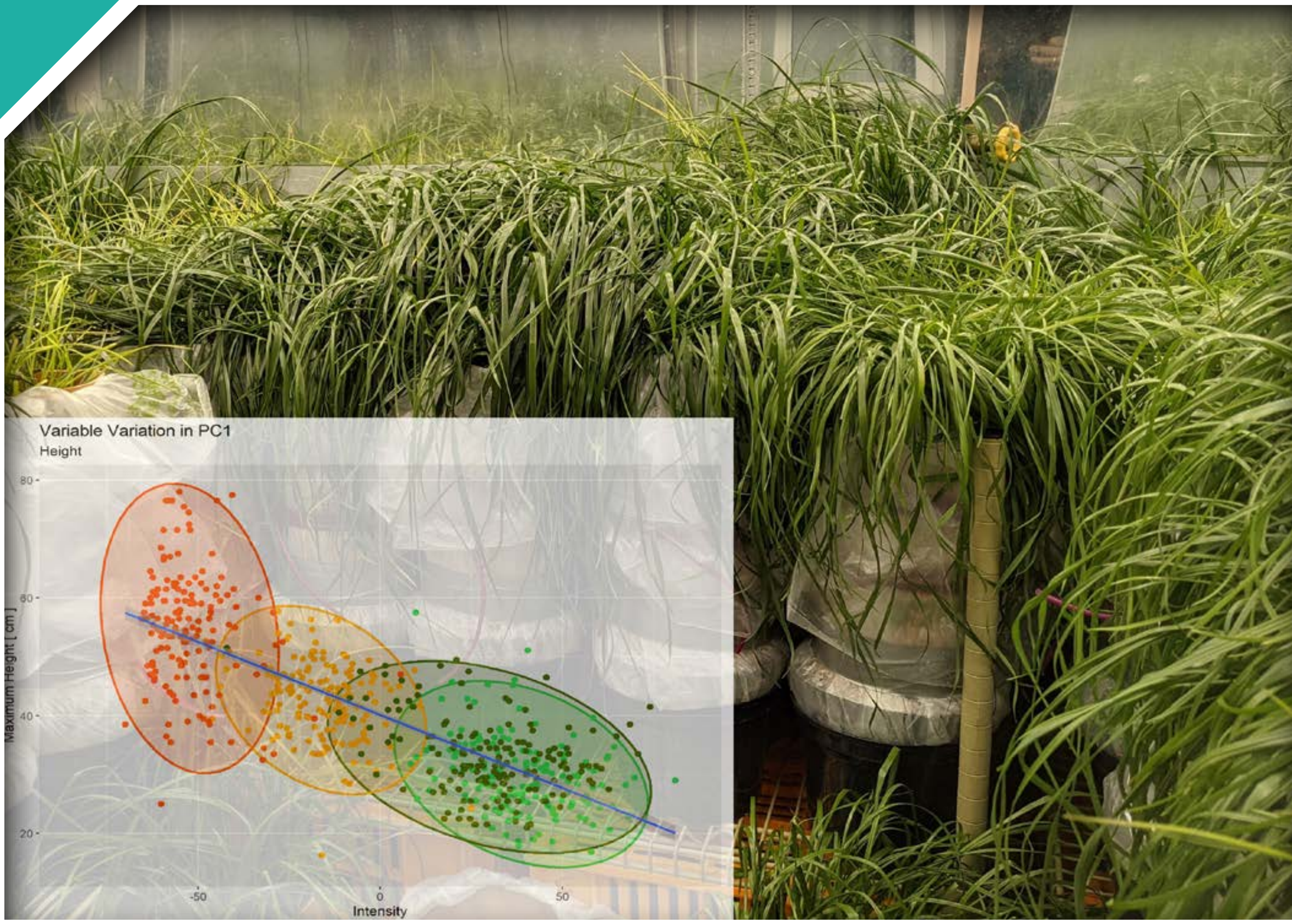
Report No.437

epaResearch

PHENOGRASS – The Phenology of Perennial Ryegrass and its Potential Contribution to Grassland Carbon Sequestration

Authors: Jonathan Yearsley, Rainer Melzer and Carl Frisk



www.epa.ie

Rialtas na hÉireann
Government of Ireland

Environmental Protection Agency

The EPA is responsible for protecting and improving the environment as a valuable asset for the people of Ireland. We are committed to protecting people and the environment from the harmful effects of radiation and pollution.

The work of the EPA can be divided into three main areas:

Regulation: Implementing regulation and environmental compliance systems to deliver good environmental outcomes and target those who don't comply.

Knowledge: Providing high quality, targeted and timely environmental data, information and assessment to inform decision making.

Advocacy: Working with others to advocate for a clean, productive and well protected environment and for sustainable environmental practices.

Our Responsibilities Include:

Licensing

- Large-scale industrial, waste and petrol storage activities;
- Urban waste water discharges;
- The contained use and controlled release of Genetically Modified Organisms;
- Sources of ionising radiation;
- Greenhouse gas emissions from industry and aviation through the EU Emissions Trading Scheme.

National Environmental Enforcement

- Audit and inspection of EPA licensed facilities;
- Drive the implementation of best practice in regulated activities and facilities;
- Oversee local authority responsibilities for environmental protection;
- Regulate the quality of public drinking water and enforce urban waste water discharge authorisations;
- Assess and report on public and private drinking water quality;
- Coordinate a network of public service organisations to support action against environmental crime;
- Prosecute those who flout environmental law and damage the environment.

Waste Management and Chemicals in the Environment

- Implement and enforce waste regulations including national enforcement issues;
- Prepare and publish national waste statistics and the National Hazardous Waste Management Plan;
- Develop and implement the National Waste Prevention Programme;
- Implement and report on legislation on the control of chemicals in the environment.

Water Management

- Engage with national and regional governance and operational structures to implement the Water Framework Directive;
- Monitor, assess and report on the quality of rivers, lakes, transitional and coastal waters, bathing waters and groundwaters, and measurement of water levels and river flows.

Climate Science & Climate Change

- Publish Ireland's greenhouse gas emission inventories and projections;
- Provide the Secretariat to the Climate Change Advisory Council and support to the National Dialogue on Climate Action;
- Support National, EU and UN Climate Science and Policy development activities.

Environmental Monitoring & Assessment

- Design and implement national environmental monitoring systems: technology, data management, analysis and forecasting;
- Produce the State of Ireland's Environment and Indicator Reports;
- Monitor air quality and implement the EU Clean Air for Europe Directive, the Convention on Long Range Transboundary Air Pollution, and the National Emissions Ceiling Directive;
- Oversee the implementation of the Environmental Noise Directive;
- Assess the impact of proposed plans and programmes on the Irish environment.

Environmental Research and Development

- Coordinate and fund national environmental research activity to identify pressures, inform policy and provide solutions;
- Collaborate with national and EU environmental research activity.

Radiological Protection

- Monitoring radiation levels and assess public exposure to ionising radiation and electromagnetic fields;
- Assist in developing national plans for emergencies arising from nuclear accidents;
- Monitor developments abroad relating to nuclear installations and radiological safety;
- Provide, or oversee the provision of, specialist radiation protection services.

Guidance, Awareness Raising, and Accessible Information

- Provide independent evidence-based reporting, advice and guidance to Government, industry and the public on environmental and radiological protection topics;
- Promote the link between health and wellbeing, the economy and a clean environment;
- Promote environmental awareness including supporting behaviours for resource efficiency and climate transition;
- Promote radon testing in homes and workplaces and encourage remediation where necessary.

Partnership and Networking

- Work with international and national agencies, regional and local authorities, non-governmental organisations, representative bodies and government departments to deliver environmental and radiological protection, research coordination and science-based decision making.

Management and Structure of the EPA

The EPA is managed by a full time Board, consisting of a Director General and five Directors. The work is carried out across five Offices:

1. Office of Environmental Sustainability
2. Office of Environmental Enforcement
3. Office of Evidence and Assessment
4. Office of Radiation Protection and Environmental Monitoring
5. Office of Communications and Corporate Services

The EPA is assisted by advisory committees who meet regularly to discuss issues of concern and provide advice to the Board.

PHENOGRASS – The Phenology of Perennial Ryegrass and its Potential Contribution to Grassland Carbon Sequestration

Authors: Jonathan Yearsley, Rainer Melzer and Carl Frisk

Identifying pressures

Agricultural grasslands are a crucial part of Ireland's agroeconomy. Within these grasslands, perennial ryegrass (*Lolium perenne*) is the dominant species and underpins much of their performance, of which the timing of growth (i.e. the phenology of grasslands) is an important aspect. A late start or an early end to the growing season requires grazing livestock to be housed for longer, which in turn requires farmers to have additional reserves of forage. These reserves also depend on the length of the grass growing season. Seasons with exceptionally late starts and those with poor forage harvests have been major contributory factors to past fodder crises, such as the 2013 fodder crisis, with broad consequences for the economy, animal welfare and human well-being.

Informing policy

It is imperative to ask: how will future climate conditions, including increases in temperature and CO_2 and the increasing possibility of extreme weather events such as flooding, impact the performance of perennial ryegrass?

Ryegrass was found to be very resilient to increased temperatures and CO_2 concentrations. Biomass accumulation and leaf appearance did not change substantially under modelled future climatic conditions. We observed a small positive effect on ryegrass growth towards the end of the growing season in September under elevated temperatures, leading to a slight lengthening of the growing season.

However, waterlogging had a far more dramatic effect on biomass accumulation and leaf appearance. All cultivars tested showed a drastically reduced performance when waterlogged.

Taken together, our study shows that small beneficial effects resulting from increases in temperature and CO_2 cannot compensate for yield losses due to severe flooding events.

Developing solutions

We were able to study ryegrass performance under future climatic conditions with great precision using growth chambers that allowed targeted manipulation of temperature and CO_2 levels. We also included data from satellite images of Irish ryegrass pastures in our analysis. Satellite imagery provides data on every part of Ireland and could contribute to national monitoring schemes.

Future breeding programmes should focus on waterlogging resilience as an important trait. Some small gains in yield might be possible if the impact of extreme weather conditions such as flooding can be mitigated. Substantial yield gains due to increased temperatures and CO_2 concentrations should not be expected.

