 3.2b). Furthermore, we did not find evidence for a log-linear relationship between variance in ANPP and mean ANPP for each plot (ρ = 0.42, p = 0.07), increasing our confidence in our observation of reduced stability (Figure S3.5).

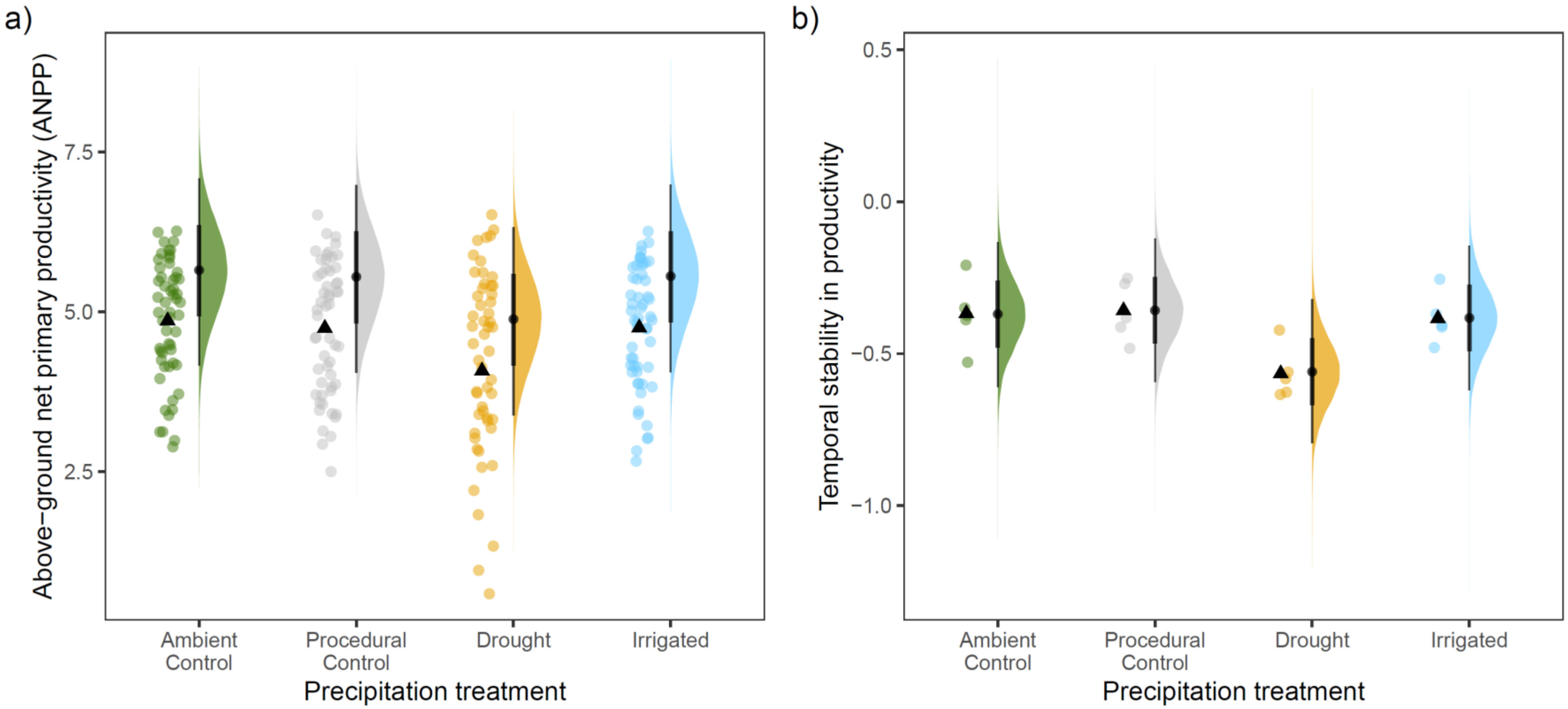

Figure 3.2 Drought reduces ANPP and its temporal stability. a) Total above-ground annual net primary productivity (above-ground ANPP) with respect to precipitation treatments, where ANPP is the natural log-transformed above-ground biomass in g m^{-2}. b) Temporal stability in productivity (inverse of interannual coefficient of variation in biomass) with respect to precipitation treatment. Coloured points give raw data across blocks and years and black triangles give the mean total ANPP. There is negative skew in raw ANPP data, which leads to reductions in mean ANPP values relative to predictions, but this skew did not impact model convergence or influence our findings (Figure S3.3). Distributions are derived from 8000 draws of the full posterior distribution including random effects, with probability density function boxplots giving the posterior mean and uncertainty.

The reductions in overall ANPP were driven primarily by decreases in the biomass of graminoids and legumes (Table S3.2; Figure S3.4). Forbs did not exhibit ANPP reductions in the drought treatment (β = -0.03 [-0.46; 0.39]), but reductions were accentuated in both graminoids (β = -1.00 [-1.41; -0.59]) and legumes (β = -0.87 [-1.29; -0.46]) (Figure S3.4). Therefore, we observed mean reductions in ANPP of 36.1% and 36.4% for graminoids and legumes, respectively. Both mean total ANPP and group-level ANPP were strongly overlapping between ambient control and procedural control treatments (β = 0.11 [-0.17; 0.38], β = 0.31 [-0.11; 0.72]; differences in posterior means between ambient control and procedural control). Furthermore, we did not find substantial variance in total biomass between blocks (ICC or σ_{block} = 0.01 [0.00; 0.05]) although the ability of the design to estimate this variance component is limited given the limited replication of five blocks.

3.3.2 Community diversity and composition were resistant to drought and irrigation

We found evidence that species composition in these calcareous grassland communities are resistant to precipitation treatments, both in terms of broad diversity indices and community composition (Figure 3.3). For species richness, the Shannon-Weiner index, and the Simpson's index, we did not observe differences in indices between precipitation treatments (Figure 3.3a-c). For all three indices, the $\Delta elpd$ compared to the base model was below 0.6, indicating no clear association between the indices and precipitation treatments, so we retained models excluding precipitation treatment (Tables S3.4-6). Similarly to ANPP, there were no clear differences in ambient control and procedural control treatments for richness, Shannon-Weiner index, or Simpson's index. Furthermore, the mean posterior block-level variance (ICC or σ_{block}) was below 0.03 for all indices.

Community composition was also not clearly associated with the precipitation treatments. There was no clear association between NMDS axes one and two (NMDS1 and NMDS2) and precipitation treatment (Figure 3.3d; Figure S3.8), or between precipitation treatments and NMDS3 (Figure S3.8). A lack of community composition differences between treatments was further supported by the analysis of similarities, for which within sample dissimilarity was broadly comparable to dissimilarity between samples (Figure S3.9; marginally significant relationship).

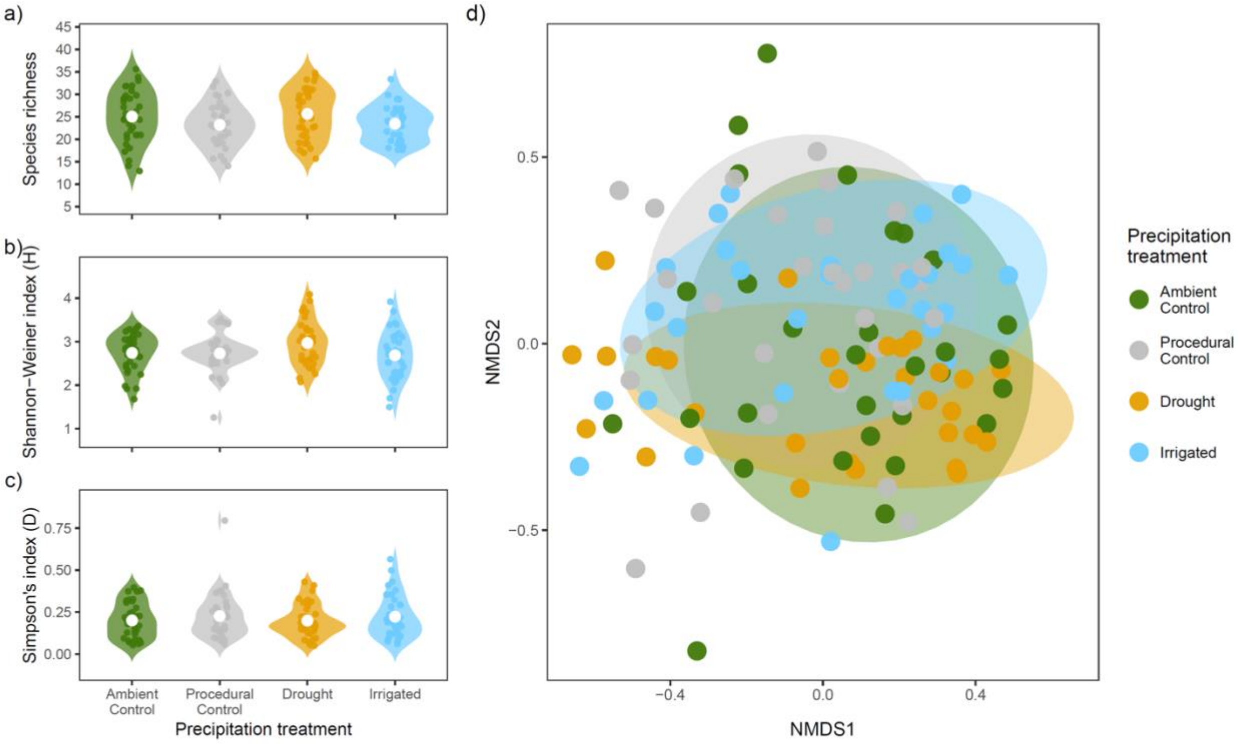

Figure 3.3 Calcareous grassland community diversity is resistant to precipitation manipulations. a-c) raw data distributions for species richness (a), Shannon-Weiner index (b) and Simpson's index (c) with respect to precipitation treatment. Coloured points indicate raw data, and violins give an estimate of data density across each index. White points indicate mean biodiversity index values. d) Non-metric multi-dimensional scaling (NMDS) results for community composition, where the first two axes (NMDS1 and NMDS2) are displayed with respect to precipitation treatments. Ellipses are the 80% two-dimensional quantiles of the NMDS axes.

3.3.4 Shifts in community composition and richness increases across the study period

Instead of an effect of precipitation treatment, we found evidence for overall temporal changes in the calcareous grassland communities, with increases in species richness and changes in community composition (Figure 3.4). However, these changes were not affected by the precipitation treatments. We recorded a total of 109 vascular plant species between 2016 and 2021 across all plots. Across all plots the annual total number of species recorded did not change substantially, ranging between 54 (2017) and 64 (2019). At the quadrat level, species richness ranged between 13-36 species m^{-2} with a mean of 24.4 ± 5.14 (S.D.) species. We found a positive association between species richness and observation year, such that there were increases in richness over the study period (Figure 3.4a; β = 0.10 [0.04; 0.16]). Overall, between 2016 and 2021, there was an increase in mean richness at the plot-level from 20.3, to 27.4 species (Figure 3.4a). Furthermore, we found a strong association between community composition and observation year (Figure 3.4b). The analysis of similarities indicated a large difference in within-year community differences compared to between year community differences (Figure S3.9). Furthermore, we observed a strong positive association between NMDS1 and observation year, which was a substantially better predictive model compared to the base model ($\Delta elpd$ = 58.4). There was a consistent increase in NMDS1 over the study period (β = 0.23 [0.20; 0.27]), indicating a shift in community composition (Figure 3.4c).

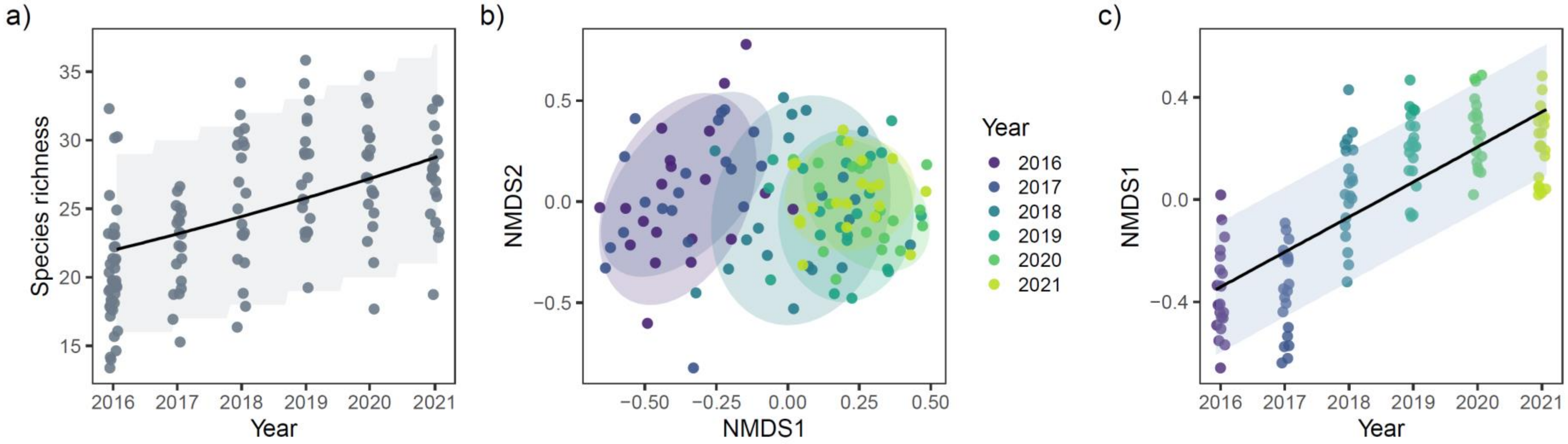

Figure 3.4. Changes in the grassland diversity and community composition 2016-2021. a) Increases in species richness between 2016-2021. Grey points give raw observations of species richness for each quadrat in each treatment. Solid line indicates the posterior mean prediction with 90% credible intervals, which include random effects. b) Non-metric multi-dimensional scaling (NMDS) results for community composition, where the first two axes (NMDS1 and NMDS2) are displayed with respect to observation year. Ellipses are the 80% two-dimensional quantiles of the NMDS axes. c) Increases to NMDS1 over the observation period. Points give observed NMDS1 scores for each quadrat for a given treatment and block across the study period. Solid black line indicates the posterior mean, with the 90% credible interval.

In spite of observed temporal trends, we did not observe an association between productivity and average interannual weather conditions. Average spring and summer weather conditions were not strongly associated with any of the biodiversity indices or ANPP (Figure S3.6-S3.7). Finally, we explored which species had the largest impact on community change across years. Four species had significant positive temporal trends in abundance across plots (*Bromus commutatus*, *Medicago lupulina*, *Trisetum flavescens* and *Viola hirta*; Figure S10), but generally species richness increases at the plot level were not repeatedly associated with a specific group of species. We then used similarity percentages across species to investigate the species that were most influential in driving differences in community dissimilarity between years. Ten species had mean percentage contributions to dissimilarity above 2% (with *Potentilla reptans* lying on the boundary), of which four were graminoids, four were legumes and one was a forb (Figure 3.5a). Of these species however, *Arrhenatherum elatius* (graminoid), *Brachypodium pinnatum* (graminoid) and *Lotus corniculatus* (legume) had mean contributions of over 5% (median > 6.5%) to community dissimilarity across years (Figure 3.5a and b). These three species were common, and varied substantially across the study period, with large interannual shifts, but displayed no clear pattern in relative abundance through time or with treatments (Figure 3.5b). In particular *Arrhenatherum elatius*, which is a common and dominant species, varied between an average abundance of 13.3% (relative abundance = 0.133) across all quadrats in 2018 to 2.3% in 2021 (Figure 3.5b).

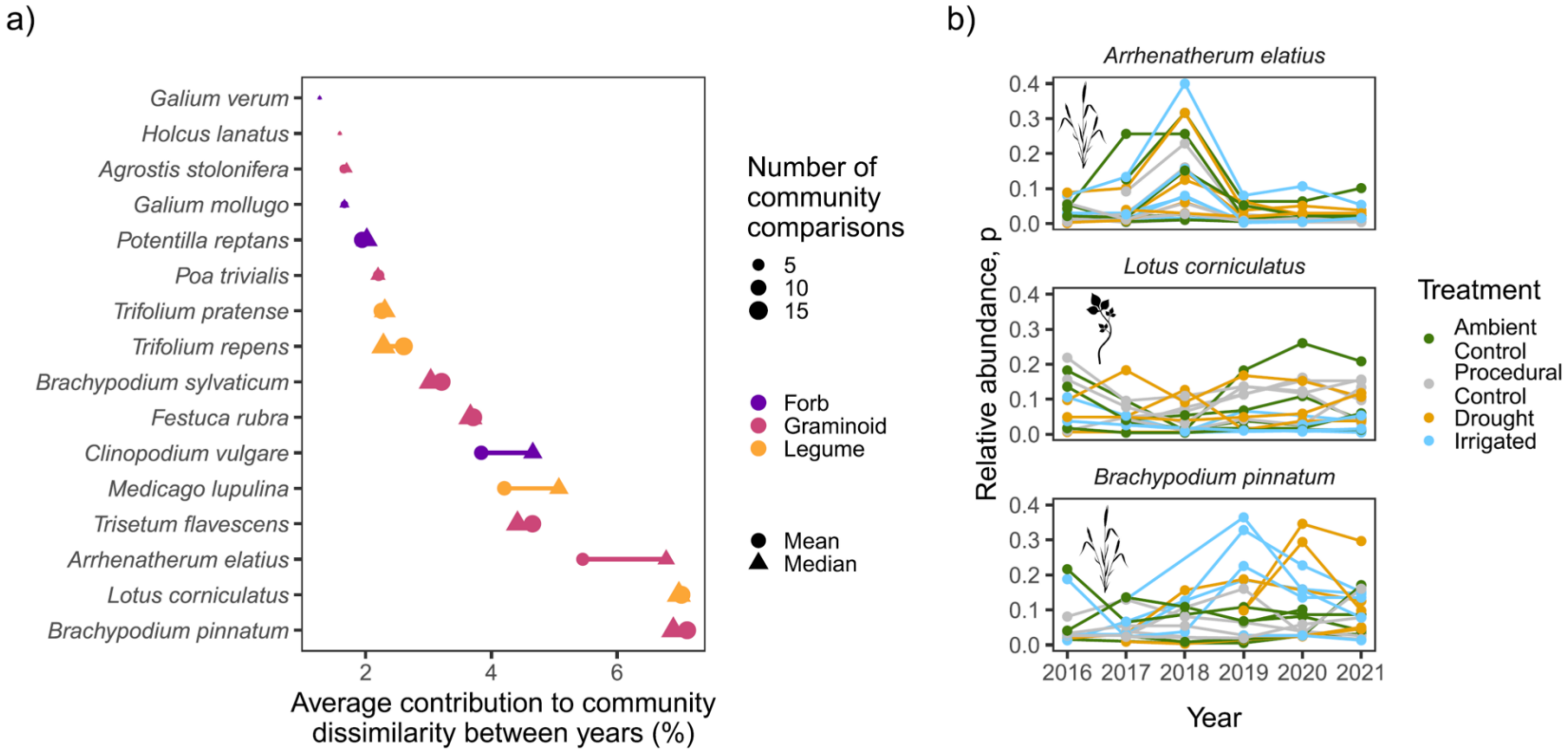

Figure 3.5 Commonly occurring graminoid and legume species drive community dissimilarity. a) Similarity percentage results for species driving community dissimilarity across years. Only 16 species (of 109) with the highest contributions are shown. Circles give mean percent contribution, and triangles mean percent contribution, with the size of the point indicating the number of community comparisons and the colour denoting the functional group. b) Change in relative abundance across years for three species with high contribution to dissimilarity across years. Points and lines give raw estimates of relative abundance in each quadrat for each species. High variability in the relative abundance of the three most influential species across the study.

3.4 Discussion

From our six-year study of precipitation manipulation in a calcareous grassland, we show that simulated drought reduced productivity and its temporal stability, but that species diversity and composition were generally resistant. These results add to global findings of decreased grassland productivity with drought (Kröel-Dulay *et al.,* 2022; Smith *et al.,* 2024), but we add a key perspective that the reductions in productivity are coupled with reduced temporal stability, and an accentuated pattern in graminoids and legumes. Despite accentuated productivity loss in graminoids and legumes, we find that overall community composition in this calcareous grassland was resistant to six years of drought conditions, a finding consistent with other studies on calcareous grasslands (Craine *et al.,* 2013; Grime *et al.,* 2000, 2008). Increases to precipitation were not associated with any change in productivity or community composition, which may indicate a lack of efficacy in the irrigation treatment. Our study emphasises the need to explore detailed temporal dynamics in grassland communities alongside changing precipitation patterns, to understand how climate change will impact these vital ecosystems and the services they provide.

In the present study, experimental drought reduced the productivity of the calcareous grassland habitat and its inter-annual temporal stability, with 25% reductions in above-ground annual net primary productivity, and a 54% increase in productivity’s coefficient of variation. Furthermore, despite a strong overall effect, reductions in productivity were driven primarily by losses in graminoid and legume biomass, but forb biomass did not change substantially. Reductions of primary productivity and biomass in response to drought have been widely reported in plant communities globally (Haddad *et al.,* 2002; Kröel-Dulay *et al.,* 2022; Smith *et al.,* 2024). Reduction in productivity may be driven by water limitation, altered patterns of nutrient cycling following precipitation extremes (Haddad *et al.,* 2002), or a diversion in the allocation of resources from survival to reproduction (Weißhuhn *et al.,* 2011). A lack of biomass reduction in the forbs may be a result of deeper rooting depth in forbs relative to graminoids and legumes increasing access to water (Sydes and Grime, 1984), or increased water limitation relative to nitrogen limitation in legumes. Kröel-Dulay *et al.* (2022) found that experimental manipulations of precipitation often underestimate the impact of drought on biomass, failing to capture other abiotic processes that are associated with drought in real-world settings, including the interaction between drought timing and plant growth (Felton and Goldsmith, 2023). Furthermore, this calcareous grassland is a mesic system with moderate levels of annual precipitation, and productivity losses are likely to be amplified in more arid environments *(*Huxman *et al., 2004;* Moran *et al.,* 2014). Reduced productivity observed here could also be linked to the aftereffects of previous agricultural management and gradual loss of nutrients in the soil and a reduction in competitive, resource-acquisitive plants (Smits *et al.,* 2008). Ultimately, reduced precipitation threatens grassland productivity worldwide, and thus many ecosystem services that grasslands provide.

In addition to changes in mean productivity, drought also decreased the temporal stability by increasing the variance of productivity. Measures of stability in abundance (here biomass) can provide comparable metrics that forecast broader community-level changes as early warning signals (Clements and Ozgul, 2016; Wu *et al.,* 2020). While previous studies have explored stability metrics in grasslands over short timescales (He *et al.*, 2022), we add an important longer-term perspective to the narrative of grassland stability with changing precipitation patterns. Reduced stability could be the result of resource limitation (drought) increasing dependence on natural variation in precipitation, where productivity mediated through survival and reproduction is more reactive to limited precipitation levels *(Grime et al.,* 2008; Weißhuhn *et al.,* 2011). Worryingly, the early warning signal of reduced stability in productivity is supported by below-ground data from the calcareous grassland in Buxton, UK, where the seedbanks are highly altered by drought conditions, which may forecast rapid future community change (Basto *et al.,* 2018). Further studies explicitly exploring metrics of community stability with respect to climate change in plant communities will provide valuable forecasts of the changing state of our ecosystems.

Importantly, over six years the diversity and composition of communities in this calcareous grassland were largely resistant to drought, despite the group-specific declines in the productivity of grasses and legumes. It is currently unclear why the composition metrics were largely unaffected by the productivity declines in grasses and legumes. Key findings from other long-term studies on calcareous grasslands support the composition resistance findings (Craine *et al.,* 2013; Grime *et al.*, 2000, 2008), but we add further temporal dynamics and investigation at the species-level. We hypothesise that the grassland resistance could be the result of two main processes, i) the maintenance of drought resistance through increased species and functional diversity over time, and ii) insufficient time for precipitation change to influence the community i.e. lagged effects. There are clear global ecological signals that higher species diversity in linked to high stability (Craven *et al.,* 2018; Hector *et al.*, 2010). Importantly, resistance to climate extremes can also be compromised by reduced species diversity (Isbell *et al.,* 2015). At the individual level, plant resistance is linked to functional traits such as leaf economic traits and root thickness (Anderegg *et al.,* 2016; Tucker *et al.,* 2011). Further, intra-specific trait variation can also contribute to stabilising populations under chronic drought as shown in *Plantago lanceolata* populations from the Buxton experiment (Ravenscroft *et al.,* 2013).

At the community level, increased functional diversity, which is linked to species diversity, can improve resilience in grasslands after drought (Griffin-Nolan *et al.,* 2019). Therefore, we hypothesise that the relatively high biodiversity of calcareous grasslands (Isbell *et al.,* 2015), creates functional redundancy and therefore buffers the community to environmental change. These patterns may also be linked with wider climatic conditions, where arid grasslands have slower functional recovery from drought compared to mesic grasslands (Moran *et al.,* 2014). Diversity in species and functional trait responses coupled with climatic variability across habitats may therefore explain why some grassland communities have responses to precipitation extremes (Harrison *et al.,* 2015), and others do not (Grime *et al.,* 2008). Long-term studies investigating coupled functional and community responses and adaptive potential to precipitation extremes will be crucial in teasing apart these patterns.

Our observations of resistance may also be explained by temporal factors. Interestingly, despite group-specific reductions in biomass for graminoids and forbs, these changes were not mirrored in community composition from percentage cover. However, the group-specific results for biomass may indicate that there will be shifts in community composition in the future, especially when there is mortality and turnover in perennial species. Understanding links between productivity and community composition, either through functional redundancy or species turnover and mortality, is a vital next step in this system. Furthermore, compared with some other grassland habitats, these calcareous grasslands have many perennial species that survive for several years. From 14 years of data from a calcareous grassland in the north of England, there was temporal drift in the community but overall resistance, which may also have been linked to longer-living species surviving but reacting in growth to interannual variation in the climate (Grime *et al.,* 2008). A delayed turnover is also supported by the seed bank, which had strong responses to drought conditions in the same system, suggesting that subsequent generations in the community will have lasting impacts of drought (Basto *et al.,* 2018). Therefore, our findings do not rule out long-term impacts from climate change on grassland communities, which may occur abruptly when tolerance limits are reached (Trisos *et al.,* 2020), or in synergy with other drivers such as habitat fragmentation (Brook *et al.,* 2008; Klaus *et al.,* 2021).

In addition to community resistance to drought, we found that neither productivity or community composition responded to irrigation in this calcareous grassland. Given that soil moisture content at the end of the growing season was not substantially increased in the irrigation treatment, the resistance of the community to irrigation could indicate failure in the irrigation treatment to successfully increase soil moisture levels on the freely draining soil and underlying bedrock. Although failure of the irrigation treatment is a likely explanation, the readings of soil moisture were taken during the late summer when precipitation is typically lower, and so further study of the irrigation treatment's efficacy is needed. Furthermore, community resistance to irrigation is a finding that has been replicated across several grassland irrigation experiments globally (DeMalach *et al.,* 2017). Nevertheless, in other systems precipitation increases have been associated with community and functional changes, also in conjunction with warming (Collins *et al.,* 2022; Kimmel *et al.,* 2019). Therefore, resistance to precipitation could also reflect an insufficiency in the extremity of the precipitation addition (Smith *et al.,* 2024), and further study of the efficacy of precipitation manipulation is needed.

Our final key finding was a general (treatment independent) temporal change in the community, where richness increased by over 7 species m^{-2} between 2016-2021 and a shift in community composition. The addition of the temporal dynamics in the community was one key advantage of the current study, and future work should focus on long term temporal patterns in community change in response to precipitation extremes. The most likely explanation is that temporal changes indicate ongoing secondary succession following the cessation of agriculture in ~1980 and sheep grazing before the experiment, with both long-term recovery and community change following grazing. An earlier climate change experiment that compared responses of a calcareous grassland at Harpur Hill in Buxton with those of the Wytham Upper Seeds found grassland diversity and composition was more stable in the established pasture at Buxton compared to the larger changes seen in Upper Seeds that the authors attribute to its secondary successional state (Grime *et al.,* 2000, 2008).

3.4.1 Caveats

There are several factors that we did not control in the current experiment that have the potential to impact community responses to precipitation extremes. First, while it is unlikely that the temporal dynamics observed are purely successional change after agriculture, grazing at the site occurring up to the start of the experiment, and other experiments at the site may have impacted the current community, (Gibson and Brown, 1991; Grime *et al.,* 2000). A switch from grazing to mowing at the start of the experiment may be responsible for the current temporal shift in the community and increase in species richness. Second, is the role of spatial dynamics and immigration from other grasslands in the area, or meta-community dynamics, which we did not control in the current study (Furey *et al.,* 2022). Immigration can maintain high levels of local species diversity in plant communities (Loreau and Mouquet, 1999), and while out of the scope of the current study, immigration may also have an impact, particularly on temporal dynamics. However, Upper Seeds is relatively isolated from other nearby areas of calcareous grassland. Third, the role of microclimate on community change, which has been demonstrated as an important mediator in grassland drought responses (Mazalla *et al.,* 2022). The drought treatment shelters in particular may have influenced light availability, and created irregular patterns of rainfall (when combined with wind) that influenced the community. However, we controlled for these structural effects using a procedural control, which is rarely available in precipitation manipulation experiments, and a replicated block design, and found no detectible differences between procedural control and ambient control plots.

3.5 Conclusion

The long-term monitoring of calcareous grassland communities with respect to experimentally controlled precipitation change can reveal detailed community responses, which are not available from snapshots of the community. Measuring and predicting the impact of climate change on plant communities can be facilitated by the approach taken here that combines long-term data on community composition and productivity. Future work should combine community and productivity data with information on functional traits and the corresponding below-ground responses.

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Data Availability Statement

All code, output and data used in the current study are archived using the Zenodo repository DOI: 10.5281/zenodo.8135588, which was created from the following GitHub repository: https://github.com/jjackson-eco/raindrop_biodiversity_analysis.

Note

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Supplementary

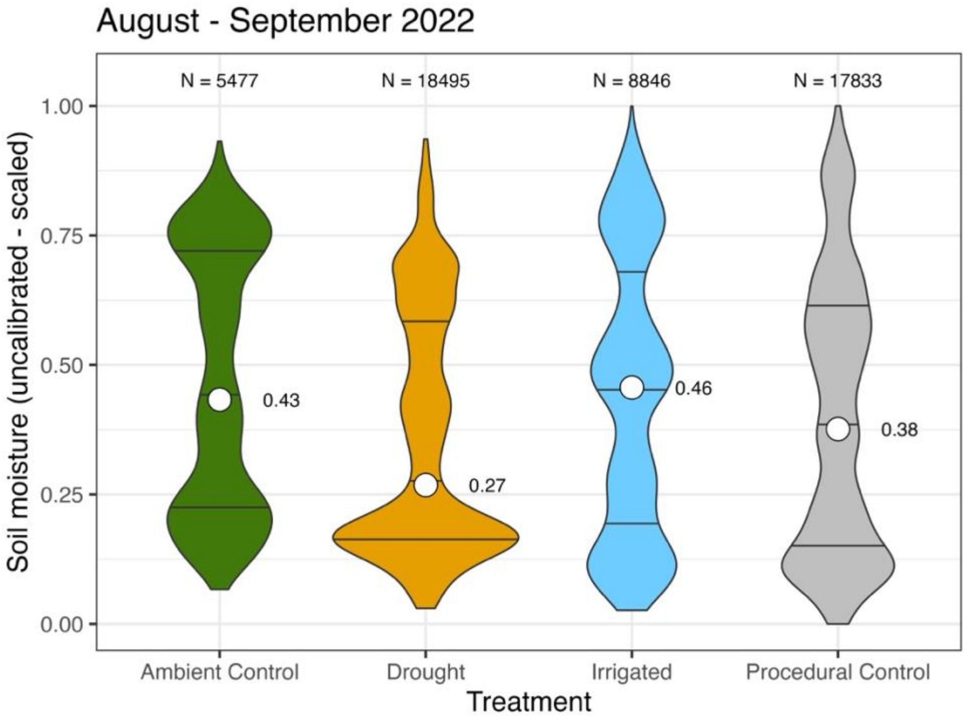


Figure S3.1 Preliminary data on soil moisture differences between treatments to validate the experimental treatments used in the current study. The density violins give an indication of the distribution of raw soil moisture values for each treatment. N values above each distribution give the number of observations, and the lines in each violin give the 25%, 50% and 75% quantiles of the data (indicating the interquartile range). The white points give the median value, which is also annotated at the side of each distribution. There is a clear reduction in the average soil moisture in the drought treatment relative to other treatments, both in the median (reduced by 37% relative to the ambient treatment) and interquartile range. Furthermore, there is a clear aggregation of low soil moisture values in the drought treatment.

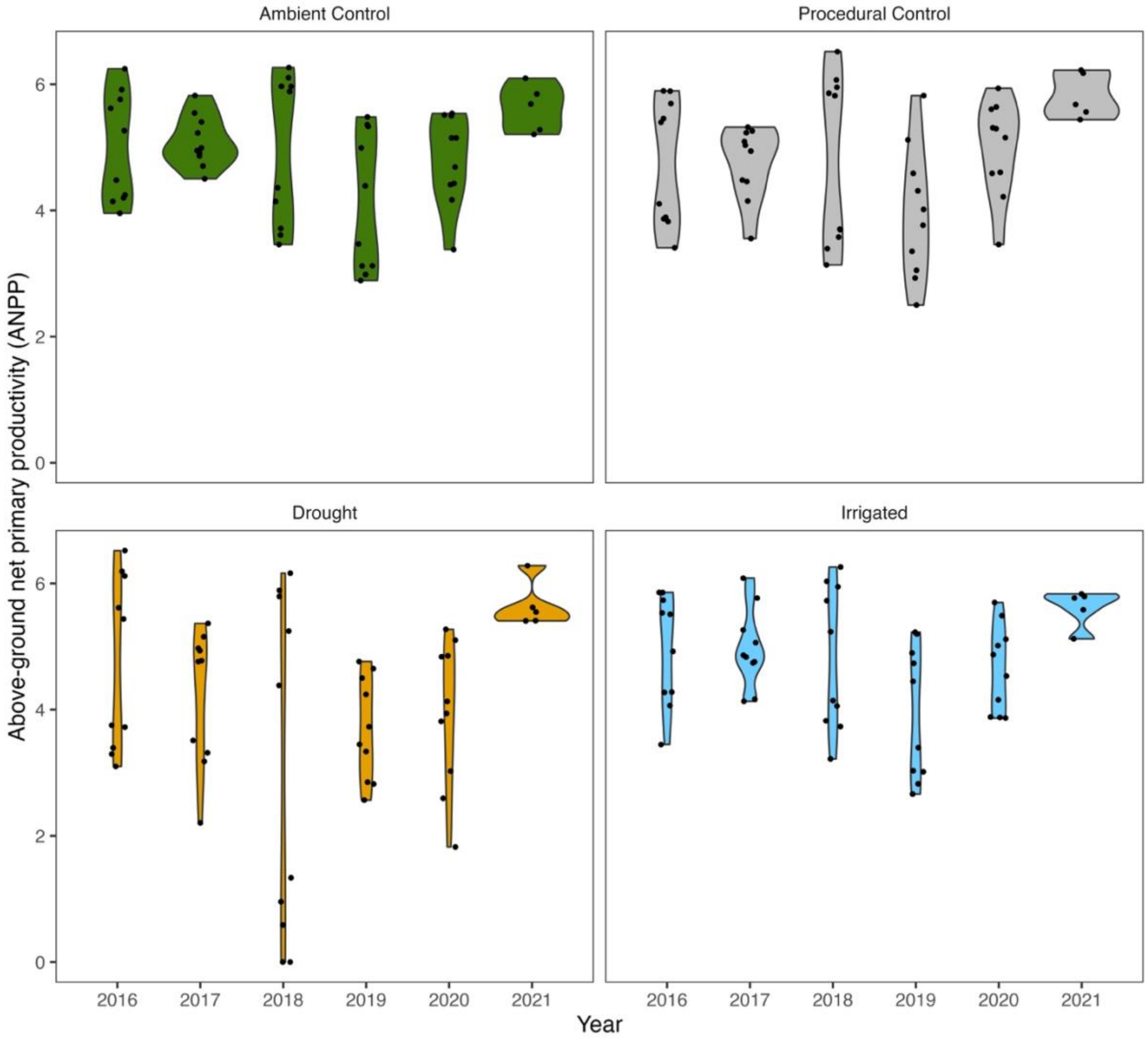


Figure S3.2 No clear temporal pattern in above-ground net primary productivity (ANPP). Although the best predictive model for total ANPP included a linear effect of observation year, the simpler model excluding the temporal trend was similar in predictive performance. Here, each point is a raw ANPP value, on the natural-log scale, for each plot in each season of each year. Density violins give an estimate of the overall distribution of data in each year. There is no clear overall temporal trend, or an interaction with the precipitation treatments (panels).

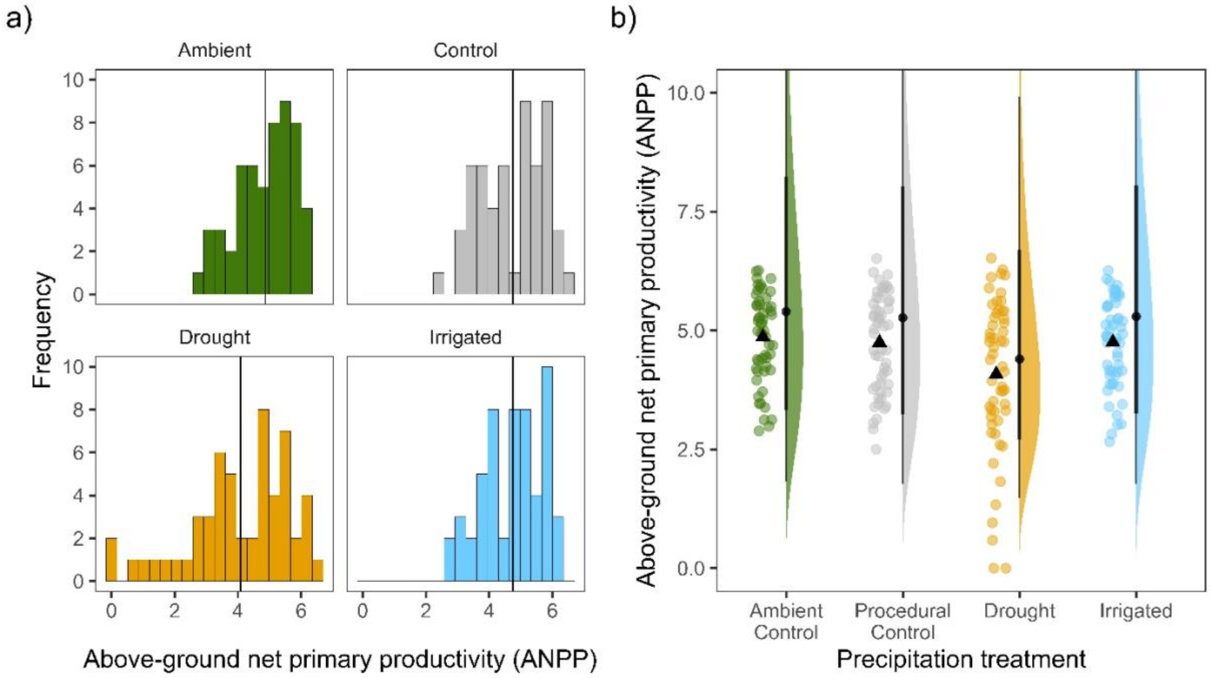


Figure S3.3 Exploring skew in above-ground annual net primary productivity (ANPP). ANPP is the natural log-transformed above-ground biomass in g m^{-2}. a) Frequency histograms for ANPP values in each precipitation treatment, indicating weak negative skew towards lower ANPP values. Based on this skew, we re-ran models of ANPP differences between precipitation treatments with gamma distribution models (shape = 5, scale = 0.8). b) Posterior predictions from gamma models of ANPP differences between precipitation treatments. Coloured points give raw data across blocks and years and black triangles give the mean total ANPP. Distributions are derived from 8000 draws of the full posterior distribution including random effects, with probability density function boxplots giving the posterior mean and uncertainty.

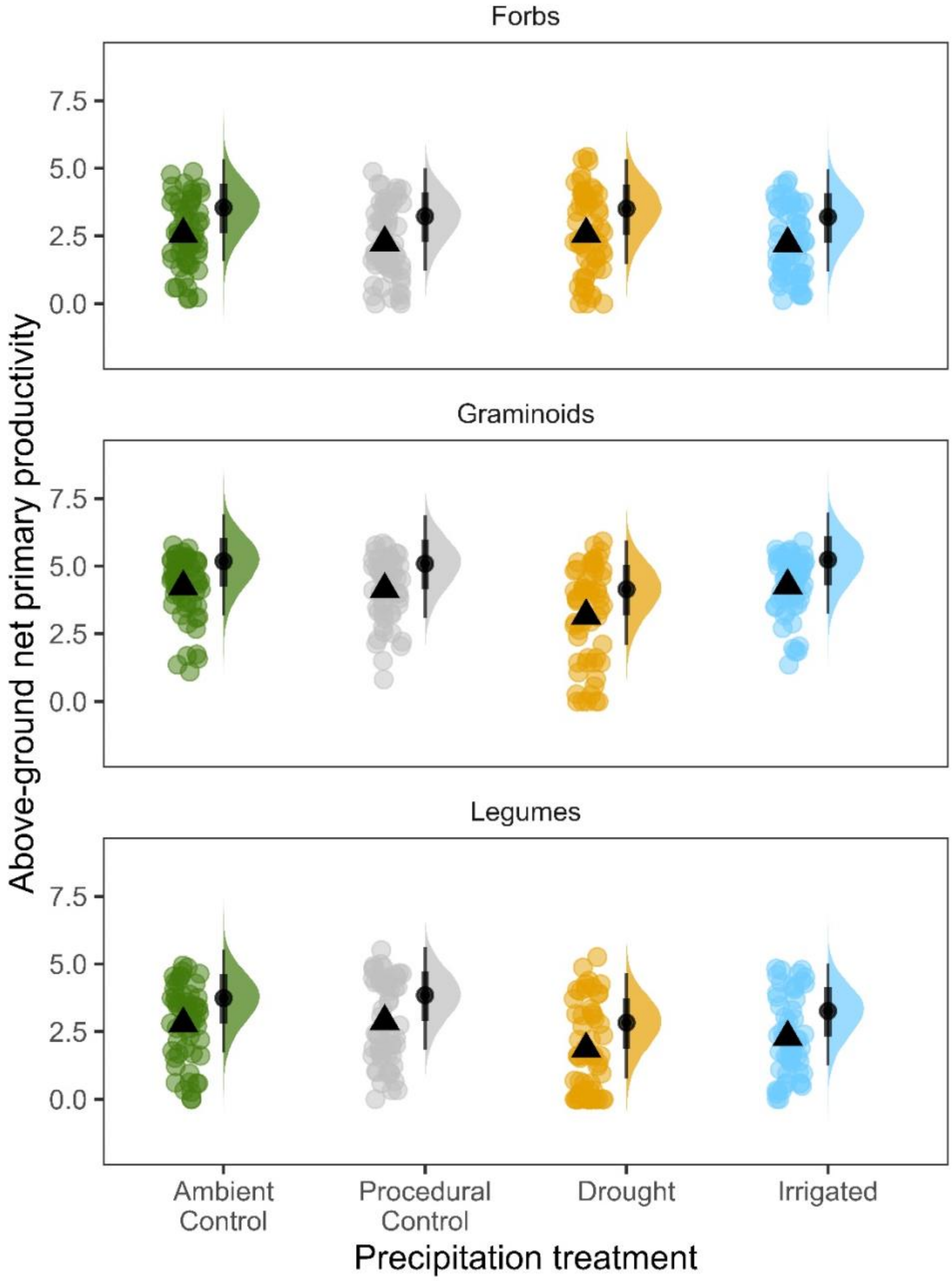


Figure S3.4 Stability reductions are driven by Graminoids and legumes. Differences in above-ground net primary productivity (ANPP) separated by functional group, between precipitation treatments. ANPP is the natural log-transformed above-ground biomass in g m^{-2}. Coloured points give raw data across blocks and years and black triangles give the mean total ANPP. Distributions are derived from 8000 draws of the posterior distribution, with probability density function boxplots giving the posterior mean and uncertainty.

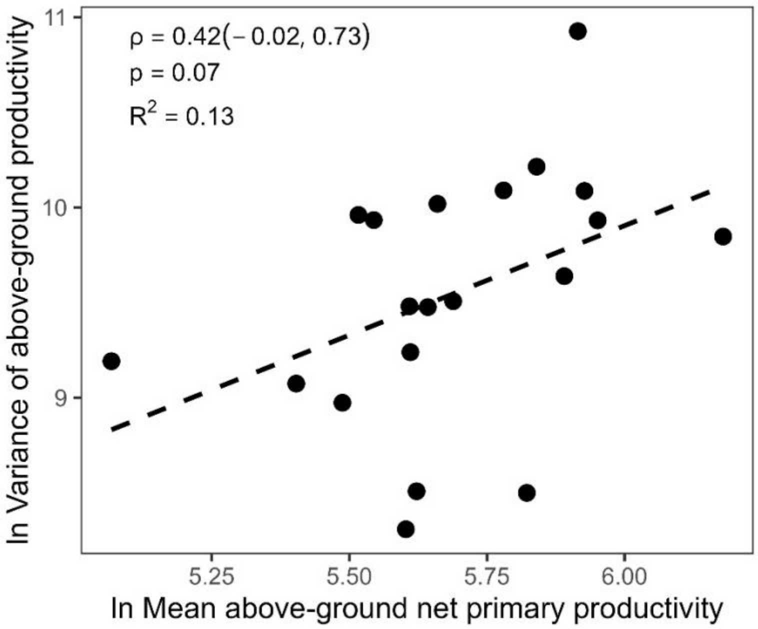


Figure S3.5 No clear association between the ln variance in ANPP and mean ANPP. Quantifying temporal stability using the coefficient of variation is often impacted by Taylors Power Law, where by decreases in variance (stability) are only observed through increases in the mean (Reckling et al. 2021). Therefore, we verified the patterns we observed using a log-linear Pearson correlation between the variance in ANPP (not the CV metric used in analyses however, which is corrected by the mean) and the mean ANPP across plots. Although the correlation coefficient was close to significance ($p = 0.07$), the 95% confidence intervals around the correlation coefficient ρ (indicated in brackets) overlapped 0, and the adjusted R^2 from a linear regression model of the same relationship was low. Therefore, we conclude that there was not clear support for the Taylors Power Law in this context.

Table S3.1. Model selection results for models of the total above ground annual primary productivity (ANPP; natural log of total biomass) total with respect to precipitation treatments and temporal trend effects. Models were compared using leave-one-out cross (LOO) validation and the expected log-wise predictive density ($elpd$). Column 'elpd difference' gives $\Delta elpd$, which was used as the measure of a model's predictive performance relative to the base model. Model formula is presented in hierarchical model syntax from the *lme4* package. *year_s* model term refers to a scaled continuous linear effect of observation year, and *year_f* refers to a categorical effect of year as a factor. The *ar()* term gives AR(1) autoregressive effects for observation year.

R object	Model formula	LOO elpd	LOO elpd error	elpd difference	elpd error difference	LOO information criterion
totbiomass_year_linear	log_tot_biomass ~ 1 + treatment + harvest + year_s + (1 l block/treatment) + (1 l year_f)	-232.07	19.55	0.00	0.00	464.15
totbiomass_treatment	log_tot_biomass ~ 1 + treatment + harvest + (1 l block/treatment) + (1 l year_f)	-232.31	19.65	-0.24	0.21	464.62
totbiomass_year_harvest	log_tot_biomass ~ 1 + treatment + year_s * harvest + (1 l block/treatment) + (1 l year_f)	-232.92	19.45	-0.85	0.38	465.84
totbiomass_year_treat	log_tot_biomass ~ 1 + treatment * year_s + harvest + (1 l block/treatment) + (1 l year_f)	-233.05	19.44	-0.97	1.03	466.10
totbiomass_base	log_tot_biomass ~ 1 + harvest + (1 l block/treatment) + (1 l year_f)	-237.34	21.85	-5.27	4.04	474.68
totbiomass_year_auto	log_tot_biomass ~ 1 + treatment + ar(gr = year_s, p = 1) + harvest + (1 l block/treatment)	-239.43	17.69	-7.36	5.87	478.86

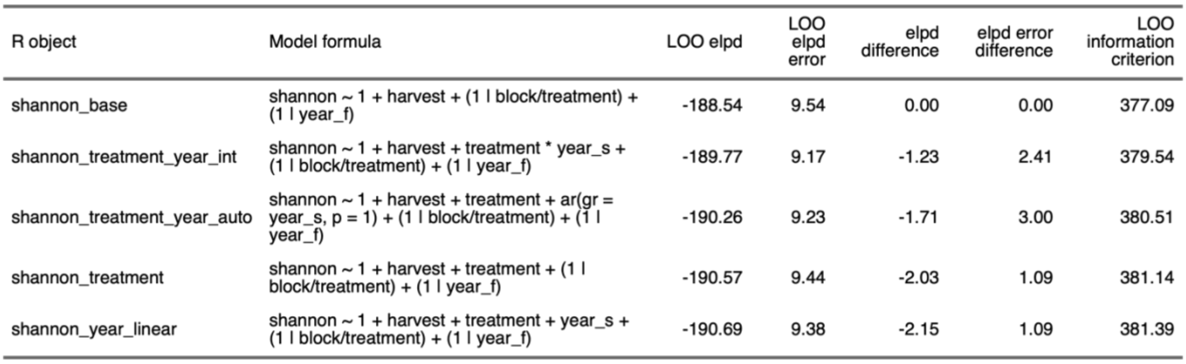
Table S3.2 Model selection results for models of the group-level above-ground biomass with precipitation treatments and temporal trend effects.

R object	Model formula	LOO elpd	LOO elpd error	elpd difference	elpd error difference	LOO information criterion
biomass_treatment_group_int	biomass_log ~ 1 + treatment * group + harvest + (1 l block/treatment) + (1 l year_f)	-816.57	20.54	0.00	0.00	1,633.14
biomass_treatment_group_year	biomass_log ~ 1 + treatment * group * year_s + harvest + (1 l block/treatment) + (1 l year_f)	-817.71	20.12	-1.14	3.82	1,635.42
biomass_treatment_group	biomass_log ~ 1 + treatment + group + harvest + (1 l block/treatment) + (1 l year_f)	-845.65	21.76	-29.08	7.71	1,691.30
biomass_group	biomass_log ~ 1 + group + harvest + (1 l block/treatment) + (1 l year_f)	-846.27	21.87	-29.70	7.96	1,692.55
biomass_treatment	biomass_log ~ 1 + treatment + harvest + (1 l block/treatment) + (1 l year_f)	-1,026.00	17.12	-209.43	15.40	2,052.01
biomass_base	biomass_log ~ 1 + harvest + (1 l block/treatment) + (1 l year_f)	-1,026.75	16.90	-210.18	15.49	2,053.49

Table S3.3 Model selection results for models of the temporal stability of productivity with respect to precipitation treatments.

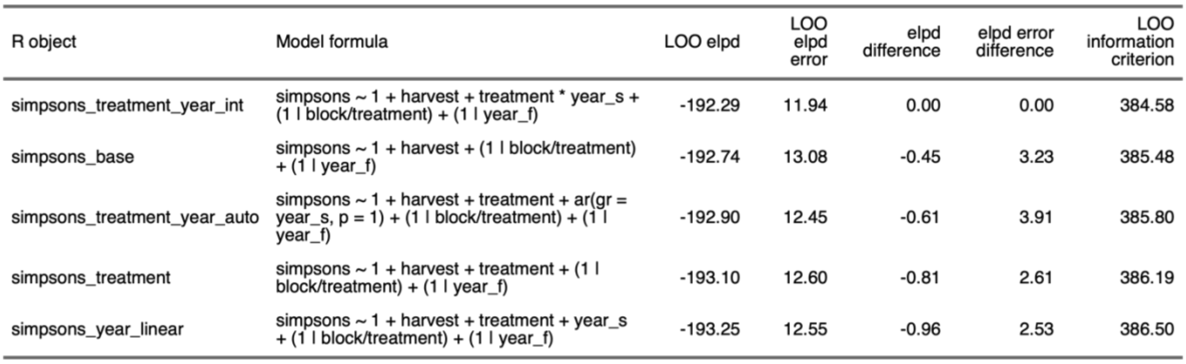
R object	Model formula	LOO elpd	LOO elpd error	elpd difference	elpd error difference	LOO information criterion
biomass_var_treat	cv_biomass ~ 1 + treatment + (1 l block)	14.43	2.72	0.00	0.00	-28.86
biomass_var_base	cv_biomass ~ 1 + (1 l block)	10.90	2.72	-3.53	2.87	-21.81

Table S3.4 Model selection results for models of the Shannon-Weiner index with respect to precipitation treatments and temporal trend effects.



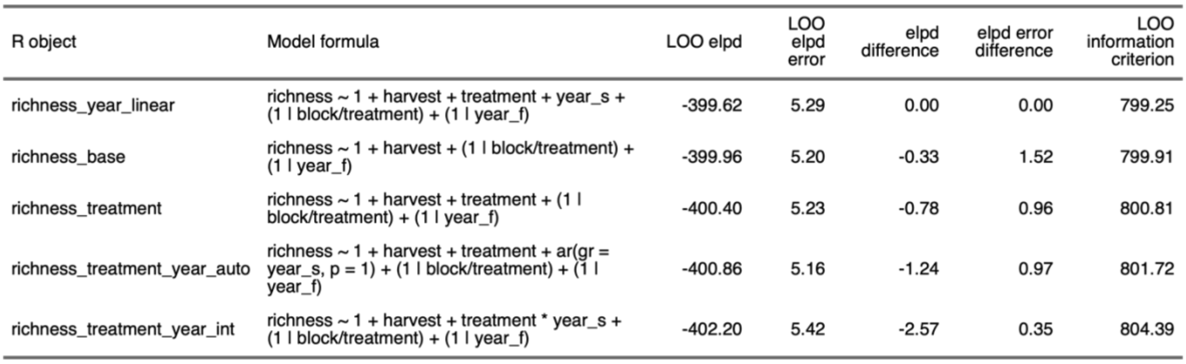
R object	Model formula	LOO elpd	LOO elpd error	elpd difference	elpd error difference	LOO information criterion
shannon_base	shannon ~ 1 + harvest + (1 | block/treatment) + (1 | year_f)	-188.54	9.54	0.00	0.00	377.09
shannon_treatment_year_int	shannon ~ 1 + harvest + treatment * year_s + (1 | block/treatment) + (1 | year_f)	-189.77	9.17	-1.23	2.41	379.54
shannon_treatment_year_auto	shannon ~ 1 + harvest + treatment + ar(gr = year_s, p = 1) + (1 | block/treatment) + (1 | year_f)	-190.26	9.23	-1.71	3.00	380.51
shannon_treatment	shannon ~ 1 + harvest + treatment + (1 | block/treatment) + (1 | year_f)	-190.57	9.44	-2.03	1.09	381.14
shannon_year_linear	shannon ~ 1 + harvest + treatment + year_s + (1 | block/treatment) + (1 | year_f)	-190.69	9.38	-2.15	1.09	381.39

Table S3.5 Model selection results for models of the Simpson’s index with respect to precipitation treatments and temporal trend effects.



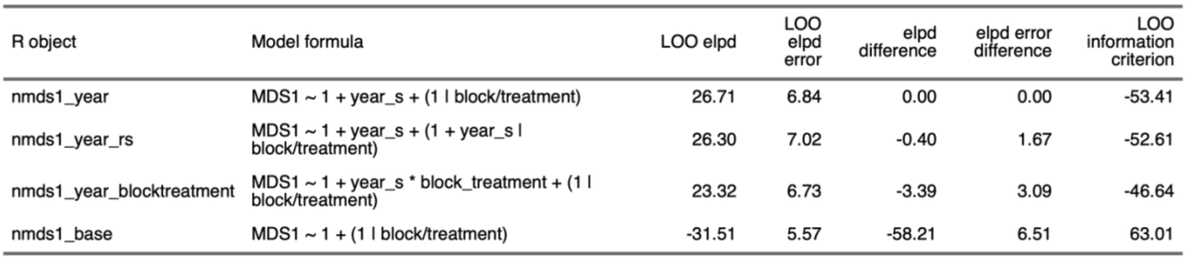
R object	Model formula	LOO elpd	LOO elpd error	elpd difference	elpd error difference	LOO information criterion
simpsons_treatment_year_int	simpsons ~ 1 + harvest + treatment * year_s + (1 | block/treatment) + (1 | year_f)	-192.29	11.94	0.00	0.00	384.58
simpsons_base	simpsons ~ 1 + harvest + (1 | block/treatment) + (1 | year_f)	-192.74	13.08	-0.45	3.23	385.48
simpsons_treatment_year_auto	simpsons ~ 1 + harvest + treatment + ar(gr = year_s, p = 1) + (1 | block/treatment) + (1 | year_f)	-192.90	12.45	-0.61	3.91	385.80
simpsons_treatment	simpsons ~ 1 + harvest + treatment + (1 | block/treatment) + (1 | year_f)	-193.10	12.60	-0.81	2.61	386.19
simpsons_year_linear	simpsons ~ 1 + harvest + treatment + year_s + (1 | block/treatment) + (1 | year_f)	-193.25	12.55	-0.96	2.53	386.50

Table S3.6 Model selection results for models of species richness with respect precipitation treatments and temporal trend effects.



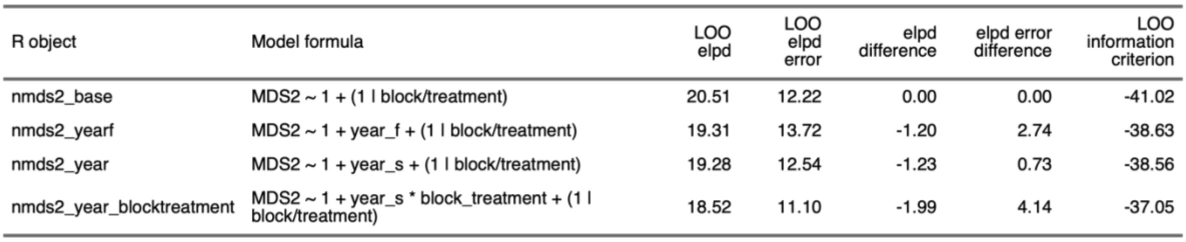
R object	Model formula	LOO elpd	LOO elpd error	elpd difference	elpd error difference	LOO information criterion
richness_year_linear	richness ~ 1 + harvest + treatment + year_s + (1 | block/treatment) + (1 | year_f)	-399.62	5.29	0.00	0.00	799.25
richness_base	richness ~ 1 + harvest + (1 | block/treatment) + (1 | year_f)	-399.96	5.20	-0.33	1.52	799.91
richness_treatment	richness ~ 1 + harvest + treatment + (1 | block/treatment) + (1 | year_f)	-400.40	5.23	-0.78	0.96	800.81
richness_treatment_year_auto	richness ~ 1 + harvest + treatment + ar(gr = year_s, p = 1) + (1 | block/treatment) + (1 | year_f)	-400.86	5.16	-1.24	0.97	801.72
richness_treatment_year_int	richness ~ 1 + harvest + treatment * year_s + (1 | block/treatment) + (1 | year_f)	-402.20	5.42	-2.57	0.35	804.39

Table S3.7 Model selection results for models of community structure, captured with the first axis of non-linear multidimensional scaling (NMDS), with respect to temporal trend effects. The *block_treatment* effect indicates a categorical effect for the specific plot (a treatment within a block).



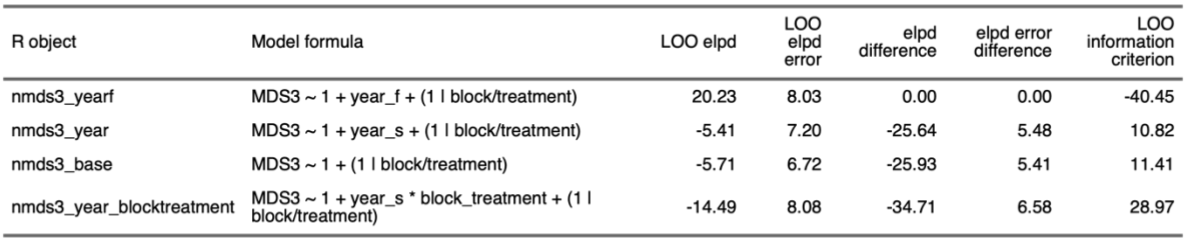
R object	Model formula	LOO elpd	LOO elpd error	elpd difference	elpd error difference	LOO information criterion
nmds1_year	MDS1 ~ 1 + year_s + (1 l block/treatment)	26.71	6.84	0.00	0.00	-53.41
nmds1_year_rs	MDS1 ~ 1 + year_s + (1 + year_s l block/treatment)	26.30	7.02	-0.40	1.67	-52.61
nmds1_year_blocktreatment	MDS1 ~ 1 + year_s * block_treatment + (1 l block/treatment)	23.32	6.73	-3.39	3.09	-46.64
nmds1_base	MDS1 ~ 1 + (1 l block/treatment)	-31.51	5.57	-58.21	6.51	63.01

Table S3.8 Model selection results for models of community structure, captured with the second axis of non-linear multidimensional scaling (NMDS), with respect to temporal trend effects.



R object	Model formula	LOO elpd	LOO elpd error	elpd difference	elpd error difference	LOO information criterion
nmds2_base	MDS2 ~ 1 + (1 l block/treatment)	20.51	12.22	0.00	0.00	-41.02
nmds2_yearf	MDS2 ~ 1 + year_f + (1 l block/treatment)	19.31	13.72	-1.20	2.74	-38.63
nmds2_year	MDS2 ~ 1 + year_s + (1 l block/treatment)	19.28	12.54	-1.23	0.73	-38.56
nmds2_year_blocktreatment	MDS2 ~ 1 + year_s * block_treatment + (1 l block/treatment)	18.52	11.10	-1.99	4.14	-37.05

Table S3.9 Model selection results for models of community structure, captured with the third axis of non-linear multidimensional scaling (NMDS), with respect to temporal trend effects.



R object	Model formula	LOO elpd	LOO elpd error	elpd difference	elpd error difference	LOO information criterion
nmds3_yearf	MDS3 ~ 1 + year_f + (1 l block/treatment)	20.23	8.03	0.00	0.00	-40.45
nmds3_year	MDS3 ~ 1 + year_s + (1 l block/treatment)	-5.41	7.20	-25.64	5.48	10.82
nmds3_base	MDS3 ~ 1 + (1 l block/treatment)	-5.71	6.72	-25.93	5.41	11.41
nmds3_year_blocktreatment	MDS3 ~ 1 + year_s * block_treatment + (1 l block/treatment)	-14.49	8.08	-34.71	6.58	28.97

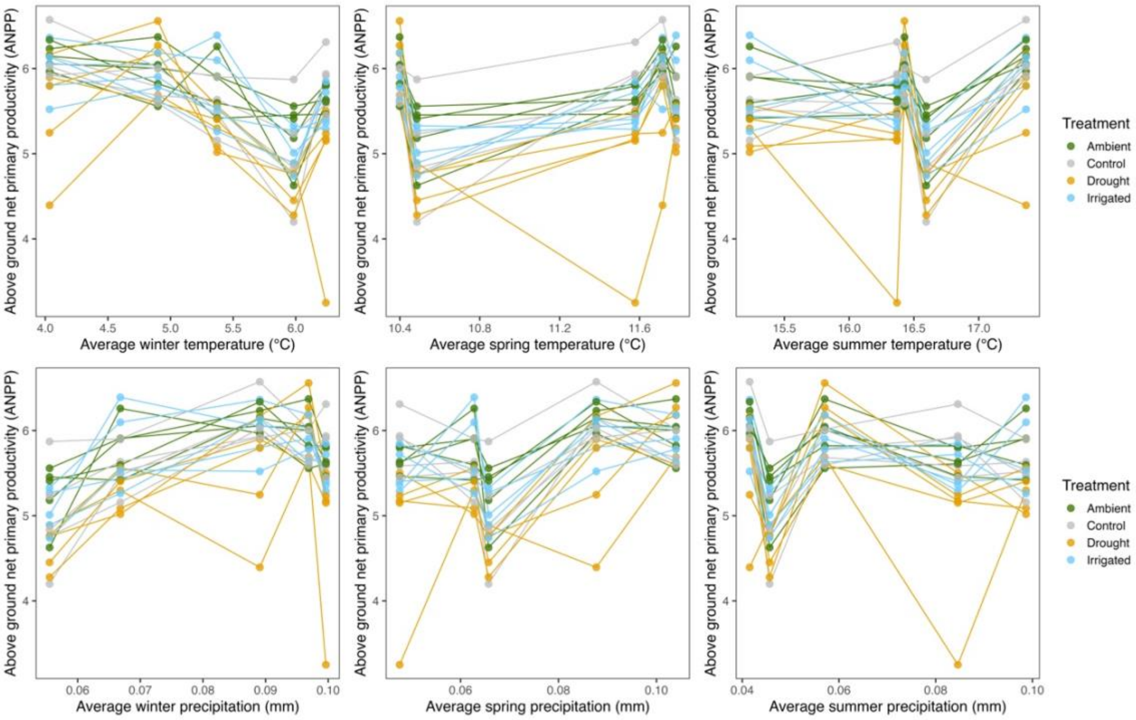


Figure S3.6 No clear effect of average daily precipitation and temperature in the winter, spring and summer on above-ground annual net primary productivity. Points are observations for each experimental plot in each year, and connecting lines are for the same plot across observation years as a visual aid.

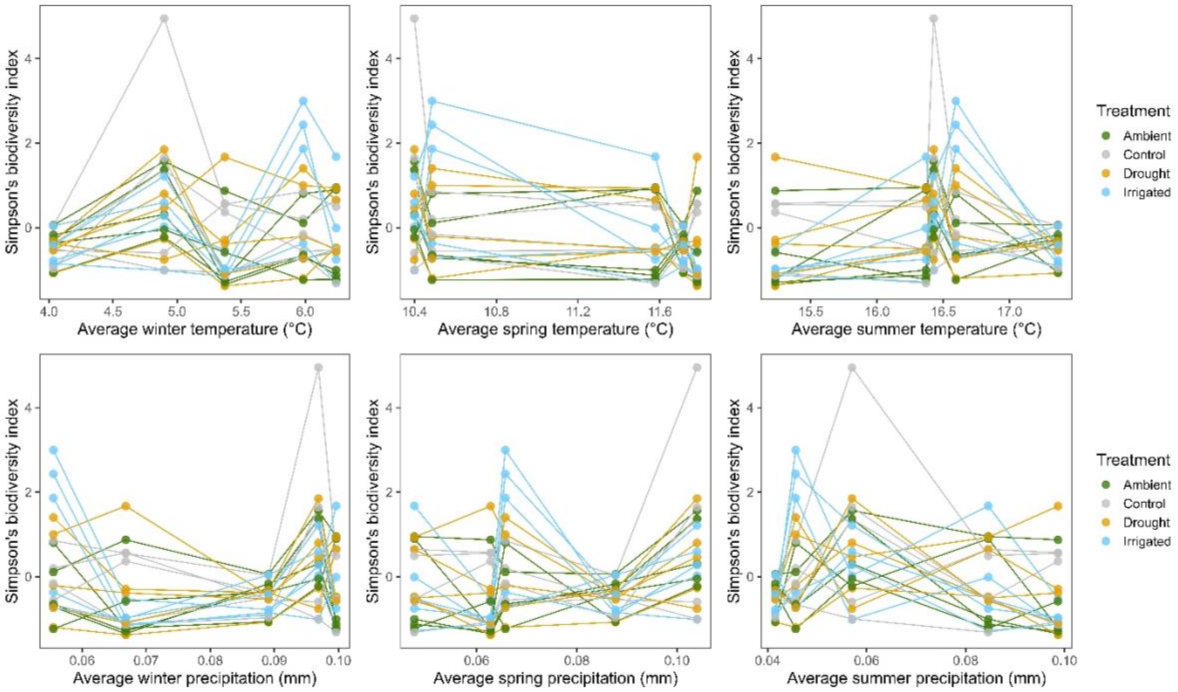


Figure S3.7 No clear effect of average daily precipitation and temperature in the winter, spring and summer on Simpson's biodiversity index. Similar patterns observed for other biodiversity metrics. Points are observations for each experimental plot in each year, and connecting lines are for the same plot across observation years as a visual aid.

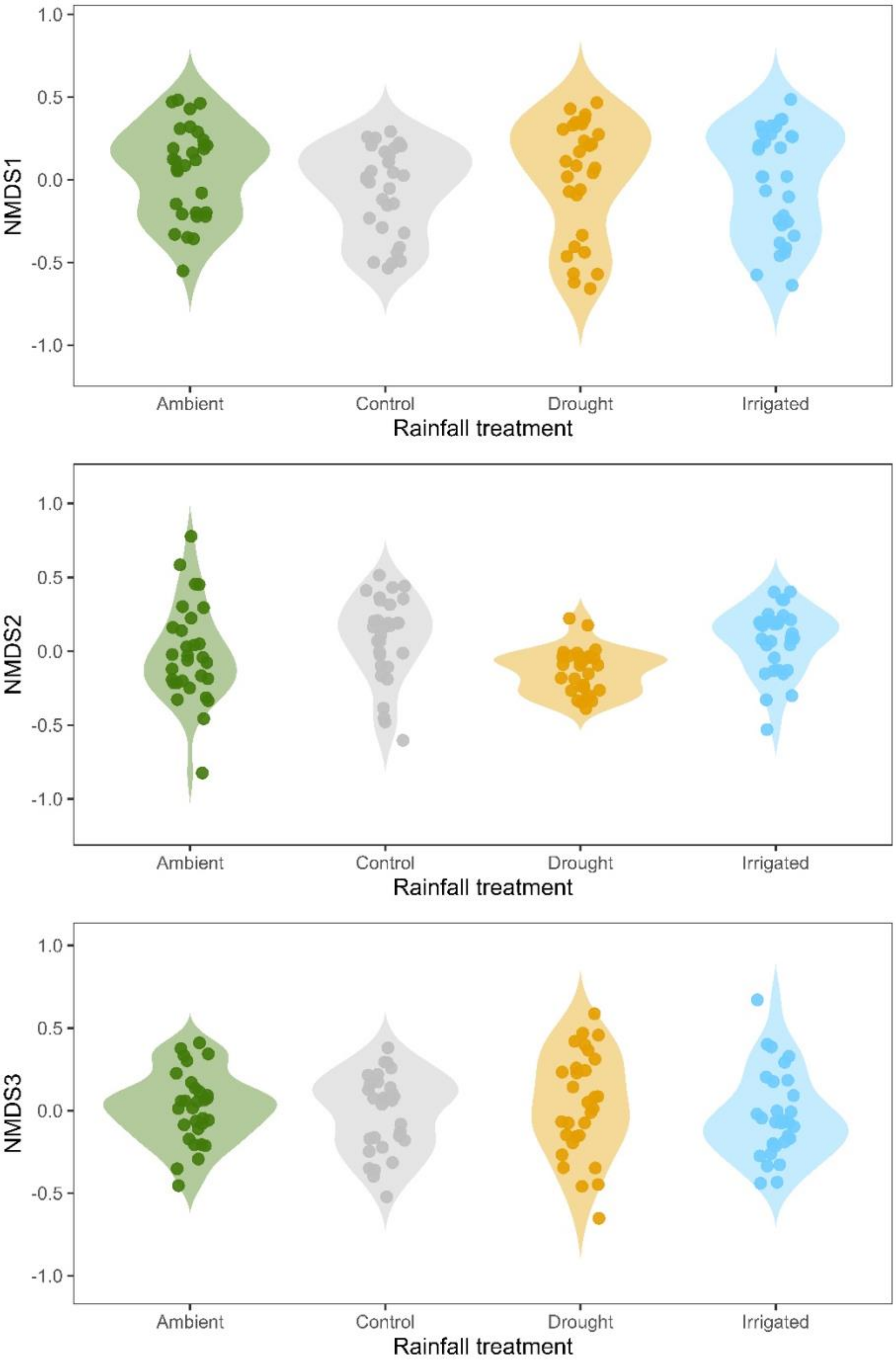


Figure S3.8 No clear difference in community composition between rainfall treatments, as calculated from three axes of non-metric multidimensional scaling (NMDS; first two axes presented in Figure 3.3d). Non-metric multidimensional scaling was performed for community composition from species percentage cover data.

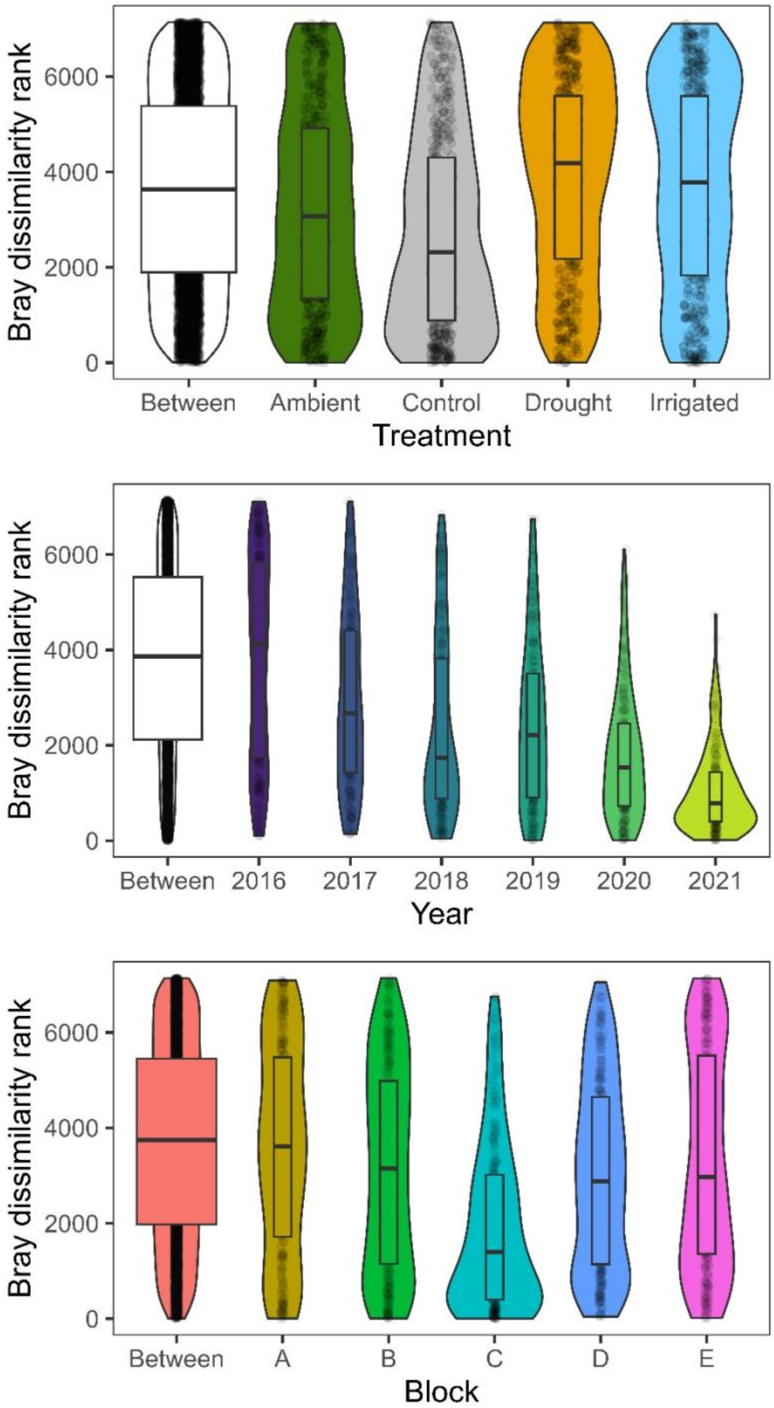


Figure S3.9 Analysis of similarities for within vs, between group community composition comparisons, based on precipitation treatment, observation year, and experimental block.

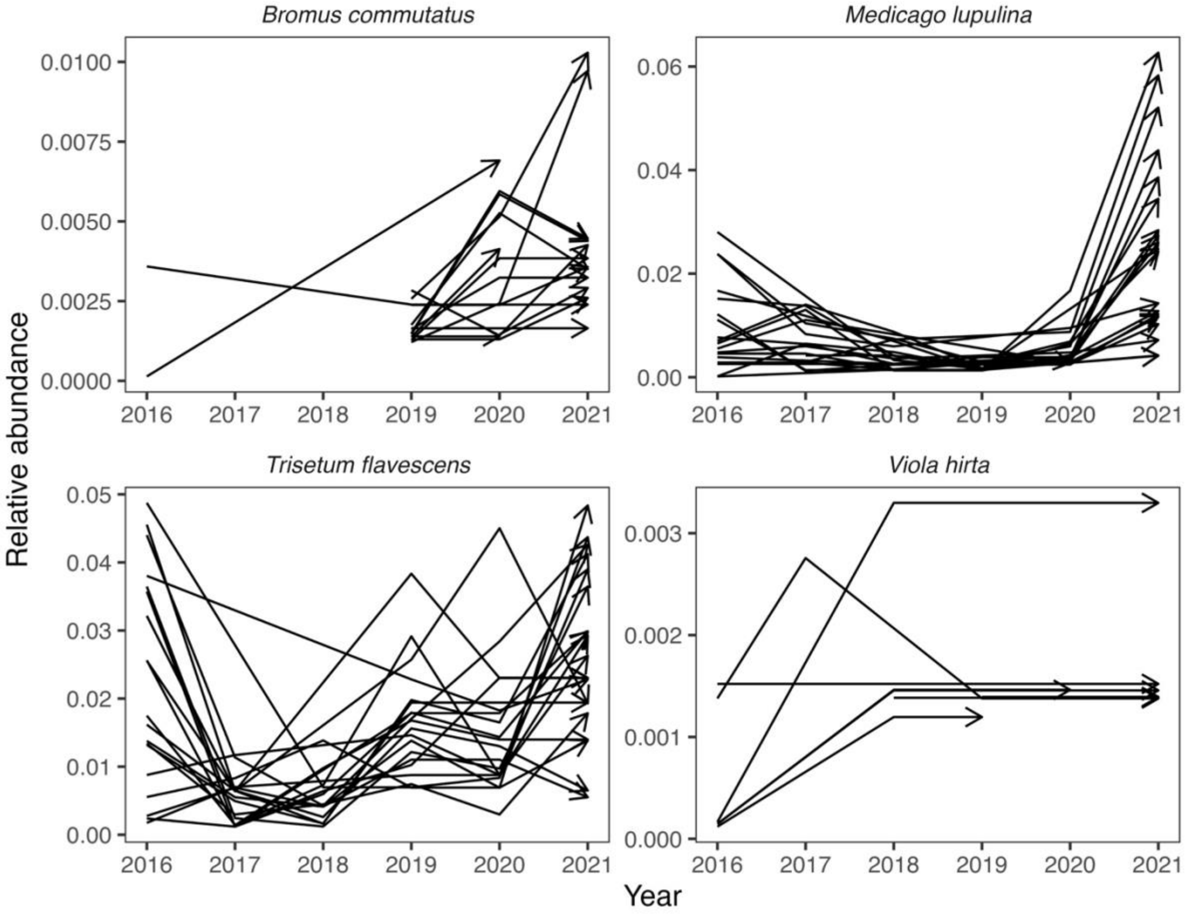


Figure S3.10 Four species with significant positive trends in relative abundance across years. Each line arrow gives the trajectory of relative abundance for a single species in a single plot. Positive trends were ascertained using linear regression of relative abundance across years. Generally, most species did not have a significant temporal trend in abundance.

Chapter 4: Demographic responses of *Brachypodium sylvaticum* to experimental precipitation manipulation

Sara L Middleton

(Image: Brooke Johnson, 2024)

Abstract

Grasslands are a major component of the terrestrial biosphere, comprising 40% of the ice-free land area. How grasslands respond to environmental change has critical significance for the integrity of the whole Earth system. Ecological droughts are a major stressor for grassland ecosystems causing physiological reactions leading to reduced plant functioning. Despite their importance and vulnerability to climatic extremes such as drought, grasslands remain relatively undervalued and understudied demographically.

Here I report on results on a three-year (2019-2021) demographic study conducted on dominant bunch grass *Brachypodium sylvaticum* in a UK calcareous grassland with experimentally manipulated (50% reduction- drought and 50% addition - irrigated) growing season rainfall. Seven demographic morphological measurements on vegetative and reproductive structures were measured on 138 individuals accounting for neighbourhood biotic interactions (plant-plant dynamics, ant and rabbit activity).

Results suggest that *B. sylvaticum* is largely resistant (in the short term) to experimentally manipulating growing season precipitation. Vital rate responses to precipitation treatments were largely undetectable, except for vegetative and reproductive height, and survival was high across treatments (93.4%, range = 85.4 – 96.4%, n = 129 in 2021). Plant height showed significant reduction with drought with evidence of lag effects and recovery over the study period. Reproductive parameters registered marginal negative treatment impacts, with some evidence of reproductive inefficiency in both droughted and irrigated individuals. There were

no clear signals of a parental penalty or treatment differences in seedling vital rates due to the low number of recruits (n = 23) recorded over the study period. Surprisingly neighbourhood biotic interactions did not seem to significantly moderate precipitation treatment effects on demographic outcomes. Variation between years and individuals and lack of efficacy in the irrigated treatment were identified as key drivers of the observed variation in demographic responses.

The outcomes highlight how demographic responses of *B. sylvaticum* to experimental precipitation treatments operate together with multiple other intrinsic (e.g. within and between individual variability) and extrinsic (e.g. neighbourhood biotic interactions and micro-environmental heterogeneity) processes which vary with time.

4.1 Introduction

How plant communities respond to global environmental change has critical significance for the integrity of the biosphere. Changes in vegetation functioning directly feed into the carbon and water cycles that mediate the climate, which in turn plants respond to, creating a feedback loop (Li *et al.,* 2023). The destabilisation of the hydro-climatic system is one of the major environmental stressors for terrestrial vegetation (Seleiman *et al.,* 2021). In many areas globally, the amount, frequency and duration of precipitation events is predicted to change (Knapp *et al.,* 2017; Lee *et al.,* 2021). As water comprises 80-95% of fresh plant biomass, extreme changes to water availability can cause alterations to the functioning, structure, and productivity of vegetation from individual to ecosystem levels (Li *et al.,* 2023; Seleiman *et al.,* 2021). Drought, defined here from the ecological plant perspective, is a deficit in water input relative to ambient levels or input relative to plant needs, reducing biomass accumulation and survival (Büntgen *et al.,* 2021; Carroll *et al.,* 2021; Swann, 2018).

Quantifying the direction and magnitude of plant responses to drought is challenging, due to different drought metrics (e.g., meteorological, ecological, or agricultural), and because vegetation can show diverging reactions across organisational (e.g. below-ground vs above-ground plant organs), geographic (e.g. xeric grasslands generally being more drought sensitive than mesic ones Griffin-Nolan *et al.,* 2019; Knapp *et al.,* 2017) and temporal scales (e.g. timing and duration of drought, Carroll *et al.,* 2021). The length of a drought event influences the magnitude of ecosystem-level responses, as shown experimentally by Carroll *et al.* (2021) in their comparisons of pulse vs press drought disturbances on ecosystem productivity in a US mesic grassland. Whilst above-ground net primary productivity was reduced in both drought durations compared to ambient conditions, there was a significantly larger reduction in acute drought conditions (Carroll *et al.,* 2021). Plant community reactions to drought in a given year are also moderated by antecedent precipitation conditions resulting in both lagged and cumulative responses (Wei *et al.,* 2022; Zhao *et al.,* 2020). Zhao *et al* (2020) reported a one-to-three-month lag in the Normalized Difference Vegetation Index (NDVI), with cumulative drought effects registering between 5 and 10 months later in Chinese Loess Plateau grasslands.

The observed macro-level vegetation responses to drought are linked to reactions to the individual-level physiological and biochemical processes which in turn affect plant demography. Typical drought symptoms include increased stomatal closure to reduce water loss at a cost of reduced gas exchange, leading to sub-optimal photosynthesis rates (Seleiman *et al.,* 2021). Persistent drought stress can result in a plant's inability to meet their metabolic needs due to the coupled mechanisms of hydraulic failure and carbon starvation, which can be lethal (Swann, 2018). Plant growth and development are dependent on cell division and differentiation which relies on optimally functioning plant apparatus which becomes hindered under drought stress. As a response, individuals, particularly perennials, can adjust their physiology to increase their survival (Salguero-Gómez and Casper, 2010). These adjustments are not always uniform in above and below ground plant organs, suggesting independent mechanisms governing their drought responses not fully captured by either the leaf or root economics spectrum (Asefa *et al.,* 2022; Wright *et al.,* 2004). To reduce energetic costs of somatic maintenance and damage, drought-stressed individuals are typically shorter, with a reduced leaf number, size and longevity (Seleiman *et al.,* 2021). This shrinkage (retrogression) presents direct and indirect impacts on plant fitness, as size is a major predictor of reproductive success (Salguero-Gómez and Casper, 2010). In addition to size, ontogeny also influences an individual's demographic responses to environmental stressors. In their early life-history stages, seedlings generally have reduced capacity to tolerate and high mortality from stressors like drought, pathogen attack and herbivory compared to more established individuals (Moles and Westoby, 2004).

Biotic interactions also moderate a plant's ability to respond to and resist drought stress. Drought-induced changes in plant-plant interactions tend to be less frequently studied compared to trophic interactions (e.g. herbivory and soil food webs, Chomel *et al.,* 2022; Ploughe *et al.,* 2019). Under intermediate abiotic stress, plant-plant interactions tend to shift from a competitive to a facilitative relationship thereby increasing community resistance to abiotic stressors (Bertness and Callaway, 1994; Grant *et al.,* 2014). Features of neighbouring plants such as above-ground architecture or nitrogen fixation ability can buffer against drought stress through reducing soil moisture evaporation via shading and nutrient additions respectively (de Jonge *et al.,* 2021; Hofer *et al.,* 2016; Ploughe *et al.,* 2019).

Grasslands are particularly sensitive to drought compared to forested ecosystems due to lower water use efficiency and the reduced ability to exploit deeper pockets of soil water owing to shallower rooting depths (Felton and Goldsmith, 2023; Kowalski *et al.,* 2023). Despite grasslands being an expansive repository for biodiversity and soil carbon, covering 40% of the Earth's land surface and nearly 70% of agricultural land area (Bardgett et al., 2021), assessing their impacts from environmental change, especially droughts are often overlooked (Parr *et al.,* 2014). Further, for high conservation priority habitats supporting up to 40 plant species per m^2, such as calcareous grasslands (Loos *et al.,* 2021, citing Ellenberg and Leuschner, 2010), even less is understood about their reactions to drought. Calcareous grasslands are characterised by a limestone bedrock overlain by shallow nutrient-poor alkaline soils and are in decline due to multiple pressures from agricultural intensification, eutrophication and changes in scrub and grazing management (Ridding *et al.,* 2020). Drought could buffer some effects of the previously mentioned threats in Central European grasslands, as drier spells (in addition to warming) can maintain community stability by filtering out poorly adapted competitive or potentially invasive species (Mazalla *et al.,* 2022).

In the context of European grasslands, within the last decade, Europe has been marked by the most severe summer drought of the last two millennia (Mazalla *et al.,* 2022). Indeed, the climate change predictions for the region indicate declines in growing season precipitation manifested via periods of more intense and prolonged droughts (Bütof *et al.,* 2012). Precipitation manipulation experiments in grasslands are a common approach to investigate this aspect of climate change and have yielded important insights into the patterns of responses to net primary productivity (Carroll *et al.,* 2021), nutrient cycling (Sardans and Peñuelas, 2007), community structure (Batbaatar *et al.,* 2022) and community functional composition (Griffin-Nolan *et al.,* 2019). Demographic approaches can extend our knowledge further through integrating ecological responses to climate extremes by examining the underlying mechanisms for the observed patterns at ecosystem and community levels (Felton and Smith, 2017; Ehrlén, 2019). Changes in demographic processes via an individual organism's vital rates (e.g. survival, growth and fecundity) can induce shifts in population structure with these effects propagating at higher organisational levels. This integrated response pathway follows the hierarchical response framework by Smith *et al.* (2009) which details how plants respond to climatic extremes from individuals to ecosystems (see also review by Felton and Smith, 2017).

Demographic approaches that focus on dominant species in particular can provide helpful perspectives, as highly abundant species are hypothesised to be major moderators of community and ecosystem structure and functions (Smith *et al.,* 2009; Whittaker, 1965). As such, dominant species can be utilised as "barometers" by studying their demographic responses to climatic extremes, creating a promising avenue to examine potential drivers of change at higher levels of organisation. Due to the complex nature of plant life-histories (e.g. retrogression, coloniality and seed dormancy), empirical data on key demographic parameters of perennial plants are limited on fine spatial scales (and long-time scales), particularly in grassland ecosystems (Lauenroth and Adler, 2008), hindering our ability to quantify and predict ecological processes. In addition, examining phenotypic variation in responses to climate extremes of key demographic processes can provide insights into populations adaptive potential and persistence in a rapidly changing world (Ravenscroft *et al.,* 2015). In a long-term climate change experiment on a calcareous grassland in northern England, evidence was found for climate-driven evolutionary change in key demographic parameters in several species at the site (e.g. vegetative and reproductive traits in *Plantago lanceolata* (Ravenscroft *et al.,*2014) and reproductive and germination phenology in *Festuca ovina* (Trinder e*t al.,* 2020)).

The present study uses three years (2019-2021) of individual demographic data combined with community-level data to examine the reduction in growing season water input in a precipitation manipulation experiment in a recovering UK calcareous grassland. The experimental site has imposed two precipitation extremes of 50% reduction and 50% addition in growing season rainfall, which hereafter will be referred to as drought and irrigated respectively. The dominant perennial bunch grass species *Brachypodium sylvaticum* was the focal study species used to addresses the following questions:
Q1: Is the survival, above-ground vegetative and reproductive components and number of new recruits of *B. sylvaticum* reduced in response to a decrease in precipitation and enhanced by an increase?

Q2: Is the size of above-ground vegetative structures of reproducing vs non-reproducing *B. sylvaticum* individuals reduced in response to a decrease in precipitation and enhanced by an increase?

Q3: Is the net effect of neighbouring plant interactions on the size of above-ground vegetative and reproductive structures of *B. sylvaticum* less negative under more stressful drought conditions and *vice versa* for irrigated conditions?

Q4: How do biotic disturbances from rabbit and ant activity affect the size of above-ground vegetative and reproductive structures of *B. sylvaticum* and do these disturbance effects vary with precipitation treatment?

The study findings on individual and micro-community measurements were then related to longer established species richness data from the site (Jackson *et al.,* 2024).

4.2 Methodology

4.2.1 Site description and experimental set up

The RainDrop experiment is a ~ 2-hectare area nested within a 10-hectare calcareous grassland (160 m a.s.l) at Upper Seeds, Wytham Woods, Oxfordshire, UK (51°46'15.7" N, 1°19'56.9" W) (Figure 4.1) (Gibson, 2011, Jackson *et al.,* 2024). The experimental site sits on a bedrock of Jurassic Corallian Rag limestone overlain with a shallow alkaline soil layer (Kirby *et al.,* 2022). Information on the site usage and management prior to the 1940s is unknown, but over the centuries the Wytham Estate has oscillated between agriculture and managed woodland (Gibson and Kirby, 2011; Gibson, 2011). Between c. 1940-1980 the site was used intensively for arable crops. Since 1980, the site has been in recovery, naturally colonised with grassland and woodland edge plant species from soil seedbank and surrounding grassland and wooded areas maintained with sheep grazing (Gibson and Kirby, 2011). There was a previous climate change experiment at the site that ran during the 1990’s that investigated winter warming and summer drought (Cummins *et al.,* 1995). Since 2015, the site has been fenced to exclude grazing animals, particularly sheep. Rabbits remain a persistent occurrence at the site and are not controlled. For the 2016-2020 period, the range in average daily temperature and total daily precipitation was −5 to 26 °C and 0-40 mm respectively (Jackson *et al.,* 2024; Rennie *et al.,* 2017).

The Upper Seeds site as a whole is species rich considering the recent agricultural land use, with 264 plant species registered at least once between 1983 and 2007 (Gibson, 2011). The RainDrop site has 109 vascular plant species based on the most recent plant list in 2022, with the graminoids *Arrhenatherum elatius, Brachypodium pinnatum* and *B. sylvaticum* found in high abundance. Since 2016, the site has been managed with two annual cuts with above-ground biomass removal in mid-July and end of September (Jackson *et al.,* 2024).

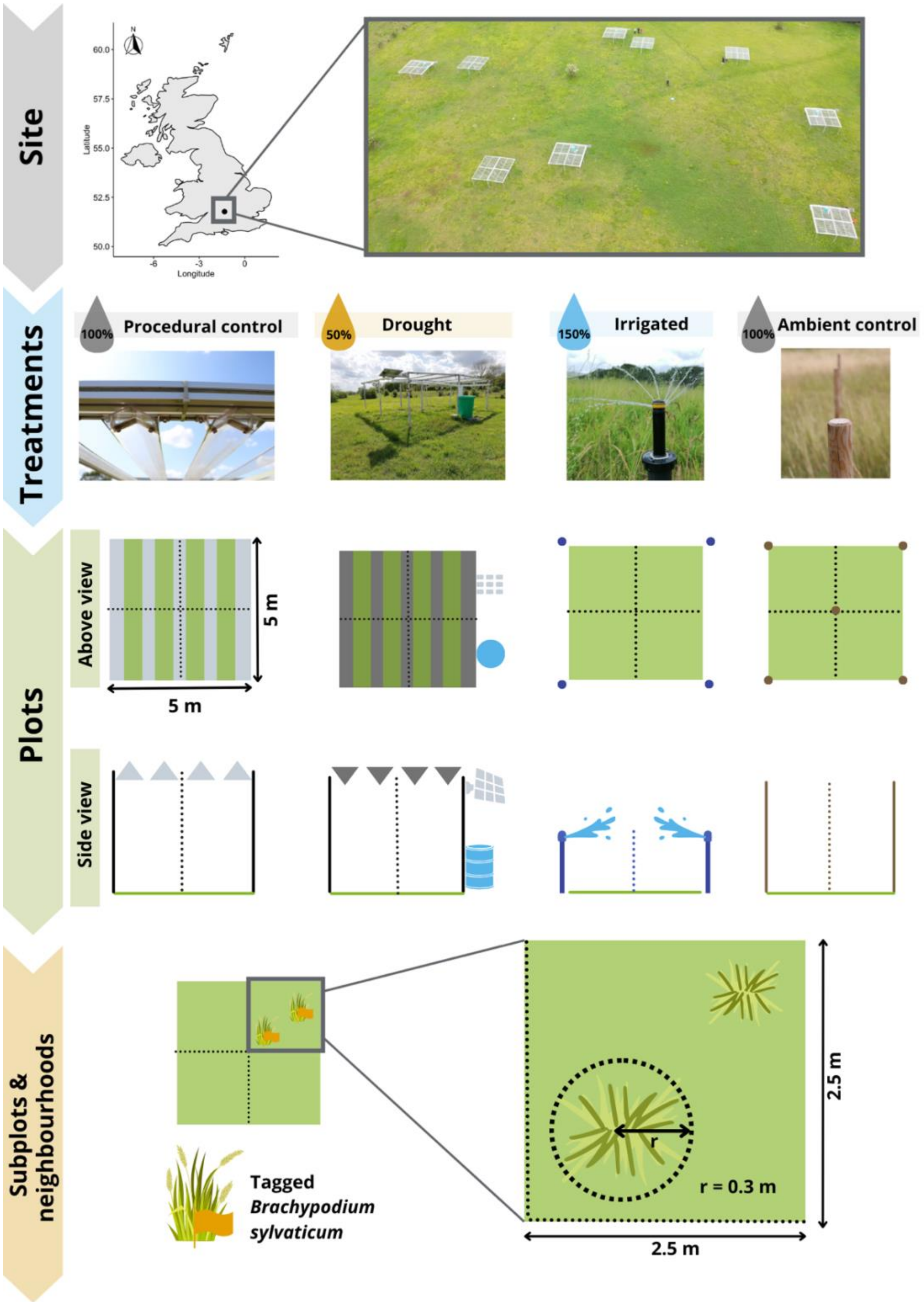


Figure 4.1 The randomised replicated blocked design of RainDrop at Upper Seeds, Wytham Woods outlining the distribution and structure of 5 x 5 m experimental plots and arrangement of the 0.3 m radius neighbourhoods around tagged *Brachypodium sylvaticum* individuals. Drone photo in top right by Rob Salguero-Gomez.

The Raindrop experiment was established in 2016 to examine how manipulating precipitation affects grassland functioning and resilience, as part of a coordinated international drought network (DroughtNet) (https://droughtnet.weebly.com/). The DroughtNet consortium consists of over 100 sites distributed across six continents where each site follows a minimum set of biotic and abiotic measurements set out in experimental protocols (Knapp *et al.,* 2017). Extreme drought is simulated through the passive interception of precipitation through fixed rainout shelters applied at the plot level. During extreme wet and dry years, a 40% and 30% departure from mean annual precipitation levels respectively can be observed (Knapp *et al.,* 2015). To ensure multisite comparisons, treatment level is applied relative to the long-term precipitation records of the site (Knapp *et al.,* 2017; Smith *et al.,* 2024).

At RainDrop, the precipitation manipulation consists of four treatments applied on 20 replicated experimental units of 5 x 5 m plots randomly arranged across five blocks. The four treatments consisted of two controls: ambient and procedural control (each receiving 100% of incoming precipitation), drought and irrigated representing 50% and 150% precipitation levels respectively (Figure 4.1). Rainout shelters exclude precipitation via v-shaped polycarbonate plastic gutters mounted onto metal structures. The height of the shelters varied from between 1.65 m to 2.10 m, reflecting the small topographic changes at the site and to reduce microclimatic effects of humidity and temperature. To maintain near-surface air exchange, all four sides of the shelter were kept open. The intercepted precipitation from drought plots was collected in an automated water collection tank, which was then redistributed to irrigated plots via a sprinkler system. Procedural control plots were used to determine the effect of the shelter on incoming photosynthetically active radiation and humidity. Although the rainout shelters and plastic gutters remain in place year-round, the drought treatment was only applied during the growing season between April 1st and October 1st each year. Outside the growing season, the drought shelter gutters are inverted to reinstate ambient precipitation levels.

4.2.2 Life history and ecology of Brachypodium sylvaticum

False brome *(Brachypodium sylvaticum)* (Huds.) is a bunch-forming perennial grass species native to Eurasia that is self-compatible, and wind pollinated (Figure 4.2) (Monroe and Des Marais, 2021; Stace, 2019). In the UK, it grows up to 1 m in height on woodland edges and scrub, favouring well-drained calcareous-neutral soils (Streeter *et al.,* 2009; Stace, 2019). The seedbank is short-lived, typically no more than one year (Davies and Waite, 1998) and individuals start out from seed usually near the parent plant, but short-distance seed dispersal can occur, typically with ungulates such as deer (Miller *et al.,* 2011; Roy *et al.,* 2011). Seedlings grow through the production of asexual tillers which usually flower after their second year (Roy *et al.,* 2011). *B. sylvaticum* flowers relatively late in the growing season, between July and August. Outside the growing season, particularly during cold winter months, plants will die back to ground level (Roy *et al.,* 2011).

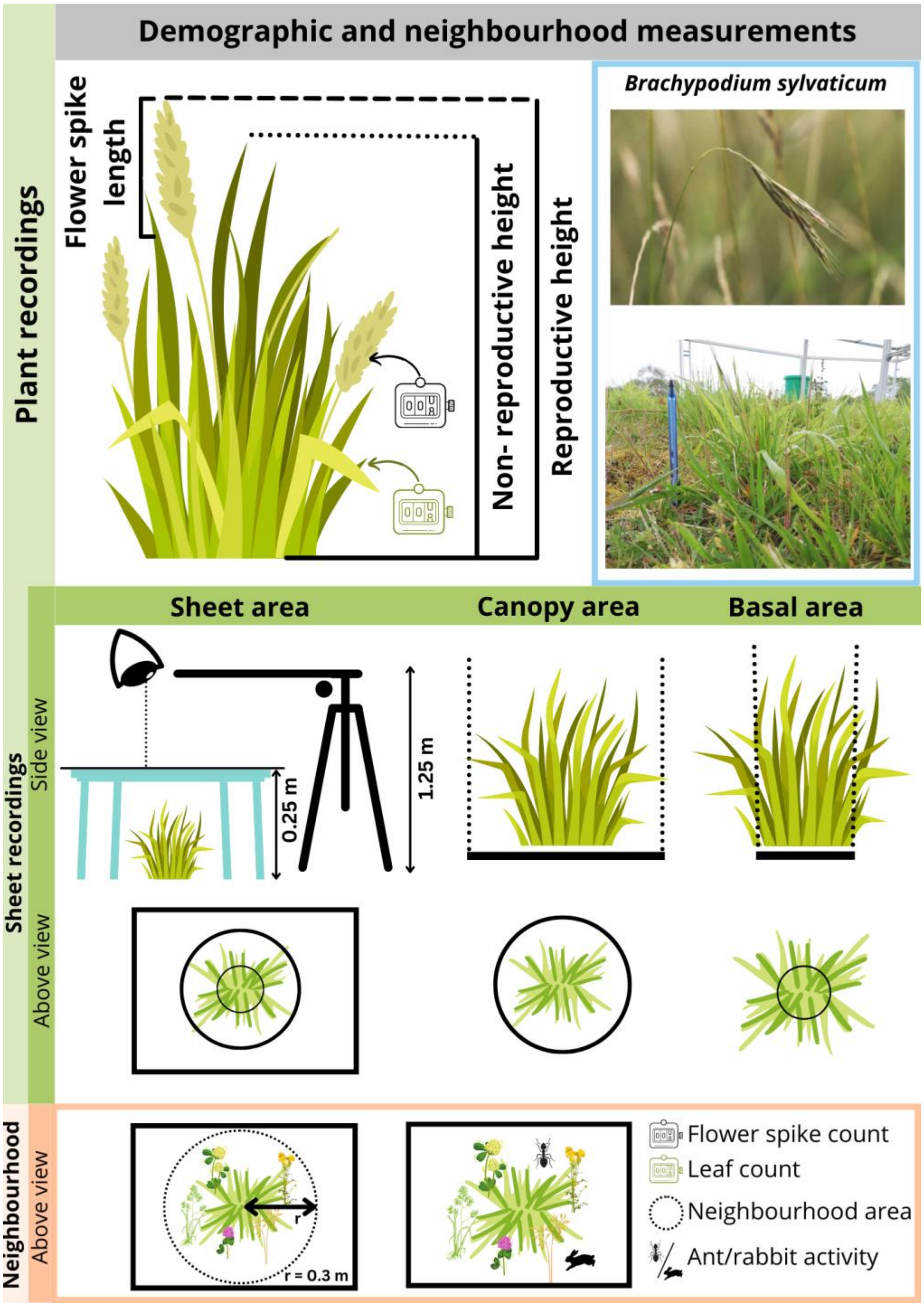


Figure 4.2. The seven measurements relating to demographic performance taken on tagged *Brachypodium sylvaticum* individuals and of their neighbourhoods during the peak growing season period between 2019 and 2021. Plant recordings refer to the five demographic measurements (non-reproductive and reproductive height, number of leaves and inflorescences (spikes) and length of inflorescence) taken directly from the physical plant. Sheet recordings denote measurements using acetate sheets (canopy and basal area). Top right (blue box): photographs of *B. sylvaticum* from the RainDrop site at Wytham Woods; top photo: close up of *B. sylvaticum* inflorescence; bottom: side view of an individual bunch taken in early May.

4.2.3 Data collection: proxies of demographic performance

The life history and high abundance of *Brachypodium sylvaticum* at RainDrop made it an ideal focal species for this study. In May 2019, 138 mature individual bunches of *B. sylvaticum* were tagged to the nearest centimetre using a cartesian coordinate system with the shelters (Figure 4.1). Given the complex nature of plant modularity and defining individuality (see section 1.5 in **Chapter 1**), for practical reasons in this study an individual was classed as a distinct bunch, which was unconnected aboveground to other bunches. To avoid edge effects, individuals were as far as practical selected beyond a 0.3 m buffer zone at the edge of the plot (~91% of tagged individuals were outside buffer zone, Figure S4.1). As far as possible an even tagging coverage between treatment types was made, where 33, 34, 43 and 28 individuals were tagged in drought, ambient control, procedural control and irrigated plots respectively (Figure S4.2).

To ascertain the effects of the drought treatments on the life history of individual *B. sylvaticum* bunches, seven variables pertaining to aspects of plant growth and reproduction were measured (reproductive and non-reproductive height, number of leaves, basal and canopy area, number of inflorescences (flowering spikes) and inflorescence length) (Figure 4.2). These measurements were taken during the peak growing season (late June to late July) between 2019 and 2021. Both reproductive and non-reproductive height were measured from ground level to the top of the tallest unstretched flowering stem (culm) and leaf blade in centimetres to one decimal place respectively. Flowering spike length was measured by first selecting the tallest culm and then measuring to the nearest centimetre to one decimal place from the base to the tip of the awns. Counting the number of leaves and culms involved using a hand tally counter. Leaves or culms with 50% or more of visible signs of senescence or disease did not contribute to the tally count. The number of leaves and inflorescences were used to estimate reproductive allocation as the ratio of number of inflorescences to the sum of leaves and inflorescences (Bazzaz *et al.,* 2000). The count of reproductive and vegetative structures was used as a proxy for biomass or energy, which have tighter links to demographic performance, but are more labour and time intensive to measure (Wenk and Falster, 2015; de Bello *et al.*, 2021). Basal and canopy area were recorded using permanent markers on 0.60 x 0.84 m acetate sheets mounted on a transparent table 0.25 m above the ground (Figure 4.2). A new acetate sheet was used at each growing season and some tagged individuals growing near each other were recorded onto the same sheet. The four corners of the table were geo-referenced in the same way as the tagged individuals and placed in the same location at each census. The 0.25 m table height was constrained for practical reasons and some of the larger individuals were slightly vertically compacted. To maintain uniformity between recordings, the recordings were made from a fixed viewing distance of 1.25 m.