Satellite remote-sensing data allows current and past grassland phenology in Ireland to be quantified across Ireland at a relatively low cost and with high precision. Work is needed to validate approaches with ground-based phenological observations and to link estimated phenology with environmental variables.

EPA RESEARCH PROGRAMME 2021–2030

PHENOGRASS – The Phenology of Perennial Ryegrass and its Potential Contribution to Grassland Carbon Sequestration

(2018-CCRP-MS.52)

EPA Research Report

Prepared for the Environmental Protection Agency

by

University College Dublin

Authors:

Jonathan Yearsley, Rainer Melzer and Carl Frisk

ENVIRONMENTAL PROTECTION AGENCY
An Ghníomhaireacht um Chaomhnú Comhshaoil
PO Box 3000, Johnstown Castle, Co. Wexford, Ireland

Telephone: +353 53 916 0600 Fax: +353 53 916 0699
Email: info@epa.ie Website: www.epa.ie

ACKNOWLEDGEMENTS

This report is published as part of the EPA Research Programme 2021–2030. The EPA Research Programme is a Government of Ireland initiative funded by the Department of the Environment, Climate and Communications. It is administered by the Environmental Protection Agency, which has the statutory function of co-ordinating and promoting environmental research.

The authors would like to acknowledge the members of the project steering committee, namely Gerry Brady (Central Statistics Office), David Dodd (Dublin Climate Action Regional Office), Emily Gleeson (Met Éireann), Colin Kelleher (National Botanic Gardens), Frank McGovern (Environmental Protection Agency), John Muldowney, Philip Blackwell and Liam Brennan (Department of Agriculture, Food and the Marine), Aoife Ní Bhroin (Met Éireann) and Seán O'Leary (Environmental Protection Agency); also Oonagh Monahan (Research Project Manager on behalf of the EPA).

The authors also thank Astrid Wingler, Fiona Cawkwell, Paul Holloway and Gourav Misra (University College Cork) from the PhenoClimate project (2018-CCRP-MS.54) for a fruitful collaboration on outreach and on reviving phenological research in Ireland. In addition we thank Susanne Barth (Teagasc) for advice on *Lolium perenne* experimental protocols.

DISCLAIMER

Although every effort has been made to ensure the accuracy of the material contained in this publication, complete accuracy cannot be guaranteed. The Environmental Protection Agency, the authors and the steering committee members do not accept any responsibility whatsoever for loss or damage occasioned, or claimed to have been occasioned, in part or in full, as a consequence of any person acting, or refraining from acting, as a result of a matter contained in this publication. All or part of this publication may be reproduced without further permission, provided the source is acknowledged.

This report is based on research carried out/data from June 2019 to April 2022. More recent data may have become available since the research was completed.

The EPA Research Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

EPA RESEARCH PROGRAMME 2021–2030
Published by the Environmental Protection Agency, Ireland

ISBN: ISBN- 978-1-80009-118-4 September 2023

Price: Free Online version

Project Partners

Dr Jonathan Yearsley
School of Biology and Environmental Science
& UCD Earth Institute
University College Dublin
Dublin 4
Ireland
Tel.: +353 (0)1 716 2265
Email: Jon.Yearsley@ucd.ie

Dr Rainer Melzer
School of Biology and Environmental Science
& UCD Earth Institute
University College Dublin
Dublin 4
Ireland

Dr Carl Frisk
School of Biology and Environmental Science
University College Dublin
Dublin 4
Ireland

Professor Bruce Osborne
School of Biology and Environmental Science
University College Dublin
Dublin 4
Ireland

Dr Stuart Green
Teagasc
Rural Economy & Development Programme
Dublin
Ireland

Dr Hannah White
School of Life Sciences
Anglia Ruskin University
Cambridge
United Kingdom

Dr Charilaos Yiotis
Department of Biological Applications and
Technologies
University of Ioannina
Ioannina
Greece

Dr Maurício Cruz Mantoani
Institute of Astronomy, Geophysics and
Atmospheric Sciences
University of São Paulo
São Paulo
Brazil

Contents

List of Figures

List of Tables

Executive Summary

Improved agricultural grasslands are an important part of Ireland's agroeconomy. Within these grasslands, perennial ryegrass (*Lolium perenne*) is the dominant species and underpins much of their performance. An important aspect of grassland performance is the timing of the growth (i.e. the phenology of grasslands). For example, a late start or an early end to the growing season requires grazing livestock to be housed for longer, which in turn requires farmers to have additional reserves of forage. Forage reserves also depend on the length of the grass growing season. Years with exceptionally late starts to the growing season and years that have resulted in poor forage harvests have been major contributory factors to past fodder crises (e.g. the 2013 fodder crisis), with broad consequences for the economy, animal welfare and human well-being.

In the context of future climate change it seems imperative to ask: how will future climate conditions impact the performance of perennial ryegrass? Which mitigation approaches might best safeguard grassland performance? And how can the functioning of our grassland ecosystems be consistently monitored at an all-Ireland scale?

Objectives

The PHENOGRASS project analysed the performance and timing of ryegrass grasslands under past, current and future climatic conditions. The project specifically aimed to:

1. Develop all-Ireland monitoring methodologies for grassland phenology based on satellite remote sensing.
2. Experimentally test the phenological response of perennial ryegrass under the climatic conditions projected for 2050 in Ireland. We focused on growth under increased temperature and carbon dioxide (CO_2) concentration (2°C above current temperature, 550 ppm CO_2). As extreme weather events are projected to increase in the future, we also analysed the effect of waterlogging on ryegrass. The research was performed in climate-controlled growth chambers that allowed simulation of future climatic conditions. Ryegrass cultivars (bred and cultivated varieties) from the current Department of Agriculture, Food and the Marine Irish Recommended List were included, but for a subset of the experiments wild varieties were also analysed.
3. Infer how environmental and genetic factors are associated with the phenological trends for perennial ryegrass varieties.
4. Infer the characteristics of ryegrass varieties and provenances that will promote resilient, future-proof grassland agriculture for Ireland.

Key Findings

- Ryegrass was found to be very resilient to increased temperatures and CO_2 concentrations. Biomass accumulation and leaf appearance did not change substantially under future climatic conditions. However, we observed a small positive effect on ryegrass growth towards the end of the growing season in September under elevated temperatures, leading to a slight lengthening of the growing season.
- Waterlogging had a far more dramatic effect on biomass accumulation and leaf appearance than did increased CO_2 concentrations and temperature. All cultivars tested showed a strongly reduced performance when waterlogged.
- The effects of waterlogging appeared to be slightly less severe under increased temperature and CO_2 conditions but still had a substantial negative effect on ryegrass performance.
- Some wild and semi-natural ryegrass varieties may have useful traits for breeding that can withstand future climatic conditions with little cost to growth performance.
- Over the last decade the start of the growing season has become more variable, with an increased frequency of exceptionally late starting seasons.

Recommendations

- Future breeding programmes should focus on waterlogging resilience as an important trait.
- Substantial yield gains due to increased temperatures and CO_2 concentrations should not be expected, although small gains might be possible if extreme weather conditions such as flooding can be minimised.
- Satellite remote-sensing data allow current and past grassland phenology in Ireland to be quantified. Work is needed to validate approaches with ground-based phenological observations and to link estimated phenology with environmental variables.

1 Introduction

1.1 Background

Phenology is the study of the timing of developmental events such as the onset of seasonal growth or the onset of flowering. Many different developmental events evolved in response to biotic and abiotic interactions. Flowering is, for example, often tuned to the presence of appropriate pollinators (Sandring and Ågren, 2009). Likewise, plants possess elaborate mechanisms to respond to changes in day length and temperature (Andrés and Coupland, 2012).

Existing populations have often evolved phenological responses over long time periods, resulting in individual species, as well as interactions between species, that are well adapted to local climatic conditions. However, climate change is likely to have a strong impact on phenology, as almost every species responds to temperature changes. Evolutionary changes may not be sufficiently fast to respond to altered climatic conditions in a synchronised way, which will potentially lead to disruptions in the timing of biotic interactions. There is already evidence that climate change alters phenology, with increased temperatures leading to extended growing seasons and shifts in the phenophases of species (Kharouba *et al*., 2018). A number of reports indicate that phenological changes associated with increased temperatures have also taken place in Ireland (Donnelly, 2018). For example, with increasing spring temperatures over the past 70 years, bud-burst in trees has shifted to earlier in the year, which may lead to the disruption of ecological interactions. This is illustrated by the observation that leaf-out in beech was 5 days earlier for every 1°C of average temperature increase in February/March/April, whereas migrant birds arrived only 2 days earlier per 1°C temperature increase (Donnelly, 2018). These phenological changes are also expected to show spatial variability, reflecting the regional variation in climate change predictions. In ecosystems where many finely tuned biotic interactions have evolved in concert, phenological shifts may have significant detrimental consequences.

Beyond ecosystem-level effects, phenological changes in plants are of special interest, as plants are also important in controlling the extent of carbon storage. Some phenological changes associated with climate change may lead to an increased capability for sequestering carbon. For example, increased spring and autumn temperatures will, in general, lead to an extended growing season, which will, in turn, increase the net carbon uptake (Richardson *et al*., 2013). The extent of this does, however, depend on local conditions. Ecosystems in which water limitation occurs during the growing season may not respond with increased carbon uptake even if temperatures rise (Hu *et al*., 2010).

Based on the above considerations, the effects of future climate changes on plant development and phenology need to be understood to allow sustainable land management. Phenology has an impact on biodiversity and carbon uptake, and therefore past and future phenological changes will be one critical factor in future land management decisions.

One of the most important plants for managed ecosystems in Ireland is perennial ryegrass (*Lolium perenne*) because it is the dominant grassland species. Approximately 60% of the land area of Ireland is covered by grassland, and perennial ryegrass is a key species in many of those habitats (O’Neill *et al*., 2013). It is found in natural and semi-natural grasslands, but is also the most important species in agriculturally improved grassland (O’Neill *et al*., 2013). In Ireland, perennial ryegrass accounts for more than 95% of the forage grass seeds sold (DAFM, 2022). This grass may have shaped Irish habitats more than any other species and thus is of extreme ecological and economic importance.