Prior to the start of each field data collection, individuals were retagged, based on 2019 coordinates, with visible markers which were removed at the end of the previous growing season. At this stage, tagged individuals were also assessed if they survived the winter period, signs of new recruits and for any visible leaf damage. Individuals with no visible above-ground biomass or total leaf senescence (i.e. brown dry leaves) were classed to have died. Attributing cause of death was not possible in the vast majority of cases, but if rabbit activity (e.g. signs of burrowing) was observed within neighbourhoods of tagged individual this was noted as a possible contributing factor. Individuals were classed to have reproduced if there were new recruits present (i.e. small seedlings) within a 0.20 m radius of the basal area outer edge of tagged plant, which were not present the year before. Accounting for immigration inside plots and genotyping was outside the scope of this study, however, it was reasonable to assign parentage of new recruits to the closest tagged plant as *B. sylvaticum* seeds typically germinate close to the parent plant and opportunities for animal-assisted seed dispersal limited due to site-fencing. New recruits were tagged using the same geo-reference system as parent plants and given a new ID number. Demographic measurements of new recruits followed the same protocols as mature individuals.

4.2.4 Plant-plant interactions and additional unmanipulated measurements in neighbourhoods

To account for the aboveground plant neighbourhood effects on the demographic performance of the target individuals, species richness (as counts) of neighbouring plants species was recorded. The neighbourhood around the target individuals was constrained to the size of the sheets to within 0.3 m radius to centre of target individuals. Plants that were rooted outside but fell into the neighbourhood area were counted, as any crowding would affect light and nutrient availability of target individuals (Cahill, 2003). The identity of plants was resolved to the lowest taxonomic level following Stace (2019). Recording of the neighbouring species of new recruits were classed as the same as their parent plant.

Additional unmanipulated variables such as presence/absence of rabbit and ant activity within 0.3 m radius neighbourhoods were also measured and are listed as covariates in Table 4.1.

These variables were not explicitly outlined in the experimental design but were important to include due to effects on demographic performance of tagged individuals.

4.2.5 Data processing

Processing image-based data to identify meaningful objects can be time and labour-intensive. Therefore, an automated image segmentation analysis was used to extract the basal and canopy areas from digitised recordings on the acetate sheets. The five key steps involved are outlined in Figure 4.3 and briefly described below. For more information on the wider context of automating image segmentation in vegetation monitoring see Middleton (2023).

After basal and canopy area recordings on the acetate sheets (384 sheets in total) were made in the field (step 1, Figure 4.3) they were photographed against a white background using a Canon 80 D camera attached to a tripod with a centimetre ruler for scale (step 2, Figure 4.3). Two photographs of each sheet were taken and individually checked for quality to ensure no glare or blurriness. The photographs with the best image quality were selected for the next stage of digitising. To extract size area data from the photographs, the NumPy, Matplotlib and OpenCV packages in Python (version 3.9.5) were used (Bradski and Kaehler, 2008; Harris *et al.,* 2020; Hunter, 2007). Each image was first cropped to locate region of interest (ROI), then the warp perspective was applied to adjust for minor skew from the angle that the photograph was taken (step 3, Figure 4.3). Edge detection and thresholding (binarization) enabled the segmentation of ROIs from each image (step 4, Figure 4.3). The number of pixels were then extracted for both canopy and basal area (step 5, Figure 4.3).

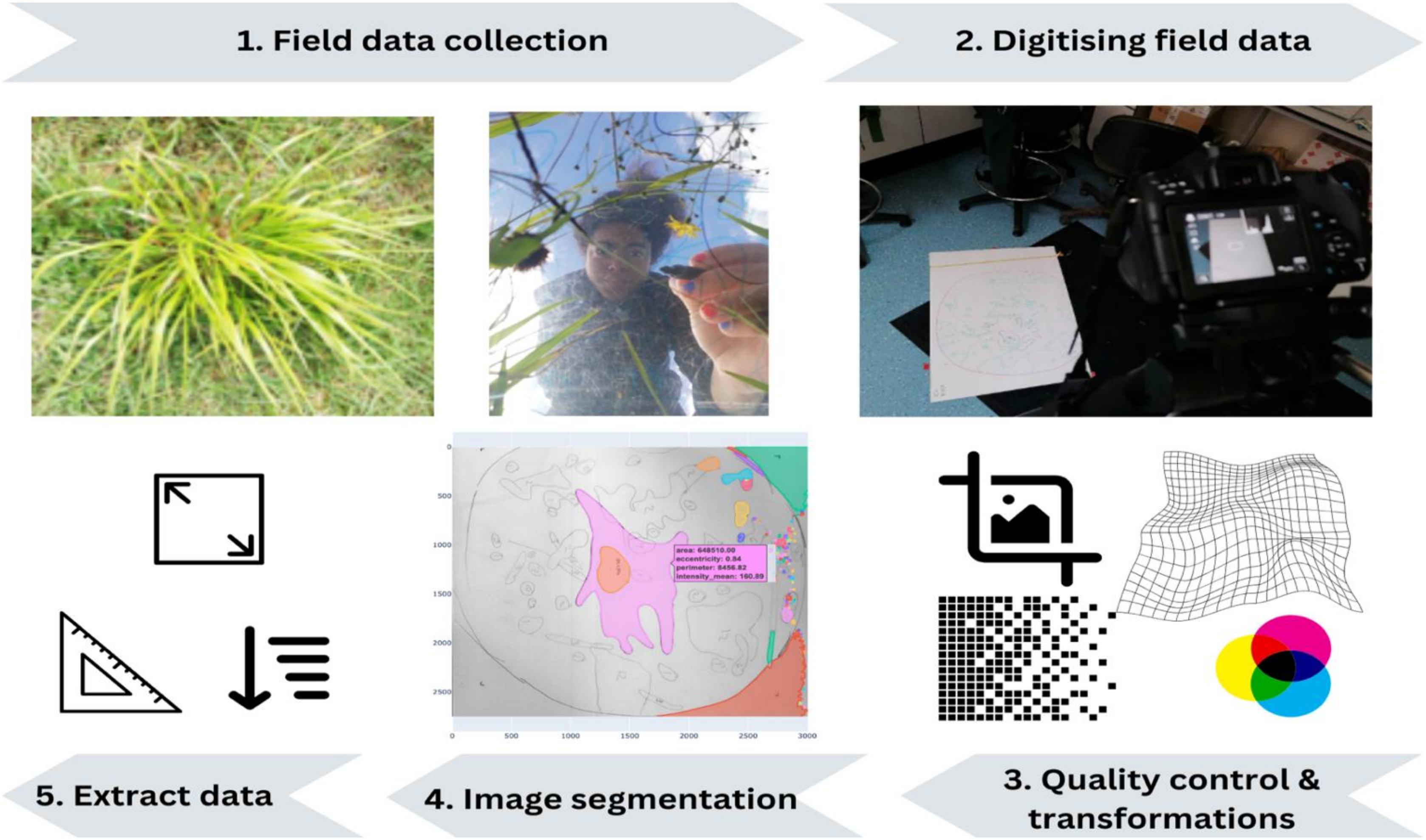


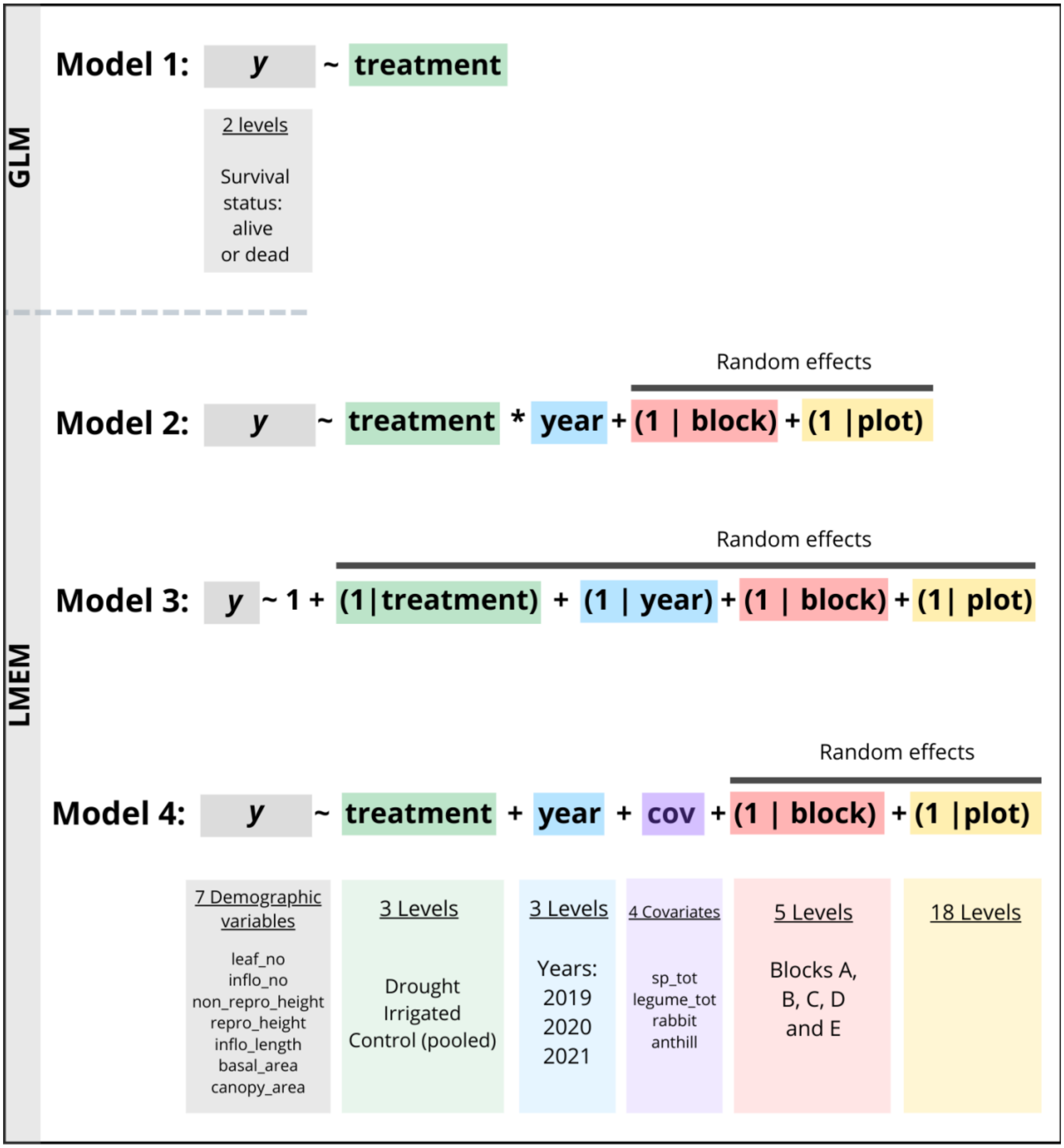
Figure 4.3. The five key steps involved in using automated image segmentation to extract *Brachypodium sylvaticum* plant size data from field recordings on acetate sheets. Numbers refer to key steps from field data collection (step 1) to extracting required data from images (step 5).

4.2.6 Data analysis

Investigating the effect of precipitation manipulation on demographic performance of *Brachypodium sylvaticum* individuals involved performing linear mixed effects models and generalised linear models (GLM) using the ‘lme4’ package in R (version 4.2.0) (Bates *et al.,* 2015; R Core Development Team, 2023*)* (see below). Explanations of model structure, variables and their biological or experimental relevance are displayed in Box 4.1 and Table 4.1. Given their similarity (Table S4.1), ambient and procedural control treatments were pooled - for simplicity and to reduce the number of model parameters, resulting in a fixed-effect treatment factor having three levels: control, irrigated and drought.

Experimental models

To investigate if treatment affected the survival of mature individuals tagged at the start of the experiment in 2019 (Q1), a GLM with a binomial logit link with the following structure from the glm() function was computed using model 1, where, *y* was the survival status of mature individuals (alive/dead) at the end of the experiment in 2021, and treatment was a fixed factor with three levels (control, irrigated, drought). It was not possible to account for block and plot as random effects in the survival model using the glmer() generalised linear mixed effects model, as this model failed to converge and so a simplified version was used.



Box 4.1. Structure of experimental and post hoc models used to assess treatment effects on survival status (model 1), each of the seven demographic measurements (model 2) and variance component analysis (model 3). Model 4 was a post hoc model to test the influence of covariate variables on each of the seven demographic measurements. GLM and LMEM refer to structure of models of generalised linear model and linear mixed effect model respectively.

Linear mixed effects models were used to assess effect of treatment on each of the seven demographic variables in turn (Q1). To improve model fit, Box-Cox transformations were computed using the 'MASS' package for leaf and inflorescence number and basal area response variables (Ripley *et al.,* 2013). λ values indicated square root and natural log transformation for leaf and inflorescence number and basal area respectively. Full results of Box-Cox transformations can be found in Table S4.2. To reflect the experimental design, models were computed with treatment and observation year both as fixed categorical explanatory variables, each with three levels, and block and plot as intercept-only random effects at five and 18 levels respectively. Models followed the general structure for the lmer() function with restricted maximum likelihood (model 2), where *y* is one of the seven demographic response variables shown in Table 4.1. The treatment:year interaction term was dropped if it reduced the model fit (see below details of model comparisons). Conditional fixed effects with 95% confidence intervals from model 2 were graphed with the ggpredict() function from the 'ggeffects' R Package (Lüdecke, 2018).

It is not straight-forward to compare the importance of fixed and random effects in mixed-effects models. Therefore, to allow for direct comparisons of all variables in model 2, treatment, observation year, block and plot were also all modelled as intercept-only random effects with just a fixed intercept (grand mean) for each response variable *y*. This approach is an extension of ANOVA proposed by Gelman (2005) that allows comparison of all variables by treating them all as random effects to allow estimation and comparison of their variance components and uncertainties (model 3).

Post-hoc models

To assess the influence of non-experimental variables (Q3 and Q4), such as rabbit and ant activity and neighbourhood species richness, on the demographic performance of *y* response variables, covariate variables, *cov* were added to model 2 and followed the structure of model 4. Each *cov* variable was modelled separately for each *y* response variable. Conditional fixed effects and 95% confidence intervals of covariate models (model 4) were graphed with the same approach using ‘ggeffects’ R Package (Lüdecke, 2018) as for experimental models (model 2).

For all models, the ‘check_model’ function in the ‘performance’ package was used to inspect model fit (Lüdecke *et al.,* 2021). *Akaike information criterion* (AIC) values were computed to compare model performance for models 2 and 4 with ‘AICcmodavg’ package (Mazerolle and Mazerolle, 2017), with only the most parsimonious models selected for presentation (Harrison *et al.,* 2018). Details of candidate models can be found in Table S4.1.

Table 4.1. Description of variables and their biological relevance included in model selection for demographic analysis in mature *Brachypodium sylvaticum* individuals. Treatment originally four levels before ambient and procedural controls were pooled for analysis. Demographic measurement categories are shown in brackets are v = vegetative, r = reproductive, s = size, a = acquisition.

Variable	**Description**	**Unit**	**Levels**	**Transformation**	**Biological/experimental relevance**
Response (y)					
alive	Survival of mature individuals at the end of the experiment in 2021	*N/A*	2	N/A	A key whole plant demographic parameter
leaf_no	Number of leaves	*n*	N/A	Square root	Indicator of resource-availability for growth (v, a)
inflo_no	Number of flowering spikes (inflorescences)	*n*	N/A	Square root	Proxy for reproductive success (r)
repro_allocation	Ratio of number of flowering spikes to total number of leaves and flowering spikes	*%*	N/A	N/A	Indicator of resources available for reproduction (r)
non_repro_height	Vegetative plant height	cm	N/A	N/A	Indicator of vegetative size (v, s)
repro_height	Reproductive plant height	cm	N/A	N/A	Measure of vertical size of vegetative structure (r, s)
inflo_length	Length of inflorescence/spikelet	cm	N/A	N/A	Proxy for reproductive success (r)
basal_area	Basal area	pixels	N/A	Natural Log	Measure of lateral size of vegetative structure (v, s)
canopy_area	Canopy area	pixels	N/A	Natural Log	Measure of lateral size of vegetative structure (v, s)
Explanatory					
treatment	Type of treatment: (Drought (D) 50%; Control (C), 100%; Irrigated (I), 150% of incoming precipitation)	-	3	N/A	Modification of incoming precipitation during growing season (April-October)

Design					
year	Observation year	Year	3	N/A	Repeated measures to assess inter-annual variability
block	Block ID	-	5	N/A	Grouping variable
plot	Plot number	-	18	N/A	Experimental unit to which treatment is applied
Covariate (cov)					
sp_tot	Total number of co-occurring species in 30 cm radius neighbourhood	*n*	N/A	N/A	Proxy for plant-plant interactions (facilitation/competition)
legume_tot	Total number of co-occurring legume species in 30 cm radius neighbourhood	*n*	N/A	N/A	Proxy for plant-plant interactions (facilitation/competition)
rabbit	Prescence of rabbit activity within 30 cm radius neighbourhood	Prescence/ absence	2	N/A	Indicator of disturbance within plots
anthill	Prescence of anthills within 30 cm radius neighbourhood	Prescence/ absence	2	N/A	Indicator of disturbance within plots

4.3 Results

4.3.1 Survival and reproduction

Of the 138 individuals tagged in 2019, 129 (93.4%) survived into the 2021 growing season. The lowest finite survival rates were observed in control plots, where 85.4% (n = 71) of individuals survived in the two years post tagging (Table S4.3). Survival rates were higher in drought and irrigated plots at 93.9% (n = 31) and 96.4% (n = 27) respectively but with a high degree of overlap of the confidence intervals. Results of the logistic regression null model indicated high odds of survival of individuals at the end of the experiment ($p < 0.001$, Log-Odds = 2.66, SE = 0.34, n = 138), however there were no detectable differences in survival odds between treatments (Figure 4.4).

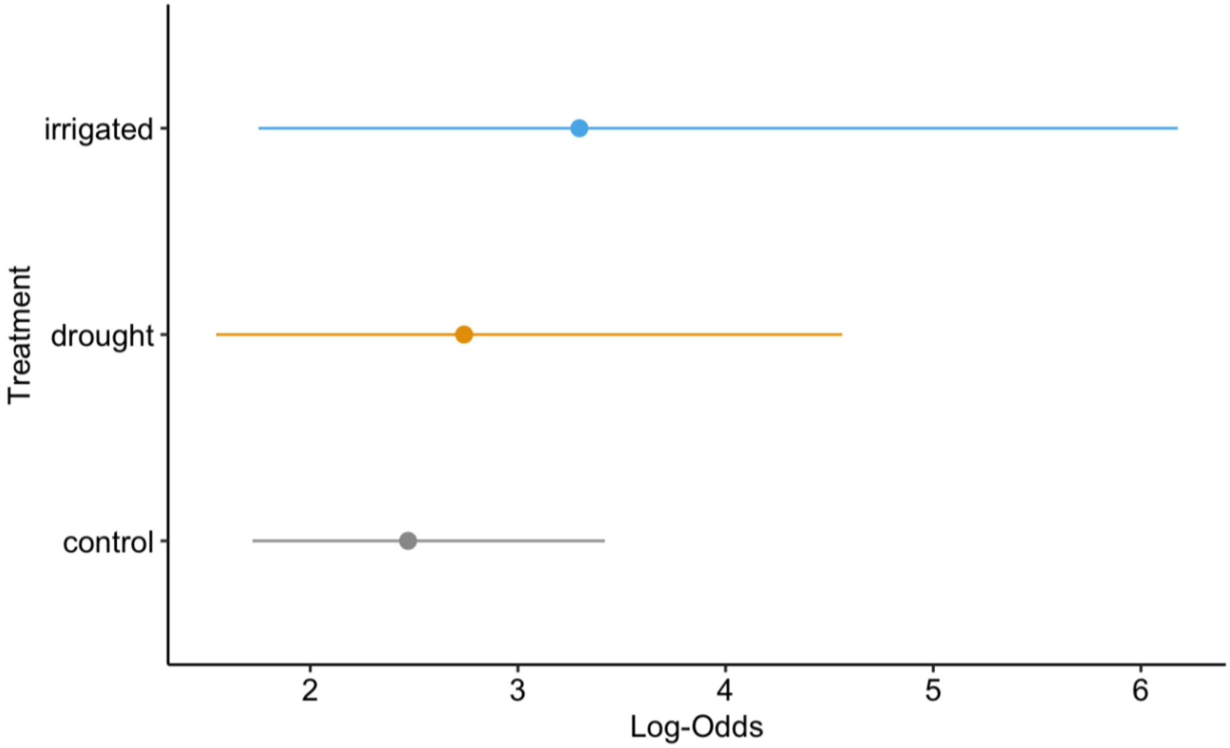


Figure 4.4 Logistic regression model results showing estimates and 95% confidence intervals of survival odds by treatment displayed on a Log-Odds scale (n = 138). Survival refers to the individuals that survived two growing seasons. A higher log-odds value indicates increased odds of survival.

Mean reproductive allocation (RA) for the study period was 17.8% (SD = 13.9, range = 0 - 77.2%, n = 397). RA in droughted individuals was generally lower (but not significant) than for control and irrigated individuals which both showed similar RA trends over study period (Figure S4.3). Between the 2020 and 2021 growing seasons, a total of 8.7% (n = 12) individuals reproduced with a range of 1-6 new recruits per reproducing individual. No qualitative differences were observed between reproducing (parent) and non-reproducing (non-parent) individuals and between treatments in the four demographic size measures: non reproductive height, number of leaves and canopy and basal area (Figure 4.5).

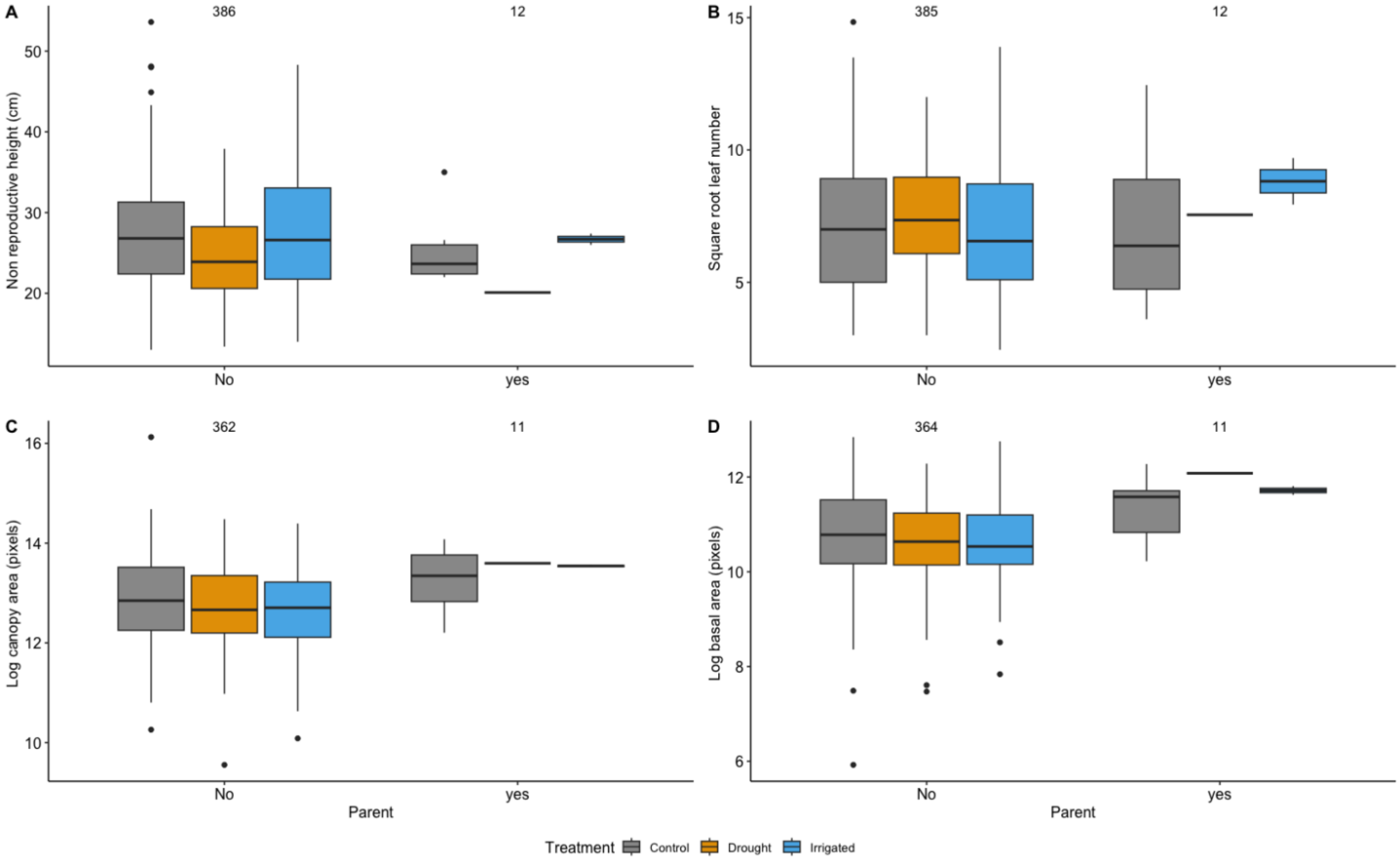


Figure 4.5 Box-and-whisker plots displaying relationship between parent status and four demographic size measures: non-reproductive height (A), number of leaves (B) and canopy (C) and basal area (D). Numbers above box-and-whisker plots indicate number of observations. Parent status equates to mature adults with at least one offspring in 2020 or 2021. Thick horizontal lines within boxes indicate median, the boxes represent the interquartile range, whiskers show minimum and maximum values, and the dots represent outliers. In some cases, sample size of parents was very low, resulting in collapsed box plots appearing to show no variation (absence of whiskers).

4.3.2 New recruits

A total of 23 new recruits were registered over the study period with 70% (n=16) found in 2020. The majority of seedlings, 83% (n = 19), were found in control plots, with irrigated and drought plots recording only two and one new recruits respectively. Five new recruits from 2020, including the one seedling from the drought plot, did not survive into the 2021 growing season. Qualitatively there was no difference in the non-reproductive height or in the number of leaves between 2020 new recruits found in irrigated and control treatments (Figure 4.6). Interestingly the single drought seedling registered almost four times the mean number of leaves ($\bar{x}$ = 3.5, SD = 2.3, n = 16) of all 2020 new recruits, despite not surviving into 2021.

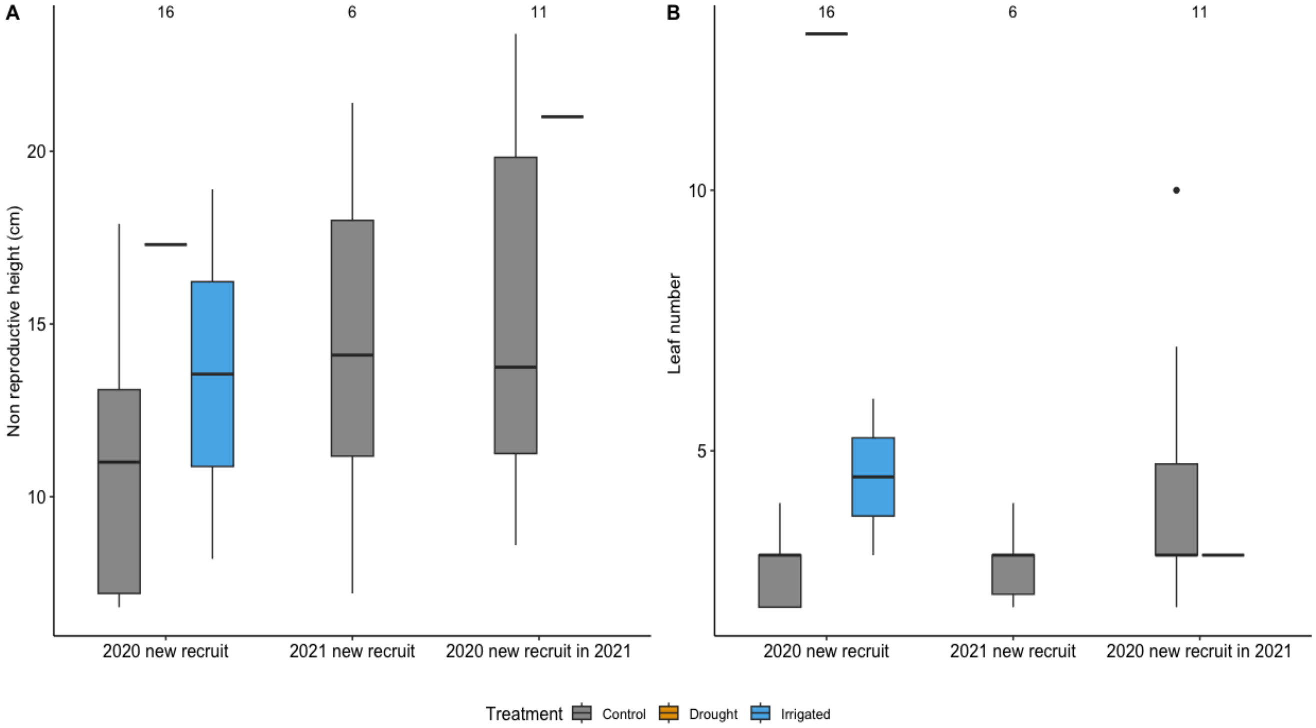


Figure 4.6 Box-and-whisker plots displaying relationship between new recruits and two size measures: non-reproductive height (A), number of leaves (B). Numbers above box-and-whisker plots indicate number of observations. Thick horizontal lines within boxes indicate median, the boxes represent the interquartile range, whiskers show minimum and maximum values, and the dots represent outliers.

4.3.3 Relationship between demographic performance proxies

Seven variables relating to demographic processes were measured on mature individuals between 2019-2021 growing seasons and generally treatment effects showed similar relationships across demographic measures. Pearson correlations between the four vegetative measures; non-reproductive height (n = 398), leaf number (n = 397) and canopy (n = 373) and basal area (n = 375) fell between -0.12 and 0.79 (Figure 4.7). Reproductive height (n = 369) showed overall significant positive correlation with the other two reproductive demographic variables inflorescence number (n = 398, $r^2 = 0.62$, p = < 0.001) and length (n = 369, $r^2 = 0.48$, p = < 0.001) (Figure 4.7). The two paired size measures: non-reproductive and reproductive height and canopy and basal area showed moderate and high positive correlations across treatments respectively. Correlations between leaf number (n = 397) and basal area (n = 375) showed diverging patterns between treatments, moderate positive ($r^2 = 0.42$, p = < 0.001) for drought and almost no association for control ($r^2 = 0.07$, p = > 0.1). This treatment divergence was particularly pronounced for canopy area and the three reproductive measures, where irrigated and drought treatments showed opposing trends. Canopy area (n = 373) and reproductive height (n = 369) showed a significant negative ($r^2 = -0.28$, p = < 0.01) and positive trend ($r^2 = 0.34$, p = < 0.01) for drought and irrigated treatments respectively, although this relationship reversed when correlations were examined by observation year (Table S4.4). Diverging treatment effect on the relationship between canopy area and reproductive variables was less pronounced for the two inflorescence measures.

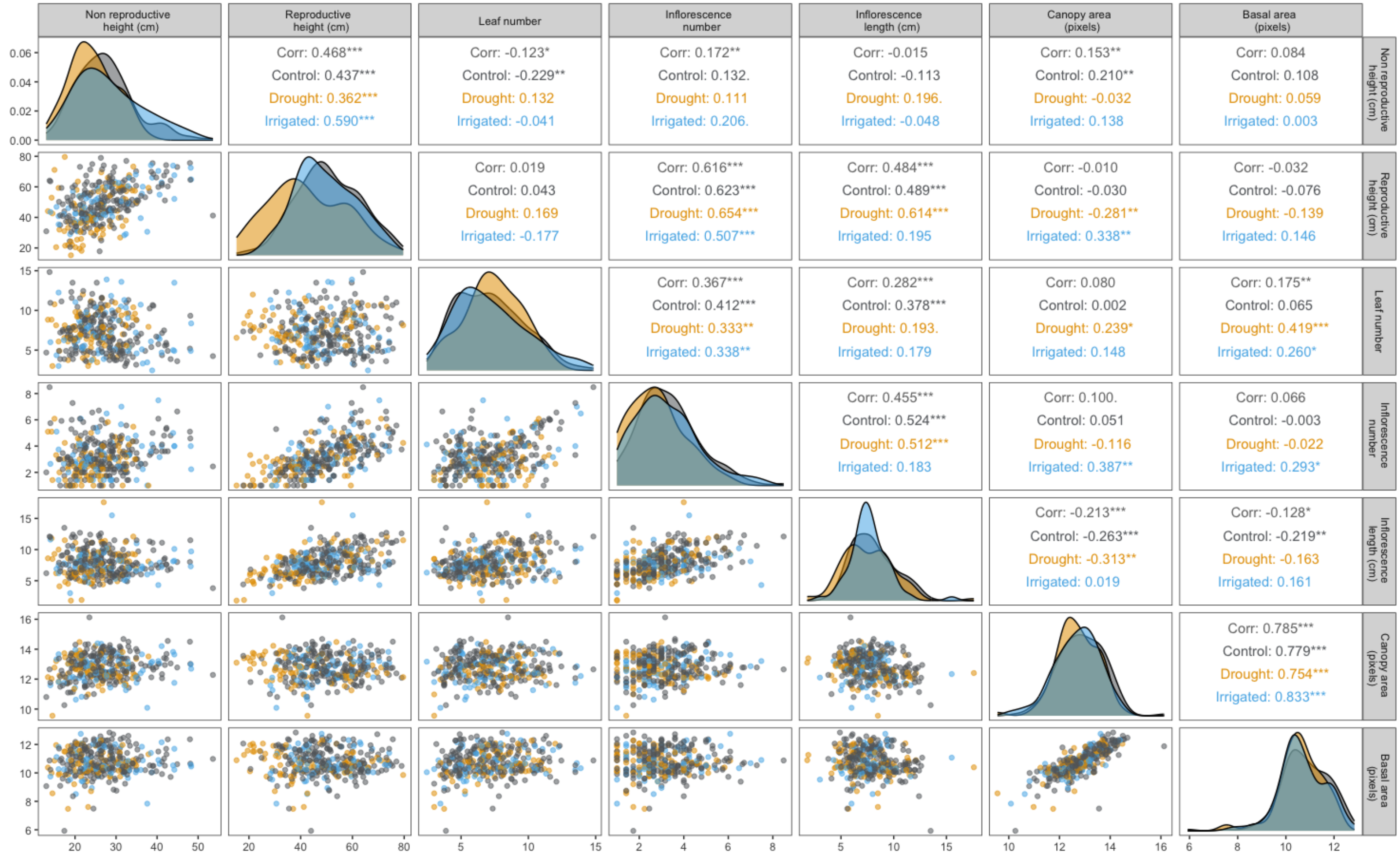


Figure 4.7 Correlation matrix plot for seven variables relating to the demographic performance of mature *Brachypodium sylvaticum* individuals between 2019-2021 for combined and separate control, drought, and irrigated treatments. Bottom left, centre and top right of matrix display scatter plots, density distributions of data and Pearson's correlation coefficients with significance levels respectively. Each significance level is denoted with a symbol to indicate p-value, where: *** = < 0.001, ** = < 0.01, * = < 0.05, and . = < 0.10. Details of variable transformations are shown in Table 4.1. Control treatment refers to pooled data from both ambient and procedural controls.

4.3.4 Overall precipitation manipulation treatment effect on demographic performance proxies

Results of the type I ANOVA for model 2 showed that the precipitation manipulation treatment had a significant effect on non reproductive and reproductive height which also varied significantly over observation years (Table 4.2). The three vegetative size variables (leaf number, canopy and basal area) and the reproductive variable inflorescence length showed no detectable treatment effect (p values ranged from 0.12 to 0.77). For all demographic performance proxies, observation year was significant ($p < 0.0001$).

Table 4.2. ANOVA (Type I sequential sums of squares) output table for model 2 fixed effects of each of the seven variables relating to demographic performance. Each significance level is denoted with a symbol to indicate p-value, where: *** = < 0.0001, ** = < 0.001, * = < 0.01.

Variable	**Degrees of freedom**	**Sum of squares**	**Mean square**	**F value**	**P value**
Non reproductive height					
treatment	2	488.20	244.11	6.69	0.0014 **
year	2	5818.90	2909.44	79.67	< 0.0001 ***
treatment x year	4	1210.50	302.63	8.29	< 0.0001 ***
Reproductive height					
treatment	2	4305.70	2152.8	17.48	< 0.0001 ***
year	2	14497.50	7248.7	58.87	< 0.0001 ***
treatment x year	4	3403.40	850.9	6.91	< 0.0001 ***
Leaf number					
treatment	2	18.94	9.47	2.15	0.12
year	2	494.96	247.48	56.06	< 0.0001 ***
treatment x year	4	32.66	8.17	1.85	0.12
Inflorescence number					
treatment	2	28.93	14.47	7.17	< 0.0001 ***
year	2	92.87	46.44	23.03	< 0.0001 ***
Inflorescence length					
treatment	2	11.19	5.59	1.87	0.15
year	2	552.30	276.15	92.55	< 0.0001 ***
treatment x year	4	43.06	10.76	3.61	0.0067 **
Canopy area					
treatment	2	1.11	0.56	1.12	0.33
year	2	135.10	67.55	135.67	< 0.0001 ***
Basal area					
treatment	2	0.43	0.21	0.26	0.77
year	2	115.74	57.87	70.92	< 0.0001 ***

4.3.5 Effect of drought and irrigation treatments on demographic performance proxies

The two plant height measures registered the most detectable responses to precipitation treatment out of the seven demographic related variables over the three-year study period (Figure 4.8). There were significant drought-induced decreases in non-reproductive and reproductive height, especially in 2019, where conditional drought effects registered a 7.6 cm and 14.0 cm reduction in non-reproductive (95% CI = 22.8 – 28.8, n = 398) and reproductive height (95% CI = 38.0 – 46.7, n = 370) relative to control respectively. For control and irrigated treatments across all seven demographic measures, there were similar direction and magnitude of responses. There was evidence of moderate inter-annual variability in demographic responses, with canopy and basal area both showing similar trends across study period. A modest (non-significant) reduction in inflorescence length was recorded for drought individuals in 2020 and irrigated individuals in 2021 relative to control.

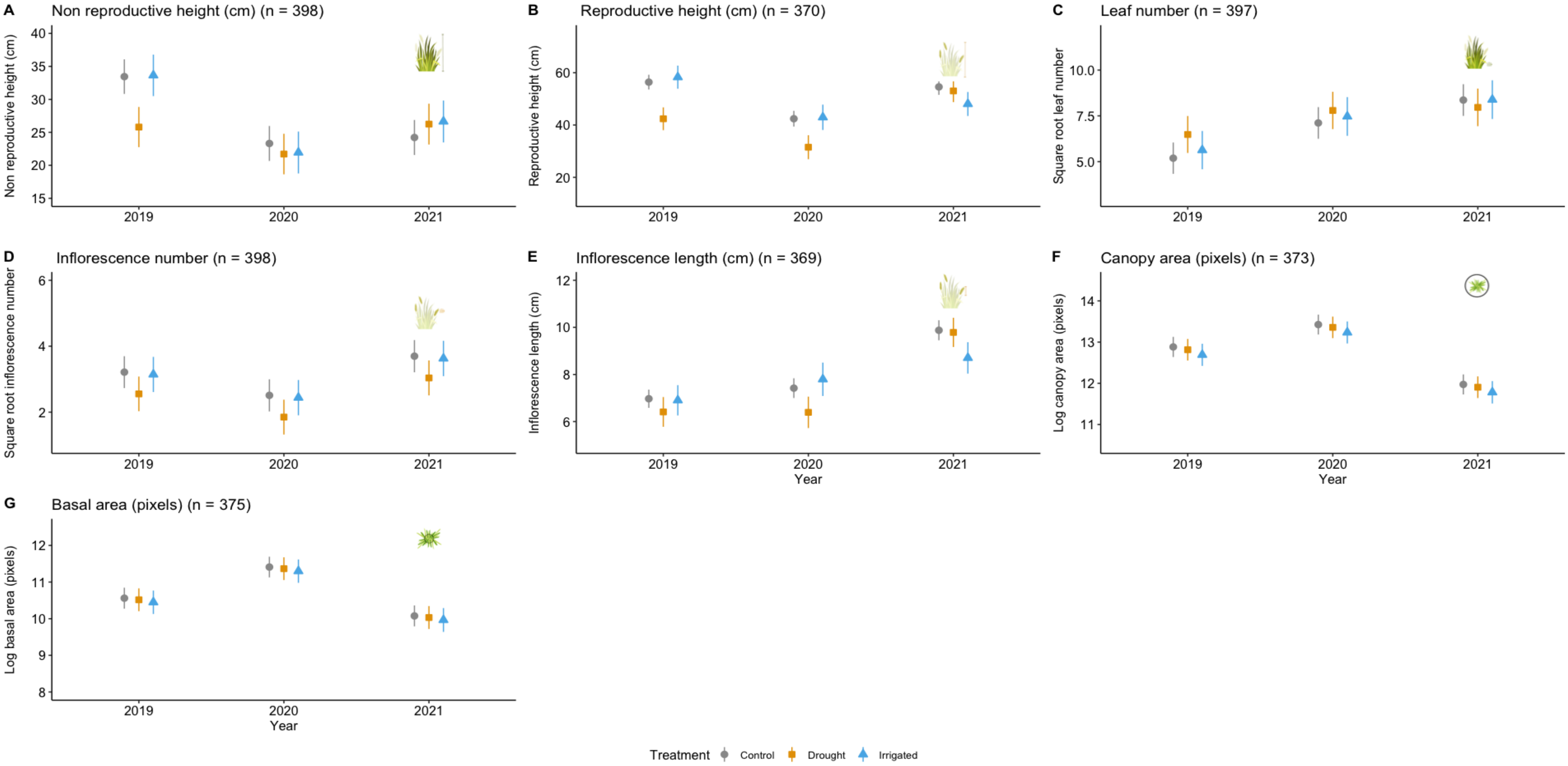


Figure 4.8 Coefficient plot for the conditional fixed effects from model 2 showing differences in demographic performance proxies measured according to precipitation manipulation treatment and observation year. Points and error bars signify conditional fixed effect estimates and 95% confidence intervals respectively. n indicates number of observations per demographic measure.

4.3.6 Variance partitioning of explanatory variables

Assessing the variance components of the random effects from model 2 revealed that for all seven of the demographic variables, residual variation was the largest contributor to the total variation (SD_{total}) (Figure 4.9). Block and plot both contributed the least to SD_{total} and their point estimates did not significantly differ from each other. Re-computing the variance partitioning analysis with the addition of treatment and observation year (model 3), showed that observation year and residual variation were again significant contributors to SD_{total} across all demographic measures (Figure 4.10, Table S4.5). For the experimental parameters, block, plot and treatment, point estimates and 95% confidence intervals did not overlap with observation year or residual except for non-reproductive and reproductive height. Observation year showed the largest range in uncertainty and had smaller point estimates than residual except for canopy area.

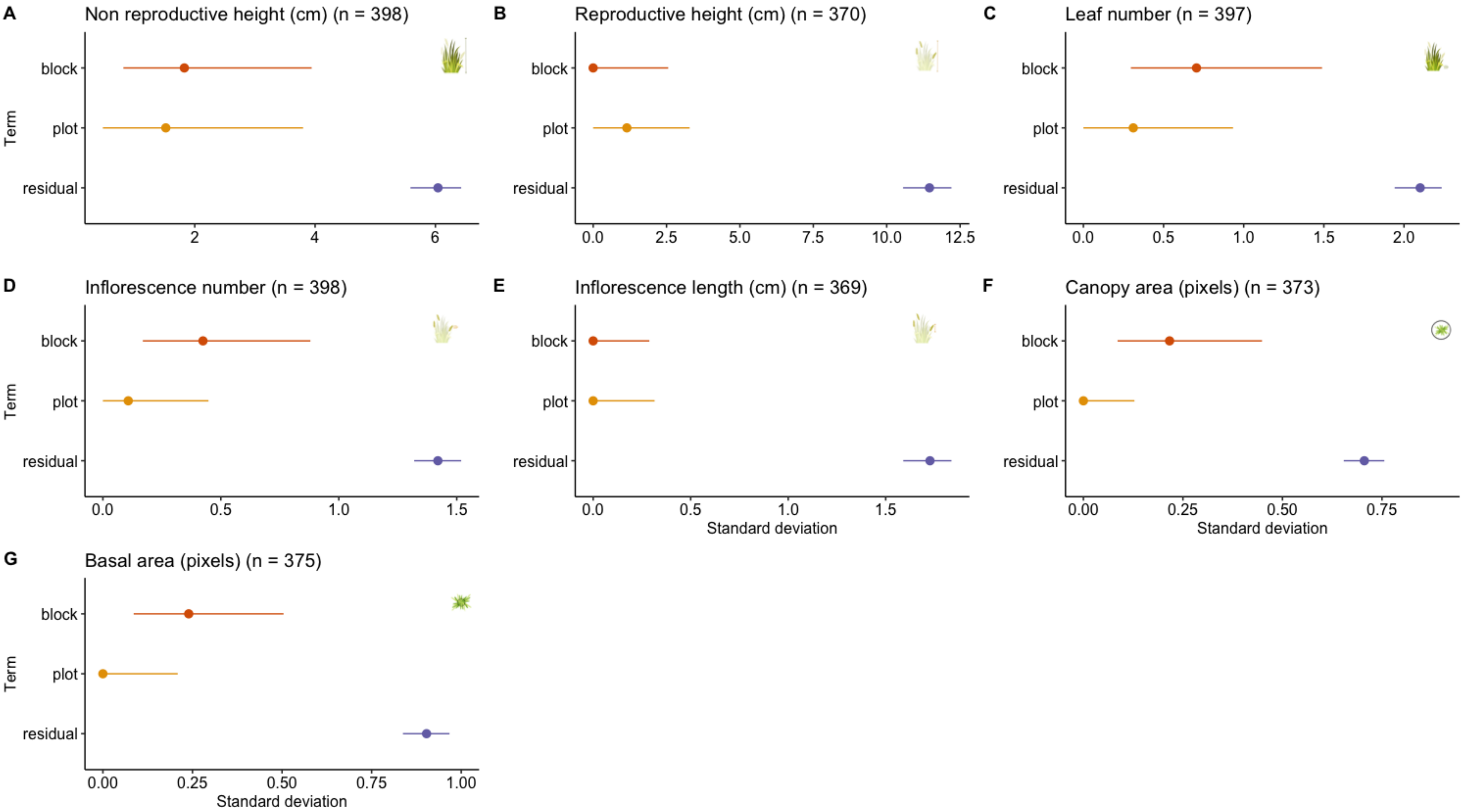


Figure 4.9 Model outputs of random effects from model 2 for each demographic performance proxy. Variance components of model terms displaying standard deviations (point estimates) with 95% confidence intervals (tails). n indicates number of observations per demographic measure.

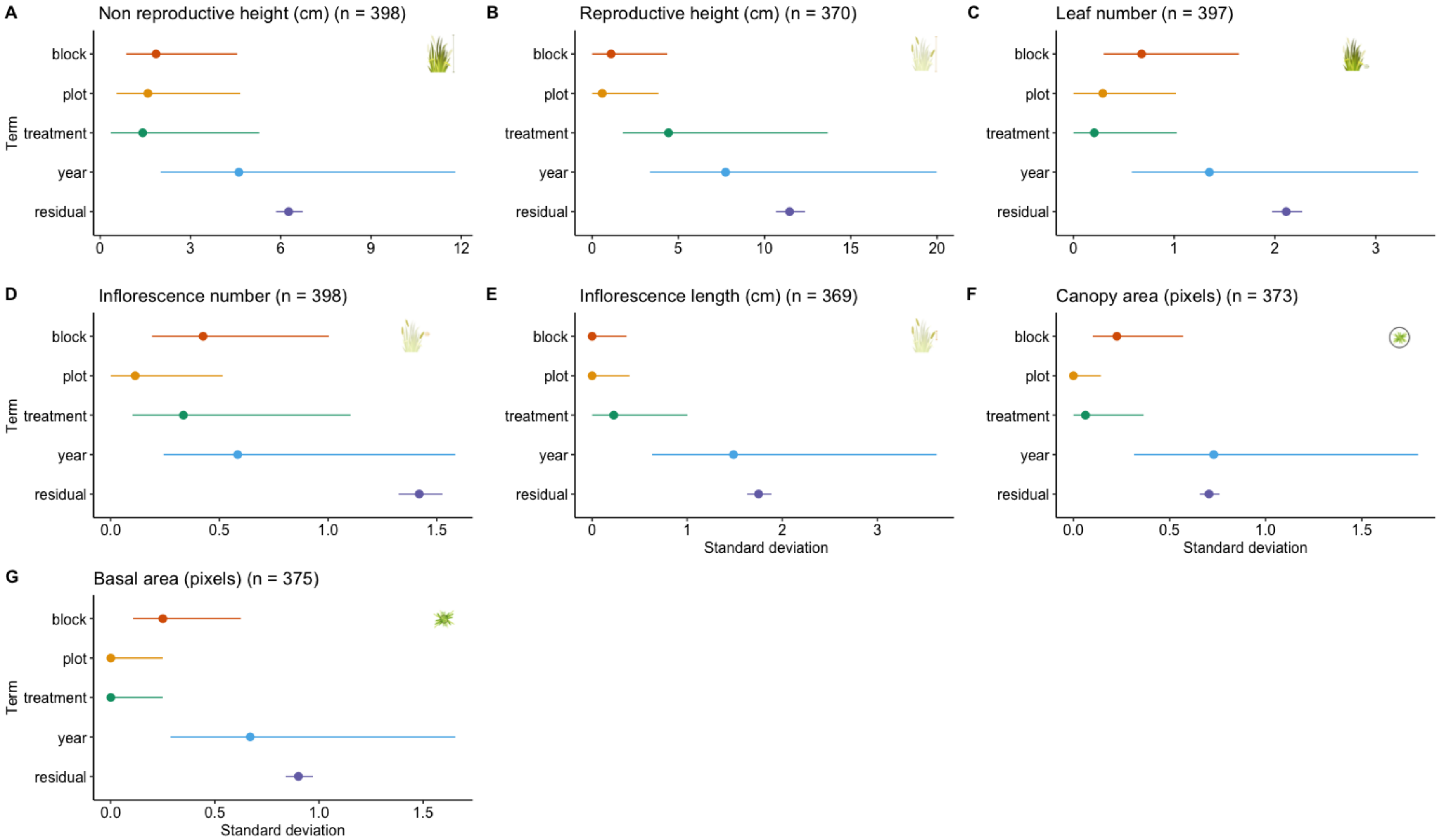


Figure 4.10 Model outputs for each demographic performance proxy from model 3. Variance components of model terms displaying standard deviations (point estimates) with 95% confidence intervals (tails). n indicates number of observations per demographic measure.

4.3.7 Neighbourhood community

The total number of vascular plant taxa recorded within the 0.3 m radius neighbourhoods (~0.28 m^2) of mature adults between 2019-2021 was 67 ($\bar{x}$ = 15.0, SD = ±3.3, n = 398). There were no significant differences in the number of species recorded within each treatment or across the study period (Figure 4.11).