Most Irish grasslands were created by human activity and are semi-natural or agriculturally improved, replacing the forests that covered Ireland until a few hundred years ago. Despite the ecological and economic importance of perennial ryegrass, little is known about the relationship between perennial ryegrass, phenology changes and climate. Equally important, yet poorly studied, is the genetic capacity of perennial ryegrass to respond to future climate changes. If temperatures increase and thus permit a longer growing season, the plants must also be capable of fully responding to the extended growth period if this is to result in any increases in productivity. If, for example, earlier growth in spring is offset by earlier senescence in autumn, the overall benefit in terms of carbon uptake might be minimal (Richardson *et al.*, 2013). Likewise, many cultivars of perennial ryegrass require a period of low temperatures to be able to flower. Warmer winters may thus have an inhibitory effect on flowering in some perennial ryegrass varieties.

Most phenological traits, such as flowering time, leaf-out and the onset of senescence, are under strong genetic control. All of these traits can vary substantially among provenances and varieties of the same species. The most obvious example might be winter and spring wheat. The former requires low temperatures to be able to flower, whereas the latter does not. The genetic control is usually quantitative in nature, which allows us to select varieties that are adapted to the specific local climatic conditions (Schilling *et al.*, 2018).

However, given that temperatures will rise, and that the frequency of extreme weather events will increase, it is equally important to understand how those changes will affect phenological traits in the future. This has important consequences for land management decisions. For example, for a projected extension of the growing season, perennial ryegrass varieties that can take full advantage of the altered environmental conditions need to be selected. Different modelling approaches to predict future phenological changes exist (Richardson *et al.*, 2013) and could be used to match a particular cultivar to local environmental conditions.

1.2 Project Overview

The PHENOGRASS project's overall objective was to develop a baseline database on the present and future phenology of Irish grasslands, with a focus on perennial ryegrass (*L. perenne*) and its response to temperature and waterlogging during the growing season. The project also worked with the EPA-funded PhenoClimate project (Wingler *et al.*, 2022) to continue the development of Ireland's National Phenology Network by organising network events and outlining priorities for sustained phenological research in the future.

The PHENOGRASS project builds on past phenological work funded by the EPA (Donnelly *et al.*, 2013) and grassland remote-sensing research funded by Science Foundation Ireland (White *et al.*, 2020, 2021, 2022). The project has two facets: (i) develop a remote-sensing methodology that can use satellite imagery for the monitoring of grassland phenology at an all-Ireland scale, and (ii) conduct controlled growth chamber experiments to quantify the phenological response of different ryegrass varieties to future climatic conditions (increased temperature, increased atmospheric carbon dioxide (CO_2) concentrations and increased waterlogging events).

1.3 Project Objectives

1.3.1 Objective 1

Objective 1 was to develop a large-scale monitoring approach to grassland phenology across the island of Ireland using satellite data. For this objective, we used MODIS (Moderate Resolution Imaging Spectroradiometer) satellite imagery from the Terra and Aqua satellites. The MODIS imagery provides a time-series back to 2002 that allows some long-term trends to be identified. MODIS also allows a medium-resolution time-series to be constructed over a year, even in regions that have frequent cloud cover (i.e. data at least every 8 days), because the two satellites take at least two images of the Earth every day.

1.3.2 Objective 2

Objective 2 set out to experimentally test the phenological response of perennial ryegrass varieties to changes in temperature, atmospheric CO_2 concentrations and waterlogging. For this objective, we used climate-controlled growth chamber experiments. The growth chambers allow temperature and CO_2 concentration to be adjusted in a targeted manner. In one set of chambers we simulated a typical Irish growing season (May to September) at current CO_2 concentrations (415 ppm). For comparison, we simulated the predicted 2050 climatological conditions according to the RCP8.5 scenario (IPCC, 2014), with a 2°C increase in temperature and an elevated CO_2 concentration of 550 ppm. A subset of the plants was exposed to 4 weeks of waterlogging. Leaf appearance, biomass and plant height were regularly measured to detect any difference between the treatments.

1.3.3 Objective 3

Objective 3 was to infer how environmental and genetic factors were associated with the phenological trends for perennial ryegrass varieties. We mainly used experimental data from objective 2 to construct a linear model to infer how waterlogging and temperature changes affect the growth of different ryegrass varieties, including the phenology of growth. The linear model allowed us to estimate the relative importance of different factors (temperature and CO_2 concentration, growth month, waterlogging) for the biomass accumulation and leaf appearance of ryegrass.

1.3.4 Objective 4

Objective 4 was to infer the characteristics of ryegrass varieties and provenances that will promote resilient, future-proof grassland agriculture for Ireland.

2 Grassland Phenology from Remotely Sensed Data

2.1 Introduction

Remote-sensing data from satellites allow some components of an ecosystem's functioning (in particular plant productivity) to be monitored across wide spatial and temporal extents (Weiss *et al.*, 2020; Burke *et al.*, 2021). These data complement ground-based observations and experiments by providing a national overview that can also give a historical perspective over several decades (e.g. data products from Landsat and MODIS), thereby providing some insight into long-term changes.

In this project we analyse time-series of the enhanced vegetation index (EVI, a commonly used proxy for plant productivity) (Huete *et al.*, 2002; Zhu *et al.*, 2021) for the period from August 2002 to April 2020 for 14 10 × 10 km squares (hereafter referred to as 10-km squares) distributed across the island of Ireland (Figure 2.1). These remotely sensed EVI data were obtained from 16-day composite images (MODIS products MYD13Q1 and MOD13Q1) with a pixel size of 250 m. These products use data from MODISv6 (http://modis.gsfc.nasa.gov/) and provide good time-series data across a year with minimal data loss due to cloud cover (typically 40 observations per year; Figure 2.2). For relatively large features, such as grasslands, these data are suitable for identifying vegetation phenophases, such as start of season (SOS), peak of season (POS) and end of season (EOS).

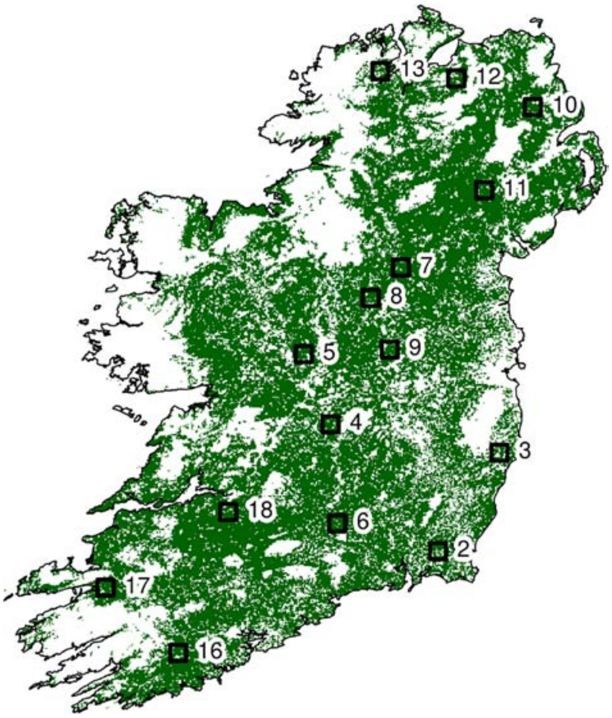


Figure 2.1. The 14 10-km squares that were used to estimate phenophases from the 250-m-resolution MODIS data (the assigned number of each square is shown to the right of the square). Green shading shows areas classified as pasture in Corine Land Cover 2018 data (https://land.copernicus.eu/pan-european/corine-land-cover/clc2018).

2.2 Estimating Phenophases from Remotely Sensed Data

2.2.1 Methodology

We use time-series of EVI values to estimate three phenophases (SOS, POS and EOS) for each year in the period 2003–2019 and for each pixel in each of the 14 squares (Figure 2.1) with more than 90% of its area covered by grassland (as classified by Corine Land Cover 2018 data: https://land.copernicus.eu/pan-european/corine-land-cover/clc2018). We also derive a fourth phenophase (length of season) by calculating the difference between EOS and SOS.

For each pixel the analysis starts by taking a time-series from the focal year and 100 days into the preceding and following years (Figure 2.2). The data are cleaned using a generalised additive model (GAM) to smooth the raw EVI data (Wood, 2017), and any EVI values more than six standard errors below the smoothed data are removed from the time-series. These cleaned data are then smoothed once again with a GAM, and the smoothed data are segmented into six linear components (Figure 2.2). The breakpoints between these six linear components are potential phenophases.

The SOS phenophase is identified as the first breakpoint in the focal year that is followed by a linear component with a positive slope. The POS phenophase is identified as the first breakpoint after

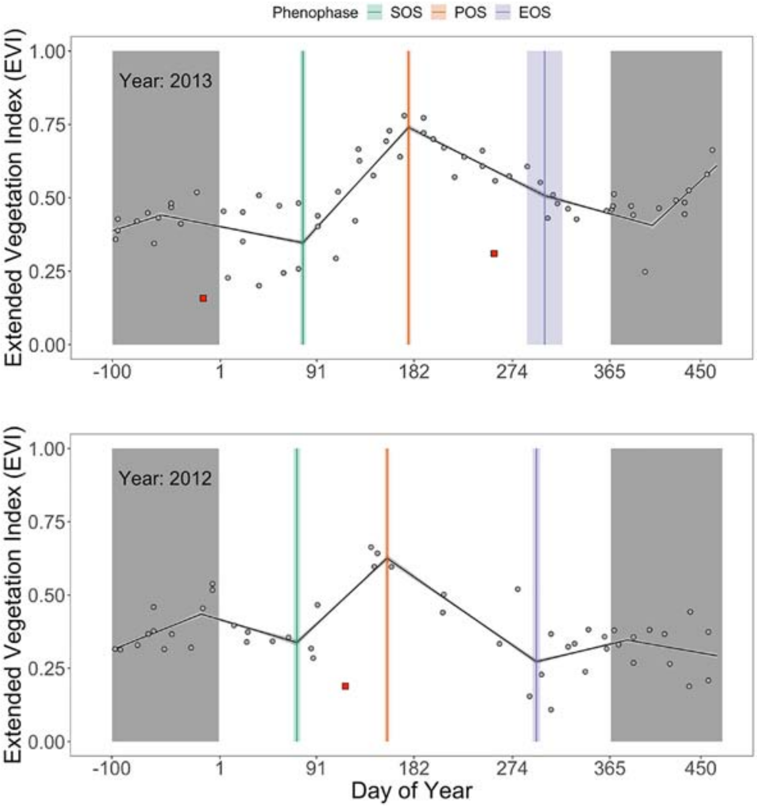


Figure 2.2. EVI time-series data (grey circles) for years 2012 (upper panel) and 2013 (lower panel) for one 250 × 250 m MODIS pixel (7.3612°W, 53.4240°N) from square 9 (see Figure 2.1). Data from the preceding and following years are shown in the grey boxed regions. Red squares show data points that were identified as outliers and removed before estimating the phenophases. The EVI time-series was smoothed using a GAM and then segmented into six linear components (solid black line, with 95% CI shown as grey shading). The breakpoints within the year show the estimates for SOS (green line), POS (orange line) and EOS (purple line). Coloured shaded regions are 95% CIs. Days of year 91, 182 and 274 correspond to 1 April, 1 July and 1 October, respectively.

the SOS in the focal year that is a local maximum (i.e. a positive slope before the breakpoint and a negative slope after the breakpoint). The EOS phenophase is identified as the first breakpoint after the POS where the slope is greater (i.e. less negative) after the breakpoint than before the breakpoint.

For each 10-km square, therefore, we have estimated phenophases for SOS, POS and EOS for each pixel that was classified as having a land cover of > 90% pasture, giving a distribution of phenophases for each 10-km square (Figure 2.3). These three phenophase estimates, along with their 95% confidence intervals (CIs), are saved for later analysis. All data processing was performed using R software, version 4.2.0 (R Core Team, 2022), and the packages segmented version 1.6-0 (Muggeo, 2003), mgcv version 1.8-40 (Wood, 2011), terra version 1.5-21 (Hijmans, 2022) and sf version 1.0-7 (Pebesma, 2018).

2.2.2 Spatial autocorrelation

The variation in the estimated phenophases within a 10-km square is spatially patterned. There is spatial autocorrelation in the estimates out to approximately 1 km (Figures 2.4–2.6). This spatial autocorrelation is consistent across all 14 10-km squares and for all three phenophases and is well described by an exponential spatial autocorrelation structure. The variograms were fitted in R using the package gstat version 2.0-9 (Pebesma, 2004).

Variation in all three phenophase estimates (the right-hand plateau in Figures 2.4–2.6) is consistently greater for more south-westerly areas (squares 4, 6 and 16) than for more north-easterly areas (squares 10 and 11).

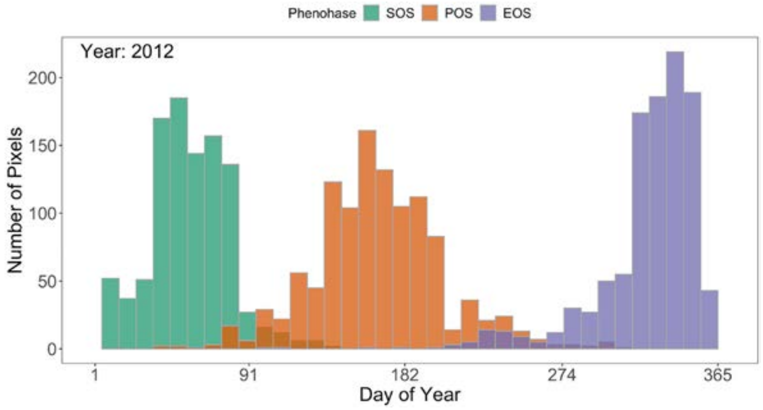


Figure 2.3. The distribution of estimated phenophases (SOS, green bars; POS, orange bars; EOS, purple bars) from 2012 for all 250 × 250 m pixels with a land cover of pasture within square 9 (a 10-km square with south-west corner 7.405°W, 53.423°N, north-east corner 7.240°W, 53.513°N; see Figure 2.1). Each bar corresponds to a 10-day period. Days of year 91, 182 and 274 correspond to 1 April, 1 July and 1 October, respectively.

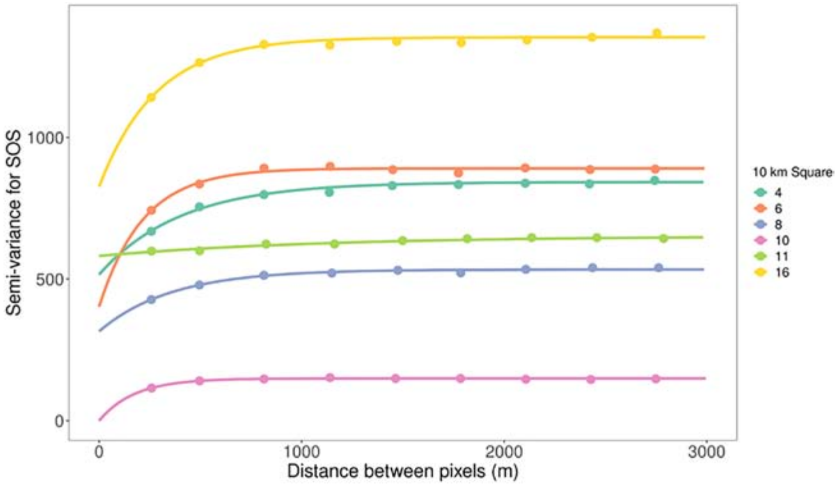


Figure 2.4. Semi-variogram for the estimated SOS for 2017 (empirical variogram shown as points; fitted exponential variogram model shown as lines). Six semi-variograms are shown from 10-km squares 16, 6, 4, 8, 11, 10, which span a transect from the south-west to the north-east, respectively (see Figure 2.1).

2.2.3 *Analysis of phenophase variation across space and time*

Variation in all three phenophases was analysed using linear models, fitted by generalised least squares. The models are described by:

$$\text{Phenophase} \sim \beta_{\text{year}} + \beta_x x + \beta_y y + \beta_{x2} x^2 + \beta_{y2} y^2 + \beta_{xy} xy + N(0, \sum) \qquad (2.1)$$

where phenophase is the day of year of an estimated phenophase (SOS, POS, EOS or length of season) for a pixel, *x* and *y* are the spatial coordinates of a pixel (eastings and northings centred on the centroid of all 10-km squares), β_{year} is the fitted fixed effect of the year, β_x, β_y β_{x2}, β_{y2} and β_{xy} are the fitted trends in phenophase due to changes in the spatial coordinates and $N(0, \sum)$ is a normal distribution describing residual variation with a covariance matrix, $\sum$, that has an exponential spatial correlation structure for each 10-km square. This correlation structure was motivated by the variogram analysis (Figures 2.4–2.6); it has no nugget and takes the form $\exp(-\Delta/d)$, where Δ is the distance between pixels and the range, *d*, is fixed at 500 m. Models were fitted using the package nlme, version 3.1–157, in R (Pinheiro and Bates, 2000; Pinheiro *et al*., 2022).

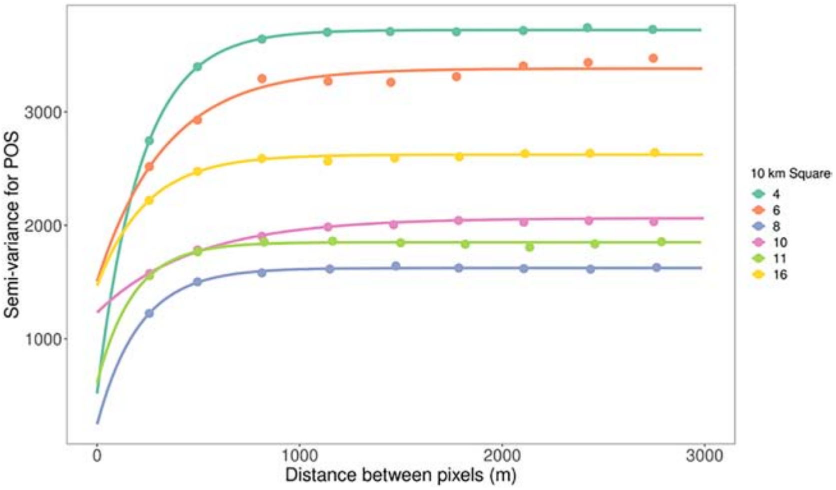


Figure 2.5. Semi-variogram for the estimated POS for 2017 (empirical variogram shown as points; fitted exponential variogram model shown as lines). Six semi-variograms are shown from 10-km squares 16, 6, 4, 8, 11, 10, which span a transect from the south-west to the north-east (see Figure 2.1).

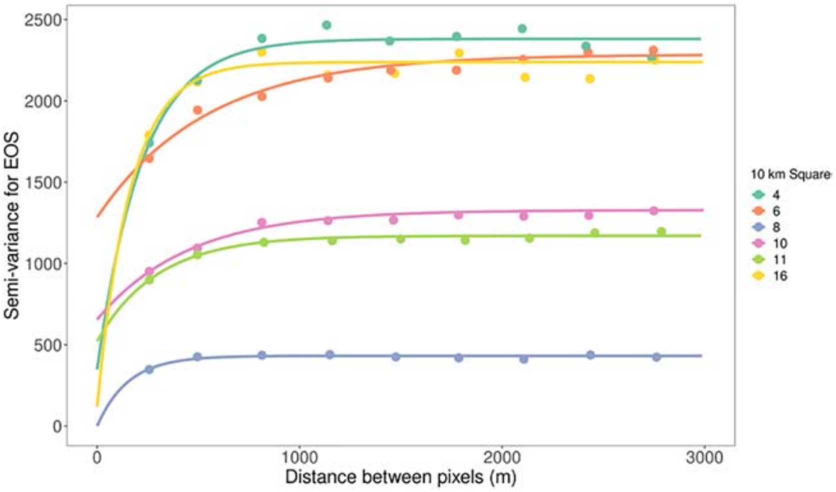


Figure 2.6. Semi-variogram for the estimated EOS for 2017 (empirical variogram shown as points; fitted exponential variogram model shown as lines). Six semi-variograms are shown from 10-km squares 16, 6, 4, 8, 11, 10, which span a transect from the south-west to the north-east, respectively (see Figure 2.1).