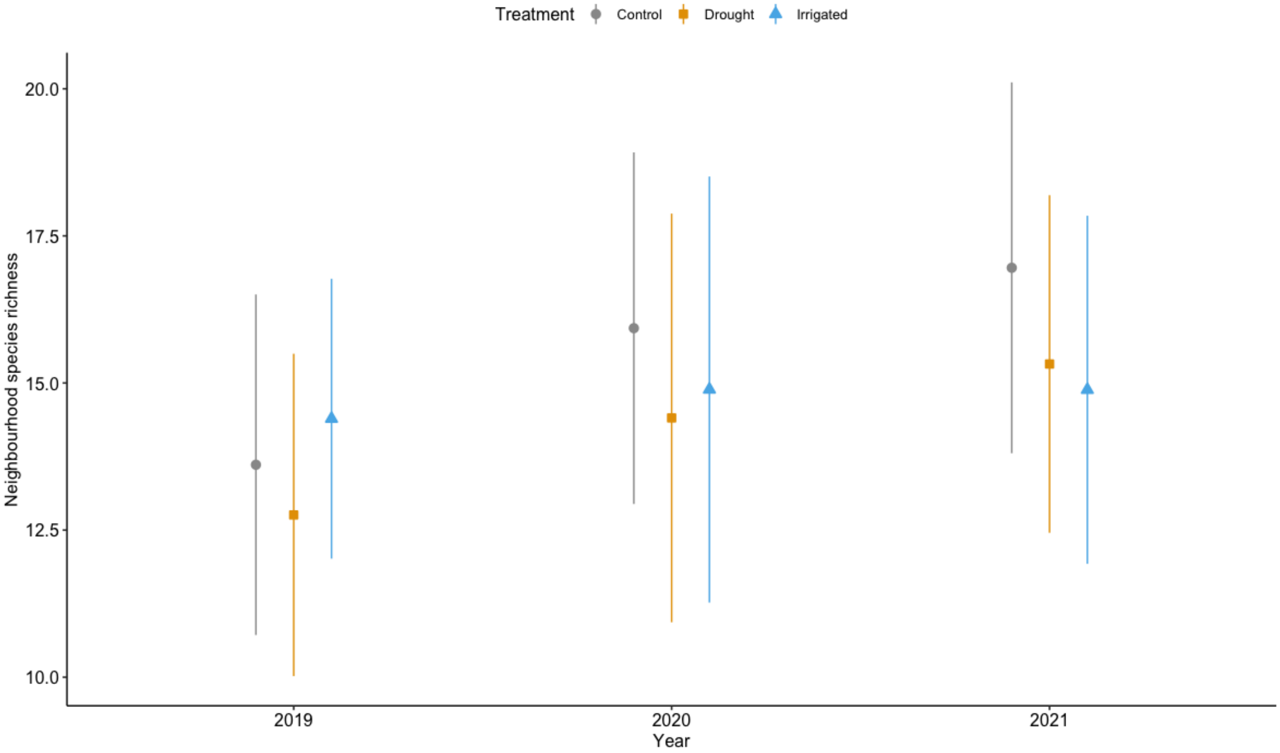


Figure 4.11 Species richness recorded in 0.3 m radius neighbourhoods showed no treatment differences or temporal shifts across study period. Points indicate mean number of species recorded and tails indicate standard deviation.

Of the 67 taxa recorded, over 80% were non-leguminous forbs and graminoid species with comparable proportions of both legumes (10.6%) and scrub/woody species (9.1%) (Table S4.6). The proportion of each functional type differed in the top ten most frequently co-occurring species with *Brachypodium sylvaticum* over the study period (Figure 4.12). Four of the top ten species were graminoids, with *Trisetum flavensens* being the most frequently co-occurring species. Legumes and forbs had three species each represented. Proportions of species present appear to remain stable over the study period, except for *Agrimonia eupatoria* which exceeded null proportions (0.25) in 2019 but decreased by approximately 50% in irrigated plots in subsequent years. The presence for the top ten species was above the 0.50 expected null proportion in control plots for all experimental years.

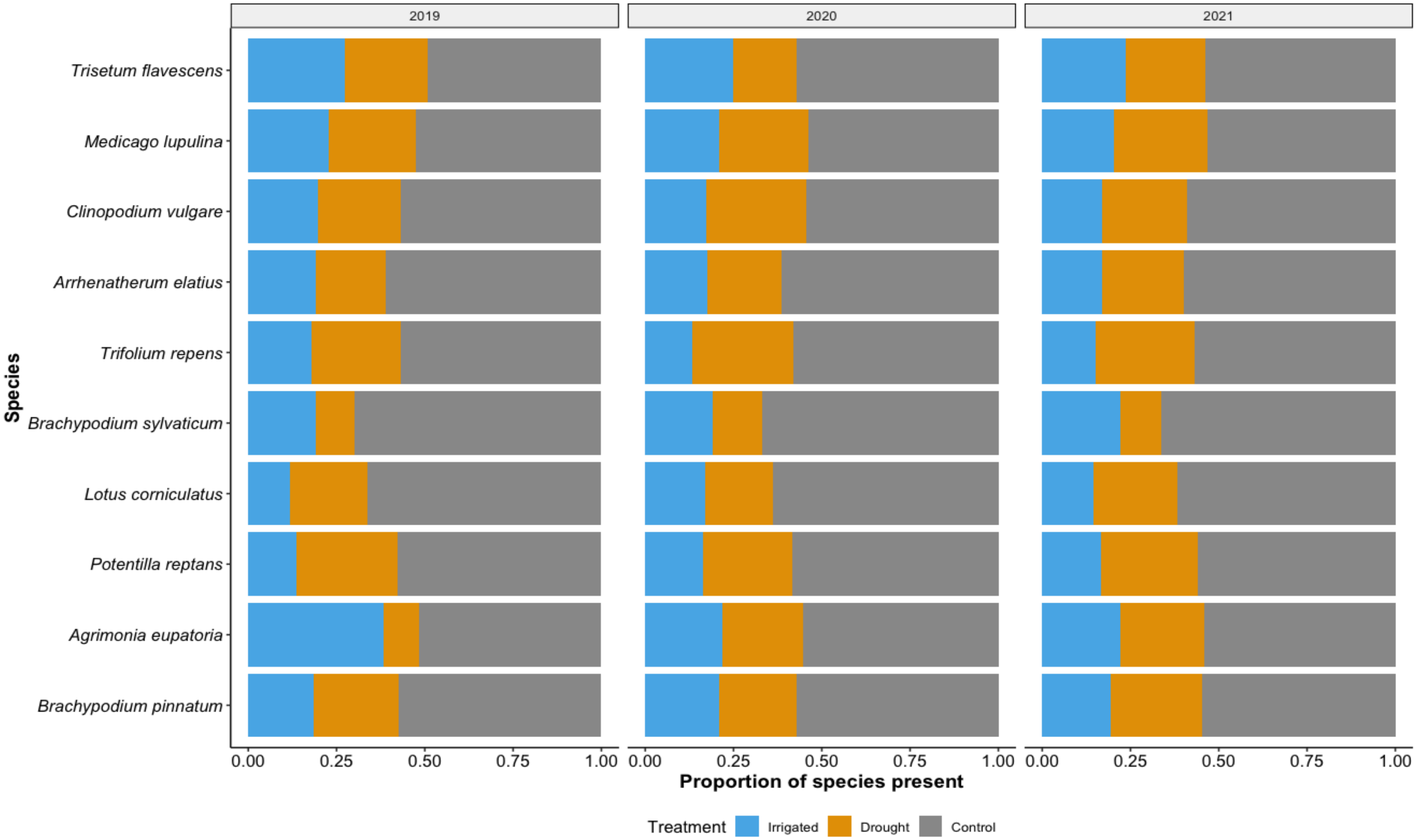


Figure 4.12 Proportion of the top ten most frequently co-occurring species within *Brachypodium sylvaticum* 0.3 m radius neighbourhoods over the study period. Species are listed in descending order from most to least frequently co-occurring. Note: control treatment combines ambient and procedural controls so that expected null proportions are 0.50, compared to 0.25 for drought and irrigated treatments

4.3.8 Effect of biotic interactions remain consistent across demographic performance proxies

Outcomes of model 4, which assessed the effect of biotic interactions on *Brachypodium sylvaticum* demography showed a consistent direction of effect across demographic measures (Figure 4.13). The presence of rabbit or ant activity generally showed negative effect on both reproductive and vegetative demographic measures. Significant negative effect from rabbit activity was registered for reproductive height -8.6 cm (95% CI = -14.1 – -3.2, n = 370) and (square root) inflorescence number -0.7 (-1.4 – -0.1, n = 398) (Figure 4.13 B and D). The effect of plant-plant interactions were marginal across demographic measures, except species total significantly negatively reduced both reproductive and non-reproductive height by 0.7 cm (95% CI = -1.2 – -0.3, n = 370) and 1.2 cm (95% CI = -2.0 – -0.3, n = 398) respectively. Legume total generally had marginal (non-significant) positive effect on the vegetative demographic measures non-reproductive height, leaf number and basal area. The presence of ants significantly reduced flowering spike length by 0.9 cm (95% CI = -1.7 – -0.1, n = 396) and marginally decreased in reproductive height 4.9 cm (95% CI = -9.8 – 0.2, n = 370. Examining the relationship of biotic interactions and demographic variables across precipitation treatments and observation year revealed broadly similar trends to model 2 (Figure 4.8), with a few instances of marginal effects of covariates (rabbit and legume total) on a few demographic variables (Figure S4.4).

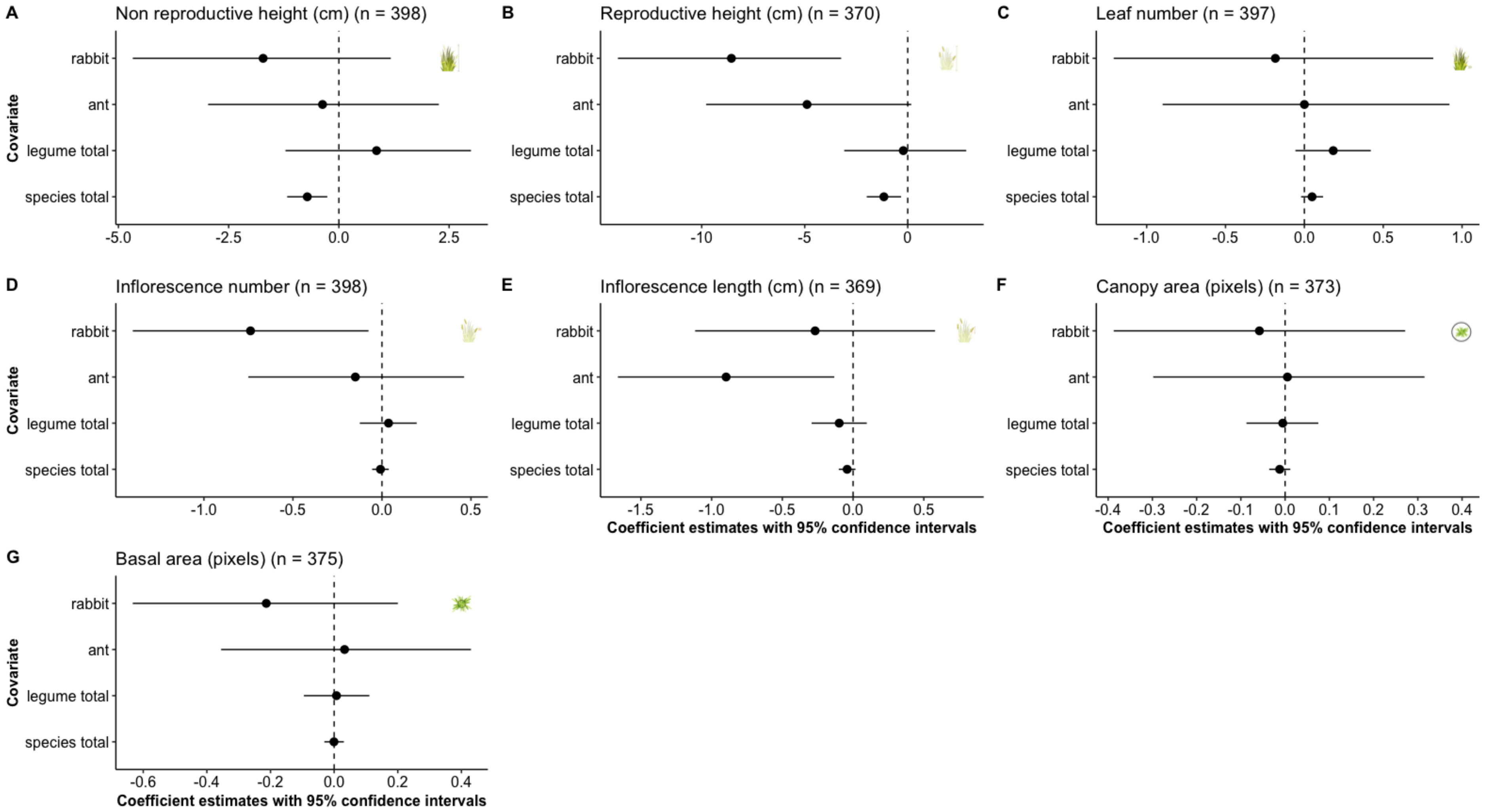


Figure 4.13 Coefficient plot for each covariate across the demographic performance proxies from model 4. Each covariate shown is a fixed effect resulting from separate models. Points and tails signify fixed effect estimates and 95% confidence intervals respectively. Estimates with a negative value indicate a reduction in the size or number of vegetative or reproductive structures. Details of variable transformations are shown in Table 4.1. n indicates number of observations per demographic measure.

4.4 Discussion

This three-year study assessed the effect of precipitation manipulation on the demographic performance dynamics of *Brachypodium sylvaticum* individuals accounting for neighbourhood biotic interactions in a calcareous grassland. The main outcome of this study was that experimentally manipulating growing season precipitation alone largely only significantly affects plant height related measures, with modest evidence for a treatment effect on reproductive parameters. Survival rate of *B. sylvaticum* individuals was high across precipitation treatments. When examining the source of variation in demographic responses, a significant driver of the variation was attributed to interannual variability and non-experimental parameters, with the former being important to consider when examining potential vital rate trade-offs. The final notable finding was that for this study system, accounting for neighbourhood biotic interactions in *B. sylvaticum* demography appeared not to significantly ameliorate or exacerbate effects of precipitation treatment on demographic outcomes.

4.4.1 Precipitation treatment effects

Reduction in vegetative and plant height were the two main demographic variables to show strong responses to precipitation treatment in mature *B. sylvaticum* individuals. It demonstrates that experimental precipitation treatments can induce detectable (meta)population-level responses in a long-lived perennial grass species on relatively short timescales. Survival and other demographic performance proxies showed no or only marginal evidence of treatment effects. There are three plausible explanations for these findings; the first relates to the efficacy of the imposed precipitation treatments, particularly irrigated, the second possibility is that the treatments were effective, but *B. sylvaticum* individuals were unaffected, based on the largely undetectable responses in the non-plant height related demographic performance proxies. The third possible explanation is that the type of imposed drought (press perturbation) did not elicit a strong response in *B. sylvaticum*. The experimental treatments were designed to passively intercept 50% of incoming precipitation (drought) and redirect the water to irrigated plots which received a 50% increase in rainfall relative to controls. This passive rainfall exclusion approach might not always achieve target precipitation inputs as it is dependent on ambient precipitation levels. This discrepancy in treatment effectiveness is particularly apparent in years when ambient rainfall is below average, as drought and irrigated treatments are less severe. To illustrate this, take an extreme hypothetical scenario of no rainfall over the growing season: all treatments would receive the same amount of rainfall (0 mm), which would result in marginal ecological responses between treatments, giving the impression of a negligible treatment effect. Smith *et al.* (2024) found a small number of sites (n = 4) reported experimental drought effect sizes near zero in their global synthesis of ecosystem functional responses in grassland and shrubland biomes. Similarly, community structure was found to be drought resistant in a long-term study at the same site reported here (Jackson *et al.,* 2024). Both Smith *et al.* (2024) and Jackson *et al.* (2024) highlight that the observed drought resistance is partially attributed to the experimental design and that additional factors (e.g. site management history, soil chemical and structural profile) are likely to also be contributing to muted ecological responses to drought.

Another plausible scenario is that the imposed precipitation treatments (regardless of effectiveness) did not extend past the species’ tolerance window, thereby resulting in weak treatment responses in mature individuals. *B. sylvaticum* is one of the dominant grass species found at the site and is characterised with a stress-competitive profile and low-to-moderate relative growth rate (Grime and Hunt, 1975; Kilinc *et al.,* 2010). These plant attributes enable this species to have wide range of abiotic tolerances, including in novel environments (Marchini *et al.,* 2018). Mingo and Mazzoleni (1997) showed *B. sylvaticum* had the capacity to tolerate multiple experimentally induced stressors indicated by the positive responses in root/shoot allocation and compensatory growth post-defoliation under drought unshaded conditions.

Finally, aside from species' drought tolerance window, two temporal dynamics of drought: drought timing and lagged effects could provide further explanations for a weak drought effect. The imposed precipitation treatments occurred only during the growing season (April to October). C_3 grasses, like *B. sylvaticum*, tend to gain the majority of biomass during the cooler months and therefore may be more vulnerable to droughts occurring earlier on in the growing season, which may not have been fully accounted for by the experiment (Felton and Goldsmith, 2023). There were signs of lagged drought effects evidenced by the significant reductions in non-reproductive and reproductive height in 2019. In 2018, the year prior to the first demographic census, there was a combined extreme drought and heatwave in the UK (Turner *et al.,* 2021), with only 71.6 mm of precipitation recorded from June to August, equivalent to a 66% reduction in the longer-term average summer rainfall (2007-2017) at the site (Rennie *et al.,* 2017; Stone, 2020). Height related plant traits are known to be highly variable (Pérez-Harguindeguy *et al.,* 2013), which might explain why the other demographic measures did not respond as strongly to treatments. Explicit measures of drought legacy effects in grasslands remain understudied, despite having the potential to increase prediction capabilities of ecological responses in these biomes (Ogle *et al.,* 2015).

Interestingly the modest treatment effects observed here suggests *B. sylvaticum* is more drought resilient than other species found in this calcareous grassland community (Jackson *et al.,* 2024). Community-level drought responses, as measured by productivity (above-ground biomass) and temporal stability, were found to be driven mainly by decreases in graminoid and legume biomass (Jackson *et al.,* 2024). Biomass measures may not always couple with fitness, especially in multispecies comparisons or in resource limited contexts (Younginger *et al.,* 2017). These differing responses to drought within the same study system highlight the importance of a complimentary approach in climate experiments, where multiple organisational-level responses are studied. In addition, it remains challenging to disentangle the temporal and spatial dimensions of drought experimentally and this study underscores the need for additional long-term empirical data to further tease apart demographic responses.

4.4.2 Variation between individuals and years as key drivers in variation of demographic responses

Assessing the variance components of experimental parameters showed that residual variation contributed the most to total variation, suggesting non-experimental parameters are largely driving the demographic outcomes. Here, the residual term in models 2 and 3 includes individual-level variation in addition to measurement error, which reflects the nested design of the experiment. It is common to observe some residual variation when assessing vital rates, which cannot be quantitively characterised, as by definition is unexplained (Kohyama *et al.,* 2018). A qualitative reason for the strong signal could be attributed to variation between individuals. This finding would follow the hierarchical response framework which posits responses to disturbances such as changes to soil nutrient or water status, occur firstly at the individual level then upscale to higher levels of biological organisation (Smith *et al.,* 2009). Variation between individuals can be due to genetic differences, plant age, developmental plasticity, and even phenotype-environment mismatching (Westneat *et al.,* 2015). Further study is required to unravel the causal mechanisms for the large residual variation observed here, which could provide valuable insights into eco-evolutionary processes.

Inter-annual variation contributed significantly to responses recorded within and amongst vital rates. These variations in demographic responses are likely driven by both biotic and abiotic factors such as environmental (and demographic) stochasticity as well as successional trajectory (Paniw *et al.,* 2021; Hilde *et al.,* 2020). Temporal shifts, including drought lag effects, suggest non-uniform demographic outcomes, indicating that short-term resistance and resilience to stressors like drought may diminish when observed over longer time scales (Paniw *et al.,* 2021) and when scaled up (Suding *et al.,* 2008; Wang *et al.,* 2021).

Year-to-year variation is also important to consider when examining relationships amongst vital rates. There was evidence to suggest a size-reproductive trade-off between canopy area and reproductive height (and to a lesser extent inflorescence number and length), with diverging trends for drought and irrigated individuals (Figure 4.7). When examining if this relationship remained constant when disaggregated by observation year (Table S4.4), demographic responses showed similar trends regardless of treatment type. Environmental conditions fluctuate between years which can influence individual vital rates (e.g. survival) and their relationship with other vital rates (e.g. survival and reproduction) asynchronously, with these differences only appearing when aggregated over multiple years. It is important to acknowledge the observed temporal differences in responses is likely also attributed to lower statistical power from the small sample sizes for each observation year. To more accurately assess mechanisms for variations within and among vital rates, longer time series of a minimum of 10 years (for plants typically reproducing yearly), has been recommended (Hilde *et al.,* 2020).

4.4.3 Brachypodium sylvaticum short-term survivorship

Contrary to expectations that survivorship would be affected by precipitation treatment, a high survival rate (93.4%) at the end of the study period was recorded across treatments with a range of 85.4 – 96.4% between the lowest survival rates in control and highest in irrigated plots. A plausible explanation for not detecting any treatment effect on survival is due to *B. sylvaticum* having a long lifespan and known to live beyond two decades (Haeggstrôm and Skytén, 1996). Haeggstrôm and Skyten (1996) also recorded comparable high annual survival rates (~90%) for this species in their long-term population study in Aland Islands (southwest Finland). Beyond the minimum of surviving winter and continued growth post flowering, long-lived polycarpic perennials in temperate regions must optimise resource (re)allocation needed for and after winter dormancy, growth, reproduction and defence without compromising fitness. This optimisation relies on individuals assessing and responding to surrounding micro-environment (e.g. soil moisture and air temperature) (Lei *et al.,* 2023). The results suggest that in the short term, individuals in drought plots were able to optimise resources under lower water input enough to maintain their survival. It also is likely the length of the study period (two demographic time intervals) was not long enough to detect a treatment effect on the study population's finite survival rate (Roy *et al.,* 2011).

4.4.4 Reproduction and new recruits

The overall low reproductive allocation (RA) (17.8%) and reproduction rates (8.7%) observed in this study can also be attributed to long life span of *B. sylvaticum* and is suggestive of a more conservative life-history strategy (Reich, 2014). These results broadly agree with a reproductive allocation experiment of 40 grass species, including *B. sylvaticum*, which showed high survival post flowering and moderately low RA (25.6%), expressed as a proportion of above-ground biomass associated with reproductive structures (Wilson and Thompson, 1989).

There was some evidence of treatment effects when comparing the components along the reproductive process to the number new recruits. Reproductive stature in droughted individuals was lower in 2019 and 2020 compared to individuals in irrigated and control plots over the same period. Despite high initial commitment to reproduction (e.g. growing flowering culms) across treatments (drought = 89%, control and irrigated ~93%), only one and two new recruits were recorded for drought and irrigated individuals respectively compared to 19 in control. Together these results suggest i) individuals in drought plots had a lower RA baseline (relative to control) particularly in 2019 and 2020 affecting reproductive output, and ii) irrigated individuals had high initial reproductive investment that was not converted into a higher number of new recruits. The possible explanation for the observed drought reproductive responses is lag effects, and for irrigated: experimental design, which are explored in the next section.

Here commitment to reproduction was evaluated by reproductive allocation, which shares conceptual links to reproductive efficiency – the resource conversion from vegetative structures to reproductive output (Bonser, 2013). There were indications of lower efficiency for both droughted and irrigated individuals, likely facilitated by aborting mechanisms. Plants can over allocate resources to the production of reproductive organs which subsequently fail to mature in circumstances of resource limitation (Bazzaz, 1997) or pathogen infection (Halbritter *et al.,* 2012). Aborting mechanisms during reproductive process can be a beneficial "cut your losses early on" strategy which trades short-term physiological losses for longer term evolutionary fitness (Bazzaz, 1997). For droughted individuals, the combined reduced water availability and lagged effects from the 2018 drought and heatwave (Turner *et al.,* 2021), could have contributed to lower reproductive efficiency (i.e. low conversion rates between reproductive stages from growing flowering culms to seedlings) observed. Interestingly, none of the four covariates measuring biotic interactions appeared to impact (e.g. facilitate - legumes and species richness or inhibit - rabbit and ant activity) the size or number of reproductive structures for droughted individuals (although see Figure S4.4 where there was a slight amelioration of drought effect on reproductive height in 2019 when legumes).

For individuals in irrigated plots, the discrepancy between initial high reproductive commitment and low number of new recruits is likely due to experimental factors: i) efficacy of irrigation treatment and ii) evaluation method of plant-plant interactions. There were negligible differences in reproductive height compared to control, and no effect when species richness was also considered (Table S4.7, Figure S4.4), suggesting water availability elicits a stronger response in plant reproduction than interspecific plant interactions. It was expected that irrigated plots receiving 50% additional rainfall would not be water limited and so plant-plant interactions (measured using species richness) would be more competitive in nature, negatively affecting reproductive structures (Bonser, 2013). The absence of a signal is likely an efficacy issue with the irrigation treatment, which was not high enough to register competitive effects (Jackson *et al.,* 2024), and the need for additional plant neighbourhood measures (e.g. biomass) to adequately assess plant-plant interactions (Eckstein, 2005). Although, high abortion rates even under optimal nutrient and water conditions have been found (e.g. wild barley, Volis *et al.,* 2004). With regards to interspecific interactions, similar findings have been observed by Olsen *et al.* (2016) where no net effect of plant-plant interactions on forb reproductive parameters along a climate gradient in a semi-natural grassland setting in Southern Norway. Olsen *et al.* (2016) did note temperature was a bigger driver of plant interactions than precipitation in their study. More studies are required to untangle how plant-plant interactions can mediate perennial plant reproductive responses in climate change experiments, especially for grass species, as this has important implications for accurately assessing impacts of climate change.

Deducing treatment effects on the relationship between parent status and vegetative size (Q3) (i.e. trade-off between somatic and reproductive investment) and the vital rates of new recruits (Q1) in this study is challenging due to the low sample size. These demographic parameters have important population-level implications for *B. sylvaticum*, as size is a major predictor of reproductive success (Salguero-Gómez and Casper, 2010) and warrant further study. Future investigations on reproductive costs should seek to account for the modular nature of trade-offs (e.g. above vs below-ground organs) apparent on different time scales, as reproductive penalties can be partially compensated or delayed (Obeso, 2002; Reekie and Sakar, 2005). With regards to seedling vital rates, tracking the survival of *B. sylvaticum* seedlings under resource limitation would be an important next step in assessing the long-term persistence of *B. sylvaticum,* especially given this species showed evidence of low reproductive efficiency and is known to have a transient seed bank (Davies and Long, 1991).

4.4.5 Neighbourhood plant community dynamics

Total plant species richness and dominant species recorded within the 0.3 m radius neighbourhoods across all plots were comparable to values reported by Jackson *et al.* (2024) at the m^2 quadrat level. Species richness was similar across precipitation treatments and over time, although there were minor increases in mean species richness for control and drought plots over time, albeit with large confidence intervals. When the same time interval is considered (2019-2021), these results measured at a finer spatial scale broadly corroborate with species richness trends recorded over a six-year period (2016-2021) by Jackson *et al*. (2024).

It was expected that neighbourhood effects, particularly plant interactions would exert stronger effects under precipitation treatments. Aside from considerations of treatment efficacy and plant interaction metrics mentioned previously, the muted response could be because the focal species, *B. sylvaticum* is a dominant species and so controls a larger proportion of resources compared to its heterospecific neighbours (Smith *et al.,* 2009; Whittaker, 1965).

4.4.6 Effects of uncontrolled covariates

There were several factors that were not directly controlled for in this experimental study, which could have affected the demographic dynamics of *B. sylvaticum* and merit further consideration in future investigations. Firstly, the role of micro-environmental parameters such as soil moisture, aspect, shade and air temperature are notable drivers of demographic responses in grassland species (Mazalla *et al.,* 2022; Corcket *et al.,* 2003). The structural design of the treatment shelters, particularly drought, could have altered air currents and incoming solar radiation resulting in subtle changes to surface air temperature and soil moisture. These micro-environmental effects were mitigated through having a procedural control treatment in addition to an ambient control to account for potential structural-induced effects, and no discernible differences between controls were recorded. Secondly, biotic interactions as potential mediators of demographic performance. The four neighbourhood variables presented here were assessed as covariates, which aided interpretation of the demographic responses. To mechanistically determine the impact of these plant-plant and plant-animal relationships, future work should seek to integrate these biotic variables in a factorial experimental design. Thirdly, the age of tagged *B. sylvaticum* individuals censused was not accounted for, as the focus was on size and ontogeny in this study. It is possible a portion of the residual variation driving demographic performance could have been due to individuals being of differing ages. Age-based plant demography is less well studied, and it remains unclear how age interacts with key vital rate components and environment (Baden *et al.,* 2021). Lastly, the genotypic diversity of individuals censused was not measured, as this was outside the scope of the study. It has been shown in a calcareous grassland community that genotypic variation within a species has fitness consequences observed at the neighbourhood community level (Whitlock *et al.,* 2007).

4.5 Conclusion

This study set out to examine precipitation treatment effects and the influence of neighbourhood biotic interactions on key demographic components (survival, size, reproduction and new recruits) of a long-lived perennial *Brachypodium sylvaticum.* The main finding was that *B. sylvaticum* is largely resistant to the effects of experimentally imposed drought, maintaining high survival in the short term. A notable exception was for vegetative and reproductive height which registered significant reductions with drought, with a one- and two-year recovery time respectively. There was marginal evidence of reproductive components being negatively impacted by reduced water input, though assessing the parent penalty and vital rates of new recruits remained inconclusive due to low reproduction rates. The lack of strong drought effect in most vegetative parameters reported here does not rule out potential long-term effects on the individual life history and population persistence of *B. sylvaticum* in calcareous grasslands. Rather it demonstrates how demographic responses to experimental precipitation treatments operate together with multiple other intrinsic (e.g. intra and inter-individual variability) and extrinsic (e.g. neighbourhood biotic interactions and micro-environmental heterogeneity) processes which vary with time. Future demographic studies should prioritise examining potential vital rate trade-offs over longer time frames by integrating belowground, inflorescence traits and seedbank dynamics to assess the threshold of this drought buffering capacity, especially given the additional environmental stressors faced by calcareous grasslands.

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Supplementary

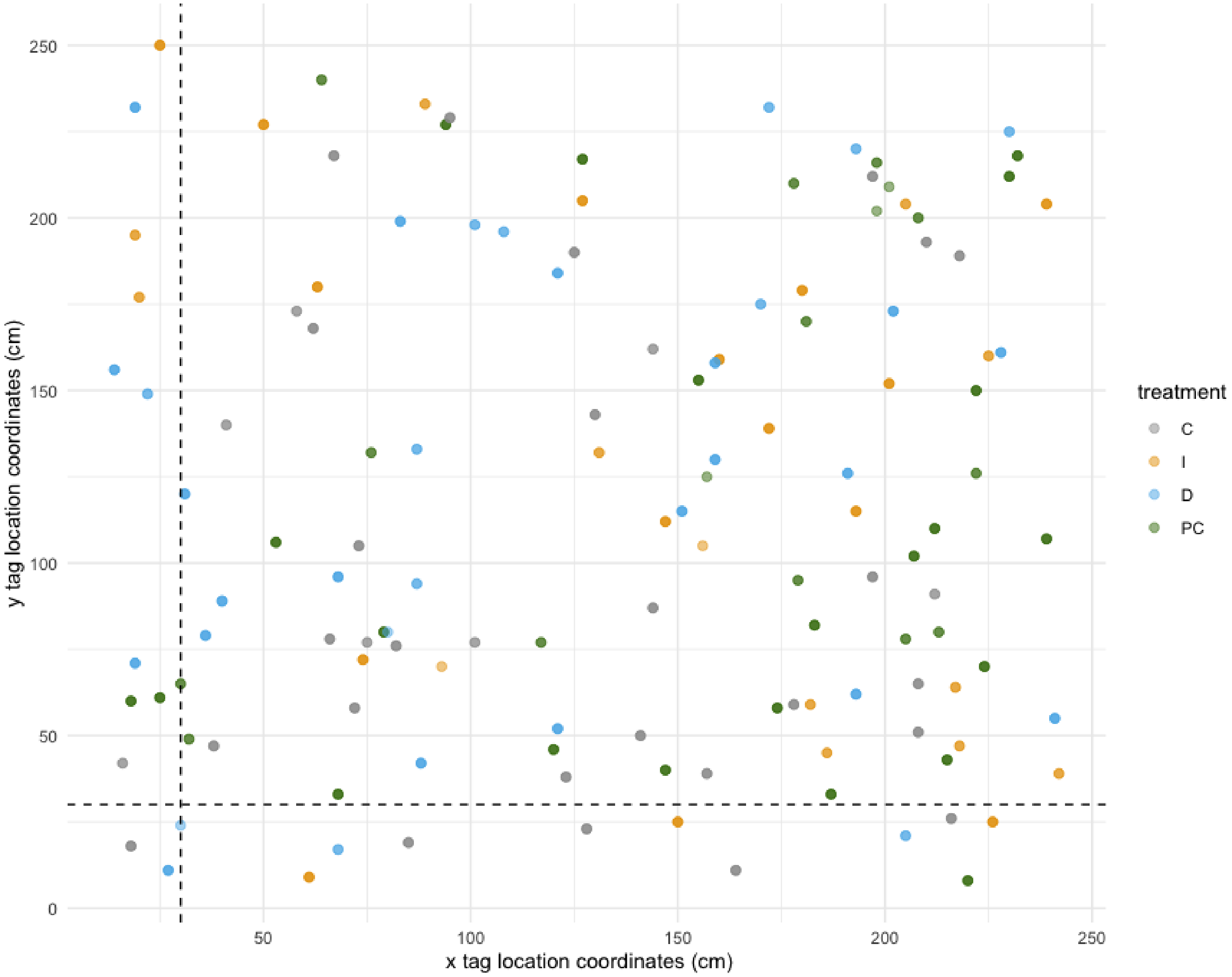
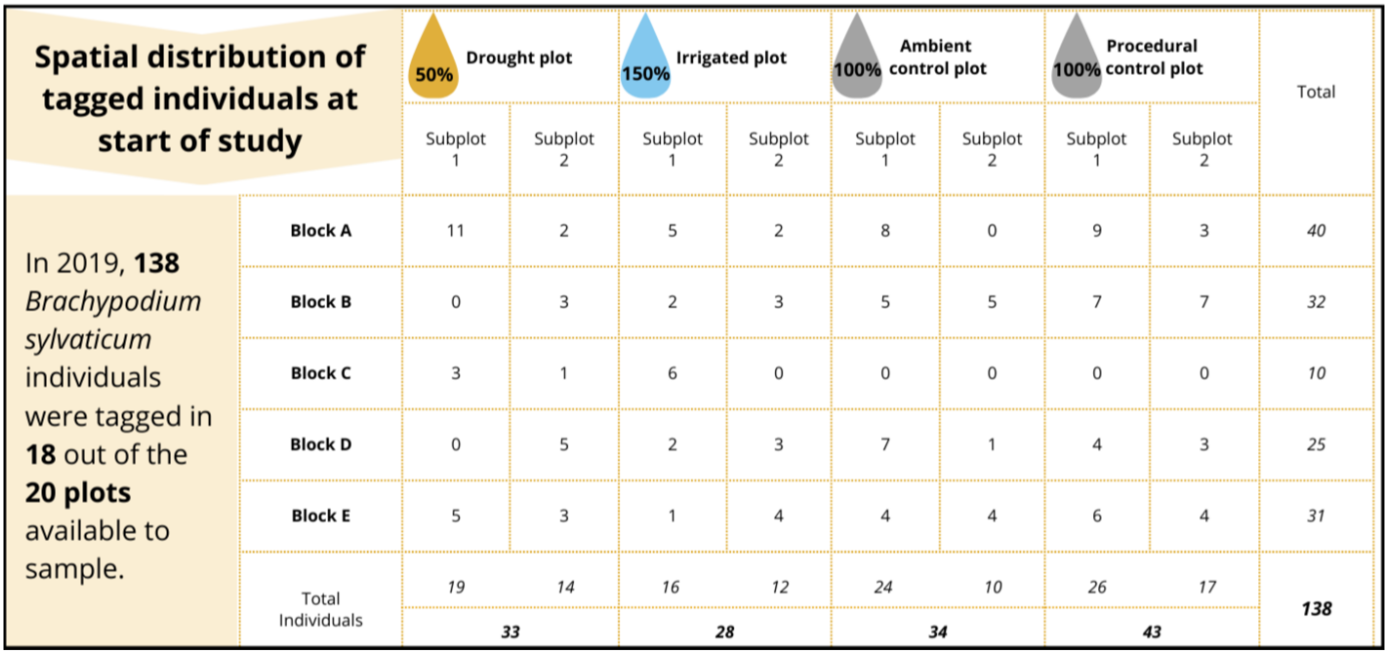


Figure S4.1 *Brachypodium sylvaticum* individuals tagged in 2019 showing x and y coordinates in each 2.5 x 2.5 m plot for each treatment. Black dashed line indicated 30 cm buffer zone with edge of plot.



Spatial distribution of tagged individuals at start of study

In 2019, **138** *Brachypodium sylvaticum* individuals were tagged in **18** out of the **20 plots** available to sample.

	50% Drought plot		150% Irrigated plot		100% Ambient control plot		100% Procedural control plot		Total
	Subplot 1	Subplot 2	Subplot 1	Subplot 2	Subplot 1	Subplot 2	Subplot 1	Subplot 2	
Block A	11	2	5	2	8	0	9	3	*40*
Block B	0	3	2	3	5	5	7	7	*32*
Block C	3	1	6	0	0	0	0	0	*10*
Block D	0	5	2	3	7	1	4	3	*25*
Block E	5	3	1	4	4	4	6	4	*31*
Total Individuals	*19*	*14*	*16*	*12*	*24*	*10*	*26*	*17*	***138***
	33		***28***		***34***		***43***		

Figure S4.2 Distribution of tagged individuals across blocks, plots, subplots and treatments at the start of the study in 2019.

Table S4.1 Summary of AIC model selection results for demographic models for both experimental and covariate models. K indicates number of model parameters. Each demographic variable was analysed using ‘lmer’ in ‘lme4’ R package with restricted maximum likelihood (REML). Models in bold were selected as being the most parsimonious based on lowest AIC values (except non reproductive and reproductive height, due to decision to pool control treatments). Note: AICs are not comparable between models of different demographic variables or types. See Table 4.1 for additional information about each variable. xy location models were dropped from final results presentation.

Demographic variable	**Model type**	**Model**	***K***	**AIC**	**ΔAICc**	**AIC weight**
Non_repro_height						
	Experiment	Unpooled control treatments, treatment x year interaction	15	2568.53	0	0.96
		Pooled control treatments, treatment x year interaction	**12**	**2574.87**	**6.34**	**0.04**
		Unpooled control treatments, treatment + year	9	2606.06	37.53	0
		Pooled control treatments, treatment + year	8	2610.13	41.6	0
	Covariate	**Pooled control treatments, treatment x year x legume total interaction**	**21**	**2546.88**	**0**	**0.88**
		Pooled control treatments, treatment x year x species total interaction	21	2551.09	4.21	0.11
		Pooled control treatments, treatment x year + legume total	13	2555.39	8.51	0.01
		Pooled control treatments, treatment x year + species total	13	2561.62	14.74	0
		Pooled control treatments, treatment x year + rabbit	13	2573.07	26.19	0
		Pooled control treatments, treatment x year + ant	13	2574.5	27.62	0
		Pooled control treatments, treatment x year + xy location	13	2586.2	39.32	0
		Pooled control treatments, treatment + year + legume total	9	2588.35	41.47	0
		Pooled control treatments, treatment + year + species total	9	2595.99	49.11	0
		Pooled control treatments, treatment + year + rabbit	9	2609.25	62.37	0
		Pooled control treatments, treatment + year + ant	9	2609.72	62.84	0
		Pooled control treatments, treatment + year + xy location	9	2621.33	74.45	0

Table S4.1 continued

Demographic variable	Model type	Model	*K*	AIC	ΔAICc	AIC weight
Repro_height						
	Experiment	Unpooled control treatments, treatment x year interaction	15	2828.7	0	1
		Pooled control treatments, treatment x year interaction	**12**	**2844.37**	**15.67**	**0**
		Unpooled control treatments, treatment + year	9	2874.88	46.18	0
		Pooled control treatments, treatment + year	8	2878.7	50.01	0
	Covariate	**Pooled control treatments, treatment x year x legume total interaction**	**21**	**2768.67**	**0**	**1**
		Pooled control treatments, treatment x year x species total interaction	21	2791.06	22.39	0
		Pooled control treatments, treatment x year + species total	13	2797.42	28.76	0
		Pooled control treatments, treatment x year + legume total	13	2798	29.34	0
		Pooled control treatments, treatment x year + rabbit	13	2803.4	34.74	0
		Pooled control treatments, treatment x year + ant	13	2808.98	40.31	0
		Pooled control treatments, treatment x year + xy location	13	2824.54	55.87	0
		Pooled control treatments, treatment + year + legume total	9	2834.51	65.84	0
		Pooled control treatments, treatment + year + species total	9	2836.06	67.39	0
		Pooled control treatments, treatment + year + rabbit	9	2841.29	72.62	0
		Pooled control treatments, treatment + year + ant	9	2846.65	77.99	0
		Pooled control treatments, treatment + year + xy location	9	2859.7	91.03	0

Table S4.1 continued

Demographic variable	Model type	Model	*K*	AIC	ΔAICc	AIC weight
Leaf_no						
	Experiment (Untransformed)	Unpooled control treatments, treatment x year interaction	15	3851.49	0	1
		Pooled control treatments, treatment x year interaction	12	3866.44	14.96	0
		Unpooled control treatments, treatment + year	9	3886.19	34.7	0
		Pooled control treatments, treatment + year	8	3890.13	38.64	0
	Experiment (Square root)	**Pooled control treatments, treatment x year interaction**	**12**	**1746.94**	**0**	**0.74**
		Pooled control treatments, treatment + year	8	1749.02	2.07	0.26
	Covariate (Square root)	**Pooled control treatments, treatment x year + legume total**	**13**	**1727.14**	**0**	**0.66**
		Pooled control treatments, treatment x year + species total	13	1729.94	2.8	0.16
		Pooled control treatments, treatment + year + legume total	9	1730.44	3.3	0.13
		Pooled control treatments, treatment + year + species total	9	1732.52	5.39	0.04
		Pooled control treatments, treatment x year x legume total interaction	21	1735.85	8.71	0.01
		Pooled control treatments, treatment x year + rabbit	13	1748.44	21.31	0
		Pooled control treatments, treatment x year x species total interaction	21	1748.66	21.53	0
		Pooled control treatments, treatment x year + ant	13	1748.77	21.63	0
		Pooled control treatments, treatment + year + rabbit	9	1750.25	23.11	0
		Pooled control treatments, treatment + year + ant	9	1750.72	23.59	0
		Pooled control treatments, treatment x year + xy location	13	1758.46	31.33	0
		Pooled control treatments, treatment + year + xy location	9	1760.58	33.44	0

Table S4.1 continued

Demographic variable	Model type	Model	*K*	AIC	ΔAICc	AIC weight
Inflo_no						
	Experiment (Untransformed)	Unpooled control treatments, treatment x year interaction	15	2919.36	0	0.91
		Pooled control treatments, treatment x year interaction	12	2924.1	4.74	0.09
		Unpooled control treatments, treatment + year	9	2935.78	16.42	0
		Pooled control treatments, treatment + year	8	2936.25	16.89	0
	Experiment (Square root)	**Pooled control treatments, treatment + year**	**8**	**1439.3**	**0**	**0.73**
		Pooled control treatments, treatment x year interaction	12	1441.29	1.99	0.27
	Covariate (Square root)	**Pooled control treatments, treatment + year + legume total**	**9**	**1424.41**	**0**	**0.41**
		Pooled control treatments, treatment x year + legume total	13	1424.61	0.2	0.37
		Pooled control treatments, treatment x year + species total	13	1427	2.58	0.11
		Pooled control treatments, treatment + year + species total	9	1427.04	2.63	0.11
		Pooled control treatments, treatment + year + rabbit	9	1437.02	12.61	0
		Pooled control treatments, treatment x year + rabbit	13	1438.49	14.08	0
		Pooled control treatments, treatment x year x legume total interaction	21	1438.6	14.18	0
		Pooled control treatments, treatment + year + ant	9	1441.67	17.25	0
		Pooled control treatments, treatment x year + ant	13	1443.17	18.76	0
		Pooled control treatments, treatment + year + xy location	9	1451.95	27.54	0
		Pooled control treatments, treatment x year x species total interaction	21	1453.58	29.16	0
		Pooled control treatments, treatment x year + xy location	13	1454.04	29.63	0

Table S4.1 continued

Demographic variable	Model type	Model	*K*	AIC	ΔAICc	AIC weight
Inflo_length						
	Experiment (Natural log)	**Pooled control treatments, treatment x year interaction**	**12**	**1472.5**	**0**	**0.59**
		Unpooled control treatments, treatment x year interaction	15	1473.39	0.89	0.38
		Unpooled control treatments, treatment + year	9	1479.86	7.36	0.01
		Pooled control treatments, treatment + year	8	1480.23	7.72	0.01
	Covariate (Natural log)	**Pooled control treatments, treatment x year + ant**	**13**	**1469.52**	**0**	**0.41**
		Pooled control treatments, treatment x year + legume total	13	1469.79	0.27	0.36
		Pooled control treatments, treatment x year + species total	13	1471.23	1.71	0.17
		Pooled control treatments, treatment x year + rabbit	13	1474.09	4.57	0.04
		Pooled control treatments, treatment x year x legume total interaction	21	1476.75	7.23	0.01
		Pooled control treatments, treatment + year + legume total	9	1478.12	8.6	0.01
		Pooled control treatments, treatment + year + ant	9	1479.71	10.19	0
		Pooled control treatments, treatment + year + species total	9	1480.49	10.97	0
		Pooled control treatments, treatment + year + rabbit	9	1481.72	12.2	0
		Pooled control treatments, treatment x year + xy location	13	1484.84	15.32	0
		Pooled control treatments, treatment + year + xy location	9	1492.64	23.12	0
		Pooled control treatments, treatment x year x species total interaction	21	1502.72	33.2	0

Table S4.1 continued

Demographic variable	**Model type**	**Model**	***K***	**AIC**	**ΔAICc**	**AIC weight**
Canopy_area						
	Experiment (Natural log)	**Pooled control treatments, treatment + year**	**8**	834.63	0	0.78
		Pooled control treatments, treatment x year interaction	12	838.47	3.84	0.11
		Unpooled control treatments, treatment + year	9	838.76	4.12	0.1
		Unpooled control treatments, treatment x year interaction	15	845.72	11.09	0
	Covariate (Natural log)	**Pooled control treatments, treatment + year + rabbit**	**9**	**838.34**	**0**	**0.38**
		Pooled control treatments, treatment + year + ant	9	838.6	0.27	0.34
		Pooled control treatments, treatment + year + legume total	9	841.24	2.9	0.09
		Pooled control treatments, treatment x year + rabbit	13	842	3.67	0.06
		Pooled control treatments, treatment x year + ant	13	842.47	4.13	0.05
		Pooled control treatments, treatment + year + species total	9	842.71	4.37	0.04
		Pooled control treatments, treatment x year + legume total	13	844.94	6.6	0.01
		Pooled control treatments, treatment + year + xy location	9	845.26	6.92	0.01
		Pooled control treatments, treatment x year + species total	13	846.22	7.89	0.01
		Pooled control treatments, treatment x year + xy location	13	849.04	10.7	0
		Pooled control treatments, treatment x year x legume total interaction	21	875.02	36.69	0
		Pooled control treatments, treatment x year x species total interaction	21	883.77	45.44	0

Table S4.1 continued

Demographic variable	Model type	Model	*K*	AIC	ΔAICc	AIC weight
Basal_area						
	Experiment (Log)	**Pooled control treatments, treatment + year**	**8**	**1020.77**	**0**	**0.47**
		Pooled control treatments, treatment x year interaction	12	1020.92	0.15	0.44
		Unpooled control treatments, treatment + year	9	1025.08	4.31	0.06
		Unpooled control treatments, treatment x year interaction	15	1026.3	5.53	0.03
	Covariate (Log)	**Pooled control treatments, treatment x year + rabbit**	**13**	**1023.32**	**0**	**0.29**
		Pooled control treatments, treatment + year + rabbit	9	1023.64	0.32	0.24
		Pooled control treatments, treatment + year + ant	9	1024.22	0.9	0.18
		Pooled control treatments, treatment x year + ant	13	1024.42	1.1	0.1
		Pooled control treatments, treatment + year + legume total	9	1026.89	3.58	0.05
		Pooled control treatments, treatment x year + legume total	13	1027.11	3.79	0.04
		Pooled control treatments, treatment + year + species total	9	1029.34	6.03	0.01
		Pooled control treatments, treatment x year + species total	13	1029.53	6.22	0.01
		Pooled control treatments, treatment + year + xy location	9	1033.6	10.29	0
		Pooled control treatments, treatment x year + xy location	13	1033.94	10.63	0
		Pooled control treatments, treatment x year x legume total interaction	21	1052.67	29.36	0
		Pooled control treatments, treatment x year x species total interaction	21	1061.79	38.47	0

Table S4.2. Box-Cox transformations λ values for three of the demographic variables using MASS package in R (Ripley *et al.,* 2013). For all variables, the vector of values of λ were set to -5, 5.

Variable	λ value	Transformation chosen
Leaf number	0.35	Square root
Inflorescence number	0.25	Square root
Basal area	0.35	Natural log

Table S4.3. The number of tagged mature *Brachypodium sylvaticum* individuals surviving and reproducing and new recruits between 2019 and 2021.

Year	Treatment	Number of mature individuals	Annual survival rate (%)	Number of individuals reproducing	Number of new recruits
2019					
	Control	77	N/A	N/A	N/A
	Drought	33	N/A	N/A	N/A
	Irrigated	28	N/A	N/A	N/A
Total		*138*	*N/A*	*N/A*	*N/A*
2020					
	Control	73	94.8	5	13
	Drought	32	97.0	1	1
	Irrigated	28	100	2	2
Total		*133*	*96.3*	*8*	*16*
2021					
	Control	71	97.3	2	7
	Drought	31	96.9	0	0
	Irrigated	27	96.4	0	0
Total		*129*	*97.0*	*2*	*7*

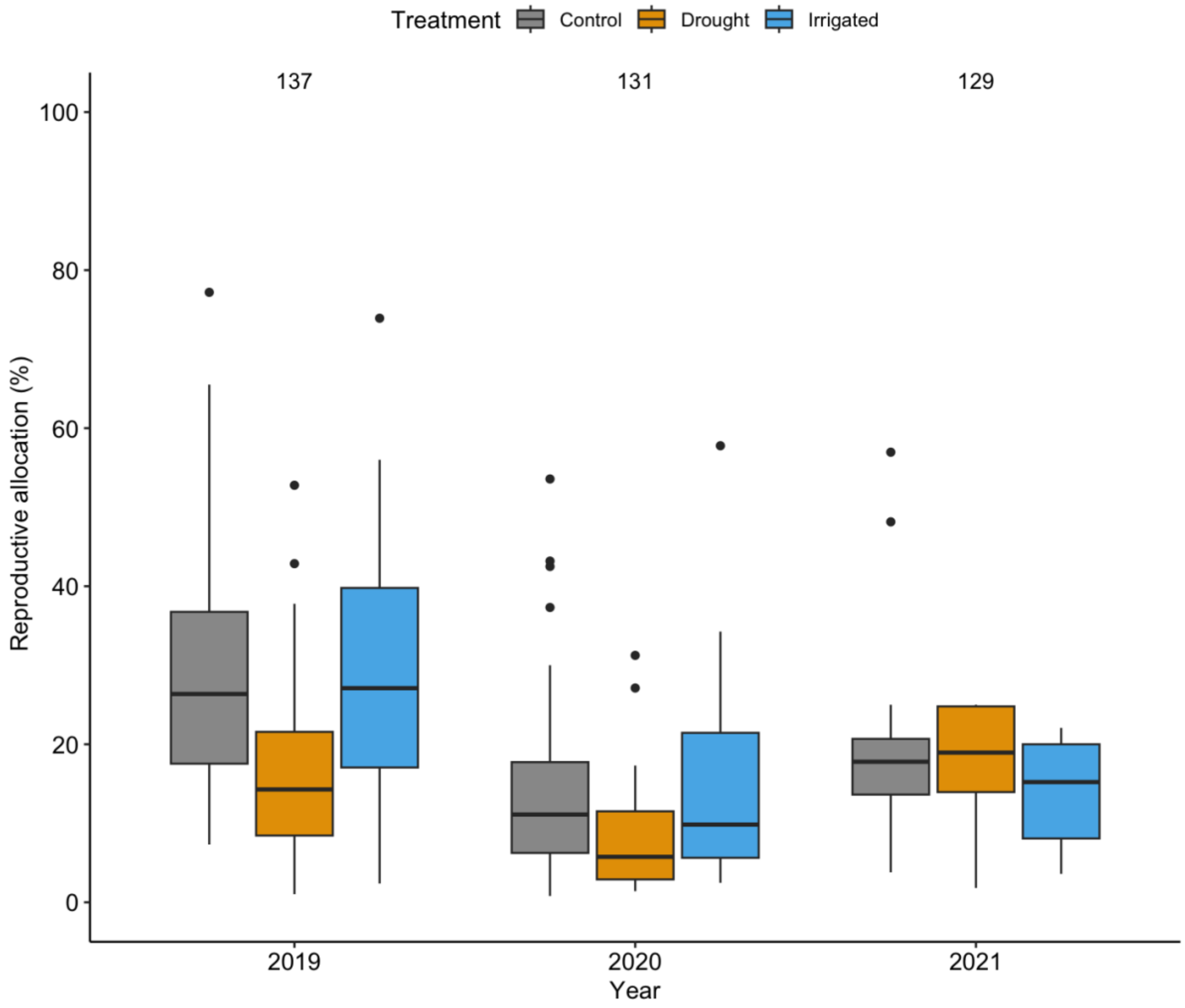
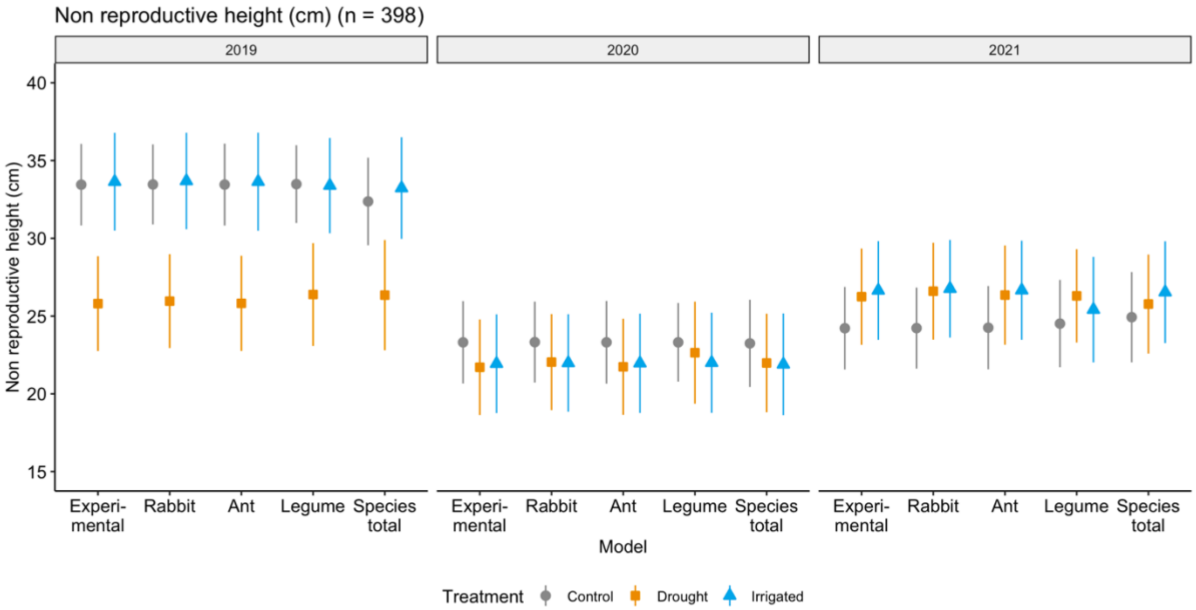
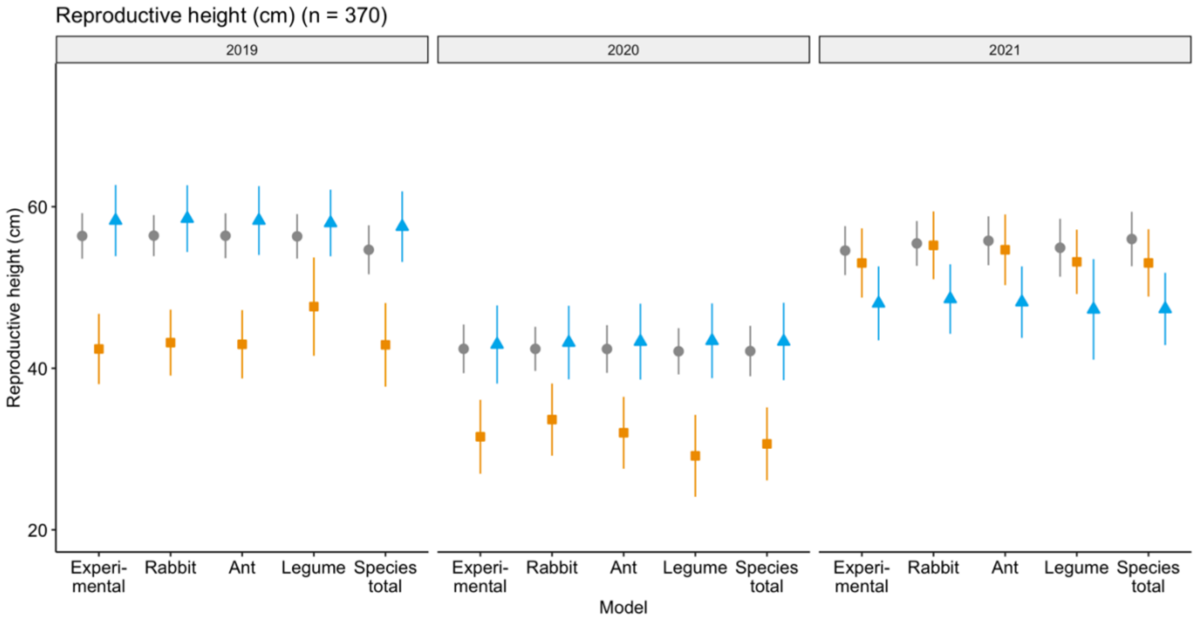


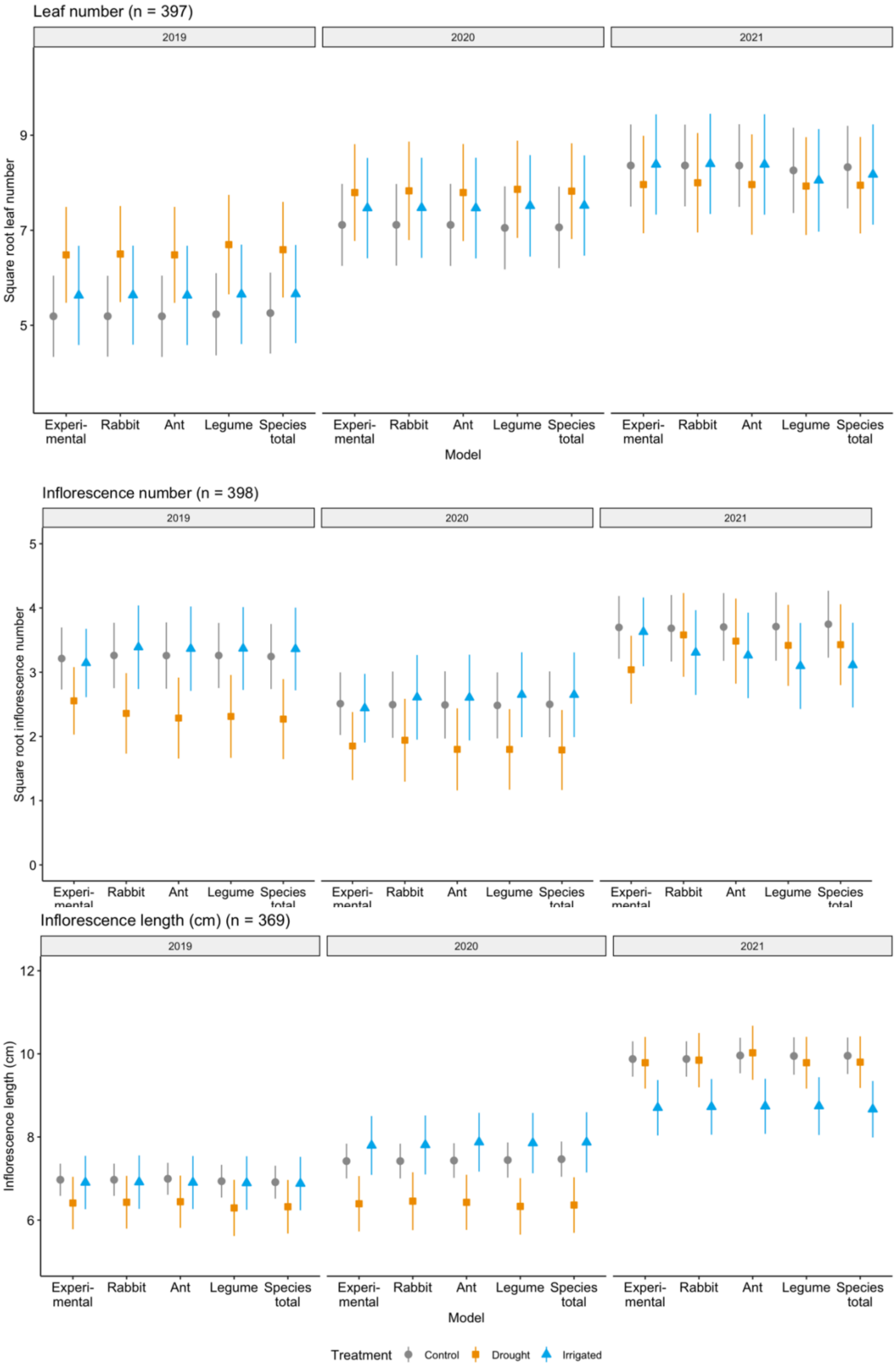
Figure S4.3 Box-and-whisker plots showing the reproductive allocation of *Brachypodium sylvaticum* adults across precipitation treatments over the study period. Reproductive allocation is expressed as a ratio of number of flowering spikes to total number of leaves and flowering spikes. Numbers above box-and-whisker plots indicate number of observations. Thick horizontal lines within boxes indicate median, the boxes represent the interquartile range, whiskers show minimum and maximum values, and the dots represent outliers.



Non reproductive height (cm) (n = 398)
2019
2020
2021
Non reproductive height (cm)
40
35
30
25
20
15
Experi-mental
Rabbit
Ant
Legume
Species total
Model
Treatment
Control
Drought
Irrigated



Reproductive height (cm) (n = 370)
2019
2020
2021
Reproductive height (cm)
60
40
20
Experi-mental
Rabbit
Ant
Legume
Species total
Model


Leaf number (n = 397)
2019
2020
2021
Square root leaf number
9
7
5
Experi-mental
Rabbit
Ant
Legume
Species total
Model
Inflorescence number (n = 398)
Square root inflorescence number
5
4
3
2
1
0
Inflorescence length (cm) (n = 369)
Inflorescence length (cm)
12
10
8
6
Treatment
Control
Drought
Irrigated

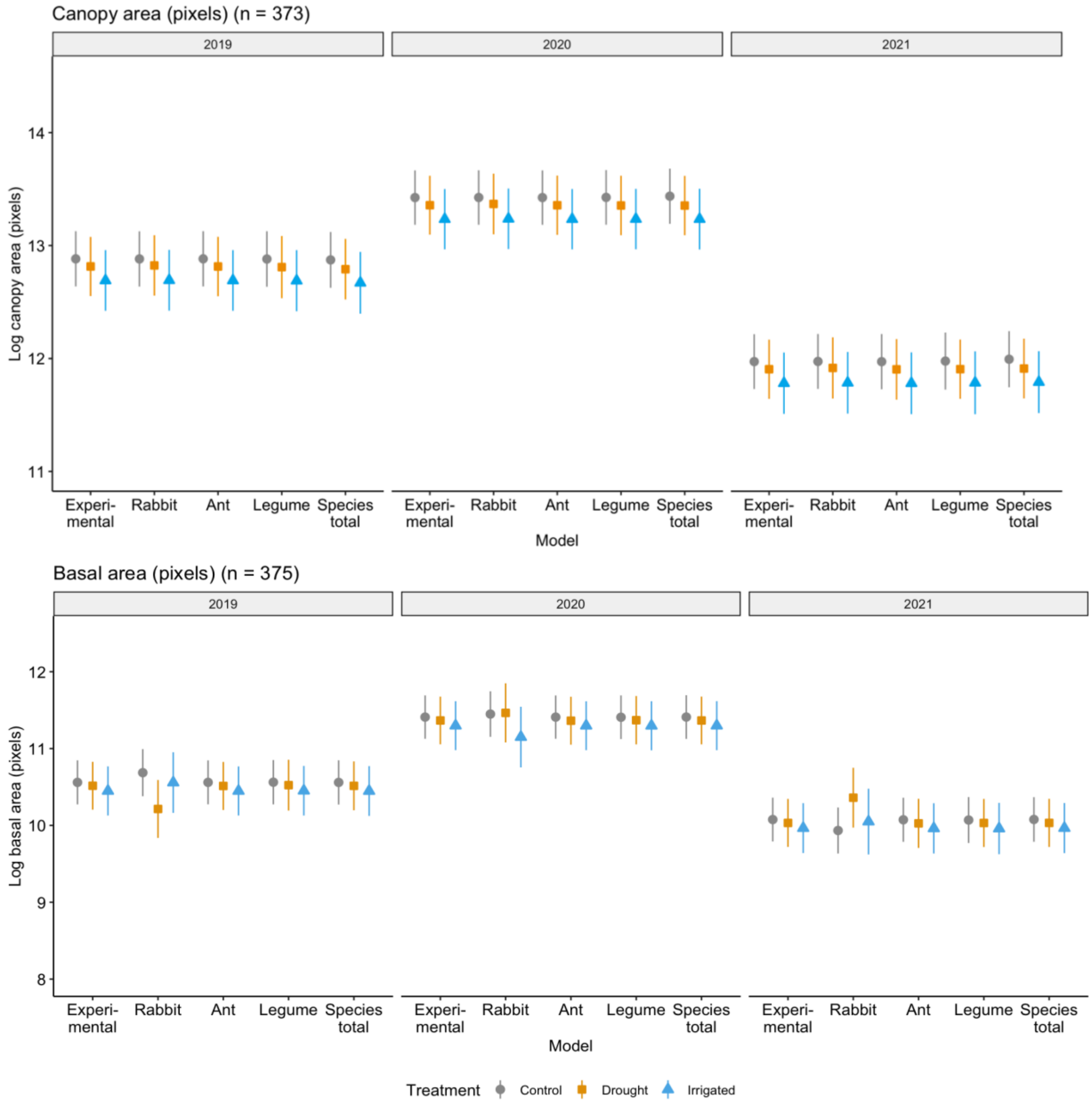


Figure S4.4 Comparison of conditional fixed effect estimates of seven demographic variables from experimental models (model 2) and covariate models (model 4) measured according to precipitation manipulation treatment and observation year. Rabbit, ant, legume and species total refer to covariates modelled separately. Points and error bars signify conditional fixed effect estimates and 95% confidence intervals respectively. n indicates number of observations per demographic measure.

Table S4.4. Pearson correlation results disaggregated by observation year for relationship between log canopy area (pixels) and reproductive measures in *Brachypodium sylvaticum* individuals. Inflorescence number has a square root transformation. Each significance level is denoted with a symbol to indicate p-value, where: *** = < 0.001, ** = < 0.01, * = < 0.05, and . = < 0.10.

Treatment	**Reproductive height (cm)**	**Inflorescence number**	**Inflorescence length (cm)**
2019			
Control	0.400 **	0.417 ***	0.061
Drought	0.203	0.091	0.003
Irrigated	0.584 **	0.485*	0.196
2020			
Control	0.334 **	0.505 ***	0.173
Drought	0.345 .	0.270	0.353 .
Irrigated	0.398 .	0.585 **	0.371 .
2021			
Control	0.478 ***	0.611 ***	0.274 *
Drought	0.441 *	0.571 ***	0.216
Irrigated	0.624 *	0.651 **	0.248

Table S4.5. Results of variance partition analysis from model 3 demographic models

Demographic variable (unit)	Model term	Standard deviation	Confidence interval: 2.5%	Confidence interval: 97.5%	Variance	Summed variance	Proportion of summed variance (%)
non_repro_height (cm)	block	1.859	0.8691877	4.557596	3.455	68.469	5.046
	plot	1.587	0.5494903	4.656819	2.518	68.469	3.678
	treatment	1.417	0.3562335	5.291413	2.007	68.469	2.931
	year	4.61	2.0139577	11.803241	21.252	68.469	31.039
	residual	6.264	5.8470499	6.733642	39.237	68.469	57.306
repro_height (cm)	block	1.1026	0	4.355522	1.216	212.157	0.573
	plot	0.5857	0	3.84482	0.343	212.157	0.162
	treatment	4.4301	1.789558	13.665168	19.625	212.157	9.250
	year	7.7389	3.351803	19.979535	59.89	212.157	28.229
	residual	11.4492	10.660286	12.340885	131.083	212.157	61.786
leaf_no	block	0.6769	0.2998376	1.641745	0.4582	6.8623	6.677
	plot	0.2917	0	1.018792	0.08506	6.8623	1.240
	treatment	0.2067	0	1.025368	0.04274	6.8623	0.623
	year	1.3488	0.5795541	3.419787	1.81917	6.8623	26.510
	residual	2.1112	1.9705471	2.269992	4.45713	6.8623	64.951
inflo_no	block	0.425	0.18925965	1.0033605	0.18063	2.66194	6.786
	plot	0.1124	0	0.5144856	0.01263	2.66194	0.474
	treatment	0.3344	0.09972676	1.1036359	0.1118	2.66194	4.200
	year	0.5841	0.242607	1.5861279	0.34114	2.66194	12.815
	residual	1.4198	1.32535295	1.5264235	2.01574	2.66194	75.724

inflo_length (cm)	block	0	0	0.3622061	0	5.33298	0
	plot	0	0	0.3939529	0	5.33298	0
	treatment	0.2278	0	1.0045298	0.05189	5.33298	0.973
	year	1.4876	0.6323759	3.6248355	2.21293	5.33298	41.495
	residual	1.7516	1.631755	1.8869622	3.06816	5.33298	57.532
canopy_area (pixels)	block	0.2263	0.1012681	0.5706016	0.051213	1.085558	4.718
	plot	0	0	0.1431311	0	1.085558	0
	treatment	0.06288	0	0.3650314	0.003954	1.085558	0.364
	year	0.72996	0.3155462	1.7929067	0.532841	1.085558	49.085
	residual	0.70537	0.6571178	0.7598743	0.49755	1.085558	45.834
basal_area (pixels)	block	0.2501	0.1070812	0.6241673	0.06254	1.32349	4.725
	plot	0	0	0.2496219	0	1.32349	0
	treatment	0	0	0.2493968	0	1.32349	0
	year	0.6696	0.2860932	1.6551813	0.4483	1.32349	33.873
	residual	0.9015	0.8400935	0.9707534	0.81265	1.32349	61.402

Table S4.6. Plant taxa recorded over the three-year study period (2019-2021) within the 0.3 m radius neighbourhoods based on Stace (2019).

Plant taxa	Functional type	Parasitic	Primary life form
Lotus corniculatus	legume	-	Perennial
Trifolium repens	legume	-	Perennial
Trifolium pratense	legume	-	Perennial
Medicago lupulina	legume	-	Annual
Agrimonia eupatoria	Forb	-	Annual
Geranium dissectum	Forb	-	Annual
Geranium columbinum	Forb	-	Annual
Hypericum perforatum	Forb	-	Annual
Hypericum hirsutum	Forb	-	Perennial
Gallium vernum	Forb	-	Perennial
Clinopodium vulgare	Forb	-	Perennial
Agrostis stolonifera	Graminoid	-	Perennial
Agrostis capilaris	Graminoid	-	Perennial
Lolium perenne	Graminoid	-	Perennial
Dactylis glomerata	Graminoid	-	Perennial
Brachypodium pinnatum	Graminoid	-	Perennial
Brachypodium sylvaticum	Graminoid	-	Perennial
Jacobeae sp.	Forb	-	Biennial
Pastinaca sativa	Forb	-	Biennial
Trisetum flavescens	Graminoid	-	Perennial
Arrhenatherum elatius	Graminoid	-	Perennial
Crataegus monogyna	Woody	-	Perennial
Holcus lanatus	Graminoid	-	Perennial
Rosa canina	Forb	-	Perennial
Convolvulus arvensis	Forb	-	Perennial
Primula veris	Forb	-	Perennial
Linum catharticum	Forb	-	Annual
Tragopogon pratensis	Forb	-	Biennial
Festuca rubra	Graminoid	-	Perennial
Potentilla reptans	Forb	-	Perennial
Veronica chamaedrys	Forb	-	Perennial
Cerastium fontanum	Forb	-	Perennial
Anacamptis pyramidalis	Forb	-	Perennial
Gernamium sp.	Forb	-	-
Viola hirta	Forb	-	Perennial
Cirsium eriophorum	Forb	-	Biennial

Taraxacum officinale	Forb	-	Perennial
Galium mullugo	Forb		Perennial
Rhinanthus minor	Forb	Yes	Annual
Fraxinus excelsior	Woody	-	Perennial
Linaria vulgaris	Forb	-	Perennial
Cynosurus cristatus	Graminoid	-	Perennial
Taraxicum sp	Forb	-	-
Graminoid sp	Graminoid	-	-
Carex sp.	Graminoid	-	-
Odontites vernus	Forb	Yes	Annual
Vicia sativa	Legume	-	Annual
Astereaceae sp	Forb	-	-
Bromus commutatus	Graminoid	-	Annual
Bromus sterilis	Graminoid	-	Annual
Crepis sp.	Forb	-	-
Clematis vitalba	Forb	-	Perennial
Rubus fruiticosus agg	Woody	-	Perennial
Prunus sp.	Woody	-	Perennial
Knautia arvensis	Forb	-	Perennial
Woody sp.	Woody	-	Perennial
Danthonia decumbens	Graminoid	-	Perennial
Trifolium dubium	Legume	-	Annual
Glechoma hederacea	Forb	-	Perennial
Trifolium campestre	legume	-	Annual
Geranium lucidem	Forb	-	Annual
Prunella vulgaris	Forb	-	Perennial
Plantago lanceolata	Forb	-	Perennial
Orobanche minor	Forb	Yes	Perennial
Bromus hordaceus	Graminoid	-	Annual
Ranunculus sp.	Forb	-	Perennial
Quercus sp.	Woody	-	Perennial
Total: Plant taxa 67	Forb = 37, graminoid = 17, legume = 7, woody = 6	Parasitic = 3	Perennial = 40, biennial = 5, annual = 15

Table S4.7. Reproductive height estimates and standard error for mixed effect covariate models (model 4). Reference level is observation year 2019 and control treatment.

Model term	**Estimate**	**Standard Error**
Intercept	72.01	6.08
Species total	-1.16	0.43
Drought x species total	1.18	0.85
Irrigated x species total	-0.07	0.99
2020 x species total	1.32	0.06
2021 x species total	0.79	0.61
Drought x 2020 x species total	-3.03	1.18
Drought x 2021 x species total	0.32	1.17
Irrigated x 2020 x species total	-0.01	1.31
Irrigated x 2021 x species total	0.31	1.31

Chapter 5: Functional trait responses of *Brachypodium sylvaticum* in a precipitation manipulation experiment

Sara L Middleton

(Image: Sara Middleton)

Abstract

Grasslands are important ecosystems, that are increasingly vulnerable to climate extremes. Climate change is altering precipitation patterns resulting in grasslands worldwide becoming more frequently exposed to both extreme wet and dry precipitation events. Trait-based approaches can be used to better understand how grassland vegetation responds to major climate stressors and how these responses feedback across organisational scales.

Here I report on results on a trait study conducted on dominant bunch grass *Brachypodium sylvaticum* (Huds.) in a UK calcareous grassland with experimentally manipulated (50% reduction- drought and 50% addition - irrigated) growing season rainfall. Six above-ground morphological traits associated with plant resource use, growth and drought tolerance (leaf area, specific leaf area, leaf dry matter content, leaf thickness, stem specific density and maximum plant height) were measured on 40 individuals.

B. sylvaticum showed high tolerance to precipitation treatments, as no significant shifts in trait means were detected. In response to precipitation deficits, individuals showed signs of trait co-ordination in leaf anatomy via marginal reductions in leaf size (~16%) and display of leaf rolling, while maintaining stability in leaf thickness and leaf dry matter content. There were similar trait variation patterns across the experimental design levels, indicating no single clear source of variation in trait responses. The relatively small study area likely had similar environmental processes operating across organisational scales within the site. Taken together these results suggest that in the short-term *B. sylvaticum* is a drought tolerant species with a conservative resource strategy, able to maintain functionality across a range of water availability conditions with only minor trait adjustments. Understanding

the long-term potential adaptive capacity of this and other co-dominant species to buffer an increasingly stochastic environment is key with implications on community structure and functioning.

5.1 Introduction

Grasslands are important ecosystems integral to the structure and functioning of the terrestrial biosphere that are increasingly under threat from climate extremes, particularly precipitation extremes (White *et al.,* 2000). Grasslands have a wide biogeographic coverage, typified by seasonal precipitation patterns and recurrent deficits in rainfall (Strömberg and Staver, 2022), as such, they are especially vulnerable to exposure to both extreme wet and dry precipitation events accelerated by climate change (Smith *et al.*, 2024). Further, the low rooting depth of grassland species and lower water use efficiency (compared to trees), make grassland ecosystems even more vulnerable during deficits of precipitation (Felton and Goldsmith, 2023). Drought defined here from the ecological plant perspective, is a deficit in water input relative to ambient levels or input relative to plant needs, reducing biomass accumulation and survival (Büntgen *et al.,* 2021; Carroll *et al.*, 2021; Swann, 2018). How grasslands respond to an increasingly destabilised hydro-climatic system has critical significance for global biodiversity, carbon storage, ecosystem provisions and socio-economic security (Bardgett *et al.,* 2021). Generally, droughts reduce net primary productivity (Carroll *et al.,* 2021). However, drought responses can vary with geography (e.g. xeric vs mesic grasslands, with the former being more drought sensitive, (Griffin-Nolan *et al.*, 2019; Knapp *et al.,* 2017) or lag, with effects registering months after drought event (Zhao *et al.,* 2020). Experimental studies reporting on vegetation responses to wet precipitation extremes are more sparce, although it is thought that excess rainfall events increase productivity, with similar responses to nutrient additions (DeMalach *et al.,* 2017). As with drought, the attributes of wet precipitation events are important to consider when examining responses (Knapp *et al.*, 2015). Increasing knowledge of the mechanisms underlying these ecological shifts is pertinent and can be used to predict and mitigate effects of future climate change on these globally important ecosystems.

Trait-based approaches are a promising avenue for examining the processes driving plant responses to climate change stressors by relating an individuals' phenotype to the environment (Gallagher *et al.,* 2020; Volaire *et al.,* 2020). Traits are measurable features (e.g. morphological, structural, physiological) typically of an individual organism that is measured at either the individual or other relevant level of organisation (Dawson *et al.*, 2021). When these traits explicitly relate to a component of an organism's fitness via vital rates (survival, growth and reproduction), they are classed as being functional (*sensu* Violle *et al.,* 2007). Studies on plant trait-environment relationships based on mean differences species' traits have yielded important insights such as the leaf economics spectrum (Wright *et al.,* 2004), integrated whole-plant trait spectrum (Reich, 2014) and a global spectrum of vascular plant traits (Díaz *et al.,* 2016). Increasingly it has been recognised that focusing only on mean trait values between species (i.e. inter-specific variation) without considering within species variation (intra-specific variation) provides only a partial picture of ecological functioning and processes, especially at lower levels of biological organisation (Castro Sánchez-Bermejo *et al.*, 2023). Frequently labelled as 'phenotypic noise' understanding the role of intra-specific trait variation both *amongst* and *within* individuals of the same species is critical for characterising plant performance and fitness particularly in the context of adaption to climate change *(*Castro Sánchez-Bermejo *et al.,* 2023). Within species trait variation was recognised by Darwin as an evolutionary mechanism for natural selection to act. In the 20th century, intraspecific variation was considered more in evolutionary ecology (e.g. niche breadth (Roughgarden, 1972)), with less theoretical and empirical research interest in ecology in the subsequent decades (Bolnick *et al.*, 2011). More recently, the importance of understanding the role of intraspecific variation for population and community stability has come to light (see review by Moran *et al.* (2016)). Experimental work has shown intraspecific trait variability can buffer against local extinction, thereby contributing to stability of communities (Whitlock *et al.,* 2007). Even more scarcely considered, within-individual trait variation refers to a plant's ability to express different trait values across modular units of the plant body (e.g. leaves, flowers or fruits) (Castro Sánchez-Bermejo *et al.,* 2023). The shape of trait distributions within and between individuals can provide rich information on the degree of trait-environment matching and the genetic, developmental, and environmental forces acting on organisms (Enquist *et al.,* 2015). The plasticity of traits and how adequately they co-ordinate determines the capacity of plants to cope with environmental stressors (Reinelt *et al.,* 2023).

Drought is a strong abiotic stressor that constrains the range of trait values found within a population or community (Jung *et al.,* 2014). The regulation of water loss through leaves can be expressed via key traits found in the leaf economic and plant water economic spectrums (Reich, 2014). Increased leaf dry matter content (LDMC) and leaf thickness, decreased specific leaf area (SLA) and leaf area are associated with higher water use efficiency, which helps plants maintain physiological processes during period of lower availability (Vitra *et al.,* 2019; Jung *et al.,* 2014). Most of the research on trait responses to precipitation extremes such as drought comes from forested systems with temperate grasslands relatively understudied given their importance to biosphere integrity and vulnerability to climate change (Kühn *et al.,* 2021, although see Trinder *et al.,* 2020). Intraspecific variation in several vegetative and reproductive traits has been shown to have potential adaptive value and confer grassland community resistance to experimentally imposed chronic drought (Ravenscroft *et al.,* 2014). Most observational and experimental studies have focused on upscaling traits to examine ecosystem or community functioning under precipitation gradients or treatments (Vitra *et al.*, 2019). Few studies have examined how precipitation mediates trait responses and consequences to plant performance or community composition (but see Jung *et al.,* 2014 and Fenollosa *et al.,* 2023).

The present study examines the variation in trait responses of a perennial bunch grass species *Brachypodium sylvaticum* growing under different precipitation manipulation treatments in a recovering UK calcareous grassland. The experimental site has imposed two precipitation extremes of 50% reduction and 50% addition in growing season rainfall, which hereafter will be referred to as drought and irrigated respectively. This work is an extension to previous studies at the site examining community-level functioning and composition (Jackson *et al.*, 2024) and demographic and micro-community dynamics (**Chapter 4**). With a focus on the scale of within and between individuals, three key research questions were addressed:

1. Do the traits of *B. sylvaticum* individuals from drought plots have narrower trait distributions, as water stress selects for increased similarity in trait values?
2. Do *B. sylvaticum* individuals in drought plots have higher trait values for LDMC, stem specific density, and leaf thickness and lower height, leaf area and SLA values compared to control plots?
3. How does variation in the response of traits to precipitation manipulation vary across spatial scales in the experimental design from block to plot to individual?

5.2 Methodology

The present study is situated at the same experimental site detailed in **Chapter 3**, with the same life history of the study species as **Chapter 4**. Key methods will be repeated here for convenience.

5.2.1 Site description and experimental set up

The RainDrop experiment is a ~ 2-hectare area nested within a 10-hectare calcareous grassland (160 m a.s.l) at Upper Seeds, Wytham Woods, Oxfordshire (UK) (51°46'15.7" N, 1°19'56.9" W (Gibson and Kirby, 2011; Jackson *et al.,* 2024). The experimental site sits on a bedrock of Jurassic Corallian Rag limestone overlain with a shallow alkaline soil layer (Kirby *et al.,* 2022). Information on the site usage and management prior to the 1940s is unknown, but over the centuries the Wytham Estate has oscillated between agriculture and managed woodland (Gibson and Kirby, 2011; Gibson, 2011). Between c. 1940-1980 the site was used intensively for arable crops. Since 1980, the site has been in recovery, naturally colonised with grassland and woodland edge plant species from soil seedbank and surrounding grassland and wooded areas maintained with sheep grazing (Gibson and Kirby, 2011). There was a previous climate change experiment at the site that ran during the 1990's that investigated winter warming and summer drought (Cummins *et al.,* 1995). The site is fenced to exclude grazing animals, particularly sheep. Rabbits remain a persistent occurrence at the site and are not controlled. For the 2016-2020 period, the range in average daily temperature and total daily precipitation was −5 to 26 °C and 0-40 mm respectively (Jackson *et al.,* 2024; Rennie *et al.,* 2017).

The Upper Seeds site is species rich considering the recent agricultural land use, with 264 plant species registered at least once between 1983 and 2007 (Gibson, 2011). The RainDrop site (five acre field) has 109 vascular plant species based on the most recent plant list in 2022, with the graminoids *Arrhenatherum elatius, Brachypodium pinnatum* and *B. sylvaticum* found in high abundance. Since 2016, the site has been managed with two annual cuts with above-ground biomass removal in mid-July and end of September (Jackson *et al.,* 2024).

The Raindrop experiment was established in 2016 to examine how manipulating precipitation affects grassland functioning and resilience, as part of a coordinated international drought network (DroughtNet) (https://droughtnet.weebly.com/). The DroughtNet consortium consists of over 100 sites distributed across six continents where each site follows a minimum set of biotic and abiotic measurements set out in experimental protocols (Knapp *et al.,* 2017; Smith *et al.,* 2024). Extreme drought is simulated through the passive interception of precipitation through fixed rainout shelters applied at the plot level. During extreme wet and dry years, a 40% and 30% departure from mean annual precipitation levels respectively can be observed (Knapp *et al.,* 2015). To ensure multisite comparisons, treatment level is applied relative to the long-term precipitation records of the site (Knapp *et al.,* 2017).

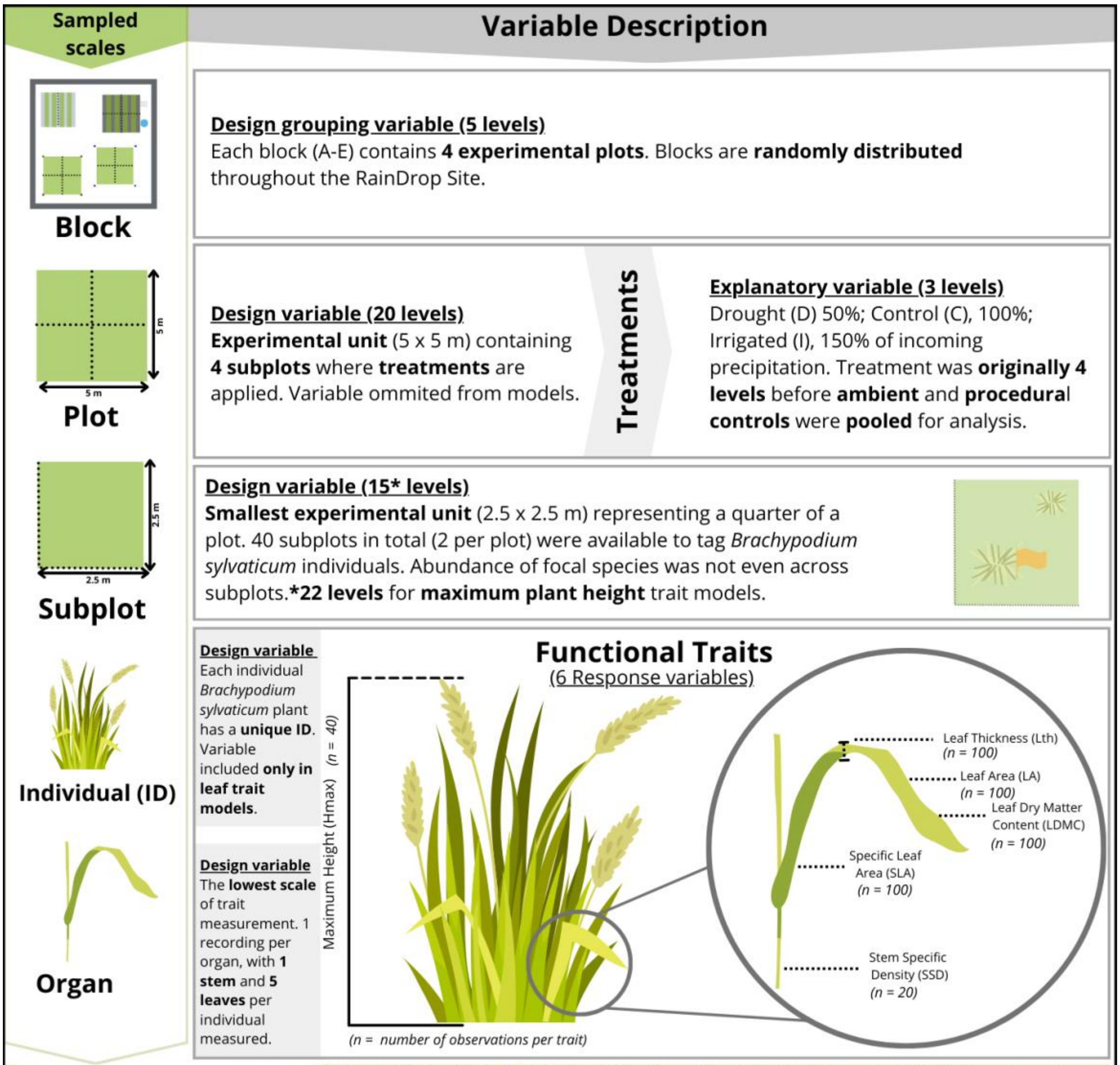
At RainDrop, the precipitation manipulation consists of four treatments applied on 20 replicated experimental units of 5 x 5 m plots randomly arranged across five blocks. Within each plot there are four 2.5 x 2.5 m subplots (Figure 5.1). The four treatments consisted of two controls: ambient and procedural control (each receiving 100% of incoming precipitation), drought and irrigated representing 50% and 150% precipitation levels respectively. Rainout shelters exclude precipitation via v-shaped polycarbonate plastic gutters mounted onto metal structures. The height of the shelters varied from between 1.65 m to 2.10 m, reflecting the small topographic changes at the site and to reduce microclimatic effects of humidity and temperature. To maintain near-surface air exchange, all four sides of the shelter were kept open. The intercepted precipitation from drought plots was collected in an automated water collection tank, which was then redistributed to irrigated plots via a sprinkler system. Procedural control plots were used to determine the effect of the shelter on incoming photosynthetically active radiation and humidity. Although the rainout shelters and plastic gutters remain in place year-round, the drought treatment was only applied during the growing season between April 1st and October 1st each year. Outside the growing season, the drought shelter gutters are inverted to reinstate ambient precipitation levels.

5.2.2 Life history and ecology of Brachypodium sylvaticum

False brome *(Brachypodium sylvaticum)* (Huds.) is a bunch-forming perennial grass species native to Eurasia that is self-compatible, and wind pollinated (Monroe and Des Marais, 2021; Stace, 2019). In the UK, it grows up to 1 m in height on woodland edges and scrub, favouring well-drained calcareous-neutral soils (Stace and Thompson 2019; Streeter *et al.,* 2009). The seedbank is short-lived, typically no more than one year (Davies and Waite 1998) and individuals start out from seed usually near the parent plant, but short-distance seed dispersal can occur, typically with ungulates such as deer (Miller *et al.*, 2011; Roy *et al.,* 2011). Seedlings grow through the production of asexual tillers which usually flower after their second year (Roy *et al.,* 2011). *B. sylvaticum* flowers relatively late in the growing season, between July and August. Outside the growing season, particularly during cold winter months, plants will die back to ground level (Roy *et al.*, 2011).

5.2.3 Trait data measurement and processing

In May of 2022, 40 randomly selected surviving *B. sylvaticum* individuals that were originally studied in the 2019-2021 demographic study at RainDrop (n = 138) (**Chapter 4**), were used for trait analyses. The 5 x 5 m plots naturally divide into 2.5 x 2.5 m quarters - subplots - due to the placement of upright supporting legs of the rainout shelters midway along each edge and in the centre (Figure 5.1, **Chapter 4**). Of the 40 individuals used for the monitoring of traits, 21 occurred within the subplot used to take the long-term annual measurements of diversity and biomass and 19 in the unused subplot (see totals in subplot 1 and 2 columns in Figure 5.1). The number of individuals located in each subplot ranged from 0-4, reflecting the natural uneven abundance of the species at the site. As discussed below (data analysis) this very small and unbalanced subsample of subplots within plots meant that trying to estimate variation of subplots within plots was likely to cause estimation problems and produce highly imprecise estimates. I therefore estimated variation between the 15 subplots (in stem/leaf trait models) and 22 subplots (in maximum plant height models).



Variable Description

Design grouping variable (5 levels)
Each block (A-E) contains **4 experimental plots**. Blocks are **randomly distributed** throughout the RainDrop Site.

Design variable (20 levels)
Experimental unit (5 x 5 m) containing **4 subplots** where **treatments** are applied. Variable ommited from models.

Explanatory variable (3 levels)
Drought (D) 50%; Control (C), 100%; Irrigated (I), 150% of incoming precipitation. Treatment was **originally 4 levels** before **ambient** and **procedural controls** were **pooled** for analysis.

Design variable (15* levels)
Smallest experimental unit (2.5 x 2.5 m) representing a quarter of a plot. 40 subplots in total (2 per plot) were available to tag *Brachypodium sylvaticum* individuals. Abundance of focal species was not even across subplots.***22 levels** for **maximum plant height** trait models.

Design variable
Each individual *Brachypodium sylvaticum* plant has a **unique ID**. Variable included **only in leaf trait models**.

Design variable
The **lowest scale** of trait measurement. 1 recording per organ, with **1 stem** and **5 leaves** per individual measured.

Functional Traits
(6 Response variables)

(n = number of observations per trait)

Sampling Across Blocks, Plots and Subplots

	Drought plot 50%		Irrigated plot 150%		Ambient 100% control plot		Procedural 100% control plot		Total
	Subplot 1	Subplot 2	Subplot 1	Subplot 2	Subplot 1	Subplot 2	Subplot 1	Subplot 2	
Block A	3	0	1	1	4	0	2	1	*12*
Block B	0	1	2	1	1	2	2	1	*10*
Block C	0	1	3	0	0	0	0	0	*4*
Block D	0	3	0	0	1	0	0	0	*4*
Block E	0	2	0	2	0	2	2	2	*10*
Total Individuals	*3*	*7*	*6*	*4*	*6*	*4*	*6*	*4*	***40***
	10		***10***		***10***		***10***		

Sampling
Out of the **138 individuals** orginally tagged in 2019, **40 individuals** were **randomly** selected for **maximum plant height** and a **subset of 20** individuals were used to measure **stem/leaf traits.**

Figure 5.1 Description of the sampled spatial scales in the functional trait study on *Brachypodium sylvaticum* at the RainDrop site. Six functional traits were measured, with a total of 460 unique trait measurements recorded on 40 individuals (maximum plant height) and a subset of 20 individuals for the remaining five stem and leaf traits. Stem specific density was measured using one stem per individual which was calculated by averaging three stem diameter measurements on the one stem.

Six above-ground traits were measured on the subset of individuals: four leaf traits: leaf area (cm^2), leaf thickness (mm), specific leaf area (cm^2 g^{-1}) and leaf dry matter content (g g^{-1}); and two “structural” traits: maximum plant height (cm) and stem specific density (g cm^{-3}) (Figure 5.1 and Table 5.1). Trait selection was based on potential to detect physiological and morphological response related to drought tolerance (Jung *et al.,* 2014; Vitra *et al.,* 2019; Seleiman et al., 2021) and practical limitations of the field site such as soil conditions. To capture within individual trait variability, five leaves per individual were sampled for five individuals in each of the four treatments. For stem specific density (SSD) and maximum plant height, only one measurement per individual was taken, with the latter sampling ten individuals across the treatments. SSD was measured using one stem per individual which was calculated by averaging three stem diameter measurements on the one stem. Traits were measured from mid-June to early July 2022 following standard protocols in Perez-Harguindeguy *et al.* (2013). After maximum plant height was measured, leaf and stem samples were harvested for a given individual. Leaf samples were cut and stored still attached to the stem, whilst samples for stem specific density, a single ~20 cm basal portion of a different stem was cut per individual. All samples were stored in plastic zip-lock bags with a wet paper towel and placed in a refrigerated opaque container and processed within 24 hours to minimise transpiration losses.

The ligule of each leaf sample was removed before trait measurements. Leaf thickness was measured using a digital micrometre (Tresna 0-25mm/0.001). Leaves were scanned (at 600 dpi) upper side facing downwards in a flatbed scanner (Canon CanoScan Lide 300), and then fresh weighed using high-precision balance (Ohaus DV215CD/0.01 mg). Some leaf samples required clear tape to correctly position them in the flatbed scanner. All leaf and stem samples were oven-dried at approximately 65 °C for 72 hours. Details of calculations of traits are shown in Table 5.1. Image J was used to extract leaf area data from the leaf scans (Schneider *et al.,* 2012). Each leaf image was spatially calibrated using a 1 cm^2 scale present in each scanned image. Each image was then converted to 8-bit and the threshold manually adjusted to ensure the leaf shadow was excluded from the leaf area calculation.

Table 5.1. The biological significance of trait selection and calculations. Details of functional trait levels and measurement replication can be found in Figure 5.1 and Table S5.1.

Trait variable (*y*)	**Unit**	**Biological significance to drought**	**Trait calculation**	**References**
Leaf traits				
Leaf thickness (L_{th})	mm	Species with higher leaf construction costs (e.g. higher L_{th} values) have increased drought resistance	Thickness of a fresh leaf lamina	Pérez-Harguindeguy *et al.,* 2013; Jung *et al.,* 2014; Petruzzellis *et al.,* 2019
Leaf area (LA)	cm^2	During drought stress, plants tend to be smaller in size, plant leaves may roll inwards to reduce further water loss	One-sided area of a fresh leaf	Pérez-Harguindeguy *et al.,* 2013; Le Bagousse-Pinguet *et al.,* 2015
Leaf dry matter content (LDMC)	$g\ g^{-1}$	Species with higher leaf construction costs (higher LDMC values) have higher drought resistance	Dry mass/fresh mass	Pérez-Harguindeguy *et al.,* 2013; Vitra *et al.,* 2019
Specific leaf area (SLA)	$cm^2\ g^{-1}$	Species with higher leaf construction costs (lower SLA values) have higher drought resistance	One-sided area of a fresh leaf/dry leaf mass	Pérez-Harguindeguy *et al.,* 2013; Poorter *et al.,* 2012; Petruzzellis *et al.,* 2019
Structural traits				
Specific stem density (SSD)	$g\ cm^{-3}$	Linked to biomechanics and hydraulics on the growth-survival trade off	Dry mass of section of stem/fresh volume of same section. Volume calculated from averaging diameter from 3 sections along stem sample.	Pérez-Harguindeguy *et al.,* 2013; Kervroëdan *et al.,* 2018
Maximum height (H_{max})	cm	An indicator of competitive vigour and reproductive size. During drought stress, plants tend to be smaller in stature	Shortest distance from ground level to top of photosynthetic tissues (unstretched)	Pérez-Harguindeguy *et al.,* 2013; Seleiman *et al.*, 2021

5.2.4 Data analysis

Investigating the effect of precipitation manipulation on the functional trait responses of *Brachypodium sylvaticum* individuals involved performing hierarchical (multilevel) Bayesian regression models computed using the 'brms' R package (Bürkner, 2017; R Core Development Team 2024) (see below). Bayesian regression was used because during initial analysis of trait data using mixed-effects models (implemented with the lmer() function from the 'lme4' R package (Bates *et al.,* 2015)) as there were issues with singularities.

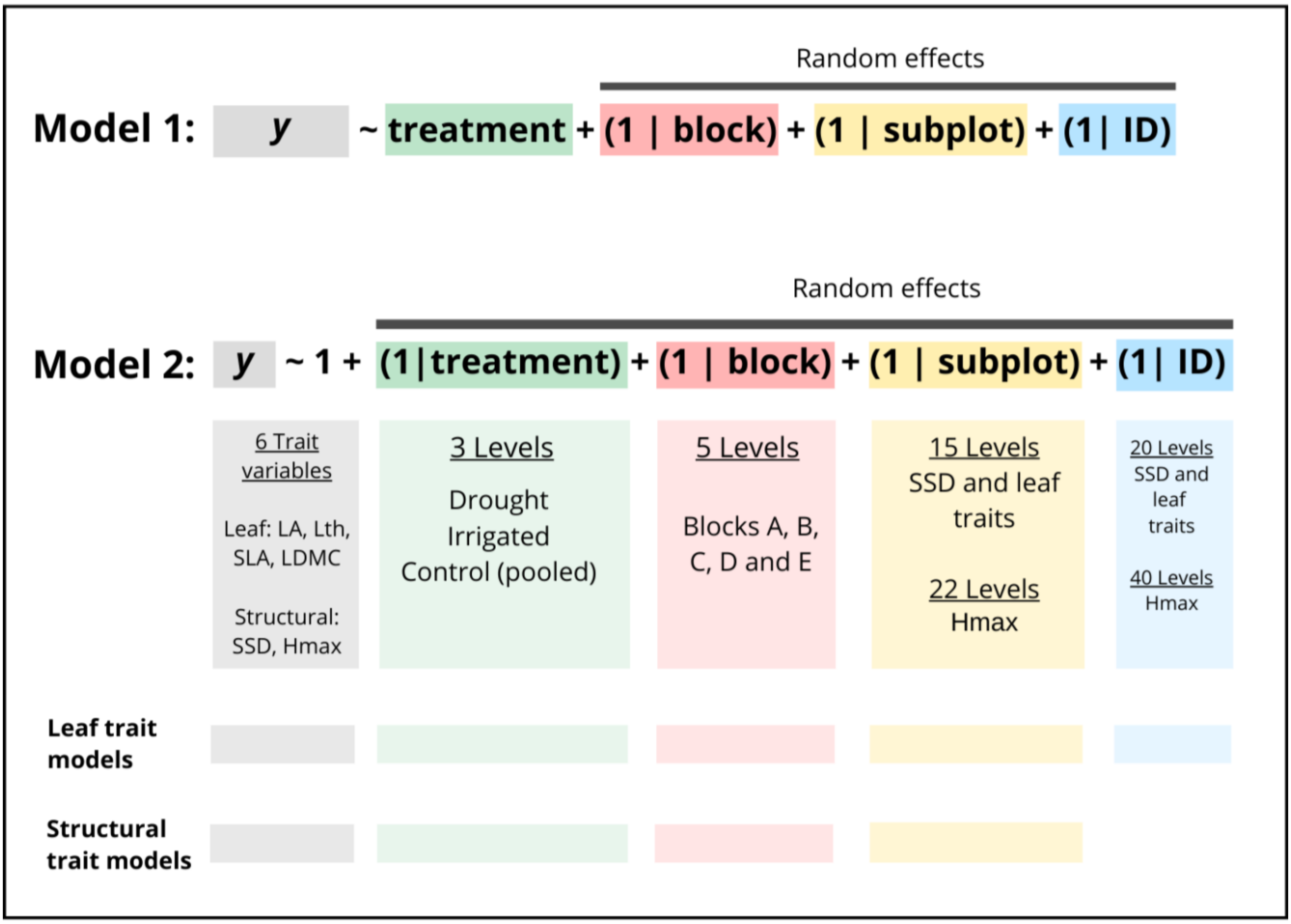
All traits were natural-log transformed before analysis and explanations of model variables and their biological or experimental relevance are displayed in Table 5.1 and Figure 5.1. To allow for comparisons between trait and demographic responses (**Chapter 4**), ambient and procedural control treatments were pooled - for simplicity and to reduce the number of model parameters, resulting in a fixed-effect treatment factor having three levels: control, irrigated and drought. To reduce chance of overfitting models, subplot was selected as the more relevant experimental level to plot in this study (Figure 5.1).

5.2.5 Trait space

Trait density distributions were plotted using raw trait values to examine the trait space (e.g. the scale and shape of distribution) across treatments. Assessing trait space in this way provides a window into ecological strategies or tolerance of the study species (Maitner *et al.,* 2023; Fernández-Murillo *et al.,* 2024). Central tendency, typically expressed as the mean, represents the average ecological strategy of a species (Gross *et al.,* 2021). The breadth of ecological strategies for a given species can be expressed via the variance (Gross *et al,* 2021). The shape of trait distributions can be characterised by the skewness and kurtosis, which refers to the symmetry and evenness (or “tailedness”) respectively (Gross *et al.,* 2021). Strong skewness can indicate either a dominant strategy or large number of individuals expressing exceptional trait values in response to an environmental filter (Fernández-Murillo *et al.*, 2024). Higher kurtosis values are suggestive of more individuals with a similar ecological strategy reflecting environmental constraints, whilst lower values indicate more diverse strategies amongst individuals (Maitner *et al.*, 2023). The ‘e1071’ package was used with the default algorithms to calculate the four moments of distribution (Meyer *et al.,* 2023), which were applied to each trait using functions in the ‘dplyr’ package (Wickham *et al.*, 2023).

5.2.6 Treatment effects

To reflect the randomised replicated experimental block design, leaf trait models were computed with treatment as a fixed categorical explanatory variable, with three levels, and block, subplot and individual (ID) as intercept-only random effects at five, 15 and 20 levels respectively. Models followed the general structure for the brm() function (following the syntax for lmer() models) and are shown in Box 5.1. Detailed descriptions of each variable and their biological relevance are given in Figure 5.1.



Box 5.1. Structure of trait models used to assess treatment effects on each of the six measured traits (model 1) and variance component analysis (model 2). Leaf trait models include the four traits: leaf area (LA), leaf thickness (L_{th}), specific leaf area (SLA), leaf dry matter content (LDMC). Stem specific density (SSD) and maximum plant height (H_{max}) are included in structural trait models and do not include the ID as a random effect.