2.3 Grassland Phenology

2.3.1 *Start of season*

We found evidence of an effect of year on the timing of SOS ($F_{16,\ 302385} = 57$, $p < 0.0001$; Figure 2.7). Comparing the period 2003–2009 with the period 2010–2019, we found no change in the average SOS (permutation test, $p = 0.07$, 10,000 permutations). However, we found evidence that the SOS becomes more variable in the period 2010–2019 (permutation test, $p = 0.007$, 10,000 permutations).

We also found evidence of a spatial trend in the SOS (Figure 2.8 and Table 2.1), with the north-west and south-east of Ireland approximately 15 days later than central and south-western parts of Ireland.

Comparison with known extremes at the start of season

We can qualitatively compare our SOS results with those for the years when grass growth was known to be late. We use information on known fodder crises and relevant major weather events (as identified by Met Éireann).

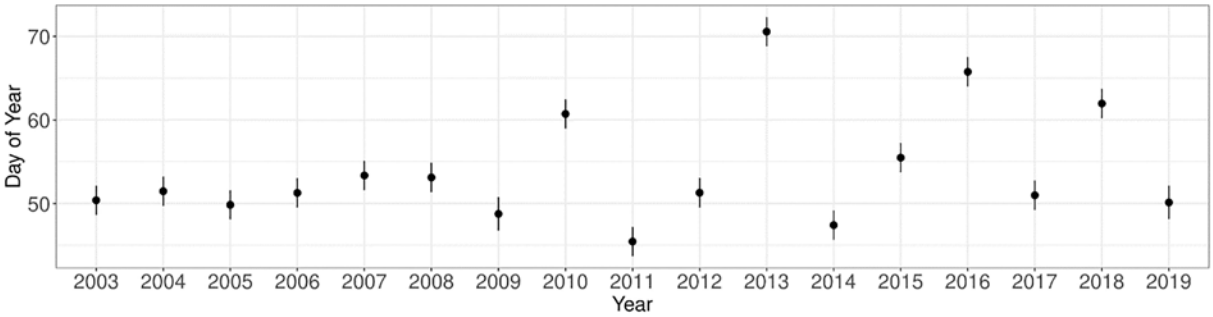


Figure 2.7. Estimated average day of year for the SOS across all 14 10-km squares (solid circles) with 95% CIs for each year over the period 2003–2019, after correcting for spatial autocorrelation and spatial trends. Day of year 60 corresponds to 1 March.

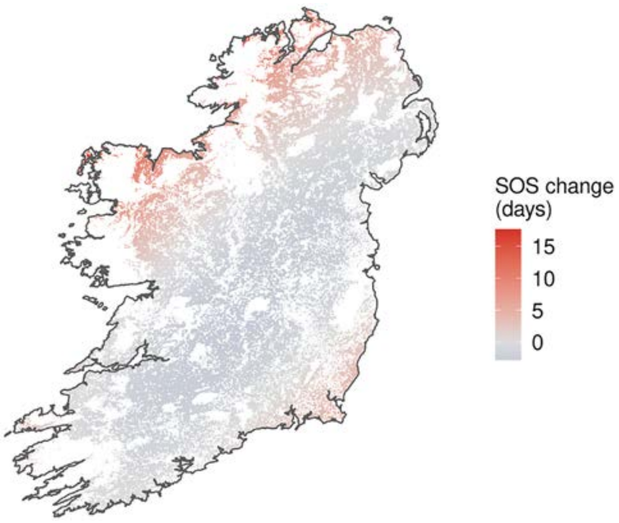


Figure 2.8. Predicted spatial trend in the SOS for grasslands relative to the average SOS across all grassland pixels. Red is a SOS later in the year and blue is a SOS earlier in the year. Only pixels with a land cover classified as pasture are coloured (see Figure 2.1).

Table 2.1. Estimated regression coefficients (see Equation 2.1) and their 95% CIs for the spatial trends in the SOS

Coefficient	Estimate	95% CI	Units
β_x	–0.5	–1.7 to 0.8	days/100 km
β_{x2}	3.2	1.9 to 4.6	days/(100 km)^2
β_y	0.7	0.0 to 1.5	days/100 km
β_{y2}	2.4	1.7 to 3.1	days/(100 km)^2
β_{xy}	–4.3	–5.9 to –2.6	days/(100 km)^2

Figure 2.7 shows four exceptionally late starts of season in 2010, 2013, 2016 and 2018. Three of these events correspond to known extreme events:

- The winter of 2009/2010 was the coldest for almost 50 years (Met Éireann, 2010).
- The spring of 2013 had extended cold and wet weather that caused a fodder crisis (Green, 2013; Seanad Éireann, 2013).
- The spring of 2018 saw another fodder crisis (Dáil Éireann, 2018; European Parliament, 2018). It was exceptionally wet and was followed by a long summer drought.

2.3.2 *Peak of season*

The POS varies between years after controlling for spatial variations ($F_{16,\ 311283} = 24$, $p < 0.0001$), but this variation shows no consistent temporal patterns across the period 2003–2019 (Figure 2.9). We found no evidence that the average day of year for the POS has changed between the periods 2003–2009 and 2010–2019 (permutation test, $p = 0.26$, 10,000 permutations) or that the variability in POS has changed between these two periods (permutation test, $p = 0.52$, 10,000 permutations).

We found evidence of spatial trends in the timing of the POS (Figure 2.10 and Table 2.2). The timing of the POS is predicted to differ by about 20 days across the island of Ireland. The earliest POS occurs in south-eastern and mid-western regions, while the latest POS occurs in the south-western and north-eastern regions.

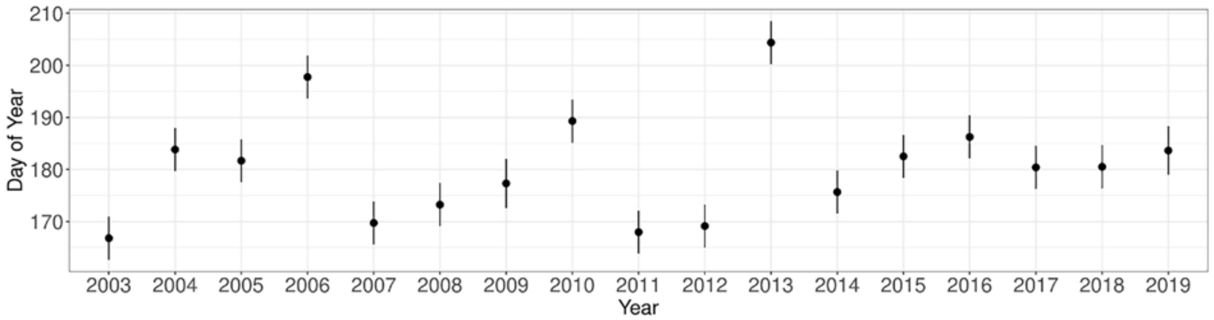


Figure 2.9. Estimated average day of year for the POS across all 14 10-km squares (solid circles) with 95% CIs for each year over the period 2003–2019, after correcting for spatial autocorrelation and spatial trends. Day of year 182 corresponds to 1 July.

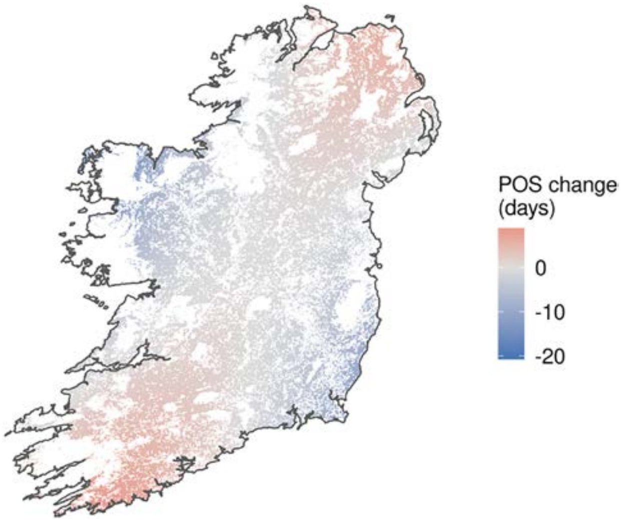


Figure 2.10. Predicted spatial trend in POS for grasslands relative to the average POS across all grassland pixels. Red is a POS later in the year and blue is a POS earlier in the year. Only pixels with a land cover classified as pasture are coloured (see Figure 2.1).

Table 2.2. Estimated regression coefficients (see Equation 2.1) and their 95% CIs for the spatial trends in the POS

Coefficient	Estimate	95% CI	Units
β_x	–1.4	–4.4 to 1.6	days/100 km
β_{x2}	–4.9	–8.2 to –1.7	days/(100 km)^2
β_y	0.08	–1.7 to 1.8	days/100 km
β_{y2}	–0.02	–1.6 to 1.6	days/(100 km)^2
β_{xy}	5.9	2.0 to 9.8	days/(100 km)^2

2.3.3 End of season

Similar to the POS, we found evidence that the EOS varies between years after controlling for spatial variations ($F_{16,\,210074} = 28$, $p < 0.0001$), but this variation shows no consistent temporal patterns across the period 2003–2019 (Figure 2.11). We found no evidence that the average day of year for the EOS has changed between periods 2003–2009 and 2010–2019 (permutation test, $p = 0.70$, 10,000 permutations) or that the variability in EOS has changed between these two periods (permutation test, $p = 0.49$, 10,000 permutations).

We found some evidence that the EOS has spatial trends (Figure 2.12 and Table 2.3). The EOS is predicted to be earliest in the south and south-east and up to 40 days later in the midlands and north.

2.3.4 Length of season

An estimate for the length of the growing season can be calculated as the difference between the SOS and the EOS. The length of season varies between years (Figure 2.13; $F_{1,16} = 36$, $p < 0.001$), but, as with POS and EOS, there is no evidence of a change in the average length of season (permutation test, $p = 0.9$, 10,000 permutations) or the year-to-year variability (permutation test, $p = 0.44$, 10,000 permutations) when comparing 2003–2009 with 2010–2019.

2.3.5 Phenophase correlations

We found evidence of correlation between SOS and POS ($r = 0.6$, 95% CI 0.2 to 0.9), SOS and LOS

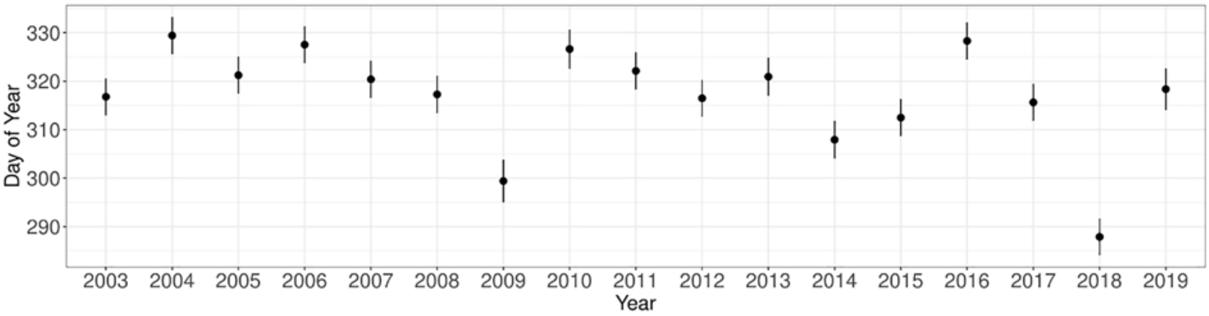


Figure 2.11. Estimated average day of year for the EOS across all 14 10-km squares (solid circles) with 95% CIs for each year over the period 2003–2019, after correcting for spatial autocorrelation and spatial trends. Day of year 305 corresponds to 1 November.

($r = -0.5$, 95% CI –0.8 to –0.02) and EOS and length of season ($r = 0.8$, 95% CI 0.6 to 0.9; Figure 2.14). Since length of season is calculated as the difference between SOS and EOS, we expect negative and positive correlations, respectively. However, the EOS has a stronger correlation with the length of the growing season than the SOS. The growing season in 2018, which saw an exceptional drought (Met Éireann, 2018), is estimated to be 230 days (95% CI 225 to 234 days), which is 44 days (95% CI 37 to 51 days) shorter than the average. This short season is primarily explained by an early EOS (31 days early, 95% CI 25 to 37 days) and to a smaller extent a late SOS (8 days late, 95% CI 6 to 11 days).

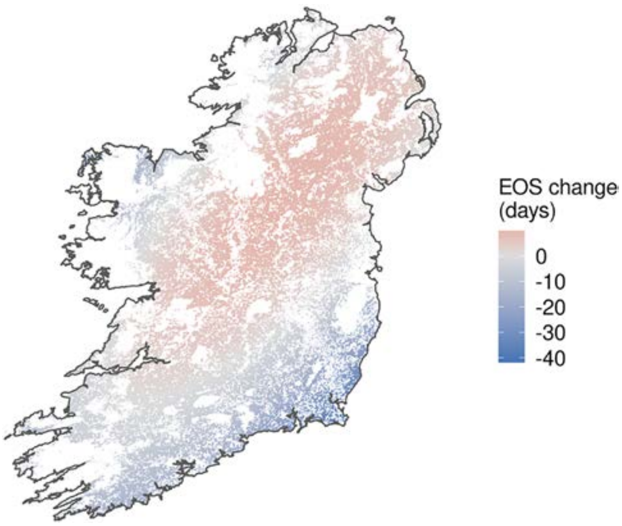


Figure 2.12. Predicted spatial trend in EOS for grasslands relative to the average EOS across all grassland pixels. Red is an EOS later in the year and blue is an EOS earlier in the year. Only pixels with a land cover classified as pasture are coloured (see Figure 2.1).

Table 2.3. Estimated regression coefficients (see Equation 2.1) and their 95% CIs for the spatial trends in the EOS

Coefficient	Estimate	95% CI	Units
β_x	–6.7	–9.5 to –3.8	days/100 km
β_{x2}	–11.4	–14.4 to –8.4	days/(100 km)^2
β_y	8.6	6.7 to 10.2	days/100 km
β_{y2}	–7.0	–8.5 to –5.6	days/(100 km)^2
β_{xy}	13.4	9.8 to 17.1	days/(100 km)^2

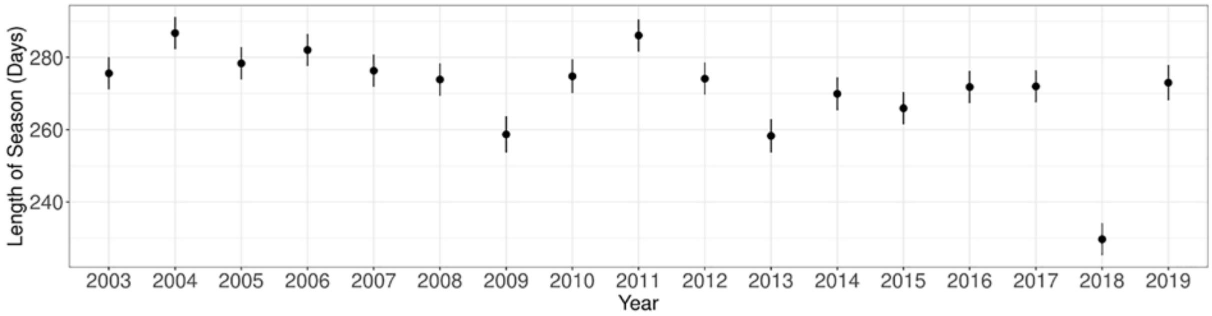


Figure 2.13. Estimated length of season (= EOS – SOS) across all 14 10-km squares (solid circles) with 95% CIs for each year over the period 2003–2019, after correcting for spatial autocorrelation and spatial trends.

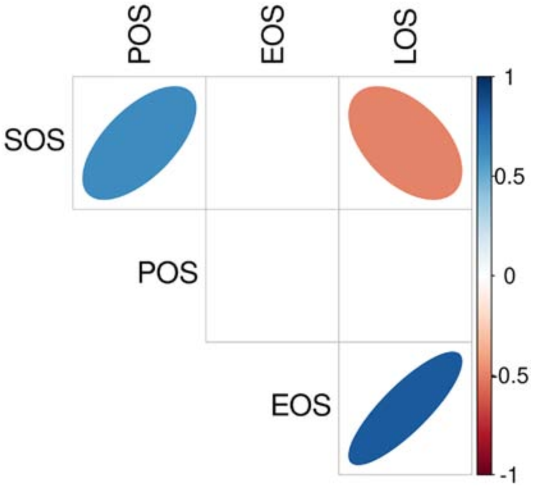


Figure 2.14. Pearson correlation coefficients between SOS, POS, EOS and length of season. The colour scale represents the magnitude of the Pearson correlation coefficient: reds are negative correlations ($r < 0$) and blues are positive correlations ($r > 0$). Correlation coefficients that do not differ from zero ($p > 0.05$) are not shown. Greater eccentricity of the ellipse corresponds to a larger absolute correlation coefficient.

3 Simulating Climate Change Conditions in Growth Chambers

Growth chamber experiments are an ideal means of studying phenological effects under controlled conditions. In this chapter, we outline the advantages of carrying out growth chamber experiments with plants, provide general recommendations for the experimental setup and briefly outline the design of our perennial ryegrass experiment.

3.1 General Considerations for Chamber Experiments

While greenhouses can be used to modify the current conditions at a location by enhancing temperature, light and humidity, growth chambers can be used to simulate special conditions that are absent from the ambient outdoor environment at the growth chamber location. Special conditions are usually related to spatial or temporal conditions that are currently not met in the ambient environment, but they can also be related to plant–animal interactions (such as herbivory) or plant–plant interactions (such as the lack of naturally occurring or co-occurring plant populations). The spatial aspect allows the chambers to simulate locations other than the current growth chamber location. The temporal aspect of the chambers allows them to simulate conditions occurring during seasons other than the current outdoor one, for example simulating summer when it is December outside. It also allows climatological reconstructions, experimentally investigating how climate in previous geological periods was likely to modulate plant growth and predicting future climate change by simulating conditions in the year 2050. The chambers can also be a way to stabilise the weather conditions, allowing equal conditions to be maintained for long periods of time, for example warm, wet summers in Ireland or cold, dry winters in the United Kingdom.

3.1.1 Climatological conditions

Alteration of multiple climatological conditions is possible in most growth chambers. This includes weather, light conditions and atmospheric gases, all of which are customisable by the user. The most common weather condition of interest is temperature, but customisable parameters also include humidity and precipitation, through the injection of water vapour and artificial irrigation systems, although manual watering is the most common method. Light conditions can be set to match a certain day/night length, with associated dawn and dusk periods, along with the strength of the solar radiation (through high-intensity lights). CO_2 concentration is by far the most common alteration in the atmospheric gas composition, but other sulfur and nitrogen gas compounds can be introduced during investigations of special conditions (e.g. to simulate acid rain). All of these alterations in the conditions allow the matching of a spatial and temporal location, for example a warm, wet and humid summer in Ireland in 2100 with increased ambient temperatures and elevated CO_2 concentrations. For ambient conditions, baseline climatological conditions can usually be obtained from meteorological stations that closely resemble the simulated location with long and accurate weather time-series. The treatment climatological conditions are then created by combining climate modelling or other weather predictions with the ambient baseline.

3.1.2 Balanced designs

To properly investigate the effects of the experimental setup, a balanced design and a reliable simulation are required. To investigate the effects of a climate change treatment, one chamber is set up with climate change conditions and another chamber with ambient conditions, and the effects are compared. Because of differences in the construction and uses of chambers, there may be small differences between them, called chamber effects. It is therefore recommended that experiments are conducted with replicate chambers to account for this variation. Therefore, to investigate the response to a whole-chamber treatment (e.g. a climate change treatment), at least four chambers are needed to reach reliable conclusions: two with climate change conditions and two with ambient conditions. We recommend testing for chamber effects before applying any type of treatment, and then randomising the treatments (climate change or ambient) between the chambers. While the Conviron BDW40 growth chambers used in our study can continuously monitor and control environmental variables to within 5% of the set points, the verification of the chamber conditions (e.g. temperature and CO_2 concentration) using independent equipment is generally recommended.