For structural traits, models were computed using Model 1, without ID as a random effect, as stem specific density and maximum plant height represent whole organism level traits and repeat measures per individual were not taken (as for other traits). All models were run using four parallel *Markov chain Monte Carlo* (*MCMC*) chains over 4000 iterations with 2000 warm-up iterations. Adequate model convergence was assessed by checking no divergent transitions occurred post warm up, visually inspecting mixing of chains using pp_check() function and ensuring all model parameters had $\hat{R}$ values ≤1.00, which quantifies degree of mixing between chains (Bürkner 2017). For all models, priors were set for the intercept and coefficients (except residual) using a similar prior predictive simulations approach as Jackson *et al.* (2024). Results from model 1 are presented as conditional fixed effects with 95% credible intervals extracted using the brms package conditional_effects() function and considering all group-level effects.

5.2.7 Variance component analysis

To determine which experimental levels were influential in driving the variation in functional trait responses, two variance component analysis approaches were used. The first approach used the random effects from model 1 outputs to compare estimates and uncertainties amongst variables. The second approach instead allowed for direct comparison of all variables by modelling treatment, block, subplot and ID as intercept-only random effects with just a fixed intercept (grand mean) for each response variable y. This approach is an extension of ANOVA proposed by Gelman (2005) that allows comparison of all variables by treating them all as random effects to allow estimation and comparison of their variance components and uncertainties (with the limitation that the variance components for the treatment variables are specific to the range of treatment manipulations used – 50, 100 and 150% ambient growing season rainfall in this case; a wider treatment range may produce larger variance components). The model structure for this analysis is shown as model 2 in Box 5.1. As with model 1, the ID term was dropped for structural traits (SSD and maximum plant height). The outputs of the two variance component analyses from models 1 and 2 are presented as standard deviations and 95% credible intervals for each random effects of each respective model.

Interpreting variable responses from model outputs was based on 95% credible intervals (CI). A significant difference was considered if there was no overlap in 95% CI between the three precipitation treatments, while noting instances of borderline significance. 95% credible intervals are akin to 95% confidence intervals, in that they describe uncertainty around a variable estimate, but the former represents the probability that a given estimate lies within the interval based on the posterior distribution - the combination of prior distribution and observed data (Hespanhol *et al.,* 2019) while the latter describe the long-term behaviour of the approach (95% confidence intervals should contain the 'true' value in 95% of their application). An additional measure of trait variation, the coefficient of variation (CV) was quantified. CV is a unitless measure calculated by dividing the sample standard deviation by the mean, allowing for the direct comparison of variability between traits with different scales.

5.3 Results

5.3.1 Variation in trait space across treatments

There were sizeable differences in intraspecific variation for the six traits measured on the *Brachypodium sylvaticum* individuals, with coefficient of variation (CV) values as low as 5% for leaf thickness and as high as 42% for stem specific density (Table S5.2). Leaf traits had a narrower CV range of 5-15%.

The analysis of trait space using the four moments of trait distributions revealed some differences between traits and treatments (Figure 5.2). The effect of precipitation treatments was more detectable in leaf traits, where mean leaf area showed significant decreases with drought treatment ($\bar{x}$ = 1.71 log cm^2, ±SE = 0.05, n = 100) relative to control ($\bar{x}$ = 1.98 log cm^2, ±SE = 0.04, n = 100), whilst irrigated individuals had significantly lower mean LDMC values ($\bar{x}$ = -1.07 log g g^{-1}, ±SE = 0.02, n = 100) compared to control ($\bar{x}$ = -1.00 log g g^{-1}, ±SE = 0.01, n = 100). Despite similar trait means across treatments, SLA registered a wider trait dispersion with drought ($\bar{x}$ = -0.98 log cm^{2} g^{-1}, SD = 0.19, n = 100) compared to control ($\bar{x}$ = -0.88 log cm^{2} g^{-1}, SD = 0.11, n = 100) and irrigated ($\bar{x}$ = -0.84 log g g^{-1}, SD = 0.10, n = 100) treatments. Except for maximum plant height, traits measured on individuals in drought and irrigated treatments showed slight negative kurtosis. Kurtosis values ranged from -0.06 (SLA) to -1.34 (SSD) and -0.36 (leaf thickness) to -0.60 (SLA) for drought and irrigated treatments respectively. The trimodal distribution of SSD measured in the irrigated treatment, is likely an artefact of low sample size and therefore uneven coverage across the range of observed values. There were two other notable findings in trait distributions that were observed irrespective of treatment: i) leaf thickness displayed a narrow distribution regardless of treatment; ii) maximum plant height registered high positive excess kurtosis (~1.8) regardless of treatment type. Generally, all traits showed approximately symmetrical distributions, as skew values fell between -0.5 and 0.5 (Table S5.2).

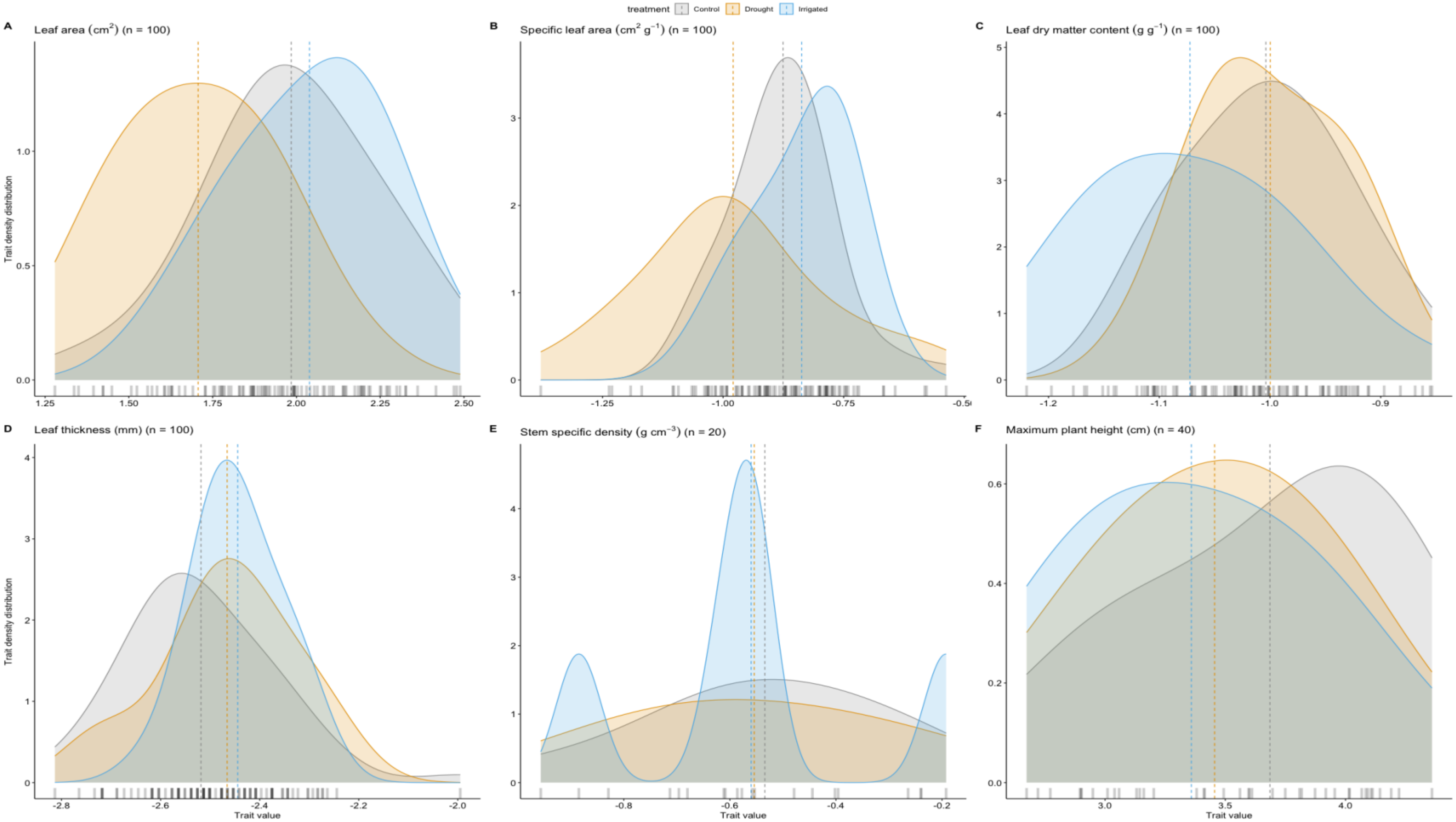


Figure 5.2 Trait density distributions for the six functional traits measured on *Brachypodium sylvaticum* across control, drought and irrigated precipitation treatments at the RainDrop experiment. All traits are natural log transformed. Dashed lines indicate mean. X-axis rug displays distribution of trait values. n shows the total number measurements, where traits A-D correspond to all sampled leaves (five per individual) and E and F show a single trait value per individual.

5.3.2 Effect of drought and irrigation treatments on trait responses

Precipitation treatments did not significantly affect mean trait responses, as shown by the overlapping 95% credible intervals based on outputs from model 1 (Figure 5.3). Leaf area showed marginal reductions in droughted plots (β = -0.31, 95% CI = 1.3, 2.0) compared to individuals measured in control plots (β = 2.0, 95% CI = 1.7, 2.3) (Figure 5.3A).

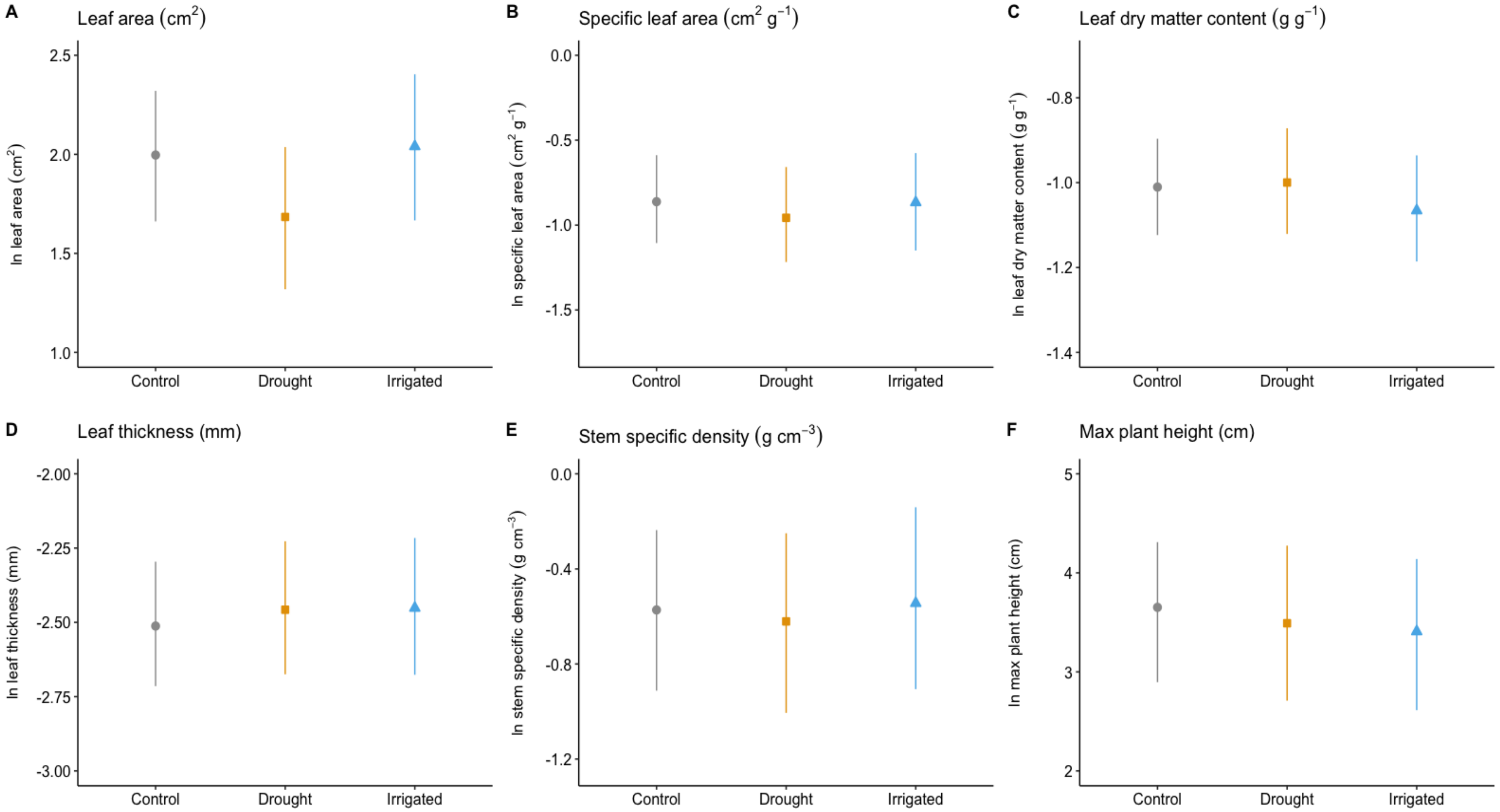


Figure 5.3 Trait responses in mature *Brachypodium sylvaticum* individuals remain largely stable with imposed precipitation treatments relative to credible intervals. Caterpillar plots show conditional fixed effects generated from 8000 draws of full posterior distribution, inclusive of random effects from model 1. Coloured shapes indicate posterior mean and bars the 95% credible intervals according to precipitation manipulation treatment.

5.3.3 Trait variation across experimental scales

Assessing which organisational level was the main source of trait variation, based on the variance component analysis of the random effects from model 1 revealed slight differences between leaf and structural traits (Figure 5.4). Noting wide intervals around the estimates of the variance components, the individual level component was a notable contributor to total variation (SD_{total}) in trait responses for leaf area, LDMC and leaf thickness, whilst for maximum plant height experimental parameters were larger drivers (Figure 5.4F). For all traits the residual component showed the widest uncertainty.

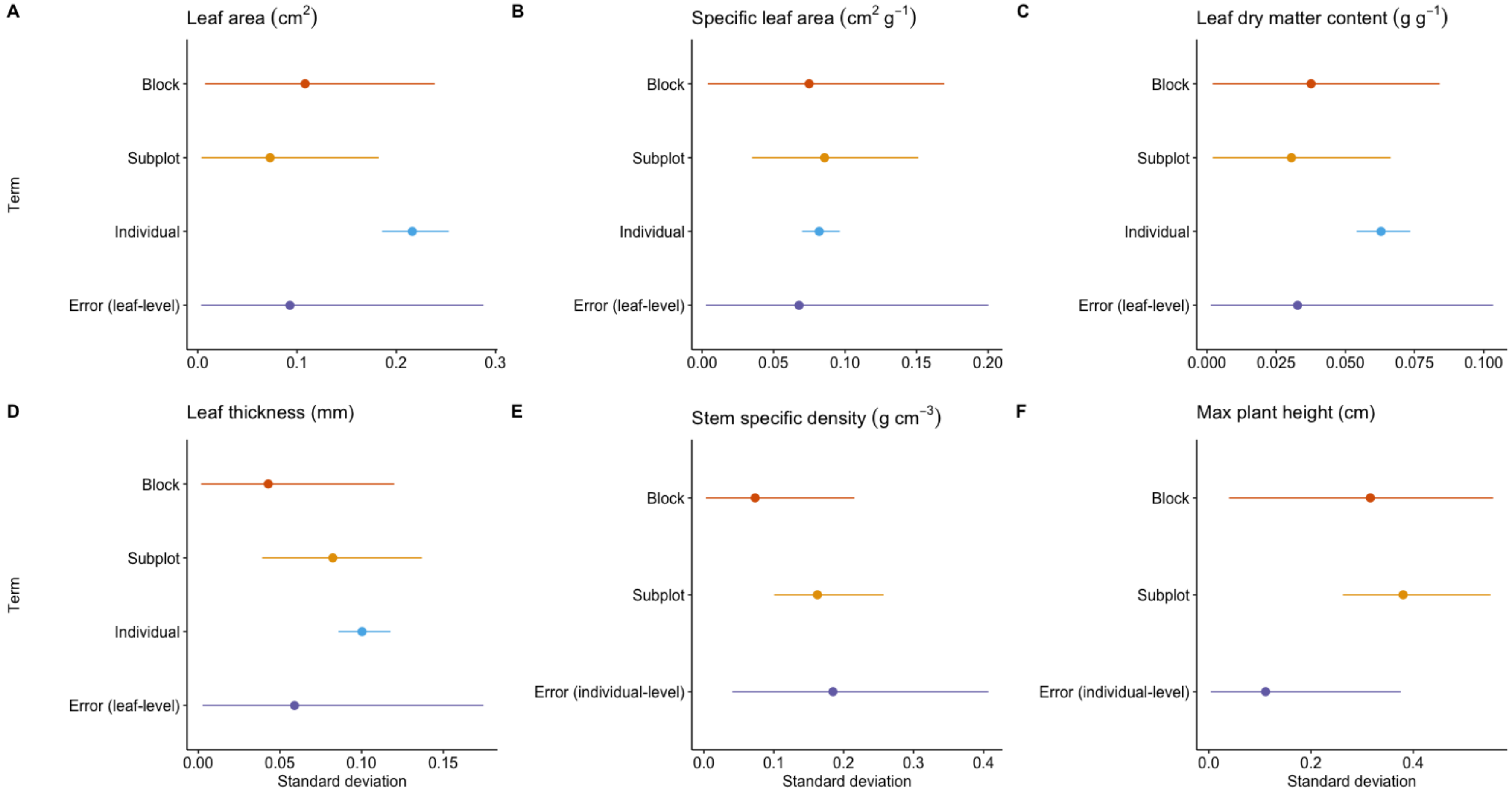


Figure 5.4. Model outputs of random effects (only) from model 1 for each trait variable. Variance components of model terms display standard deviations (point estimates) with 95% credible intervals. All traits are natural log-transformed. Stem specific density and maximum plant height are whole-organism traits and the individual level variation is incorporated into the residual error term.

Re-computing the variance component analysis with the addition of treatment (model 2, which also treats the fixed effects from model 1 as random effects), showed that there was no single dominant source of variation in trait responses (Figure 5.5). The contributions of individual and residual (leaf level) variation to the summed variance components (SD_{total}) were less marked compared to outcomes of variance component analysis from model 1 shown in Figure 5.4. These two components showed similar trends in the four different leaf traits (Figure 5.5A-D). Except for maximum plant height, the block and residual terms showed the highest levels of uncertainty as reflected in the interval widths across traits. There was some evidence to suggest the variation in maximum plant height values was attributed more at experimental design levels than the other five traits were.

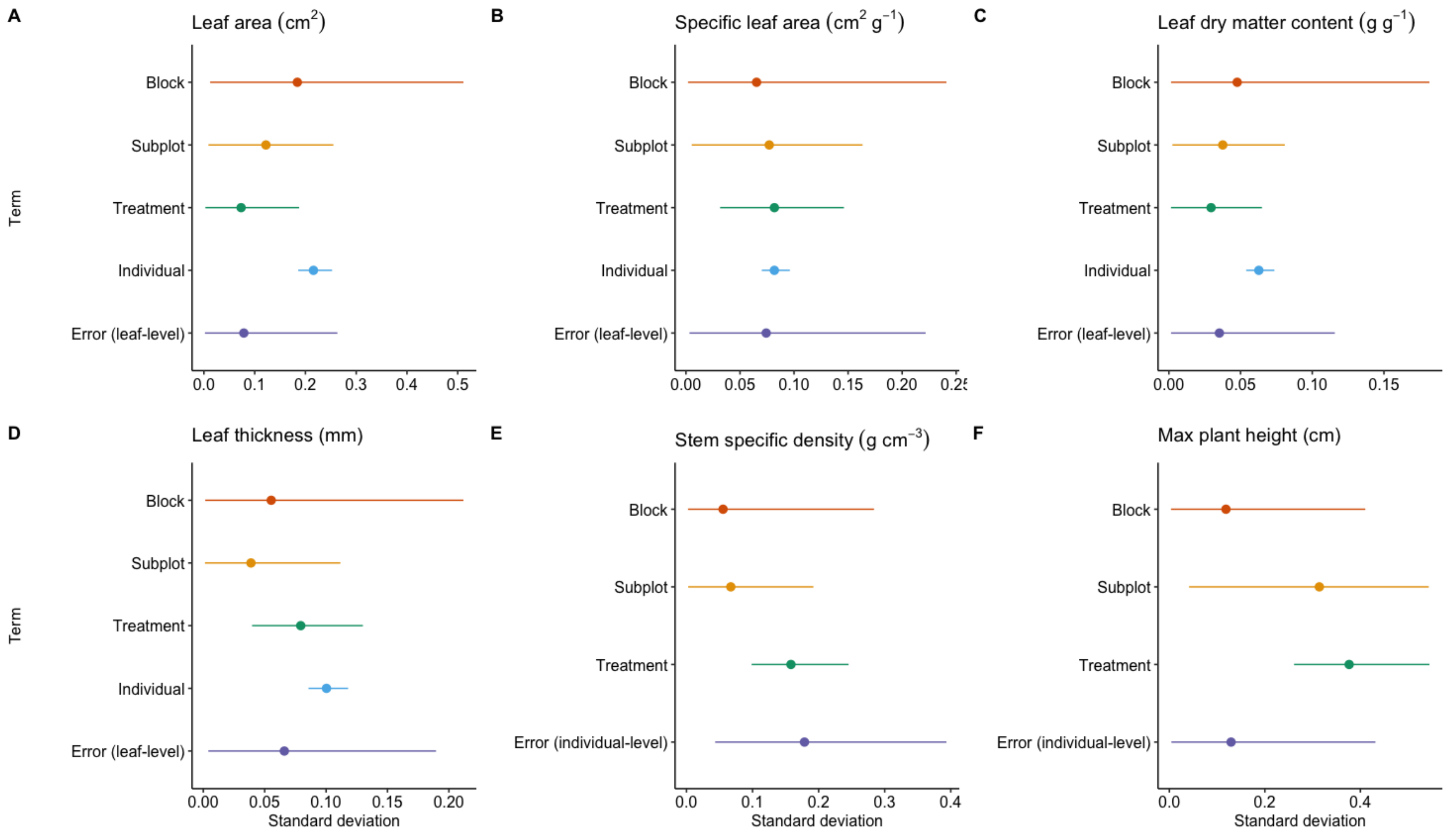


Figure 5.5 Model outputs of random effects from model 2 for each trait variable. Variance components of model terms display standard deviations (point estimates) with 95% credible intervals. Block, subplot and treatment are the three experimental design levels. All traits are natural log-transformed. Stem specific density and maximum plant height represent whole-level organism traits and the individual level variation is incorporated into the residual error term.

5.4 Discussion

This study assessed trait responses between and within individuals of the bunch grass *Brachypodium sylvaticum* growing under experimentally manipulated rainfall conditions in a calcareous grassland. The main finding was that this species showed evidence of drought tolerance, as imposed precipitation treatments largely had an undetectable effect on the six above-ground traits measured. A treatment effect was only found for leaf area, registering marginally significant reductions (~16%) in mean leaf size in drought plots compared to control. Examining the four moments of trait space revealed three interesting distribution patterns: i) drought treatment produced a wider distribution of SLA values, and regardless of treatment, ii) maximum plant height registered high positive excess kurtosis and iii) leaf thickness displayed a narrow distribution. Overall, there was no clear single source of variation in trait responses, as proportions of variance explained across organisational levels were similar based on both variance component analyses. There was some evidence to suggest variations in leaf traits were driven more by individual and organ-level components (including unexplained variance), while maximum plant height was influenced more by experimental design variables. Taken together these results suggest that *B. sylvaticum* is a drought tolerant species with a conservative resource strategy, able to maintain optimal functionality across a range of water availability conditions with only minor shifts to leaf traits.

5.4.1 Precipitation treatment effects on leaf traits

Manipulating growing season rainfall did not appear to induce significant adjustments in (mean) trait values for *Brachypodium sylvaticum,* with only a marginal reduction in leaf area in the drought treatment. These results align with a study at the same experimental site, where there were largely insignificant treatment differences in the community weighted trait means calculated for 22 calcareous grassland species (Fenollosa *et al.,* 2023). The overlap in trait means suggests *B. sylvaticum* and the wider grassland community is tolerant (at least in the short term) to a wide range in water inputs with adequate matching between phenotypes and the local environment (Gross *et al.,* 2017; Maitner *et al.,* 2023). The minor reduction in leaf size in droughted individuals likely indicates a functional trade-off between traits, where individuals modulate different combination of traits to limit drought damage (Wellstein *et al.,* 2017). Smaller leaves are a key drought tolerance strategy, as having a reduced surface area minimises evaporative losses enabling plants to remain within hydraulic safety limits and has been observed in other grassland precipitation experiments (Májeková *et al.*, 2021). Interestingly, this size reduction in leaves was not matched by a reduction in whole plant size, measured here as maximum plant height. This suggests plant height was not strongly related to water loss and therefore did not adjust. It is unclear whether these adjustments in leaf area are an active or passive response (*sensu* Schneider, 2022) to the experimental drought treatment. In other words, do smaller leaves emerge due to changes in biochemical signalling pathways via gene regulation or from the direct response to imposed environmental conditions? There is emerging work on the genetic regulation pathways to abiotic stressors in *B. sylvaticum* that could help clarify possible mechanisms in trait responses (Lei *et al.*, 2023).

5.4.2 Trait space largely stable across treatments

Contextualising trait responses to precipitation manipulation beyond the mean values uncovered some interesting distributional patterns in SLA, leaf thickness and maximum plant height. Contrary to expectations, SLA exhibited a wider trait dispersion of trait values (three times the variance) in droughted individuals compared to control. This result was surprising as environmental stressors such as drought should lead to trait convergence (tighter dispersion around the mean) (Anderegg, 2015). SLA is a key component in the leaf economics spectrum, representing a trade-off between carbon gain relative to water loss (Pérez-Harguindeguy *et al.*, 2013). Here the wider distribution was driven by a few outliers in both directions with the highest value (0.56 cm^2 g^{-1}) over twice the lowest value (0.25 cm^2 g^{-1}) and a slight positive skew. Generally, variations in SLA expression with water availability, can be understood via examining the relationship between leaf thickness and LDMC components (SLA = 1/leaf thickness x LDMC) (Vile *et al.,* 2005). Here, SLA showed non-significant weak positive ($r^2 = 0.31$, $p = 0.12$, $n = 100$) and negative correlation ($r^2 = -0.38$, $p = 0.062$, $n = 100$) with leaf thickness and LDMC respectively (Figure S5.1), suggesting other processes coordinated to produce the observed variations that were not measured (e.g. leaf age, leaf position, environmental heterogeneity) (Wilson *et al.*, 1999). A possible source of variation in SLA values in leaves from droughted individuals is from errors in measuring leaf area. Leaf rolling was observed in many droughted individuals during the trait measurement campaign. This is a common response to drought stress which reduces leaf transpiration minimising water loss and photosynthetic activity (Yavas *et al.,* 2023). During leaf area measurement, some leaves were not always truly flat in the scanner and small folds and curvatures could have influenced leaf area. The wide intervals of the combined leaf and error components shown in both variance component analyses (Figures 5.4B and 5.5B) would also support this explanation.

The similar trait space across treatments registered for leaf thickness likely reflects the conservative nature of the trait and developmental constraints compared to the other traits studied here (Coneva and Chitwood, 2018). Constancy in leaf thickness values within species was also observed in all seven grassland species studied, including the congeneric *Brachypodium pinnatum* in a drought experiment (Fenollosa *et al.,* 2023). Although shifts in leaf thickness in perennial grasses have been recorded with increasing aridity (Guo *et al.,* 2017), the lack of variation in this trait here could be adaptive (i.e. advantageous to fitness) and another part of the leaf anatomy (e.g. leaf rolling, stomatal density) could have expressed plasticity that was not measured here (Yavas *et al.,* 2023). Maximum plant height showed moderately low variability (CV = 13%) and was characterised by high kurtosis (~1.8, leptokurtic distribution), regardless of treatment. The low variability coupled with high kurtosis suggests a non-treatment induced shift towards functional similarity with the presence of some individuals at the edge of trait space with extreme heights (Maitner *et al.,* 2023). Local environmental factors like air temperature, soil nutrient status or biotic interactions can also influence trait variation. It is possible the combined heatwave and drought that coincided with the field trait campaign in 2022 (Barker *et al.*, 2024), could partially explain this trait convergence. High temperatures in combination with lower ambient rainfall across treatments, can further increase risk of dehydration from increased water demand and evaporation of soil moisture affecting plant stature (Sato *et al.,* 2024). Future analyses using multi-stressor experiments to characterise relationship between environmental heterogeneity and degree of intraspecific variability of this key trait are needed to understand the species capacity to resist environmental change (De Boeck *et al.,* 2016). Examining the different components of trait space in addition to mean trait responses provides a richer characterisation of the potential underlying processes and within plant trade-offs. It also emphasises that a lack of trait variability does not necessarily mean intolerance to environmental stressors and could indicate developmental constraints or adaptive value in the expression of phenotypes close to the optimal trait value. This study also underscores importance of taking the integrated phenotype approach, to further help disentangle trade-offs under environmental change (Castro Sánchez-Bermejo *et al.,* 2023).

5.4.3 Similar trait variation patterns across experimental scales

This study assessed the relative contributions of experimental levels in mediating trait responses within a single species irrespective of the underlying mechanisms. The fairly equal partitioning of variance across the organisational scales (e.g. block, subplot, treatment and individual) and traits could be attributed to two scenarios: i) multiple processes leading to similar spatial trait variation patterns or ii) spatially similar environmental conditions generating comparable trait variation patterns (Biswas *et al.*, 2024). Given the relatively small study area (2 ha), it is likely the various organisational scales would have been influenced by similar biotic and abiotic processes present within the site, consistent with scenario (ii) (Douce *et al.,* 2023). Scenario (i) appears to be more evident in tropical tree studies (e.g. Messier *et al.,* 2010; Biswas *et al.,* 2024), and is unclear how these spatial trait variation patterns generalise to other plant functional types or other ecological settings and warrants further investigation.

Intra-specific variation arises from genetic variability and phenotypic plasticity (Violle *et al.,* 2012). At the individual level, it is likely plasticity is at play here, given the length of the RainDrop experiment and this study is shorter than the lifespan of the species (Kramp *et al.,* 2022). At the block and plot/subplot level several biotic and abiotic environmental processes are likely driving trait variation at this scale relating to soil nutrient and water status, topography and neighbouring plants (Messier *et al.,* 2010, 2017). Although, a recent study in grasses and woody legumes suggests that a sizeable portion of intra-specific variation in leaf traits strategies (e.g. acquisitive vs conservative) are shaped by genetic/developmental factors (e.g. architectural constraints) (Gorné *et al.,* 2022).

The temporal component of trait variation such as plant age and phenological stage was not formally considered here and likely contributed to the combined individual and residual variation components registered. Studies have documented seasonal variations in LDMC values in woody plants (Palacio *et al.,* 2008) and ontogenic shifts in forb and grass seedling traits from acquisitional to conservative strategies under manipulated water availability (Havrilla *et al.,* 2021). Therefore, a natural next step would be to assess trait variance across time, in addition to space, helping to disentangle processes operating at different scales, improve the functional characterisation of this species, and determine species persistence and upscale consequences (Schneider, 2022; Umaña and Hulshof, 2023).

5.4.4 Future directions

There are a number of caveats to this study that merit further study. The nature of the sampling strategy meant traits were measured only on a subset of individuals that had survived (31% of the tagged individuals alive in 2021) during a single growing season. Linking past survival and performance of individuals in the demographic study (**Chapter 4**) to the traits measured here, suggests *B. sylvaticum* has a life history strategy that gives it the ability to tolerate drought through small adjustments in leaf traits. However, the degree to which these traits are liable and adaptive for *B. sylvaticum* to persist in the imposed treatments against the background of environmental stochasticity remains to be studied. If this species shows high temporal variability (e.g. within and between several growing seasons) in traits due to plasticity rather than genetics this would suggest high tolerance and resilience to buffer environmental change (Anderegg, 2015).

This study focused on above-ground traits, future studies should explore belowground traits such as root dry matter content and rooting depth, as root responses can be uncoupled from shoot traits and have been shown to be important in assessing drought tolerance in a temperate grassland species (Lozano *et al.,* 2020; Reinelt *et al.*, 2023). Further, integrating hydraulic traits (e.g. leaf turgor loss point) and assessing their relationship to the traits measured here would provide a deeper understanding of drought tolerance of the species (Májeková *et al.,* 2021). Lastly, quantifying the genotypic diversity of individuals was not carried out on the 40 individuals measured here, as this was outside the scope of the study. The inclusion of genotyping would help further disaggregate source of intraspecific trait variation.

5.5 Conclusion

The study aimed to characterise within and among trait variation in individuals of the bunch grass *Brachypodium sylvaticum* in a calcareous grassland under manipulated growing season rainfall. Intraspecific trait studies assessing multiple components of trait distributions (e.g. mean, variance, skewness and kurtosis) and their drivers remain understudied, despite the potential cascading effects on community structure and functioning (Enquist *et al.,* 2015; Maitner *et al.,* 2023). Here *B. sylvaticum* showed high tolerance to precipitation treatments, as no significant shifts in trait means were detected, indicating this species adopts a conservative resource use strategy. This result aligns more broadly with conservative grass species having lower variability in trait expression (Streit *et al.*, 2022). It appears individuals were able to co-ordinate their leaf anatomy through minor reductions in leaf size and display leaf rolling, whilst maintaining stability in other leaf traits in response to precipitation deficits. The comparable trait variation patterns across experimental design scales indicated no prominent source of variation in trait responses. The relatively small study area likely had similar environmental processes operating across organisational scales within the site, which contributed to the similar spatial trait variation patterns observed. Short-term drought tolerance was shown by *B. sylvaticum* and future work adopting an integrated phenotype approach is needed to assess the long-term capacity and adaptive potential of this species in response to an increasingly stochastic environment.

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Supplementary

Table S5.1. Overview of trait sampling across organisational scales in the *Brachypodium sylvaticum* trait study. Maximum levels available to sample refers to the maximum levels inherent in the randomised replicated experimental design at RainDrop. Due to the natural variation in abundance of the study species across the site, not all plots or subplots contained individuals. At the trait sampling stage, treatment had four levels, where at the data analysis stage, ambient and procedural controls were pooled resulting in three levels. * Stem specific density was measured using one stem per individual which was calculated by averaging three stem diameter measurements on the one stem.

Trait	**Sampled scale (maximum levels available to sample)**			**Number of measurements per:**			
	Block (5)	*Plot (20)*	*Subplot (40)*	*Treatment (4 levels)*	*Individual (ID)*	*Organ*	*Total*
Leaf Area (LA)	5	16	15	5	5	1	100
Leaf Thickness (L_{th})	5	16	15	5	5	1	100
Leaf Dry Matter Content (LDMC)	5	16	15	5	5	1	100
Specific Leaf Area (SLA)	5	16	15	5	5	1	100
Stem Specific Density (SSD)	5	16	15	5	1	1*	20
Maximum Plant Height (H_{max})	5	16	22	10	1	NA	40
							460

Table S5.2. Descriptive statistics for each trait measured on *Brachypodium sylvaticum* individuals, based on raw natural log-transformed values. The ‘e1071’ package was used with the default algorithms to calculate the four moments of distribution (Meyer *et al.,* 2023), which were applied to each trait using functions in the ‘dplyr’ package (Wickham *et al.,* 2023). n = total number of trait measurements per treatment, SE = standard error, SD = standard deviation, CV = coefficient of variation.

Trait	Treatment	n	Mean (± SE)	Variance	Kurtosis	Skewness	Overall SD	Overall absolute CV (%)
Leaf area (cm^2)	Control	50	1.984510 (0.037)	0.0628	0.1111554	-0.2808920	0.2398714	12.6
	Drought	25	1.706835 (0.046)	0.0466	-0.5829554	0.1031002		
	Irrigated	25	2.038942 (0.045)	0.0437	-0.3926122	-0.2998093		
Specific leaf area (cm^2 g^{-1})	Control	50	-0.8757334 (0.015)	0.0111	0.86426595	0.4612953	0.1354172	15.1
	Drought	25	-0.9791087 (0.039)	0.0354	-0.06489291	0.3161616		
	Irrigated	25	-0.8370464 (0.021)	0.00991	-0.60637439	-0.5873449		
Leaf dry matter content (g g^{-1})	Control	50	-1.0037094 (0.010)	0.00475	-0.6419985	0.09803534	0.07523386	7.3
	Drought	25	-0.9998265 (0.012)	0.00329	-0.8380749	0.05103186		
	Irrigated	25	-1.0723084 (0.018)	0.00761	-0.4183628	0.43836168		
Leaf thickness (mm)	Control	50	-2.518501 (0.021)	0.0200	1.7467931	0.8585738	0.1220475	5.0
	Drought	25	-2.465690 (0.027)	0.0167	-0.3255265	-0.3673734		
	Irrigated	25	-2.444335 (0.017)	0.00642	-0.3573102	0.1004975		
Stem specific density (g cm^{-3})	Control	10	-0.5341333 (0.069)	0.0471	-0.4165291	-0.3943264	0.2334175	42.5
	Drought	5	-0.5540049 (0.106)	0.0560	-1.3396880	0.2028437		
	Irrigated	5	-0.5599692 (0.110)	0.0609	-0.5174769	0.2846073		
Maximum plant height (cm)	Control	20	3.685730 (0.113)	0.257	1.846423	-0.5111002	0.4770819	13.6
	Drought	10	3.455645 (0.143)	0.204	1.946738	-0.1129634		
	Irrigated	10	3.359020 (0.150)	0.223	1.733680	0.1408589		

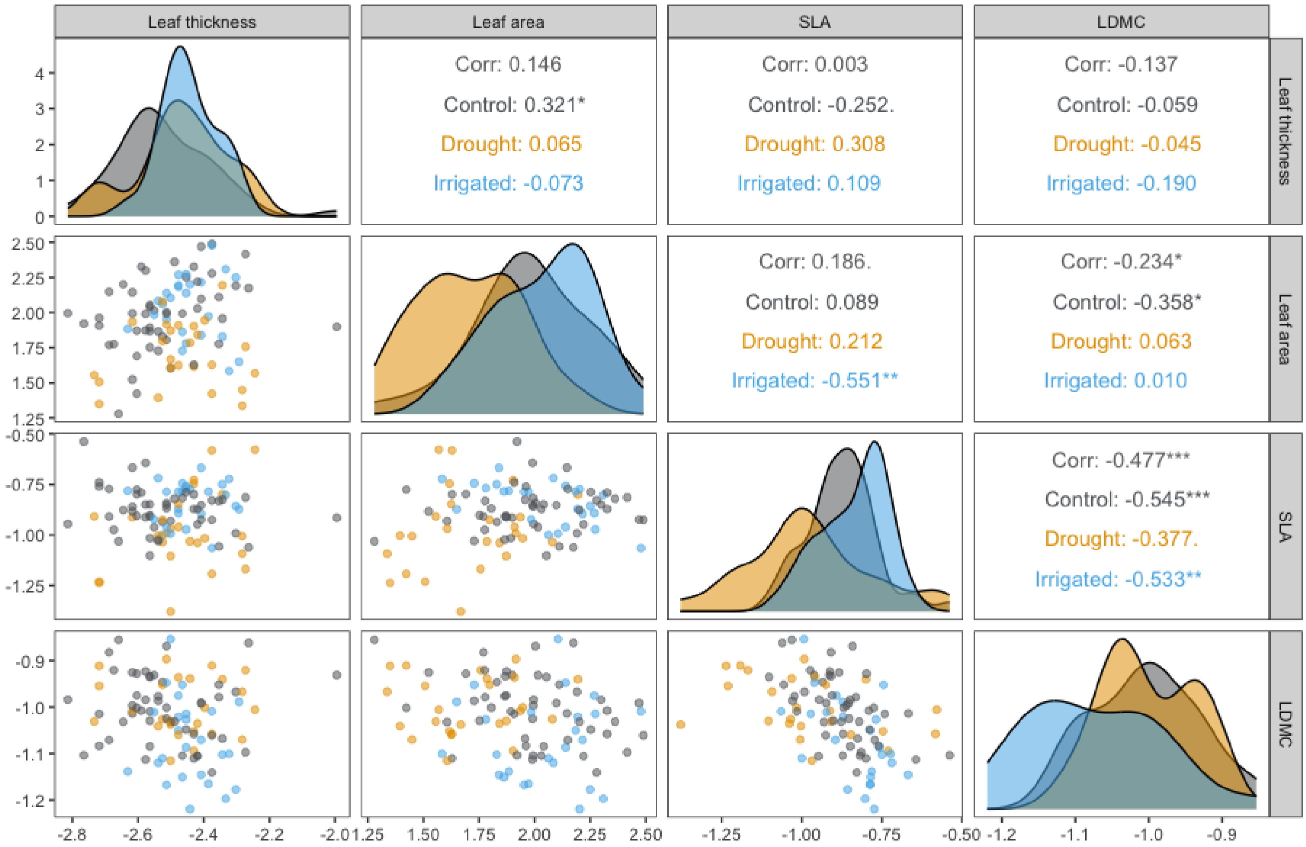


Figure S5.1 Correlation matrix of four leaf traits of *Brachypodium sylvaticum* detailing trait-trait relationships for combined and separate control, drought, and irrigated treatments. Bottom left, centre and top right of matrix display scatter plots, density distributions of data and Pearson's correlation coefficients with significance levels respectively. Each significance level is denoted with a symbol to indicate p-value, where: *** = < 0.001, ** = < 0.01, * = < 0.05, and . = < 0.10. Control treatment refers to pooled data from both ambient and procedural controls. Traits are natural log transformed and based on 100 leaf samples measured on a total of 20 individual *B. sylvaticum*.

Chapter 6: General discussion and conclusions

Sara L Middleton

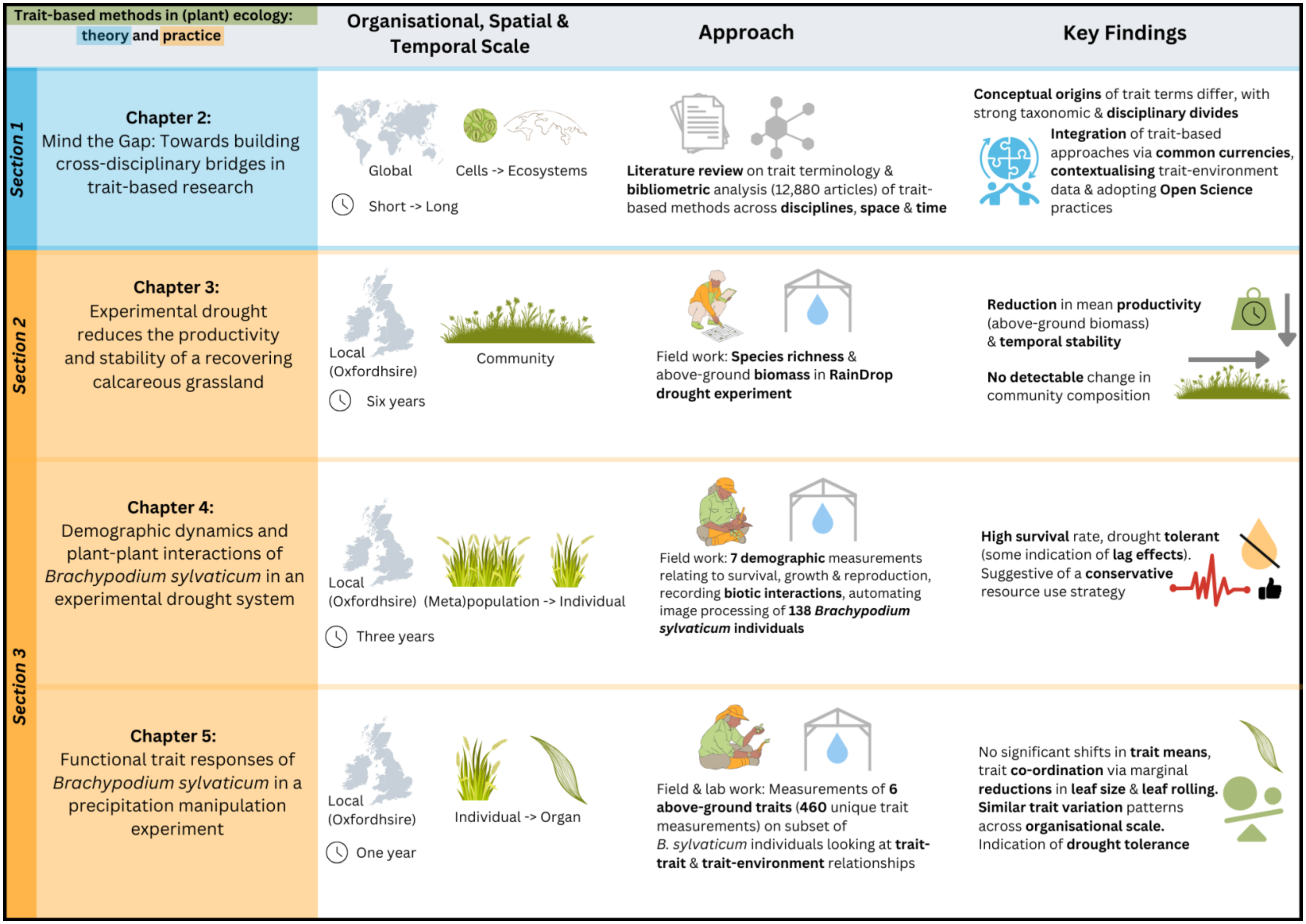
(A word cloud based on text from abstracts from chapters in this thesis showing recurring themes)

6.1 Summary of key findings

The four research chapters in this thesis have combined a wide range of methodological approaches from bibliometric, experimental to analytical techniques to critically examine trait-based ecology from a conceptual and applied perspective. The thesis was arranged into three thematic sections where the overall aim was to examine how trait-based methods can be used to assess plant community to individual-scale impacts in the context of global environmental change.

The thesis begins with taking a wider perspective of traits, where I conducted a literature review and bibliometric analysis to examine the use of traits across space, time and disciplines. This assessment revealed several conceptual and empirical challenges such as ambiguity in terminology and issues integrating trait and environmental data hindering research synthesis and synergy across eco-evo-demographic fields. With this in mind, the focus of the rest of the thesis was to examine plant functioning, trait-trait and trait-environment relationships, using a UK grassland climate change experiment as a case study.

The key outcomes of each research chapter are synthesised below and shown in Figure 6.1. I then discuss the emerging themes, situate the findings to the wider context and outline some of the outstanding questions and suggestions of future work in trait-based approaches in plant ecology.



	Trait-based methods in (plant) ecology: theory and practice	Organisational, Spatial & Temporal Scale	Approach	Key Findings
Section 1	**Chapter 2:** Mind the Gap: Towards building cross-disciplinary bridges in trait-based research	Global Cells -> Ecosystems Short -> Long	**Literature review** on trait terminology & **bibliometric** analysis (12,880 articles) of trait-based methods across **disciplines**, **space** & **time**	**Conceptual origins** of trait terms differ, with strong taxonomic & **disciplinary divides** **Integration** of trait-based approaches via **common currencies**, **contextualising** trait-environment data & adopting **Open Science** practices
Section 2	**Chapter 3:** Experimental drought reduces the productivity and stability of a recovering calcareous grassland	Local (Oxfordhsire) Community Six years	Field work: **Species richness** & above-ground **biomass** in **RainDrop drought experiment**	**Reduction** in mean **productivity** (above-ground biomass) & **temporal stability** **No detectable** change in community composition
Section 3	**Chapter 4:** Demographic dynamics and plant-plant interactions of *Brachypodium sylvaticum* in an experimental drought system	Local (Oxfordhsire) (Meta)population -> Individual Three years	Field work: **7 demographic** measurements relating to survival, growth & reproduction, recording **biotic interactions**, automating image processing of **138 *Brachypodium sylvaticum* individuals**	**High survival** rate, drought **tolerant** (some indication of **lag effects**). Suggestive of a **conservative** resource use strategy
	Chapter 5: Functional trait responses of *Brachypodium sylvaticum* in a precipitation manipulation experiment	Local (Oxfordhsire) Individual -> Organ One year	Field & lab work: Measurements of **6 above-ground traits** (**460** unique trait measurements) on subset of *B. sylvaticum* individuals looking at **trait-trait** & **trait-environment** relationships	No significant shifts in **trait means**, trait **co-ordination** via marginal **reductions** in **leaf size** & **leaf rolling.** **Similar trait variation** patterns across **organisational scale.** Indication of **drought tolerance**

Figure 6.1 Schematic summarising key approaches and findings of the thesis. Schematic designed in Canva (Sara Middleton)

6.1.1 Building cross-disciplinary bridges in trait-based research

Chapter 2 research question:

What are the conceptual genealogies of "functional", "phenotypic" and "life history" trait terms, the main challenges and possible solutions to improve research synthesis and synergy between trait-based disciplines?

Main findings:

Focusing on three frequently used trait terms "functional", "phenotypic" and "life history", the literature-based analysis revealed the three terms have different conceptual origins and developments. Commonalities lay in the primary trait measurement unit - individual organisms (where life history traits are derived from population growth models) and their contemporary applications of assessing trait-environment relationships.

Addressing the conceptual and empirical challenges (such as issue of semantics, scale and standardisation) along the life cycle of trait data will likely come from returning to basics. By this I mean simplifying trait terminology to include a broader definition of the biological unit of measurement and referring to traits as simply "traits" unless specifically measuring a function or process. Focusing on common trait currencies (e.g. energy-mass based or body size traits) and contextualising trait data (e.g. sampling both traits and environmental variables in the same location) are two other ways to build closer empirical links between disciplines.

Overall, the epistemological approach taken in this review provides a wider perspective of trait-based research across eco-evolutionary and demographic fields, which has seldom been investigated. By examining the evolution of the trait research landscape, identifying ways for building stronger disciplinary links and highlighting emerging trait-based applications this review contributes to improving the operationalisation of traits.

6.1.2 Assessing the drought impacts on the temporal dynamics of a calcareous grassland community

Chapter 3 research question:

Under an imposed precipitation treatment, what are the temporal dynamics in the structure and functioning of a calcareous grassland community?

Main findings:

The six-year study at RainDrop showed diverging trends on calcareous grassland community structural and functional properties to drought treatment. Above-ground net primary productivity (ANPP) and temporal stability decreased with drought, whilst species diversity and composition were resistant. There was a non-uniform response in plant functional groups driving reductions in ANPP, with graminoid and legumes showing the greatest biomass losses. The reduced productivity in these two functional groups was not reflected in community composition data which could be an early warning signal for delayed species turnover or mortality prompting future structural changes in this perennial dominated grassland.

A precipitation treatment response was largely undetected in the three measures of community structure: i) biodiversity indices, ii) community dissimilarity and iii) individual species community contribution. Two processes that could be contributing to community resistance is functional redundancy from increased functional diversity and species richness, and lagged drought effects whereby drought effects were delayed and not registered within the study period. Interestingly, greater water inputs did not appear to effect productivity or composition, which was thought to be due to issues with irrigation treatment efficacy. Finally, community dissimilarity was not driven by any particular functional group and temporal shifts were linked to the long-term recovery of the site from arable farming and sheep grazing.

Taken together these results add to the improved understanding of these high conservation value calcareous grasslands in the UK. It also emphasises that grassland community functional group dynamics at this site may not be synchronous and therefore there is a need for long-term vegetation monitoring to changes in rainfall patterns. This study also contributes to the global synthesis of grassland drought responses as part of the DroughtNet consortium of 120+ experimental sites (Smith *et al.*, 2024).

6.1.3 A demographic perspective on plant functioning under drought in the bunch grass Brachypodium sylvaticum

Chapter 4 research question:

How does drought influence the survival, above-ground vegetative and reproductive components of the bunch grass Brachypodium sylvaticum and in addition do biotic interactions mediate demographic responses?

Main findings:

This three-year study at RainDrop showed that *Brachypodium sylvaticum* is largely resistant to experimentally manipulating growing season precipitation, as demographic responses were largely undetectable, except for plant height measures, and survival which was high across treatments. Plant height showed significant reduction with drought with evidence of lag effects and recovery over the study period. Other reproductive parameters showed marginal signs of reproductive inefficiency in both droughted and irrigated individuals. A large component of the variation in demographic outcomes were attributed to differences between individuals and inter annual variation.

Beyond treatment effects, accounting for biotic interactions (non-leguminous and legume species, rabbit and ant activity) within the neighbourhood did not seem to significantly moderate precipitation treatment effects on demographic outcomes. This was unexpected and was explained by i) *B. sylvaticum* being a dominant species within the community and so likely controlled a larger proportion of resources compared to its heterospecific neighbours and ii) the evaluation method of the biotic interactions as covariates in this study.

Overall, these results point to *B. sylvaticum* having a conservative life history strategy, characterised by high survival, low reproduction rates linked to the species' long life span. In addition, it demonstrates how demographic responses to experimental precipitation treatments operate together with multiple other intrinsic (inter-individual variability) and extrinsic (micro-environmental heterogeneity) processes which vary with time. Empirical data on key demographic parameters of perennial plants are limited on fine spatial scales, particularly in grassland ecosystems and this study contributes to the wider grassland demographic knowledge. It also provides an improved characterisation of the life history of this model (Lei *et al.*, 2023) and invasive grass species (Arredondo *et al.,* 2018) in its native range.

The image processing methods of extracting plant size data in this work showcased an example of working at the intersections of plant ecology and computer science. It highlights the broad applicability and utility of automating image segmentation in plant-environment studies.