3.2 Recommendations for Conducting Plant Chamber Experiments

The benefit of using growth chambers is that most conditions can be controlled and accounted for, providing information about plants through individual and specific aspects of their biology and ecology. However, most plant traits follow some type of response distribution to stimuli, meaning that a representative sample of multiple plants is needed to quantify how the plants on average respond to a stimulus.

3.2.1 Sample preparation and setup conditions

To isolate the average response of plants in a chamber to a stimulus, it is necessary that all other potential causes of variation are minimised or accounted for. The elimination of unnecessary variation is ensured by using clean equipment; acquiring soil and seeds from the same batches; using setup protocols that ensure that the setup procedure is as similar as possible between samples; acclimatising soil, seeds or plants before use; and handling the samples the same way (e.g. watering each plant equally or applying the same amount of additional nutrients or fertilisers to all plants). In other cases it is not possible to eliminate variation, but protocols are required to account for it properly. Appropriate protocols for doing this will depend on what should be accounted for. Variation within the chamber caused by edge effects, closeness to the door of the chamber or uneven brightness of lights can be accounted for by knowing the exact location of all the plants in the chamber. Treatments within chambers, such as waterlogging, drought treatment or similar, can be accounted for by randomising which plants are subjected to this treatment (Figure 3.1). It is recommended that this same approach be used if multiple types of plants or varieties are used in the same chamber, since this minimises between-variety variation caused by any within-chamber differences.

3.2.2 Assurance and redundancy

However, regardless of how careful and specific the design and setup is, the unforeseen can still occur because of the inherent uncertainty associated with biological experiments. This is coupled with the time-sensitive aspect of long-term experiments and the slow growth of many plants. Therefore, it is necessary to include redundancy in the experimental design by sowing multiple seeds in germination experiments to ensure that at least one seed will germinate and to grow additional experimental plants in case some die due to unaccounted-for circumstances or just bad luck. General back-up plans are also needed in cases of massive equipment failure (e.g. breakdown of chambers) or unwanted visitors (e.g. aphid and other pest infestations).

3.3 Chamber Setup and Plant Preparation for the Perennial Ryegrass Experiments

We conducted two sequential sets of chamber experiments using perennial ryegrass and simulating climate change conditions. Both sets used four growth chambers, with two of each treatment category randomly assigned to the chambers. The experiments simulated 5 months of growth starting in May and ending in September.

The baseline temperatures used were averaged for each month from climatological data from Cork Airport (averaged over the years 1989–2018; data are publicly accessible from Met Éireann). The baseline CO_2 concentration was 415 ppm. To simulate climate change conditions, monthly temperatures were set 2°C above the baseline and CO_2 concentration was set to 550 ppm. The height of the plants was continuously measured and at the end of each simulated month the plants were harvested to imitate grazing. The harvested material was then dried and weighed to estimate the dried biomass. In the first experiment (experiment A), 14 perennial ryegrass varieties (Table 3.1) were equally distributed between the chambers, with six replicates of each variety per chamber (336 plants in total). In the second experiment (experiment B) four varieties were used

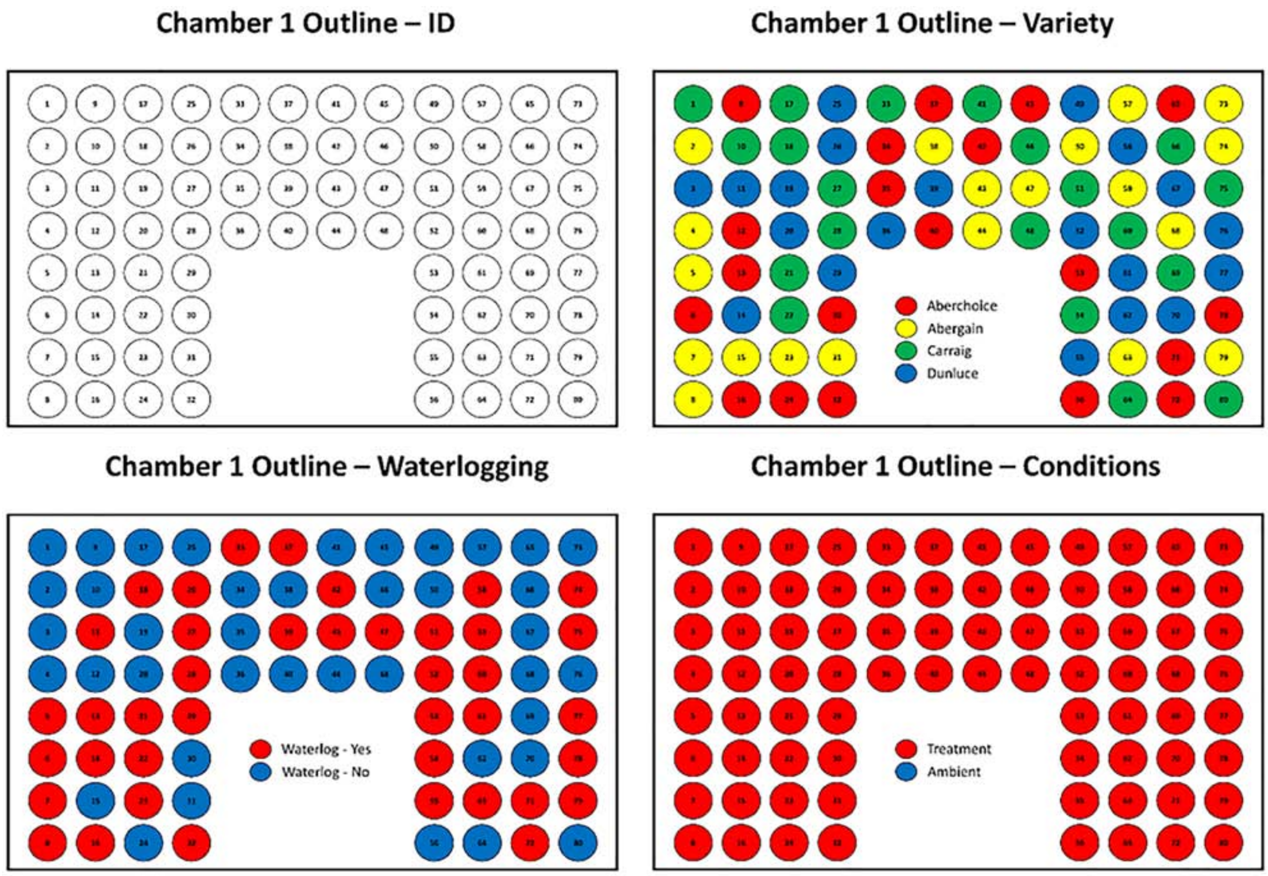


Figure 3.1. Example chamber design showing individual plant identification, randomised between-chamber climatological conditions and randomised within-chamber variety and waterlogging placement.

Table 3.1. Perennial ryegrass (*L. perenne*) varieties used in the chamber experiments

				Chamber experiment	
Variety	**Status**	**Country of origin**	**Ploidy**	**First (A)**	**Second (B)**
Aberchoice	Cultivar	-	Diploid	X	X
Abergain	Cultivar	-	Tetraploid	X	X
Aspect	Cultivar	-	Tetraploid	X	
Carraig	Cultivar	-	Tetraploid	X	X
Dunluce	Cultivar	-	Tetraploid	X	X
Lilora	Cultivar	-	Diploid	X	
Moy	Cultivar	-	Diploid	X	
Solomon	Cultivar	-	Diploid	X	
Semi-natural 6	SN	Austria	Diploid	X	
Semi-natural 7	SN	Poland	Diploid	X	
Semi-natural 11	SN	Portugal	Diploid	X	
Wild 4	Wild	Bulgaria	Diploid	X	
Wild 6	Wild	Italy	Diploid	X	
Wild 7	Wild	Spain	Diploid	X	

the same way, with 20 replicates of each variety per chamber (320 plants in total; Figure 3.1). Half of these replicates were subjected to waterlogging (i.e. the soil surface was continuously under water) at the beginning of June. This waterlogging was actively enforced for 1 month (i.e. the soil surface was continuously under water) and the water level was then allowed to naturally subside.

4 Quantifying the Effects of Waterlogging on Perennial Ryegrass

4.1 Waterlogging and Image Analysis Approach

The content of this chapter has been published as a *bioRxiv* preprint (Frisk *et al*., 2022) pending journal publication. Amendments to the preprint version have been made.

During the second chamber experiment (experiment B), half of the plants were subjected to a waterlogging treatment (i.e. the soil surface was continuously under water) to investigate the effect waterlogging of the soil has on plant performance, yield and health (see Chapter 3). Pre-harvest, in the simulated months June and July, multiple plant parameters were measured to understand how the initial waterlogging treatment affected plant physiology and to what degree the plants recovered the following month. In addition to maximum height and dried biomass, chlorophyll was measured using a SPAD (soil plant analysis development) device and soil moisture was measured to quantify the waterlogging treatment. During harvest, all harvested material was photographed to determine if the waterlogging had influenced the colours of the leaves (Figure 4.1). Waterlogging has previously been found to reduce the concentrations of plant pigments and therefore the photosynthetic capacity of the plant (Smethurst and Shabala, 2003; Ou *et al*., 2011; Barickman *et al*., 2019). The harvested material was placed on a white background and photographed using a high-resolution camera (Panasonic Lumix DC-G9) and a ColorChecker Classic chart (Calibrite; https://calibrite.com), which allowed the colours to be corrected for changing lighting conditions (Figure 4.1). All images were further processed and the leaf pixels were isolated in the software MATLAB (2021), which can handle the data extraction of images using the image processing toolbox. This approach can isolate and extract the red, green and blue (RGB) hues of every pixel of the harvested perennial ryegrass leaf, which can be further statistically analysed for patterns.

Figure 4.1. Photographic processing of the harvested material from one of the perennial ryegrass plants.