6.1.4 A trait perspective on plant functioning under drought in the bunch grass Brachypodium sylvaticum

Chapter 5 research question:

How do above-ground trait responses vary between and within Brachypodium sylvaticum individuals across precipitation treatments?

Main findings:

This study at the RainDrop drought experiment revealed that *Brachypodium sylvaticum* has tolerance (in the short term) to a range of water availability conditions with only minor trait adjustments. In response to precipitation deficits, individuals showed signs of trait co-ordination in leaf anatomy via marginal reductions in leaf size and display of leaf rolling, while maintaining stability in leaf thickness and leaf dry matter content. Unexpectedly, specific leaf area (SLA) showed a wider distribution of values in drought versus control treatments, suggesting other processes coordinated to produce the observed variations that were not measured (e.g. leaf age, leaf position, environmental heterogeneity). This result was partially explained by measurement challenges with scanning leaves sampled from individuals in drought treatment. Their leaves exhibited leaf rolling which influenced the leaf area measurement which was used to calculate SLA. Overall, the higher moments of trait distributions provided additional evidence to suggest that *B. sylvaticum* had adequate trait-environment matching across treatments in the 2022 growing season, as most traits had distributions close to the central tendency.

Beyond examining trait mean values, this study assessed the relative contributions of experimental levels (block, subplot, treatment and individual) in mediating trait responses. Results indicated no prominent source of variation in trait responses., likely attributed to the relatively small study area where similar environmental processes operated across organisational scales within the site.

This study was able to provide detailed insights into the trait-trait and trait-environment relationships within a single species at one site. There remains a scarcity of studies that consider the role of intra-specific trait variation both *amongst* and *within* individuals of the same species, as mean values obtained on different individuals and locations are typically used to characterise these trait relationships. Results from this study suggest that mean trait values of *B. sylvaticum* would adequately characterise this species' trait-environment relationship and could cautiously be used to approximate this species' dynamics in other studies of similar environmental contexts.

6.2 Synthesis and emerging themes

Understanding vegetation responses to environmental stressors is an important and pressing issue in ecological research, agricultural policy and conservation management. Assessments of vegetation functioning (e.g. above-ground productivity) tend to focus on a single organisational, spatial or temporal scale, which provides only a partial view of the underlying mechanisms governing reactions to environmental stressors. Measuring traits can be used to quantify plant-environment relationships over multiple scales. However, several conceptual and empirical challenges in adopting trait-based approaches exist which is hindering our ability to adequately characterise and synthesise research findings.

This thesis aimed to contribute to trait-based plant ecological research in two main ways: Firstly, by providing a historical and contemporary review of the trait research landscape and applications in environmental change research. Secondly, by conducting multi-scale assessments of plant functional responses to precipitation extremes in a calcareous grassland experimental system to address some of the knowledge gaps identified in the review. The main message from this thesis is the importance of taking an integrated approach across organisational, spatial, temporal scales and disciplines to studying vegetation responses to environmental change.

6.2.1 A matter of time?

Over the time scales analysed in the experimental work from six (**Chapter 3**), three (**Chapter 4**) and one (**Chapter 5**) years, drought was shown to reduce net above-ground biomass production (but not species composition) at the community level, whilst at the dominant species level, *Brachypodium sylvaticum* showed largely undetectable responses to drought (except for plant height measures). There were also no clear temporal trends in community functioning across the treatments over the six-year study period. It might be tempting to conclude that because the community composition at RainDrop appears stable, this grassland is ok. But a deeper analysis of the abundance and trait-demographic dynamics suggests a potential early warning signal for population and/or community instability (Clements and Ozgul, 2016). Three areas that point towards instability: i) dominant species are unable to compensate for the shift towards survival in subordinate species rather than biomass accumulation, ii) high survival rates/long life span is currently buffering drought effects, iii) a delay in individual and community drought responses.

Reduced community productivity, as measured by above-ground biomass was driven mainly by decreases in graminoid and legume functional groups than forbs, which corroborates with findings by Fenollosa *et al.* (2023) in the same system, but contrasts with trait-demographic responses of *B. sylvaticum* (**Chapter 5**). The more stable productivity levels in forbs could come from increased rooting depth, allowing plants to exploit deeper water sources than the shallower and more fibrous roots in graminoids (Mommer *et al.*, 2010). A possible explanation for the reduced legume biomass with drought is due to the water sensitive nature of the nitrogen-fixing rhizobia and legume partnership (Arfin Khan *et al.*, 2014). Focusing investigations on dominant and functionally important community members can provide finer grain details of responses, as the stability of dominant species' populations is a key attribute of community stability (Felton and Smith, 2017; Lisner *et al.*, 2024). While taking note that biomass does not always correlate with fitness (Younginger *et al.*, 2017), the results suggest other community members were shifting further along the spectrum of conservative resource use. These less drought-tolerant species where likely diverting resources towards survival, through changes in biomass partitioning (increase root:shoot ratio) which decreased contributions to the community above-ground biomass pool via growth and reproduction (Zia *et al.*, 2021). A greenhouse drought experiment of an herbaceous legume showed that increased root:shoot ratio was driven by a reduction in above-ground biomass allocation, as root biomass was the same across precipitation treatments (Pang *et al.*, 2011). It is energetically costly to cope with drought. A higher root allocation comes with a carbon tax via a reduction in plant growth. In addition, many grass species retrogress or shrink to reduce energetic costs of somatic maintenance and damage, which would have also decreased community productivity (Seleiman *et al.*, 2021).

In grassland systems, subordinate species tend to have low individual relative contribution to pooled biomass but have a higher number of individuals (and vice versa for dominant species) (Mariotte, 2014). The asynchronous drought responses observed here, even within the same functional group suggests that greater attention needs to be paid in quantifying the role of non-dominant species to community functioning and stability. Overall community productivity and stability can be compensated by dominant species (i.e. insurance effect) (Hector *et al.*, 2010), however the decreased temporal stability registered here suggests dominant species were not able to buffer against reduced productivity of other species. If *B. sylvaticum* is drought resistant, or at least more resistant than other species, longer term trends should show an increase in dominance (e.g. increase in above-ground percentage cover) of this species. It is also likely that the more competitive or ruderal community members will be more vulnerable to drought (Grime, 1979).

The temporal instability in productivity was not matched with shifts in community membership (i.e. community composition was drought resistant). This result has been found in other drought experiments in calcareous grasslands over varying time scales: (e.g. two-year study in south-western France (Maalouf *et al.*, 2012), a 13-year study in Northen England (Grime *et al.*, 2008). Interestingly, Evans *et al.* (2011) registered the resistance limit in community structure only after four years into an 11-year drought experiment in a semi-arid US grassland. Taken together these results indicate grasslands have capacity to buffer drought impact but also highlights the mismatch in experimental timeframes and species response times such that impacts are likely to be underestimated. In calcareous grasslands, due to the presence of long-lived perennial species characterised by stress-adapted life histories, responses are likely to be slow and lagged (Grime *et al.*, 2008). There was evidence to suggest a delayed drought response in *B. sylvaticum* (**Chapter 4**), where vegetative and reproductive height was significantly reduced in 2019. This reaction was likely driven by weather conditions in 2018, the year prior to start of demographic study. In 2018 there was a combined extreme drought and heatwave in the UK (Turner *et al.*, 2021), where only a third of the longer-term average summer rainfall fell at the site (Rennie *et al.*, 2017; Stone, 2020). *B. sylvaticum* showed signs of recovery in plant height where vegetative height aligned with individuals in control plots in 2020 and reproductive height the following year in 2021.

The increased instability in productivity and drought lag effects reported here concurs with below-ground dynamics of seedbanks in a calcareous grassland in Buxton, UK (Basto *et al.*, 2018). The response above-ground was largely independent to changes below-ground and over the span of the 14-year study, severe reductions and even the loss of two species from soil seedbanks were registered (Basto *et al.,* 2018). It was theorised that a reduction in reproductive effort via aborting mechanisms was a possible driver in the decrease in soil seed banks (Basto *et al.,* 2018). This result links with observations of *B. sylvaticum* in **Chapter 4**, where a reduction in reproductive allocation was recorded in individuals in drought plots. This is concerning, and points to the need to further evaluate grassland drought resilience by integrating above and below-ground measurements.

The trait analysis in **Chapter 5** showed *B. sylvaticum* to be drought tolerant with only minor adjustments to leaf anatomy. A similar muted response to water limitation in leaf traits of this species was shown in a greenhouse study (Marchini *et al.,* 2018). Both the greenhouse and **Chapter 5** studies provided a snapshot into the species' functioning in a single growing season and it is unclear how liable or adaptive these morphological traits are. If this species shows high temporal variability (e.g. within and between several growing seasons) in traits due to plasticity rather than genetics this would suggest high tolerance and resilience to buffer environmental change (Anderegg, 2015). A temporal shift in community weighted trait means in individuals with smaller stature and leaves between the mid and late growing season was recorded at the same site (Fenollosa *et al.*, 2023). It was hypothesised that the drought in combination with the mid-season mowing contributed to the shift in community specific leaf area (SLA) and leaf dry matter content (LDMC) values observed in the late season (Fenollosa *et al.*, 2023). This highlights, the important role of accounting for within season trait variability and that stressors/ disturbances, in this case drought and mowing, can act in tandem to influence community functioning.

Both at the individual species and community level, the calcareous grassland at RainDrop showed resistance and capacity to recover from drought, although the long-term impacts should not be ruled out. The site is still in the recovery phase, following agricultural abonnement in the 1980s, and early successional grasslands have been shown to be more vulnerable to environmental change (Grime *et al.*, 2000). The grassland community could abruptly reach a drought tolerance threshold, and perhaps more rapidly with additional environmental stressors such as changes in management and/or nutrients acting synergistically (Brook *et al.,* 2008). Beyond studying this single grassland, the likely underestimation of drought responses on longer ecological time scales is of concern for ecosystem service provisions from grassland systems at a global scale (Smith *et al.*, 2024).

The experimental work in this thesis, focused principally on manipulating the amount of incoming rainfall during the growing season, where a 50% reduction in rainfall induced significant changes to community productivity, with only modest trait-demographic reactions in the dominant grass species *Brachypodium sylvaticum*. Based on the results to date, including from this thesis, an additional drought dimension will be investigated at the RainDrop site – timing. Changes in the occurrence and length of rainfall events have been shown to influence biomass production, especially in temperate grasslands which show the highest biomass accumulation rates earlier in the growing season (Felton and Goldsmith, 2023). To investigate this aspect of drought, the procedural control plots are being converted to a drought treatment of 50% reduction in incoming rainfall over 12 months. Another option could be to make the drought treatment more extreme (75% reduction of incoming rainfall during the growing season instead of 50%). Investigating additional drought dimensions will help elucidate further the species drought tolerance thresholds in this calcareous grassland.

6.2.2 A matter of characterising space

Spatial scales can also shape trait variation. A sizeable portion of the variation in drought responses was either unexplained or similar across experimental spatial scales in the two *B. sylvaticum* studies (**Chapter 4** and **5**). These results highlight the complex nature of trying to evaluate factors shaping plant responses in grassland systems, even in experimental settings. The relatively equal contributions of experimental scale variables to variation in responses, likely point to similar biotic and abiotic environmental processes occurring at the 2-ha site (i.e. filtering processes inside the community, sensu Violle *et al.* 2012). Casual inferences cannot be made here as decomposing the underlying mechanism of spatial trait variation was not within the scope of the study, so I will share some considerations. Upper seeds is relatively topographically level and throughout the site there are exposed calcareous rocks protruding from the soil from previous ploughing (during the use for cereal production from the second world war to the late 1970s) and thus on the meso-scale (several meters) it is likely abiotic processes would be similar, which differs to the steeper slopes at Buxton (Grime *et al.*, 2000). The role of micro-climate (e.g. soil nutrients/moisture and structure) on trait-environment relationships, are not often explicitly investigated, with more literature existing in forested systems (Messier *et al.,* 2010; Zhang *et al.*, 2018; but see Deák *et al.* (2024) for example in grasslands). At the community level, micro-climate has been shown to have a notable influence on species richness in calcareous grasslands (Grime *et al.*, 2000; Mazalla *et al.,* 2022). Interestingly, in some tagged *B. sylvaticum* individuals in drought plots, I observed bunches shift a few centimetres towards the outer edges of the plots between each growing season demographic census (Middleton, 2022). This is likely a case of positive hydrotropism, where roots sense and respond by growing towards increased water sources (Kalra *et al.,* 2024). This observation suggests that micro-site heterogeneity plays a role in mediating plant responses and that in addition to plant size traits, the tracking the location of plants should be considered in drought responses.

Although the effect of neighbouring plants was not statistically significant in mediating demographic responses in **Chapter 4**, it has been shown to be important in other vegetation communities. Le Bagousse-Pinguet *et al.* (2015) showed that the traits of neighbouring plants had a significant impact in addition to water stress on variation in focal plant trait responses along a rainfall gradient in a semi-arid shrub land. It can be challenging to disentangle the abiotic and biotic processes occurring at fine spatial scales (sub meter scales), as they often interact (e.g. the presence of legumes in neighbourhood can change the soil nutrient status) (Violle *et al.*, 2012). This highlights the need for additional studies on examining the processes operating at small scales as this would aid interpretations of trait and demographic responses which has implications for driving community assembly (Funk *et al.*, 2017).

Two other aspects of spatial dynamics which are important to consider in maintaining local species and genetic diversity is immigration from other grasslands (>km scale) and seed transfer from outside treatment plots (m scale) (Loreau and Mouquet, 1999). The former is likely to be a negligible factor in this study, as the site is relatively isolated from other calcareous grasslands. Seed transfer between individuals of different treatment origin or from the rest of the field (effectively ambient control conditions) is a factor that was not controlled and could lead to drought impacts to be underestimated. There was an indication of reduced reproductive allocation in *B. sylvaticum* and other studies have shown that seed quantity and quality can reduce with drought (Bastos *et al.,* 2018). It is possible that seed transfer from outside the 5 x 5 m drought plots could increase seedling performance via increased seed nutrient reserves and/or parental environmental effects (transgenerational phenotypic plasticity) (Bell and Hellmann, 2019; Mojzes *et al.,* 2021). If enhanced performance is maintained into adult stages, this could indicate adaptive transgenerational plasticity. This would result in an underestimation of drought effects relative to if drought was applied to the whole field site (as with future climate change). However, if the performance of drought seedlings from a parent of non-drought origin is not enhanced or reduced into adulthood (e.g. neutral or maladaptive transgenerational effects respectively) then it could lead to an overestimation of drought effects. The extent to which transgenerational effect dominates has wider implications for productivity and genetic diversity and remains relatively understudied despite the eco-evolutionary consequences under global change (Mojzes *et al.,* 2021).

Characterising space from an analytical perspective revealed some interesting patterns in the trait-environment relationships of *B. sylvaticum* in **Chapter 5**. Trait-environment relationships are often based on mean trait values, especially in macro-ecological studies (Funk *et al.*, 2017). Trait means are not everything when analysing trait space. Inadequately accounting for intra-specific trait variation such as the other moments of trait distributions (variance, skewness and kurtosis) can miss important insights into ecological processes underlying plant response dynamics (Maitner *et al.*, 2023). The trait study (**Chapter 5**) showed mean trait values across the six leaf (leaf area, leaf thickness, SLA, LDMC) and structural (stem specific density and maximum plant height) traits to be relatively stable across the three precipitation treatments. Analysing the higher moments of trait distributions provided additional evidence to suggest that *B. sylvaticum* had adequate trait-environment matching across treatments in the 2022 growing season, as most traits had distributions close to the central tendency (except SLA in droughted individuals which had a large variance partly attributed to measurement issues). There were indications of possible developmental constraints in leaf thickness, as this trait showed the least variability (Coneva and Chitwood, 2018). This low phenotypic plasticity in drought related traits has been recorded in this species when comparing individuals from native vs invasive populations (Marchini *et al.,* 2018).

Based on results from **Chapter 5**, mean trait values for *B. sylvaticum* should give an adequate approximation of this species' dynamics for use in other studies of similar environments (e.g. calcareous grasslands) but would urge caution for the use of mean trait values when applied to other vegetative contexts. The relatively low intraspecific variability (ITV) observed here by no means indicates that accounting for ITV is not a worthwhile exercise in other climate change vegetation studies. ITV has been shown to stabilise short term drought responses in the composition of an alpine grassland community (Jung *et al.*, 2014). Another study showed that fixed trait means (particularly plant height) underestimated grassland community responses when ITV was unaccounted for in a factorial fertilisation x mowing experiment (Lepš *et al.*, 2011). In addition, both experimental studies reported that ITV was often a larger influence than species turnover to the functional trait space of the grassland communities. Caution is also warranted in drawing conclusions, given that the results from **Chapter 5** are based on this one-time measure of the subset in surviving individuals studied in **Chapter 4** and relatively low sample size, where the latter has been shown to influence trait variance (Jiang *et al.,* 2021). As mentioned previously the temporal dynamics would need to be further quantified to assess if and how trait space might shift in this species.

6.2.3 A matter of time and space: challenges and trade-offs

With environmental change, scaling across time and space is a necessary but complex challenge in assessing vegetation functioning. Trait-approaches vary in how space and time are considered. The bibliometric work (**Chapter 2**) showed how the different trait approaches (functional, phenotypic and life history) diverged in characterising and analysing organisms across space and time (Figure 6.2). Evolutionary and demographic approaches tend to be more mechanistic and view traits as temporally dynamic with respect to ontogeny with many repeated trait measurements on a small number of individuals to then understand the genetic or population processes (Salguero-Gómez and Laughlin, 2021). On the other hand, ecologists tend to view traits from a more integrative perspective analysing many species (e.g. community weighted means), where traits are treated as static functional snapshots used to assess functional strategies within a single spatial context or upscaled (Volaire *et al.,* 2020). A cultural analogy would be the difference between how much information (functioning or environmental context) can be gleaned about movie characters (individual organisms) from looking at a movie poster of the main characters (static traits) vs watching the entire movie and seeing how the lives of characters play out in different environmental settings (dynamic traits). Just as a movie poster and movie are related but serve different purposes, so do the various trait approaches (although there are frequent cases of overlap like body size is a functional, phenotypic and life history trait).

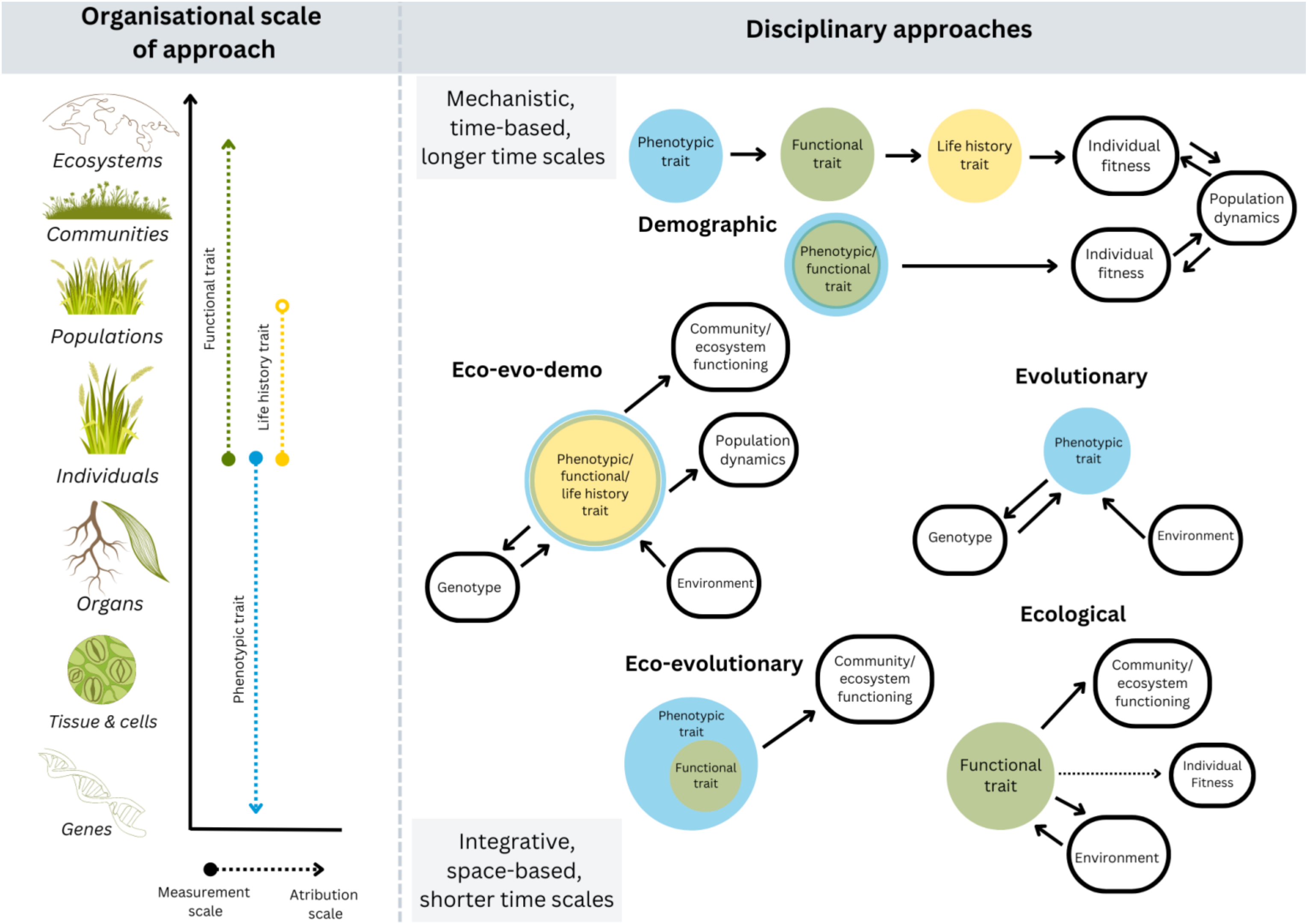


Figure 6.2 A schematic comparing functional, phenotypic and life history trait approaches across organisational scale and disciplines. Solid arrows indicate trait links. Dotted arrow for ecological functional trait approach indicates link that is not always explicitly studied.

The experimental work in this thesis has adopted a complementary eco-demographic approach to studying plant functional responses to precipitation manipulation in a grassland by linking across organisational/spatial and temporal scales. Here trait and demographic drought responses were aligned (low trait variability and conservative life history strategy) but differed to community level responses (reduction in productivity driven by decrease in graminoid and legumes). The trait-demographic consensus was not totally unsurprising, as traits were measured on a subset of surviving individuals, which from a back-casting perspective implied the traits measured were functional. Continuing with the movie analogy, this approach is akin to making a short movie trailer, where information of how main and supporting characters interact with each other and their environment in a short timeframe. This work fits within the grassland research landscape, which has seen a move towards adopting a broad spectrum of ecological metrics from plant traits, above-ground biomass, species richness and soil biochemistry, reflecting a shift in research priorities towards more practical applications *(*Liu *et al.,* 2024). Within a single study however, it remains rare to combine multiple spatial and temporal scales (Felton and Smith, 2017; Biswas *et al.*, 2024).

A major challenge in adopting multi-scale trait approaches is the labour and resource-intensive nature of collecting enough traits to make meaningful inferences on vegetation functioning (Asner *et al.*, 2015). The annual demographic census on ~150 *Brachypodium sylvaticum* adults and seedlings took approximately 200 field hours, 100 hours digitising plant size data (photographing recording sheets) and many months developing algorithms to automate the extraction of plant size data (see methods in **Chapter 4**, Middleton, 2023). In the trait study, a trade-off was made between the number of traits, replicates and individuals sampled (31% of individuals alive in 2021), which followed sampling protocols outlined in Pérez-Harguindeguy *et al.* (2013). This level of time and resource commitment is not always feasible or in alignment with conservation timeframes (Salguero-Gomez *et al.*, 2018). In addition to decision flow charts for identifying efficient trait sampling strategies (e.g. de Bello *et al.*, 2021), future work should seek assess ways of automating trait data collection and compare quality of data outputs. There is already work underway at the RainDrop site investigating the feasibility and efficiency of automating vegetation data such as hyperspectral drone imaging (Jackson *et al.*, 2022) and autonomous robots (Gadd *et al.*, 2024).

6.3 Future research needs

The experimental research conducted in this thesis showed that trends in drought responses do not always align across organisational scales. To extend the insights gain from this thesis work, there are three areas that merit further study: i) characterising plant (trait) responses to drought over longer temporal scales; ii) integration of belowground, genetics, biotic interactions and multiple stressors, iii) examining applications of trait-demographic relationships (Figure 6.3).

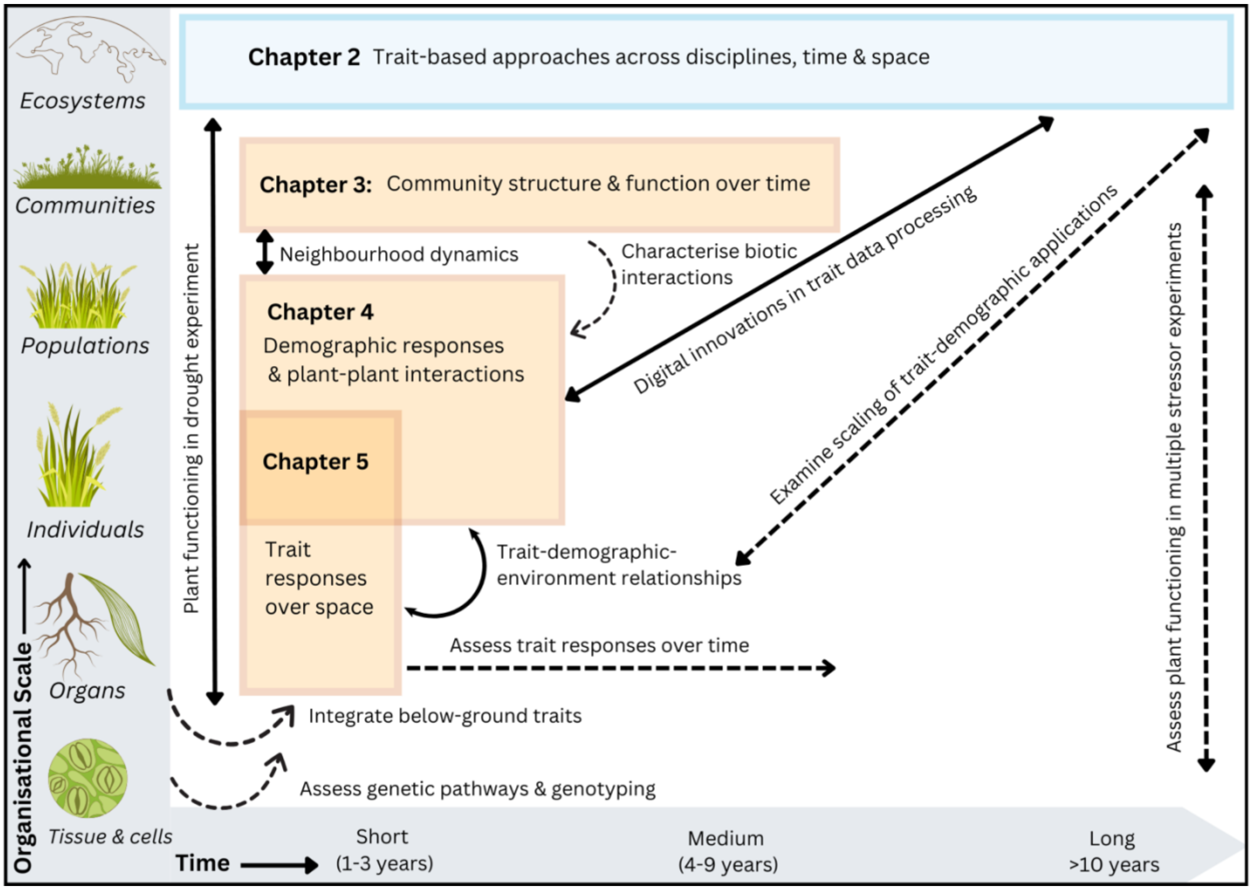


Figure 6.3 How future work relates to and extends the findings from this thesis across temporal and organisational scales. Solid and dashed arrows relate to themes in this thesis and areas of future work respectively. Time axis conveys ecological time scales.

6.3.1 Towards a more integrated outlook on plant functioning

Assessing the epistemology of (functional) trait–demographic approaches

In **Chapter 2**, I provided a broad overview of the conceptual origins and development of three frequently used trait terms (functional, phenotypic and life history) and set out ways to improve research synthesis and synergy across researchers/disciplines. To extend the work in **Chapter 2** of finding common ground in trait approaches, future bibliometric analyses could assess plant (functional) trait–demographic approaches by looking at which traits researchers' sample and link to which vital rates (i.e. assess "functionality" of functional traits). Preliminary work has been carried out in a pilot study of 32 primary research papers covering 510 plant species and 73 functional traits looking at trait-vital rate links and organisational scale of approach. Early results indicate that researchers are mostly measuring morphological traits (e.g. leaf area, leaf mass area, wood density) in tree species where the measurement-attribution trait link is at the level of individuals. Most studies focused on assessing survival and growth vital rates rather than reproductive parameters and 25% (n = 8) studies had undifferentiated or unclear life stage. Expanding this work to wider sample of studies can provide an indication of data gaps and help set future research priorities.

Characterising plant (trait) responses to drought over longer temporal scales

There is a need to better understand the time scales of drought responses and recovery in grassland systems. The multi-scale assessment of drought responses in the calcareous grassland indicated lagged effects. Outstanding questions remain as to how best to integrate the variation in response times of the different vegetation components. For example, how do the shorter physiological response times in leaf anatomy translate to the longer reaction times of demographic process in reproduction and recruitment? Future work at the RainDrop site should seek to ascertain drought tolerance limits over at least a 15-year period. Long-term vegetation monitoring is a labour and resource-intensive activity, and future studies could couple on-the-ground botanical measurements with remote sensing data (e.g. drones fitted with hyperspectral imaging equipment), to provide more rapid assessment of vegetation functioning (Jackson *et al.*, 2022).

Below-ground traits

I focused on above-ground trait measurements on *Brachypodium sylvaticum* which showed tolerance to drought in the short term with only minor adjustments to leaf anatomy. There is growing research showing that plants do not always co-ordinate responses above and below-ground, suggesting independent mechanisms governing drought responses not fully captured by either the leaf or root economics spectrum (Wright *et al.*, 2004; Asefa *et al.*, 2022). Roots represent an interface between soil and above-ground organs in the transfer and storage of water and nutrients and therefore act as first responders to environmental stressors like drought. Roots traits have been shown to exhibit plastic drought responses and therefore comparing above and below-ground responses would be a valuable next step (Lozano *et al.*, 2020). Belowground traits (e.g. rooting depth and root dry matter content) were considered during the initial planning of design and sampling strategy for **Chapter's 4** and **5.** However, due to the challenging physical structure of the soil related to previous site management, it was not possible to excavate plants in a minimally invasive way. An assessment of root dynamics at the site would likely require ex-situ investigations through growing plant seedings in isolated pots in a greenhouse environment. As this species exhibits hydrotropism behaviour in field settings (Middleton, 2022), this factor would need to be considered when growing individuals in pots. I would hypothesise that individuals under drought conditions would display higher root biomass and root tissue density than in control treatment (Fort *et al.,* 2013).

Biotic interactions

The demographic analysis in **Chapter 4** was primarily focused on quantifying precipitation treatment effects on survival, size and reproduction on *Brachypodium sylvaticum*, where biotic interactions were included in the models as covariates. Plant-plant interactions within the 30 cm radius neighbourhoods were based on species identity, which showed neither an amelioration nor hinderance to demographic outcomes. To more concretely disentangle the trade-offs between (drought) stress tolerance and competition in this species, additional empirical work is required. Experimental work has shown that calcareous grassland species (including *B. sylvaticum*) can have negative survival outcomes in the presence of plant neighbours in both wet and dry years (Nemer *et al.*, 2021). Future work could characterise these relationships through a plant neighbourhood crowding analysis that accounts for size, spatial distribution and species identity of neighbours (e.g. Aujla, 2020) or through implementing a factorial experimental design manipulating precipitation treatment and plant community composition (Grant *et al.*, 2014).

Multiple stressors

The experimental work in this thesis focused on a single environmental stressor – drought and future work should seek to understand how multiple stressors (e.g. nutrient additions x drought) mediate vegetation responses. Drought is one of several environmental stressors calcareous grasslands are experiencing and incorporating additional treatments into a factorial experimental design would help better characterise responses in these important ecosystems (Diekmann *et al.*, 2014). Indeed, multiple stressors are being increasingly recognised in grassland ecosystems with the establishment of global distributed experiments such as Disturbance and Recovery Across Global Grasslands Network (DRAGNet - https://dragnetglobal.weebly.com/).

Characterising genetic pathways and genotypic variation

Intra-specific variation was shown to be important in influencing variation in both demographic and trait drought responses (**Chapters 4** and **5** respectively), however the underlying genetic mechanisms governing this variation were not quantified. Investigating genotypic variability can help gain a more complete picture of the species' demographic performance and adaptive potential with respect to environmental stressors (Whitlock *et al.,* 2010). There is a growing body of genetic work on the species, which could help in this regard: *B. sylvaticum* is a model organism for perennial grasses used to examine the genetic pathways (e.g. determining active vs passive trait responses) to abiotic stressors (Lei *et al.*, 2023). Extensive genotyping studies have been done to understand genetic differences between the populations in its native and invasive range in North America (Holmes *et al.*, 2010). What is more, incorporating more genetic components to plant ecological studies would also help bridge the eco-evolutionary disciplinary gap identified in **Chapter 2** between functional traits and phenotypic trait approaches.

6.4 Conclusion

This thesis set out to examine how trait-based methods can be used to assess plant community to individual-scale impacts in the context of global environmental change. My bibliometric analysis demonstrated that trait-based approaches across disciplines vary in their conceptual origins and how space and time is accounted for, but areas of overlap exist in their practical applications in environmental change research. Using eco-demographic methods, my experimental work at the calcareous grassland at RainDrop showed that by assessing community-level functions (productivity) with trait-demographic responses deeper insights into vegetation functional dynamics can be obtained than by considering responses at each organisational scale in isolation. Community productivity and temporal stability decreased with imposed growing season drought driven by biomass losses in graminoid and legume species. There were indications of possible lagged effects as community composition was resistant to drought likely buffered by high survival rates and long life spans of species. Contextualising the community-level responses with the trait-demographic analysis of the dominant grass species *Brachypodium sylvaticum* suggested that this species is more drought tolerant than other members of the graminoid functional group, as differences in above-ground traits were largely undetectable across precipitation treatments. Where responses were registered, there were delayed drought reactions in reproductive parameters, which showed signs of recovery over the following two growing seasons. Both at the individual species and community level, the calcareous grassland showed resistance and capacity to recover from drought. Although it is unclear how the observed shorter term drought resistance via reducing biomass and re-allocation of resources towards survival will affect dominant and subordinate species in the longer term. Thus, long-term impacts should not be ruled out, as this early successional grassland community could abruptly reach a drought tolerance threshold.

More broadly, throughout this thesis, I have shown the need for interdisciplinary perspectives in trait-based research, which I have demonstrated by incorporated thinking from ecology, evolution, demography, conservation, crop science, computer science and philosophy. By weaving together theory and practice from these fields and beyond, we might get closer to yielding on the promise of trait-based research as a tool to quantify and predict environmental change impacts.

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Appendix

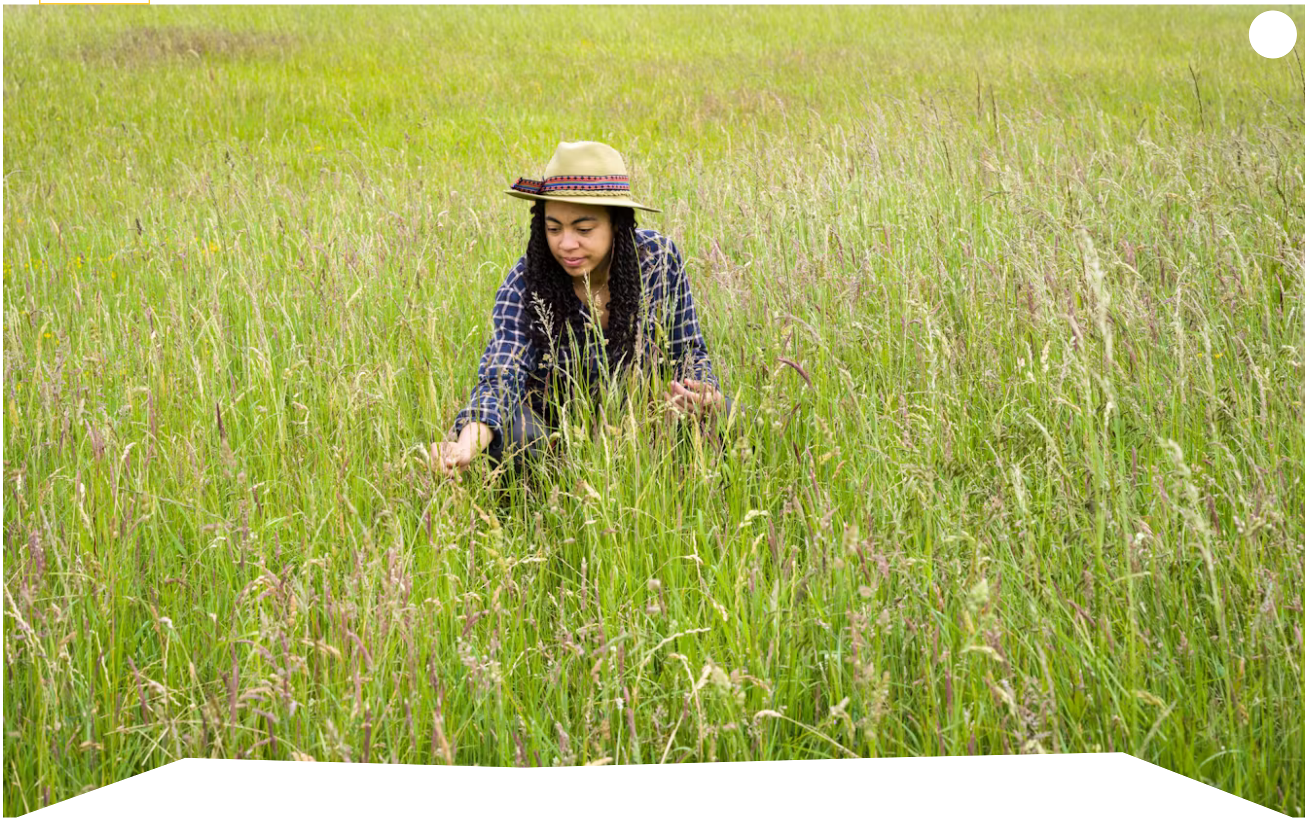
Publications resulting from this thesis

Beyond a green carpet

From a distance, grassland might look like a simple stretch of green, but this apparently uniform environment conceals a hugely diverse, living world. Plant ecologist Sara Middleton gives us a close-up view of a complex network, and ponders its future under the stresses of climate change.

Words by **Sara Middleton** | photography by **Steven Pocock** | average reading time **7 minutes**
17 August 2022

Article



Despite covering 40 per cent of the Earth's surface, grasslands are often seen as just green carpets, a homogeneous expanse with not much going on. In fact, if you were to scale up grasslands to the size of tropical forests, plant diversity would rival their tall floristic cousins.

In the last three years, I have come to appreciate how wonderful grasslands are, through my research as a plant ecologist. I intimately follow the lives of about 150 individual plants and record how the environment shapes their life in a grassland drought experiment.

Before engaging in this research, I didn’t know very much about grasslands except that they were nice to gaze out at on long train journeys across the UK and that they had the potential for carbon storage.

During the yearly summer census at my field site at Wytham Woods in Oxfordshire, I have come to learn some fascinating plant life stories. Grassland plants showcase a myriad of survival strategies, from magnificent mimics that play tricks on their pollinators to plants that make a living by befriending bacteria.

“During the yearly summer census at my field site at Wytham Woods in Oxfordshire, I have come to learn some fascinating plant life stories.”

I learned about most of the 107 plant species at my field site during hours spent lying belly down with a face full of plants, a plant identification book in one hand and a hand lens in the other. At the end of the day, I would review the species of the day and research them online.

Out of the 100-odd plant species at the site, there are two that always put a beaming smile on my face, and I feel humbled to be in their presence: the bee orchid and the common broomrape.

Magnificent mimics

June is the peak blooming period, and the grassland is awash with pinks, purples and yellows. At the start of each field day, I pay a visit to ‘Buddy’, a bee orchid I’ve nicknamed that pops up every June in the same spot.

Bee orchids (*Ophrys apifera*) are one of nature’s magnificent mimics. Against the backdrop of 50 shades of green are swarms of shin-high spikes with half a dozen bees perched on top. These static, browny-yellow ‘bees’ with outstretched pink wings resemble female bees settling on a flower. A keen male bee comes buzzing in to attempt to mate, only to find that there is no female, but he has ended up pollinating the flower.

> “Out of the 100-odd plant species at the site, there are two that always put a beaming smile on my face, the bee orchid (left) and the common broomrape (right).”

Bee orchids can’t see, so how do they pull off this uncanny resemblance? This sexual swindling trick has been perfected over millennia after a random mutation. Their ancestors relied on bees to pollinate them in exchange for sweet nectar. By chance, some flower forms were more appealing to bees and were pollinated more. Over time, the flower forms that looked more like female bees were more successful at reproduction. In the UK, bee orchids self-pollinate, as the bee species that pollinates bee orchids is not found here.

Each time I see a bee orchid I am in awe by their aesthetic and evolutionary beauty, almost like it is my first encounter with them.

Rule breakers and double agents

Parasites provoke a dual response of disgust and fascination for me. In the parasitic animal world, I despise ticks, mostly from the effort it takes to scan myself for ticks at the end of each field day.

But the parasitic plant world is mesmerising to me. Most plants look green and make all their own food, but some don't play by these rules. Members of the broomrape (*Orobanchaceae*) family survive by syphoning off nutrients from neighbouring plants. These ghostly-looking plants stab the roots of a plant host with their haustoria (a root-like structure) and draw out nutrients.

"Each time I see a bee orchid I am in awe by their aesthetic and evolutionary beauty, almost like it is my first encounter with them."

Some *Orobanche* have broad plant-host preferences while others are species-specific. The common broomrape (*Orobanche minor*) has a taste for plants from the legume and daisy family.

Other members of this plant family act more like double agents. They appear to play by the rules, as above ground they are green, because they contain the food-making pigment chlorophyll, but below ground they supplement their nutrition by stealing nutrients from their neighbours.

Some of these double agents, like the yellow rattle (*Rhinanthus minor*) are also "meadow managers". They suppress the growth of neighbouring species, particularly grasses, leaving open pockets for other species to grow.

> "Another way to survive in challenging environments is to form cooperative partnerships. A lesson I take away from these plants is the importance of collaboration with others from different communities."

Community collaboration

Another way to survive in challenging environments is to form cooperative partnerships. Plants like clovers are legumes, and they engage in a nutrient exchange by befriending nitrogen-fixing bacteria in their roots. The bacteria, known as rhizobia, supply a legume plant with nitrogen in a bio-available form and in return, the bacteria receive sugars produced by the plant.

Some grasses can make fungi friends below ground. The fungi help them access more nutrients in the soil, provide a shield against pathogens and offer a buffer against stressful situations such as drought. A lesson I take away from these plants is the importance of collaboration with others from different communities.

Shrinking and scrambling

As I criss-cross my field site, pushing a wheelbarrow overflowing with field gear, I occasionally stop and admire all the wonderful ways plants make a living while (mostly) rooted to the ground. Aside from those of bee orchids and broomrapes, there are other survival strategies.

The common centaury (*Centaurium erythraea*) is a pop-pink petalled plant, able to sense micro-meteorological changes such as an imminent rain shower. They seem to use their weather-forecasting abilities to close their flowers as a protective measure against potential weather damage. Often these plants are a better weather indicator than my phone app!

“As I criss-cross my field site, I occasionally stop and admire all the wonderful ways plants make a living”

Several plants can shrink or retrogress in challenging conditions, like drought, as being smaller requires fewer nutrients to wait out the tough times. My study species, a bunch grass called false brome (*Brachypodium sylvaticum*) does this. I’ve also observed some of the bunches in the drought plots ‘creeping’ a few centimetres each year towards the outer edges of the plots, where conditions are better.

The objective scientist in me can’t wait to do a deep dive into these data points, but the excitement is veiled by both sadness and admiration. Sadness at seeing these living beings scrambling for survival. Admiration at their resilience to adversity.

> “These 100 or so bunches of grass have taught me not to overlook how much of the environment where we grow up shapes the trajectory of our lives.”

I’ve grown fond of these 100 or so bunches of grass after closely following them for four years. I can even recognise many of them by sight now. They have taught me not to overlook how much of the environment where we are born and grow up shapes the trajectory of our lives.

The unravelling of the green carpet

Walking through grasslands, we are often oblivious to the multitude of partnerships and strategies going on beneath our feet that have been fine-tuned over generations.

To some extent, grasslands *are* a green carpet: a tightly interwoven network of living strands, a plant community. This fabric of life connects species to each other through complex above- and below-ground relationships.

> “Through my research, I have recorded the many plant winners and losers in the battle for water. When I think of the wider context of my work, I do worry.”

With environmental change, we are simultaneously pulling the green carpet in multiple directions via extreme weather events and land-use change, while also slowly pulling out threads as species become extinct, creating strains and holes. The carpet can still function with some bumps and holes, but it will look different as the plants that comprise it shift or vanish. Too many changes affect the integrity and resilience of the carpet.

Through my research, I have recorded the many plant winners and losers in the battle for water. When I think of the wider context of my work, I do worry. Many of the UK’s grasslands are already hanging by a thread, and we haven’t experienced the worst of climate change yet.

As the green carpet begins to unravel, will the plant strategies refined over millennia and their stories written into the patterns in the fabric keep up with the pace of climate breakdown? We will need to tread carefully to preserve these plant life stories before there is not much left to salvage.

About the contributors

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Sara Middleton is a plant ecologist, science communicator and intersectional justice advocate. She is currently a doctoral researcher at the University of Oxford looking at drought impacts on grasslands. Her interests also branch out into examining human-nature relationships, plant awareness disparity, agricultural food systems and raising the profile of historically excluded racial minorities in biology. Her research is funded by the Natural Environment Research Council, with logistical and maintenance support from the Wytham Woods team, Ecological Continuity Trust, and John Fell Fund.

Steven Pocock

Photographer

Steven is a photographer at Wellcome. His photography takes inspiration from the museum’s rich and varied collections. He enjoys collaborating on creative projects and taking them to imaginative places.

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RESEARCH ARTICLE

Experimental drought reduces the productivity and stability of a calcareous grassland

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Abstract

1. Grasslands, which encompass 40% of terrestrial ecosystems, hold global significance for food production, carbon storage and other ecosystem services. However, grasslands across the biosphere are becoming increasingly exposed to both wet and dry precipitation extremes resulting from climate change.
2. Therefore, understanding how grasslands will respond to precipitation extremes is a pressing issue for managing changes to biodiversity and ecosystem service provision.
3. Here, we use experimental manipulations of precipitation (50% increase and 50% decrease in growing-season precipitation) over 6 years to investigate the stability and resistance of both productivity and community diversity in a calcareous grassland community.
4. We found that decreased growing-season precipitation led to reductions in mean productivity (25% decrease in peak above-ground biomass) and its temporal stability (54% increase in the coefficient of variation of biomass across years). Productivity losses were more pronounced for graminoids and legumes, but this was not reflected in species per cent cover. Community composition was resistant to the precipitation manipulations, with no clear differences in overall community compositional turnover, dissimilarity or biodiversity indices. There was also no detectible effect of increased precipitation on productivity or community composition, which may indicate a lack of efficacy of the irrigation treatment in increasing soil moisture. Finally, the precipitation manipulations had no effect on temporal trends of community change, with an overall increase in richness and shift in grassland community composition across the study period independent of treatment.
5. *Synthesis*. While the diversity and composition of this calcareous grassland was resistant to precipitation extremes (at least in the short term), sustained reductions in growing-season precipitation reduced productivity and its temporal stability, particularly for graminoids and legumes. Therefore, we highlight that different properties of grasslands can vary in their responses to changes in precipitation.

R. Salguero-Gómez and A. Hector—Shared authorship.

KEYWORDS
biodiversity change, climate change, community resilience, DroughtNet, field experiment, precipitation

1 | INTRODUCTION

As a dominant terrestrial biome important for ecosystem services and food security, understanding the response of grasslands to climate change, and in particular changing precipitation patterns, is critical (Fay et al., 2008; Franklin et al., 2016; Hopkins & Prado, 2007). Despite the threat of climate change, its effects on plant communities are not uniform, and complex responses highlight the need for targeted community-level experiments (Gupta et al., 2020; Harrison et al., 2015; Kardol et al., 2010; Parmesan & Hanley, 2015). Key components of climate change that are expected to influence plant communities are more changeable, and more extreme, precipitation patterns (Hopkins & Prado, 2007; Knapp et al., 2015). For grasslands, which occupy 40% of terrestrial ecosystems and provide many ecosystem services (Abberton et al., 2010; O'Mara, 2012; Petermann & Buzhdygan, 2021), declines in use and extent after agricultural intensification, conversion and grazing may be exacerbated by precipitation shifts (Meng et al., 2021; Peeters, 2009). Thus, studying how grassland communities will respond to precipitation change will aid in safeguarding these globally important habitats.

In grasslands, one way that community responses to precipitation change have been explored is through productivity. Productivity in grasslands is typically measured through biomass (Fahey & Knapp, 2007). Indeed, there have been wide efforts to understand how grassland biomass changes with precipitation (Grime et al., 2008; Knapp et al., 2017; Kröel-Dulay et al., 2022; Smith et al., 2024). Generally, drought conditions reduce primary productivity in grasslands (Herben et al., 1995; Kardol et al., 2010; Smith et al., 2024; Wang et al., 2007), which can impact ecosystem services such as pollination (Phillips et al., 2018). However, despite overall reductions in productivity, grassland biomes with different baseline climatic conditions react differently, where arid or xeric biomes are more susceptible to losses in productivity with drought than mesic biomes (Cleland et al., 2013; Huxman et al., 2004; Moran et al., 2014). Productivity may also recover quickly when drought conditions subside, even in semi-arid grasslands (Luo et al., 2023). Conversely, there are relatively fewer studies investigating increases in precipitation (or irrigation), but a global meta-analysis (although largely in North America) revealed that ecological experiments with supplemented watering alone did not change biomass (DeMalach et al., 2017). Overall, there are grassland productivity declines in drought conditions, but understanding temporal dynamics in productivity with respect to precipitation is crucial in a wider range of grassland habitat types.

Community composition differences driven by changing precipitation patterns are less clear in grasslands. Harrison et al. (2015) presented findings that reductions in midwinter precipitation over 14 years reduced biodiversity in Californian grasslands. Furthermore, a recent study found that after 11 drought years, grassland communities in a German semi-arid grassland shifted to favour dry-grassland specialists and ruderal species, and composition change was mediated by microclimatic conditions of site topology (Mazalla et al., 2022). However, another long-term study of experimental precipitation manipulation in an infertile UK grassland found that although communities in drought (but not irrigated) conditions differed from control plots, interannual variation in natural precipitation and broad shifts in the community through time were better explanations for community change (Grime et al., 2008). Several studies have found that grassland communities are resistant to both experimentally induced drought and natural variation in precipitation (Cleland et al., 2013; Craine et al., 2013; Grime et al., 2000; Sternberg et al., 1999). A key mechanism proposed for community resistance to precipitation extremes is buffering via increased species diversity, whereby more species create redundancy in functional composition that is better adapted to withstanding precipitation extremes (Craine et al., 2013; Craven et al., 2018; Griffin-Nolan et al., 2019; Wagg et al., 2017). Furthermore, perceived resistance may be observed due to the lifespan of perennial plant species in a community, with much greater community differences in the seed bank resulting in a delayed response (Basto et al., 2018). To fully elucidate the temporal dynamics of community composition as precipitation patterns change, we need further long-term monitoring of grassland systems.

A notable case study of a grassland that is vulnerable to climate change are the calcareous grasslands, which we focus on here. Calcareous grasslands are typified by alkaline soils, often occurring on limestone or chalk bedrock, supporting up to 700 vascular plant species in Europe, and providing a wide range of ecosystem services such as pollination, carbon sequestration and recreation (Gibson & Brown, 1991; Grêt-Regamey et al., 2014; Klaus et al., 2021; Willems, 1990). There have been declines in the extent of many European calcareous grasslands, following agricultural intensification, scrub encroachment and grazer management (Grêt-Regamey et al., 2014; Ridding et al., 2020), which may accentuate effects of climate change. To preserve high levels of species diversity, calcareous grasslands are listed as recovering ecosystems and part of wider conservation management schemes in countries such as the United Kingdom (Gibson, 1986; Gibson & Brown, 1991; Maddock, 2008; Poschlod et al., 1998).

Here, we use 6 years (2016–2021) of biodiversity data from an experimental manipulation (50% increase and 50% decrease) of precipitation in a calcareous grassland to examine productivity and community resistance to precipitation change, an important component of climate change. We extend previous work on calcareous grasslands (Grime et al., 2000, 2008) through an analysis of detailed temporal dynamics in community composition and productivity, using annually replicated data. Specifically, we answered three key questions: (i) whether changes in precipitation affect above-ground annual net primary productivity (above-ground ANPP) and its temporal stability across years, (ii) whether calcareous grassland communities have a high resistance to drought conditions, and which species are important for driving community differences, and (iii) whether the effect of precipitation manipulation change over time, and whether these temporal dynamics affected by natural precipitation patterns. We address these questions by quantifying the stability of productivity and resistance of community composition, which are useful conceptual terms to integrate community responses to climate change and tools to compare communities from different ecosystems (Allen et al., 2019; Donohue et al., 2013; Pimm, 1984; Van Meerbeek et al., 2021). For stability, we monitored shifts in the mean and variability of productivity through time with respect to precipitation treatments. Shifts in abundance or productivity serve as early warning signals of community shifts and indicate decreased stability in ecological communities (Clements & Ozgul, 2016; Pimm, 1984). Then, we use the definition of resistance for community composition from Van Meerbeek et al. (2021), where community resistance is the ability of community composition to resist changes in system variables in response to a perturbation, which here is drought or increase in precipitation through time.

2 | METHODS

2.1 | Study site

The RainDrop (rainfall and drought platform) experiment is situated in a ~2-ha area ('five-acre field') in the Upper Seeds grasslands (51°46′16.8″ N 1°19′59.1″ W, 166 m a.s.l.) of the University of Oxford's Wytham Woods estate, Oxfordshire, UK (Figure 1). Upper Seeds is a recovering calcareous grassland, which was intermittently used for arable agriculture from the Second World War until the late 1970s, before the site was managed as a grassland beginning in 1978 (Gibson, 1986; Grime et al., 2000). Grazing by sheep has been excluded throughout the experiment but did occur before the experiment. Upper Seeds, as with other calcareous grasslands, has a high level of floral biodiversity, in which graminoids constitute ~60% of species by biomass. Management consists of mowing all above-ground vegetation in mid-July at the peak of the growing season, and again in early October, coinciding with the end of the growing season. Biomass is removed following mowing. The site has a shallow soil depth (300–500 mm), alkaline soils (Gibson & Brown, 1991), a daily average temperature range of −5°C to 26°C (2016–2020) and a daily total precipitation range of 0–40 mm (2016–2020) (Rennie et al., 2017).

2.2 | Experimental design

We explored grassland biodiversity responses to precipitation in the context of the global drought network (DroughtNet) international drought experiment. DroughtNet's international drought experiment is a coordinated distributed experiment with over 100 sites globally (https://droughtnet.weebly.com/) (Smith et al., 2024). The goal of the DroughtNet experimental network is to explore ecosystem sensitivity to precipitation extremes through experimental manipulations of precipitation (Knapp et al., 2017). Precipitation manipulation is carried out by modifying natural precipitation patterns in each plot with rainout shelters, acting as a press disturbance (continuous change in the environment) maintained across several years. The manipulation was implemented in RainDrop as a randomised, replicated block design in which four

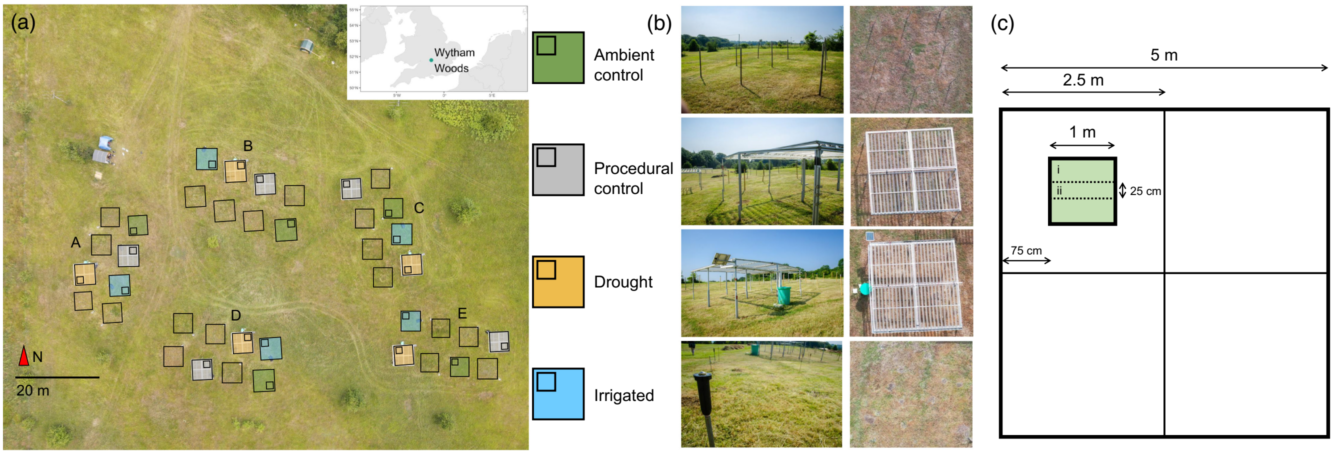

FIGURE 1 Experimental schematic of RainDrop on Upper Seeds, Wytham Woods with DroughtNet coordinated distributed experiment plots. (a) DroughtNet treatments are denoted by the colour of each 5 m × 5 m plot, and include Ambient control (green; no manipulation), Procedural control (grey; rainfall shelter but no change in rainfall), Drought (orange; −50% rainfall shelter) and Irrigated (blue; +50% rainfall with sprinklers). Letters indicate the five replicated and randomised experimental blocks A–E. Smaller squares indicate the biodiversity data collection area in each plot; the positions of which were randomised. (b) Photographs including ground-level (left) and aerial (right) views of each treatment type. (c) Experimental design schematic for each plot. Full 5 m × 5 m plot area, subdivided into quarters (2.5 m × 2.5 m), one of which contained the observation quadrat (green square—1 m × 1 m) used in this experiment. Species percentage cover (i) and biomass (ii) were collected from the observation quadrat. Each quadrat had a buffer of 75 cm.

treatments were repeated across five blocks (*n* = 20 experimental plots; Figure 1a, blocks A–E), in order to account for spatial variation and site topography, which varies by 5 m within the experimental area. Plots were therefore spatially nested within each block, which we accounted for with nested hierarchical random effects of plots within blocks in analyses.

Each replicated unit of 5 m × 5 m plots had one of four experimental treatments: ambient control plots (Ambient control), −50% precipitation rainout shelters to simulate drought (Drought), +50% irrigated plots with sprinklers to simulate increased precipitation (Irrigated) and procedural controls (Procedural control; precipitation shelter with no change to precipitation; Figure 1a). Biodiversity data collection occurs in the central 1 m × 1 m quadrat in one-quarter of each 5 m × 5 m plot, where the data collection quarter was randomised at the beginning of the experiment (Figure 1c). Therefore, each experimental quadrat has a buffer of 75 cm, which prevents wind-driven rain from entering the experimental quadrat (Figure 1c).

Rainout shelters consist of metal structures 1.5–2 m above the ground with transparent Perspex guttering. In the drought treatment, the rainout shelter gutters are approximately 10.4 cm wide and spaced such that 50% of the surface area of the plot is blocked by guttering. Irrigated treatment plots are supplied by water containers from the drought treatment, which collects precipitation with the transparent Perspex gutters, such that 50% additional precipitation is comprised of precipitation lost to the shelter (Gherardi & Sala, 2013). Procedural control plots were crucial to test the potential confounding effect of the drought treatment, in which similar structures and guttering are in place to simulate the microclimatic conditions of the shelter, but with guttering inverted to allow natural precipitation levels (Figure 1b). For example, although Perspex guttering was transparent to allow light to reach the plot, it reduced the light available to the grassland community to some degree. Thus, the procedural control enabled us to account for any such microclimatic effects. Precipitation manipulation percentages were selected through an assessment of long-term precipitation records, which found that extremes of annual precipitation differed from average years by ~40% (Knapp et al., 2015). At RainDrop, all drought treatments are removed between October and March of each year, when the Perspex gutters are inverted to restore natural precipitation levels, such that the experimental treatments are active during the growing season. However, rainout shelters and guttering remain throughout the year. To further test the validity of the experimental treatments at this site, we used preliminary data on soil moisture recorded from 13 of the 20 plots between August and September 2022. We found that simulated drought reduced median raw soil moisture by 37%, while there was only a modest soil moisture increase of 7% in the Irrigated treatment (Figure S1).

2.3 | Data collection

The core experimental protocol consists of biodiversity and productivity monitoring within experimental plots, namely species diversity and abundance, and above-ground net biomass production. To explore how precipitation manipulation influences grassland dynamics and composition, we monitored three main features of biodiversity: total community above-ground ANPP, functional group-level above-ground biomass and species-level percentage cover of vascular plants, for each 1 m × 1 m quadrat in each year between 2016 and 2021. We define ANPP as the total above-ground biomass of vascular plants across the growing season in each year, which estimates the investment of energy into growth of plant structures. We collected biomass at the peak of the growing season, 20 June–14 July, and at the end of September, such that ANPP was estimated for the entire growing season. We estimated ANPP using a 'clip strip' of all vascular plant material in a 1 m × 0.25 m strip in the centre of each quadrat, collected after percentage cover data. The size of clip strips was chosen to efficiently measure biomass across all plots in sufficient time, and representativeness of the biomass samples was ensured through spatial replication in blocks. Clip strips were gathered using hand trimmers ~1 cm above the soil surface. Within 1 day of collection, we sorted clip strips into five functional groups: graminoids, legumes, non-leguminous forbs, woody species, and bryophytes and dried them at 70°C for 48 h, before weighing the dry biomass with an accuracy of ±0.1 g. Forbs are defined as any herbaceous flowering plant other than a graminoid. We used both functional group-level estimates of biomass and summed values of ANPP in analyses, which were scaled by a factor of four to the standardised measure of $\mathrm{g\,m^{-2}}$. Due to smaller biomasses estimated for woody (1.65% of total biomass) and bryophyte groups (1.20% of total biomass), we only included graminoids, legumes and forbs in subsequent ANPP analyses.

Percentage cover data collection occurred before the peak of the growing season in each year, in mid to late June. We estimated the percentage cover of all vascular plant species in each quadrat. Because species overlapped spatially, percentage cover estimates exceed 100%. Species names follow the International Plant Names Index (IPNI, 2022).

We added environmental context and explored how biodiversity changes are influenced by local weather patterns using weather data from the National Environment Research Council (NERC) Environmental Change Network (Rennie et al., 2017). A meteorological station was present in the five-acre field within 100 m of all experimental quadrats. Raw meteorological data consisted of 16 weather variables, which were gathered at hourly intervals between 2016 and 2020, but data were not available in 2021. We used the mean hourly precipitation and temperature in the spring (21 March–20 June) and summer (21 June–22 September; the growing season) for each year of study as weather variables of interest.

2.4 | General analysis

We analysed the experimental data with hierarchical Bayesian regression models using the *brms* package (Bürkner, 2017) in R version 4.1.3 (R Core Team, 2022). To perform model selection, we estimated the out-of-sample predictive performance of candidate models relative to base models that excluded predictor variables of interest. For each candidate model, we performed leave-one-out cross-validation with the *loo* criterion and the expected log-wise predictive density (*elpd*, where Δ *elpd* gives the change in *elpd* relative to another explanatory model; Vehtari et al., 2017). Therefore, *elpd* gives an estimate of predictive performance that is analogous to an information criterion. Where two candidate models were comparable in *elpd* (Δ *elpd* <2), we reported the model with fewer explanatory variables and explored the posterior coefficients of the model to make inference. Models were run across four Markov chain Monte Carlo chains for 4000 iterations with 2000 warm-up iterations, and the convergence of the model across chains was assessed by inspecting $\hat{R}$ values, which assess the degree of mixing between chains (Bürkner, 2017). Model priors were selected using prior predictive simulations, where ranges of coefficient values were assessed for plausibility relative to prior distribution parameters. For the full set of model priors, please refer to model code (https://doi.org/10.5281/zenodo.8135588). Following model selection, we performed a set of Bayesian hypothesis tests (Bürkner, 2017) to investigate whether there were consistent differences in biodiversity and biomass measures between procedural control and ambient control treatments, and the proportion of variance explained by the random effect of experimental block. Differences between control treatments were evaluated by comparing posterior distributions between ambient control and procedural control groups. We used the intraclass correlation coefficients (ICC) (Nakagawa & Schielzeth, 2010) to assess the proportion of variance explained by the random effect of block relative to the total population-level variance.

2.5 | Testing the effect of precipitation on grassland productivity and its temporal stability

To answer our first key question, we analysed how precipitation manipulation influences ANPP and its temporal stability. We examined responses at both the whole community level and at the functional group level, as well as the interannual temporal stability of above-ground ANPP. We define the temporal stability of productivity as the inverse of the interannual coefficient of variation (CV) of above-ground ANPP (He et al., 2022) for each quadrat. Where the response variable was the annual total and group-level ANPP, raw ANPP values were transformed using the natural logarithm, which were then modelled using a Gaussian distribution (although similar results were obtained using a Gamma distribution; Figure S3). In the ANPP models, the key predictors of interest were precipitation treatment and observation year. We used model selection to test the predictive performance for a set of candidate models including precipitation treatment (categorical variable, four levels), a linear effect of the observation year (continuous variable, *z*-scored), an autoregressive term for the observation year (order = 1), and two-way interactions between precipitation treatment and observation year (linear). We investigated linear effects of observation year to capture broad changes in the community through time, but with insufficient temporal sampling to ascertain non-linear dynamics. Additional annual fluctuations were tested with effects of local environmental variables, and random effects of observation year. We compared candidate models to base models that excluded predictor variables of precipitation treatment and observation year. ANPP models estimated at the level of functional group also included terms for functional group (categorical variable, three levels). The full set of candidate models for each ANPP response variable is detailed in Tables S1–S3. We also included a categorical predictor term for the month of harvest (middle or later part of growing season).

In all models, we included a nested (hierarchical), intercept-only random effect of the precipitation treatment (four levels) within block (five levels) to account for the experimental structure of RainDrop, and an intercept-only random effect of observation year (six levels) to capture additional interannual variability. Nested intercept-only random effects were appropriate for this study due to the static spatial organisation of plots (each with one replicate of each treatment) within blocks in the current experiment (Figure 1a), for which we aimed to account for additional variance from the treatments x block interaction. Models of the stability of ANPP do not include temporal effects, because the metric captured interannual variability in biomass for each quadrat resulting in a single value per plot. Thus, for temporal stability models we tested a candidate model with the precipitation treatment to the base model with no predictor variables (Table S3). When analysing temporal stability of ANPP, we validated the patterns in stability by testing the log-linear relationship between variance in ANPP and mean ANPP for each plot using Pearson's correlation, to test the role of Taylor's Power Law in driving these effects (Reckling et al., 2021; Figure S5). In ANPP models, we used weakly informed normal priors of $N(3.5, 0.5)$ for the global intercept term and $N(0, 1)$ for predictor variables. The intercept-only random effects were fitted using exponential priors with rates between 4 and 8.

2.6 | Testing the resistance of grassland communities to precipitation treatments

We answered our second key question by investigating whether the grassland community was resistant to precipitation treatments in three ways: with broad diversity indices, using community composition and turnover, and exploring individual species contributions to community change. First, we explored how broad diversity indices at the quadrat level were influenced by the precipitation treatments using linear hierarchical mixed-effects model selection, in an identical procedure as described in Section 2.4. We calculated biodiversity indices using the relative proportions, *p*, of each species from percentage cover estimates. The three biodiversity indices included were vascular plant species richness, the Shannon–Weiner diversity index, $H = -\sum p \ln p$ (Shannon & Weaver, 1963), and the Simpson's diversity index, $D = \sum p^2$ (Simpson, 1949). For the Shannon–Weiner and Simpson's indices, response variables were z-scored (mean and variance centred on 0) for analyses and models were fitted using a Gaussian distribution. Models with species richness counts were fitted using a Poisson distribution and a log link. Shannon–Weiner and Simpson's models were fitted with regularising priors of $N(0, 0.5)$ for both intercept and predictor terms, and Richness models were fitted with a prior of $N(3, 0.25)$ for the intercept.

To assess how the grassland community composition varied between precipitation treatments, we tested community dissimilarity using non-metric multi-dimensional scaling (NMDS) implemented in the *vegan* package (Clarke, 1993; Oskanen et al., 2022). Community-level data consisted of species percentage cover data for each quadrat, which is a single treatment in a given plot each year. We fitted the NMDS using the Bray–Curtis dissimilarity index with a dimension of three, and up to 1000 random starts to reach convergence in stress values. Then, we compared the first two dominant NMDS axes between precipitation treatments. We used hierarchical linear mixed-effects models with a response variable of the NMDS axis scores, with model selection as described in Section 2.4. We fitted these models with regularising priors of $N(0,0.1)$ for both the global intercept and predictor variables. Then, in addition to the linear modelling framework, we explored statistical differences in Bray–Curtis similarities between precipitation treatments using analysis of similarities. Analysis of similarities tests differences in dissimilarity within sampling units compared with between sampling units (Oskanen et al., 2022). We also explored block-level community effects by testing the dissimilarity between experimental blocks by pooling data from the replicates within each block.

To further explore the drivers of community composition differences between sampling groups of precipitation treatments and observation years, we investigated which species were most important for community differences using Similarity Percentage analysis. Similarity Percentage is an extension of the Bray–Curtis dissimilarity measure for individual species within a community, for which the dissimilarity metric per species is averaged across pairs of sampling groups to give an overall contribution (standardised to sum to 1) of each species to differences for a set of sampling groups of interest (Clarke, 1993; Oskanen et al., 2022). We tested Similarity Percentage for the sampling groups of precipitation treatment, observation year and blocks. Then, following Similarity Percentage analysis, we explored variation in the relative abundance, p, of influential species between communities.

2.7 | Testing the temporal dynamics of the community and its drivers

We answered our third key question by exploring the temporal dynamics in the stability and resistance of the grassland community, and its potential climatic drivers. We estimated temporal trends in productivity, diversity indices and NMDS axes, as well as the similarities in floral communities between observation years. We extracted temporal trends from linear models of biodiversity specified in Section 2.5, which included linear or autoregressive effects of observation year, as well as two-way interactions between precipitation treatments and observation year. In addition to model selection, we also performed analysis of similarity tests for the NMDS communities between observation years and Similarity Percentage to identify influential species (Oskanen et al., 2022). Therefore, by assessing the temporal change in diversity indices and NMDS scores, we tested the temporal dynamics in this calcareous grassland. Furthermore, we explored which species had the largest changes in abundance over the study period, and thus which may have contributed most to temporal dynamics. For each species, we estimated the linear association between observation year and relative abundance, and explored which species had significant changes in relative abundance across plots.

Finally, to explore whether environmental drivers mediated temporal dynamics in the community, we tested how local weather variables influenced ANPP and biodiversity indices. Following the model selection framework in Section 2.4, we implemented linear models to investigate the impact of mean temperature and precipitation across the spring and summer-influenced biodiversity indices. In these models, linear terms for observation year were replaced with annual mean weather variables. We included both weather data for the current year and the previous year relative to biodiversity data collection, to test for current and lagged impacts of local weather on biodiversity differences.

3 | RESULTS

3.1 | Simulated drought reduces biomass production and its temporal stability

We found that simulated drought substantially reduced both above-ground ANPP and productivity's temporal stability (Figure 2). Across all observation years and experimental quadrats, there was a mean total ANPP of 303 ± 138 (S.D.) $\mathrm{g\,m^{-2}}$. Graminoids were the dominant functional group by biomass, with 63.6% of all biomass measured, compared with 20.4% for legumes and 16.0% for forbs. For total annual above-ground ANPP between 2016 and 2021, the model with the highest predictive performance, and fewest parameters, was the model including only the categorical effect precipitation treatment ($\Delta\,elpd = 5.03$ relative to the base model; Table S1). Total above-ground ANPP was substantially reduced in the drought treatment ($\beta = -0.77$ [−1.04; −0.49]; β coefficients give the posterior mean difference compared with the ambient control treatment on the log-scale, with the 95% credible intervals), with a mean total above-ground ANPP of 137 ± 156 (S.D.) $\mathrm{g\,m^{-2}}$ compared with 182 ± 137 (S.D.) $\mathrm{g\,m^{-2}}$ for the ambient control treatment (Figure 2a). Thus, compared with ambient conditions, the mean ANPP was reduced by 24.7% in the drought treatment. In contrast, irrigated plots did not have any substantial change in ANPP compared with the ambient control ($\beta = -0.10$ [−0.37; 0.18]; Figure 2a). We did not find evidence for either an overall linear temporal trend in

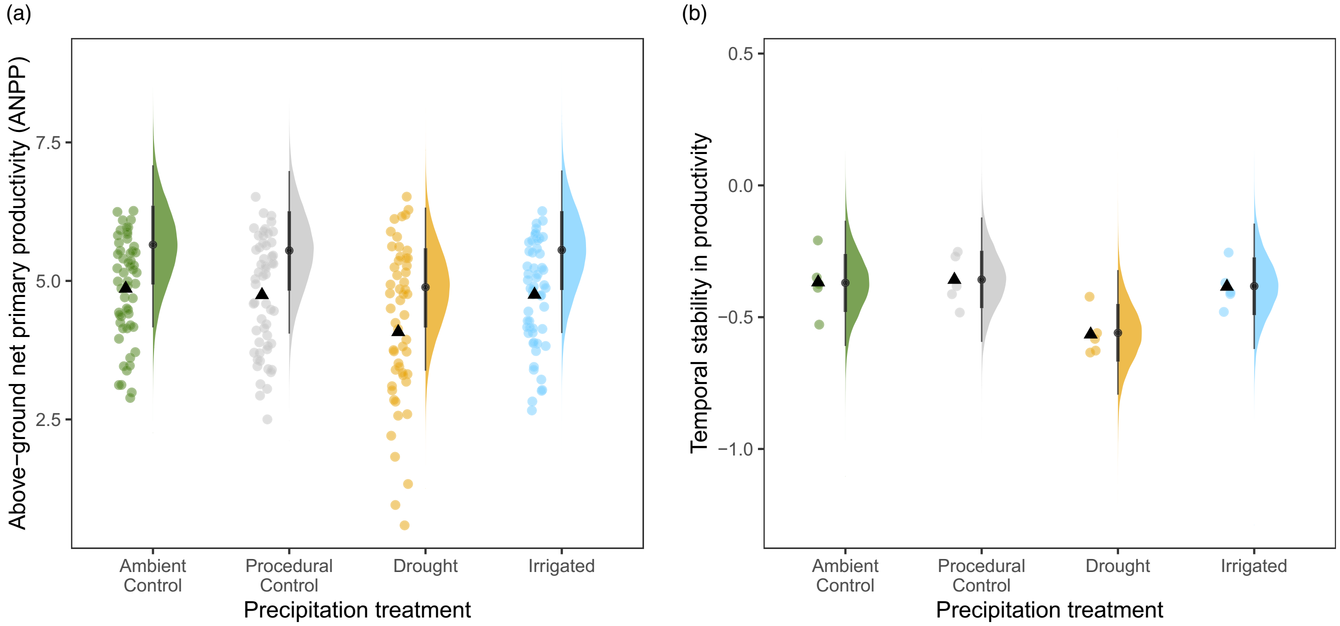

FIGURE 2 Drought reduces annual net primary productivity (ANPP) and its temporal stability. (a) Total above-ground ANPP with respect to precipitation treatments, where ANPP is the natural log-transformed above-ground biomass in $\mathrm{g\,m^{-2}}$. (b) Temporal stability in productivity (inverse of interannual coefficient of variation in biomass) with respect to precipitation treatment. Coloured points give raw data across blocks and years and black triangles give the mean total ANPP. There is a negative skew in raw ANPP data, which leads to reductions in mean ANPP values relative to predictions, but this skew did not impact model convergence or influence our findings (Figure S3). Distributions are derived from 8000 draws of the full posterior distribution including random effects, with probability density function boxplots giving the posterior mean and uncertainty.

ANPP, or for an interaction between observation year and treatment (Table S1; Figure S2). The model with the highest predictive performance included the linear effect of observation year, but the *elpd* was not substantially increased relative to the simpler model including only the effect of treatment (Table S1), and the raw data showed no clear temporal pattern in ANPP (Figure S2). There was a weak negative skew in raw ANPP values, but skew did not impact model convergence (Figure S3). Furthermore, we re-ran model selection using a gamma distribution, which did not change the qualitative results (Figure S3b). We observed a similar pattern for temporal stability in productivity, with a $\Delta\,elpd$ of 3.15 for the model including precipitation treatment compared with the base model (Table S3). Temporal stability in productivity was substantially reduced in the drought treatment ($\beta = -0.19$ [−0.32; −0.06]), equating to a 53.5% increase in the coefficient of variation of biomass (CV = 0.56) relative to the ambient control treatment (CV = 0.37) (Figure 2b). There were no discernible differences in temporal stability in productivity for both ambient control and procedural control treatments or the irrigation treatment (Figure 2b). Furthermore, we did not find evidence for a log-linear relationship between variance in ANPP and mean ANPP for each plot ($\rho = 0.42$, $p = 0.07$), increasing our confidence in our observation of reduced stability (Figure S5).

The reductions in overall ANPP were driven primarily by decreases in the biomass of graminoids and legumes (Table S2; Figure S4). Forbs did not exhibit ANPP reductions in the drought treatment ($\beta = -0.03$ [−0.46; 0.39]), but reductions were accentuated in both graminoids ($\beta = -1.00$ [−1.41; −0.59]) and legumes ($\beta = -0.87$ [−1.29; −0.46]) (Figure S4). Therefore, we observed mean reductions in ANPP of 36.1% and 36.4% for graminoids and legumes, respectively. Both mean total ANPP and group-level ANPP were strongly overlapping between ambient control and procedural control treatments ($\beta = 0.11$ [−0.17; 0.38], $\beta = 0.31$ [−0.11; 0.72]; differences in posterior means between ambient control and procedural control). Furthermore, we did not find substantial variance in total biomass between blocks (ICC or $\sigma_{block} = 0.01$ [0.00; 0.05]) although the ability of the design to estimate this variance component is limited given the limited replication of five blocks.

3.2 | Community diversity and composition were resistant to drought and irrigation

We found evidence that species composition in these calcareous grassland communities is resistant to precipitation treatments, both in terms of broad diversity indices and community composition (Figure 3). For species richness, the Shannon–Weiner index and the Simpson's index, we did not observe differences in indices between precipitation treatments (Figure 3a–c). For all three indices, the $\Delta\,elpd$ compared with the base model was below 0.6, indicating no clear association between the indices and precipitation treatments, so we retained models excluding precipitation treatment

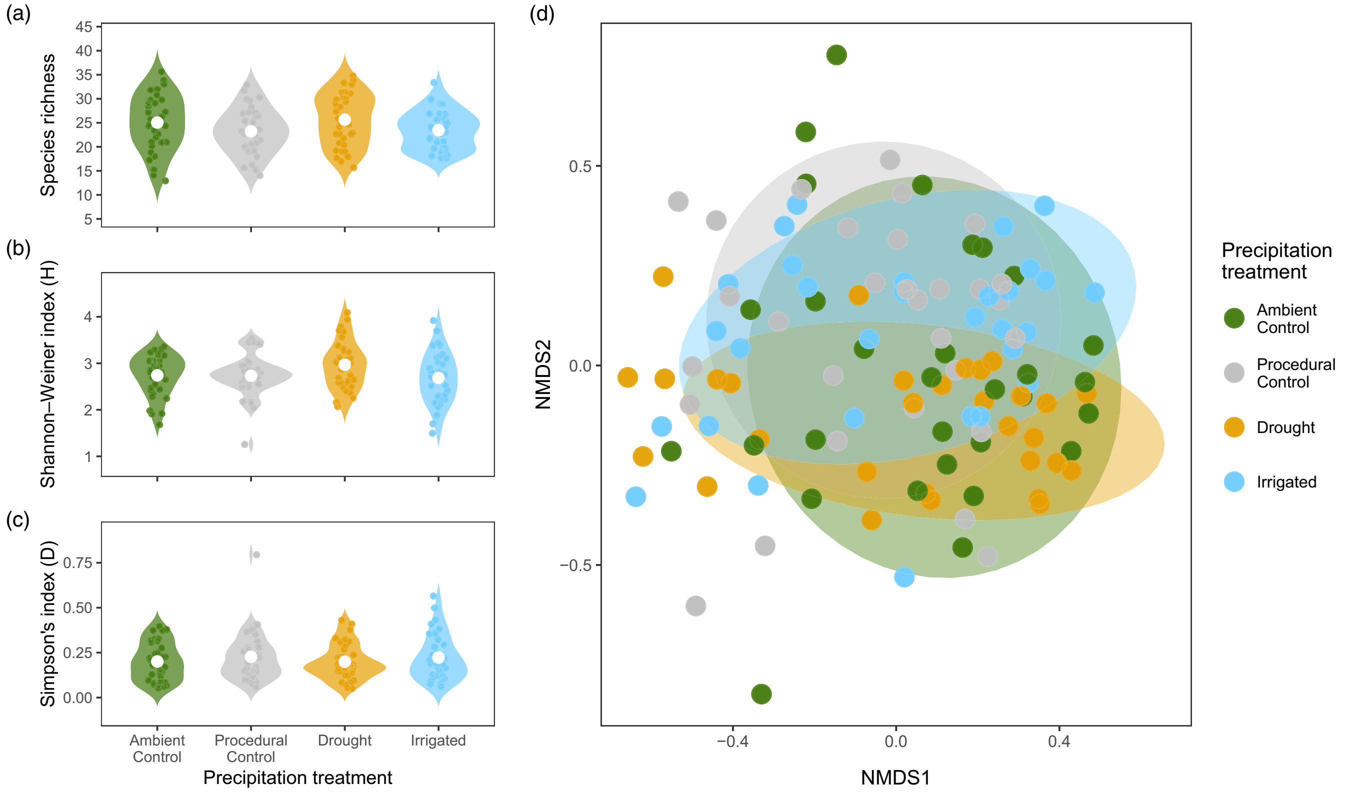

FIGURE 3 Calcareous grassland community diversity is resistant to precipitation manipulations. (a–c) Raw data distributions for species richness (a), Shannon–Weiner index (b) and Simpson's index (c) with respect to precipitation treatment. Coloured points indicate raw data, and violins give an estimate of data density across each index. White points indicate mean biodiversity index values. (d) Non-metric multi-dimensional scaling (NMDS) results for community composition, where the first two axes (NMDS1 and NMDS2) are displayed with respect to precipitation treatments. Ellipses are the 80% two-dimensional quantiles of the NMDS axes.

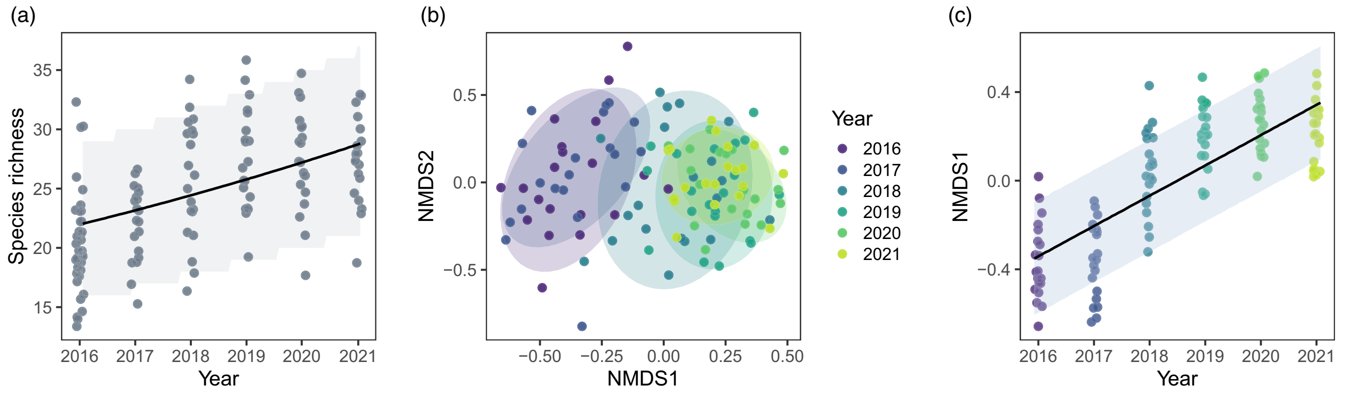

FIGURE 4 Changes in the grassland diversity and community composition 2016–2021. (a) Increases in species richness between 2016 and 2021. Grey points give raw observations of species richness for each quadrat in each treatment. Solid line indicates the posterior mean prediction with 90% credible intervals, which include random effects. (b) Non-metric multi-dimensional scaling (NMDS) results for community composition, where the first two axes (NMDS1 & NMDS2) are displayed with respect to observation year. Ellipses are the 80% two-dimensional quantiles of the NMDS axes. (c) Increases in NMDS1 over the observation period. Points give observed NMDS1 scores for each quadrat for a given treatment and block across the study period. Solid black line indicates the posterior mean, with 90% credible interval.

(Tables S4–S6). Similarly to ANPP, there were no clear differences in ambient control and procedural control treatments for richness, Shannon–Weiner index or Simpson's index. Furthermore, the mean posterior block-level variance (ICC or σ_{block}) was below 0.03 for all indices.

Community composition was also not clearly associated with the precipitation treatments. There was no clear association between NMDS axes one and two (NMDS1 and NMDS2) and precipitation treatment (Figure 3d; Figure S8), or between precipitation treatments and NMDS3 (Figure S8). A lack of community composition differences between treatments was further supported by the analysis of similarities, for which within-sample dissimilarity was broadly comparable to dissimilarity between samples (Figure S9; marginally significant relationship).

3.3 | Shifts in community composition and richness increase across the study period

Instead of an effect of precipitation treatment, we found evidence for overall temporal changes in the calcareous grassland communities, with increases in species richness and changes in community composition (Figure 4). However, these changes were not affected by the precipitation treatments. We recorded a total of 109 vascular plant species between 2016 and 2021 across all plots. Across all plots the annual total number of species recorded did not change substantially, ranging between 54 (2017) and 64 (2019). At the quadrat level, species richness ranged between 13 and 36 species m^{-2} with a mean of 24.4 ± 5.14 (S.D.) species. We found a positive association between species richness and observation year, such that there were increases in richness over the study period (Figure 4a; $\beta = 0.10$ [0.04; 0.16]). Overall, between 2016 and 2021, there was an increase in mean richness at the plot level from 20.3 to 27.4 species (Figure 4a). Furthermore, we found a strong association between community composition and observation year (Figure 4b). The analysis of similarities indicated a large difference in within-year community differences compared with between-year community differences (Figure S9). Furthermore, we observed a strong positive association between NMDS1 and observation year, which was a substantially better predictive model compared with the base model ($\Delta\, elpd = 58.4$). There was a consistent increase in NMDS1 over the study period ($\beta = 0.23$ [0.20; 0.27]), indicating a shift in community composition (Figure 4c).

In spite of observed temporal trends, we did not observe an association between productivity and average interannual weather conditions. Average spring and summer weather conditions were not strongly associated with any of the biodiversity indices or ANPP (Figures S6 and S7). Finally, we explored which species had the largest impact on community change across the years. Four species had significant positive temporal trends in abundance across plots (*Bromus commutatus*, *Medicago lupulina*, *Trisetum flavescens* and *Viola hirta*; Figure S10), but generally species richness increases at the plot level were not repeatedly associated with a specific group of species. We then used similarity percentages across species to investigate the species that were most influential in driving differences in community dissimilarity between years. Ten species had mean percentage contributions to dissimilarity above 2% (with *Potentilla reptans* lying on the boundary), of which four were graminoids, four were legumes and one was a forb (Figure 5a). Of these species however, *Arrhenatherum elatius* (graminoid), *Brachypodium pinnatum* (graminoid) and *Lotus corniculatus* (legume) had mean contributions of over 5% (median >6.5%) to community dissimilarity across years (Figure 5a,b). These three species were common, and varied substantially across the study period, with large interannual shifts, but displayed no clear pattern in relative abundance through time or with treatments (Figure 5b). In particular *Arrhenatherum elatius*, which is a common and dominant species, varied between an average abundance of 13.3% (relative abundance = 0.133) across all quadrats in 2018 to 2.3% in 2021 (Figure 5b).

4 | DISCUSSION

From our 6-year study of precipitation manipulation in a calcareous grassland, we show that simulated drought reduced productivity and its temporal stability, but that species diversity and composition were generally resistant. These results add to global findings of decreased grassland productivity with drought (Kröel-Dulay et al., 2022; Smith et al., 2024), but we add a key perspective that the reductions in productivity are coupled with reduced temporal stability, and an accentuated pattern in graminoids and legumes. Despite accentuated productivity loss in graminoids and legumes, we find that overall community composition in this calcareous grassland was resistant to 6 years of drought conditions, a finding consistent with other studies on calcareous grasslands (Craine et al., 2013; Grime et al., 2000, 2008). Increases in precipitation were not associated with any change in productivity or community composition, which may indicate a lack of efficacy in the irrigation treatment. Our study emphasises the need to explore detailed temporal dynamics in grassland communities alongside changing precipitation patterns, to understand how climate change will impact these vital ecosystems and the services they provide.

In the present study, experimental drought reduced the productivity of the calcareous grassland habitat and its interannual temporal stability, with 25% reductions in above-ground ANPP, and a 54% increase in productivity's coefficient of variation. Furthermore, despite a strong overall effect, reductions in productivity were driven primarily by losses in graminoid and legume biomass, but forb biomass did not change substantially. Productivity losses are concerning given calcareous grasslands are important ecosystems that are already vulnerable to other human impacts (Maddock, 2008). Reductions in primary productivity and biomass in response to drought have been widely reported in plant communities globally (Haddad et al., 2002; Kröel-Dulay et al., 2022; Smith et al., 2024). Reduction in productivity may be driven by water limitation, altered patterns of nutrient cycling following precipitation extremes (Haddad et al., 2002), or a diversion in the allocation of resources from survival to reproduction (Weißhuhn et al., 2011). A lack of biomass reduction in the forbs may be a result of deeper rooting depth in forbs relative to graminoids and legumes increasing access to water (Sydes & Grime, 1984), or increased water limitation relative to nitrogen limitation in legumes. Kröel-Dulay et al. (2022) found that experimental manipulations of precipitation often underestimate the impact of drought on biomass, failing to capture other abiotic processes that are associated with drought in real-world settings, including the interaction between drought timing and plant growth

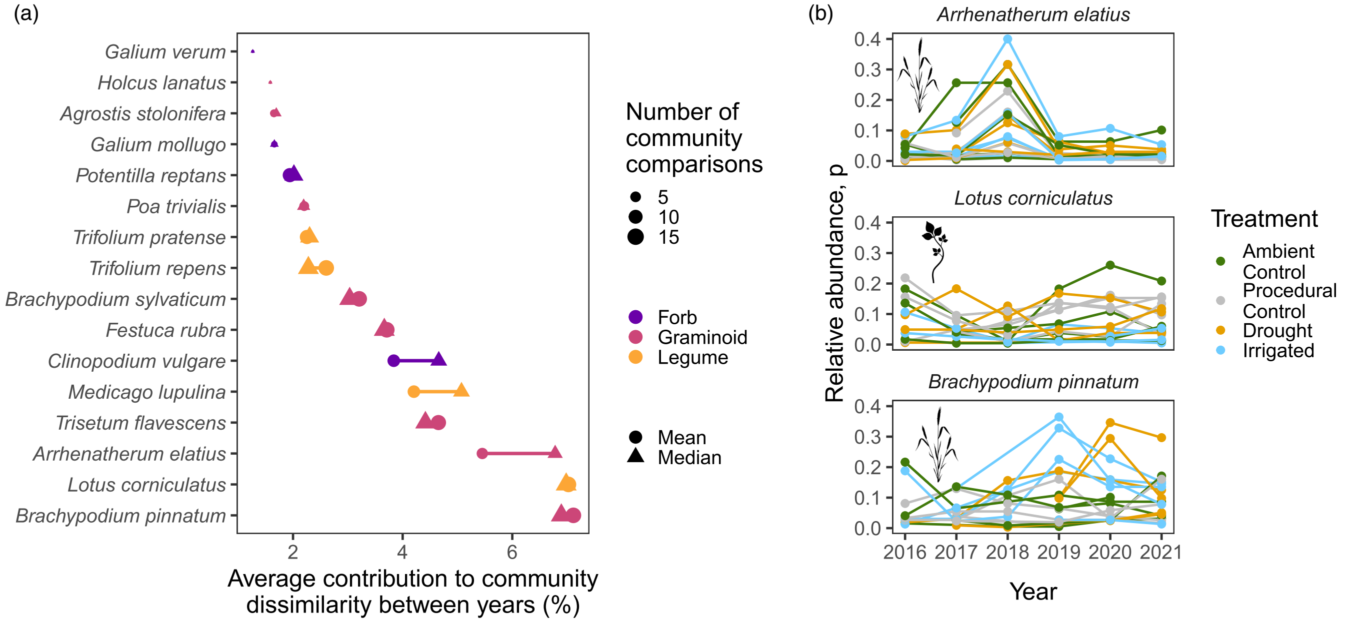

FIGURE 5 Commonly occurring graminoid and legume species drive community dissimilarity. (a) Similarity percentage results for species driving community dissimilarity across years. Only 16 species (of 109) with the highest contributions are shown. Circles give mean per cent contribution, and triangles mean per cent contribution, with the size of the point indicating the number of community comparisons and the colour denoting the functional group. (b) Change in relative abundance across years for three species with high contribution to dissimilarity across years. Points and lines give raw estimates of relative abundance in each quadrat for each species, indicating high variability in the relative abundance of the three most influential species across the study.

(Felton & Goldsmith, 2023). Furthermore, this calcareous grassland is a mesic system with moderate levels of annual precipitation, and productivity losses are likely to be amplified in more arid environments (Huxman et al., 2004; Moran et al., 2014). Ultimately, reduced precipitation threatens grassland productivity world-wide, and thus many ecosystem services that grasslands provide.

In addition to changes in mean productivity, drought also decreased the temporal stability by increasing the variance of productivity. Measures of stability in abundance (here biomass) can provide comparable metrics that forecast broader community-level changes as early warning signals (Clements & Ozgul, 2016; Wu et al., 2020). While previous studies have explored stability metrics in grasslands over short timescales (He et al., 2022), we add an important longer-term perspective to the narrative of grassland stability with changing precipitation patterns. Reduced stability could be the result of resource limitation (drought) increasing dependence on natural variation in precipitation, where productivity mediated through survival and reproduction is more reactive to limited precipitation levels (Grime et al., 2008; Weißhuhn et al., 2011). Worryingly, the early warning signal of reduced stability in productivity is supported by below-ground data from the calcareous grassland in Buxton, UK, where the seedbanks are highly altered by drought conditions, which may forecast rapid future community change (Basto et al., 2018). Further studies explicitly exploring metrics of community stability with respect to climate change in plant communities will provide valuable forecasts of the changing state of our ecosystems.

Importantly, over 6 years the diversity and composition of communities in this calcareous grassland were largely resistant to drought, despite the group-specific declines in the productivity of grasses and legumes. It is currently unclear why the composition metrics were largely unaffected by the productivity declines in grasses and legumes. Key findings from other long-term studies on calcareous grasslands support the composition resistance findings (Craine et al., 2013; Grime et al., 2000, 2008), but we add further temporal dynamics and investigation at the species level. We hypothesise that the grassland resistance could be the result of two main processes: (i) the maintenance of drought resistance through increased species and functional diversity over time, and (ii) insufficient time for precipitation change to influence the community, that is, lagged effects. There are clear global ecological signals that higher species diversity in linked to high stability (Craven et al., 2018; Hector et al., 2010). Importantly, resistance to climate extremes can also be compromised by reduced species diversity (Isbell et al., 2015). At the individual level, plant resistance is linked to functional traits such as leaf economic traits and root thickness (Anderegg et al., 2016; Tucker et al., 2011). At the community level, increased functional diversity, which is linked to species diversity, can stabilise grasslands after drought (Griffin-Nolan et al., 2019). Therefore, we hypothesise that the relatively high biodiversity of calcareous grasslands (Isbell et al., 2015), creates functional redundancy and therefore buffers the community to environmental change. These patterns may also be linked with wider climatic conditions, where arid grasslands have slower functional recovery from drought compared with mesic grasslands (Moran et al., 2014). Diversity in species and functional traits coupled with climatic variability across habitats may therefore explain why some grassland communities have responses to precipitation extremes (Harrison et al., 2015), and others do not (Grime et al., 2008). Long-term studies investigating coupled functional and community responses to precipitation extremes will be crucial in teasing apart these patterns.

Our observations of resistance may also be explained by temporal factors. Interestingly, despite group-specific reductions in biomass for graminoids and forbs, these changes were not mirrored in community composition from percentage cover. However, the group-specific results for biomass may indicate that there will be shifts in community composition in the future, especially when there is mortality and turnover in perennial species. Understanding links between productivity and community composition, either through functional redundancy or species turnover and mortality, is a vital next step in this system. Furthermore, compared with some other grassland habitats, these calcareous grasslands have many perennial species that survive for several years. From 14 years of data from a calcareous grassland in the north of England, there was temporal drift in the community but overall resistance, which may also have been linked to longer-living species surviving but reacting in growth to interannual variation in the climate (Grime et al., 2008). A delayed turnover is also supported by the seed bank, which had strong responses to drought conditions in the same system, suggesting that subsequent generations in the community will have lasting impacts of drought (Basto et al., 2018). Therefore, our findings do not rule out long-term impacts from climate change on grassland communities, which may occur abruptly when tolerance limits are reached (Trisos et al., 2020), or in synergy with other drivers such as habitat fragmentation (Brook et al., 2008; Klaus et al., 2021).

In addition to community resistance to drought, we found that neither productivity or community composition responded to irrigation in this calcareous grassland. Given that soil moisture content at the end of the growing season was not substantially increased in the irrigation treatment, the resistance of the community to irrigation could indicate failure in the irrigation treatment to successfully increase soil moisture levels on the freely draining soil and underlying bedrock. Although failure of the irrigation treatment is a likely explanation, the readings of soil moisture were taken during the late summer when precipitation is typically lower, and so further study of the irrigation treatment's efficacy is needed. Furthermore, community resistance to irrigation is a finding that has been replicated across several grassland irrigation experiments globally (DeMalach et al., 2017). Nevertheless, in other systems precipitation increases have been associated with community and functional changes, also in conjunction with warming (Collins et al., 2022; Kimmel et al., 2019). Therefore, resistance to precipitation could also reflect an insufficiency in the extremity of the precipitation addition (Smith et al., 2024), and further study of the efficacy of precipitation manipulation is needed.

Our final key finding was a general (treatment-independent) temporal change in the community, where richness increased by over 7 species m^{-2} between 2016 and 2021 and a shift in community composition. The addition of the temporal dynamics in the community was one key advantage of the current study, and future work should focus on long-term temporal patterns in community change in response to precipitation extremes. The most likely explanation is that temporal changes indicate ongoing secondary succession following the cessation of agriculture in ~1980 and sheep grazing before the experiment, with both long-term recovery and community change following grazing. An earlier climate change experiment that compared responses of a calcareous grassland at Harpur Hill in Buxton with those of the Wytham Upper Seeds found grassland diversity and composition was more stable in the established pasture at Buxton compared with the larger changes seen in Upper Seeds that the authors attribute to its secondary successional state (Grime et al., 2000, 2008).

4.1 | Caveats

There are several factors that we did not control in the current experiment that have the potential to impact community responses to precipitation extremes. First, while it is unlikely that the temporal dynamics observed are purely successional change after agriculture, grazing at the site occurring up to the start of the experiment, and other experiments at the site may have impacted the current community (Gibson & Brown, 1991; Grime et al., 2000). A switch from grazing to mowing at the start of the experiment may be responsible for the current temporal shift in the community and increase in species richness. Second, is the role of spatial dynamics and immigration from other grasslands in the area, or meta-community dynamics, which we did not control in the current study (Furey et al., 2022). Immigration can maintain high levels of local species diversity in plant communities (Loreau & Mouquet, 1999), and while out of the scope of the current study, immigration may also have an impact, particularly on temporal dynamics. However, Upper Seeds is relatively isolated from other nearby areas of calcareous grassland. Third, the role of microclimate on community change, which has been demonstrated as an important mediator in grassland drought responses (Mazalla et al., 2022). The drought treatment shelters in particular may have influenced light availability, and created irregular patterns of rainfall (when combined with wind) that influenced the community. However, we controlled for these structural effects using a procedural control, which is rarely available in precipitation manipulation experiments, and a replicated block design, and found no detectible differences between procedural control and ambient control plots.

5 | CONCLUSION

The long-term monitoring of calcareous grassland communities with respect to experimentally controlled precipitation change can reveal detailed community responses, which are not available from snapshots of the community. Measuring and predicting the impact of climate change on plant communities can be facilitated by the approach taken here that combines long-term data on community composition and productivity. Future work should combine community and productivity data with information on functional traits and the corresponding below-ground responses.

AUTHOR CONTRIBUTIONS

This study was established by Kadmiel Maseyk and Andrew Hector. PI support and resources were provided by Andrew Hector, Roberto Salguero-Gómez, Kadmiel Maseyk, Nick Hawes and Clare S. Lawson. Data were collected primarily by Clare S. Lawson and Andrew Hector, with contributions to data collection from all authors. John Jackson performed data analysis and exploration, with support from Sara L. Middleton, Andrew Hector and Roberto Salguero-Gómez. John Jackson wrote the initial draft with support from Sara L. Middleton, Andrew Hector and Roberto Salguero-Gómez. Critical manuscript feedback and approval were provided by all authors.

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CONFLICT OF INTEREST STATEMENT

Andrew Hector is an editor of the *Journal of Ecology*, but took no part in the peer review and decision-making processes for this paper.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14282.

DATA AVAILABILITY STATEMENT

All code, output and data used in the current study are archived using the Zenodo repository: https://doi.org/10.5281/zenodo.8135588 (Jackson et al., 2023), which were created from the following GitHub repository: https://github.com/jjackson-eco/raindrop_biodiversity_analysis.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1: Preliminary results of soil moisture differences.

Figure S2: No temporal trend in ANPP.

Figure S3: Exploring skew in ANPP.

Figure S4: Primary productivity separated by functional group.
Figure S5: The log-linear relationship between temporal stability in ANPP and mean ANPP.
Table S1: Model selection for ANPP.
Table S2: Model selection for group-level ANPP.
Table S3: Model selection for temporal stability of productivity.
Table S4: Model selection for the Shannon-Weiner index.
Table S5: Model selection for the Simpson's index.
Table S6: Model selection for species richness.
Table S7: Model selection for NMDS axis 1.
Table S8: Model selection for NMDS axis 2.
Table S9: Model selection for NMDS axis 3.
Figure S6: Weather effects on ANPP.
Figure S7: Weather effects on biodiversity.
Figure S8: NMDS precipitation treatment effects.
Figure S9: Analysis of community similarities.
Figure S10: Significant temporal trends in relative abundance for four species.

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Tools of the trade

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Automating image segmentation for vegetation monitoring

Plants represent a major component of the biosphere, influencing the flow of energy, carbon and water within the Earth system. Human-induced global environmental change is rapidly altering the form and function of vegetation from cells to canopies, with implications for the integrity of the Earth system. Therefore, vegetation monitoring is crucial, to quantify impacts, determine baselines and guide potential management options. Image capturing devices, such as satellites, drones, portable scanners and smartphones are widely used by plant scientists to monitor vegetation. However, processing these images to identify meaningful objects can be time and labour-intensive.

Open-source software can speed up image processing through automated image segmentation. Object-based segmentation identifies regions of interest by delineating meaningful groups of pixels, representing features such as plant organs or tree crowns, from each digital image. There are numerous open-source software packages for segmentation analysis available. Python's OpenCV package is a popular choice because Python is a flexible and user-friendly language. OpenCV contains algorithms to perform tasks such as cropping, perspective transformations, edge detection and thresholding (binarisation) that enable users to perform automated segmentation analyses. Perspective transformations (such as distortion correction) are particularly useful when the positioning of the equipment used to capture the image is not ideal such as in resource-limited or time-sensitive fieldwork. After segmentation processing, attributes such as the area and minimum and maximum pixel values of regions of interest can be extracted for subsequent modelling of vegetation data. For example, changes in pixel values can be used to monitor long-term tropical forest structure or detect physiological stress in plant tissues.

Image-processing tools such as OpenCV can be widely applied to many areas of vegetation science. For example, OpenCV has been used to extract digitised plant size data from drawings made in the field, to examine demographic trends of grassland species under experimentally manipulated rainfall conditions. Advances in artificial intelligence computer vision will lead to models that can be pretrained on large and diverse datasets, which will further reduce the user input and computing resources required for image processing. The use of such tools to speed up image-based vegetation monitoring could help to address many of the challenges associated with environmental change such as deforestation and drought.

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Competing interests

The author declares no competing interests.

Additional information

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