4.2 Plant Morphological Changes

We observed distinct morphological changes between the plants that were waterlogged and those that were not. These changes were mainly identified as stunted growth and alterations in the hues of the leaves, but also disrupted leaf unfolding and reduced root proliferation in waterlogged plants. During June, the leaves of many waterlogged plants were dark brown or purple shades, while the leaves of non-waterlogged plants were healthy green shades (Figure 4.2). As plants started to recover from the waterlogging in July, the leaves of only a few retained the darker brown and purple shades, while most plants' leaves were light-green shades. This is in contrast to the non-waterlogged plants in July, whose leaves were deeper dark-green shades than in June. Viewing the median colour of each plant's pixels for all the harvests reveals that this is a general response to the waterlogging treatments (Figures 4.3 and 4.4). Leaf morphology was also affected by the waterlogging; during both months the waterlogged plants experienced disrupted unfolding of leaves, which caused many leaves to

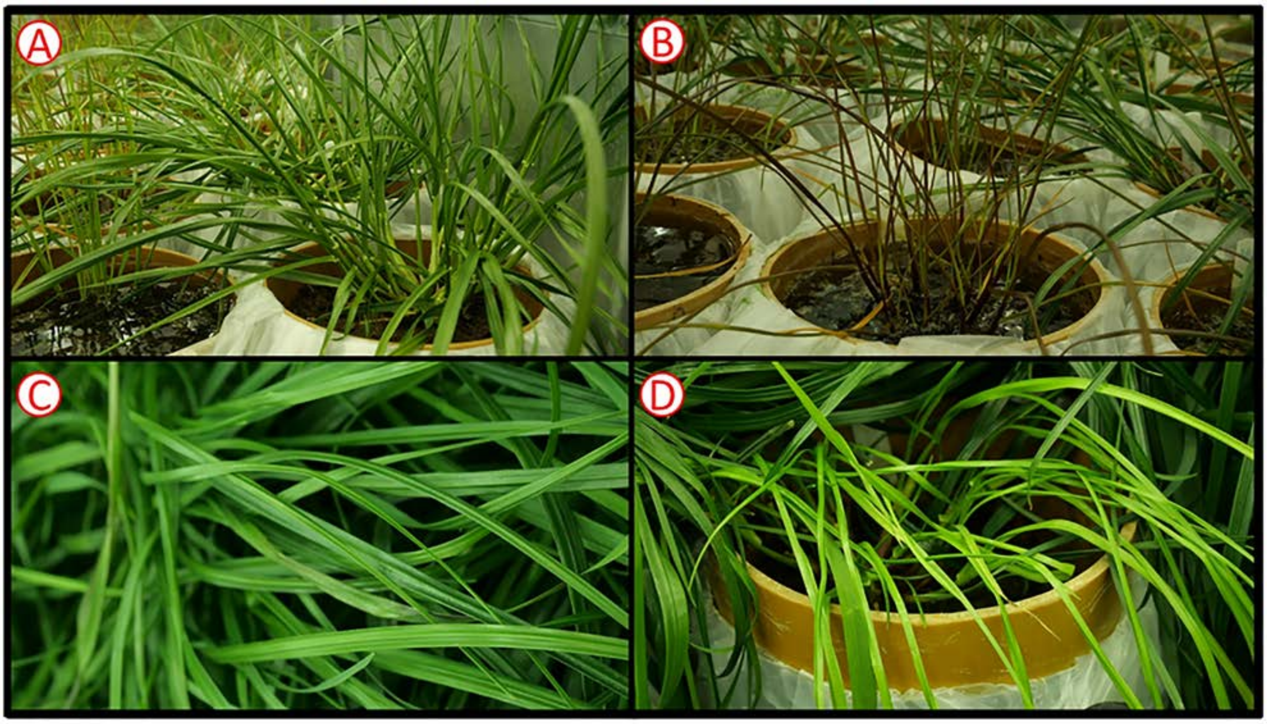


Figure 4.2. Example images showing whole plant appearance and leaf colouration of perennial ryegrass (*L. perenne*) for June and July and water status. (A) June non-waterlogged, (B) June waterlogged, (C) July non-waterlogged and (D) July waterlogged. Example images are not colour corrected.

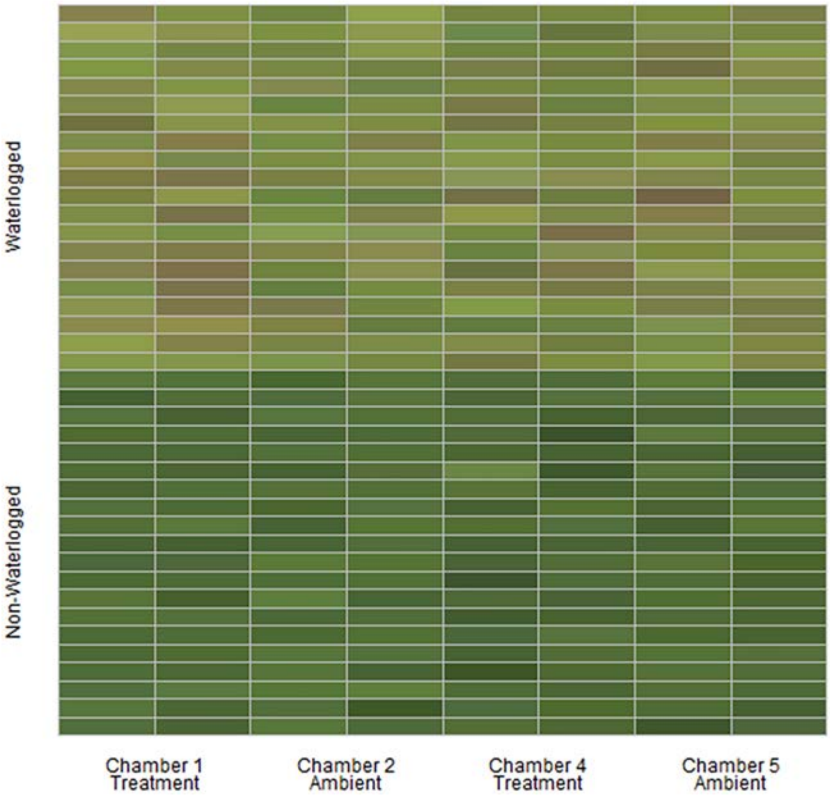


Figure 4.3. Median colour (RGB) of the harvested material from each plant in June as identified by image analysis, sorted by water status and chamber. Each cell represents the median true colour of the harvested material of a plant.

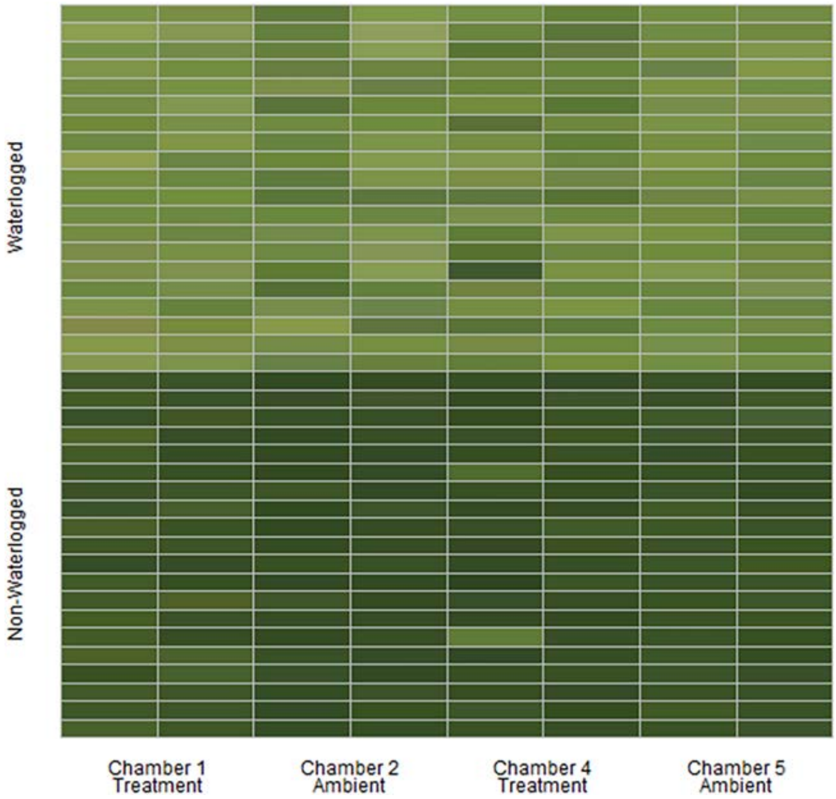


Figure 4.4. Median colour (RGB) of the harvested material from each plant in July as identified by image analysis, sorted by water status and chamber. Each cell represents the median true colour of the harvested material of a plant.

develop a concave appearance with dark brown and purple hues found on their outside (abaxial side) (Figure 4.5). Darker hues had dissipated by July, but the leaf unfolding remained disrupted. This contrasts with the leaves of the non-waterlogged plants, which had a lush and healthy appearance.

4.3 Quantifying Colours Using Principal Component Analysis

The median red, green and blue values from the harvest material of each plant for each month were extracted using the image analysis method described in section 4.1. The three median hues from each plant were then modelled using principal component analysis (PCA), which performs a linear transformation of the three colour variables (red, green and blue) to produce three new variables called principal components. The first principal component usually contains the majority of the variation, while the last principal component contains the least variation. Each principal component can then be used to aid interpretation and modelling of general relationships within the data, in our case waterlogging.

The PCA modelling showed that 97.3% of the overall variation was found in the first principal component (PC1), illustrating that most of the colour variation between all plants and months could be simplified into one variable (Figure 4.6). The three hues showed similar loadings for this first principal component axis, showing that the axis describes a divergence from a light-beige to a dark-brown hue, which we interpret as an overall colour intensity. Positive values on the axis were classified as light intensities while negative values were classified as dark intensities. Modelling these PC1 values using Kendall’s tau correlation and Wilcoxon’s signed-rank test showed that waterlogged plants were lighter in colour than non-waterlogged plants. It also showed that plants became darker over time overall, with the non-waterlogged plants likely to develop more and denser colour pigments as the growing season progressed. Waterlogging has previously been shown to reduce plant pigment accumulation (Close and Davidson, 2003), which is

Figure 4.5. Example images showing leaf morphology and leaf colouration of perennial ryegrass (*L. perenne*) for June and July and water status. (A) June non-waterlogged, (B) June waterlogged, (C) June waterlogged, (D) July non-waterlogged and (E) July waterlogged. Example images are not colour corrected.

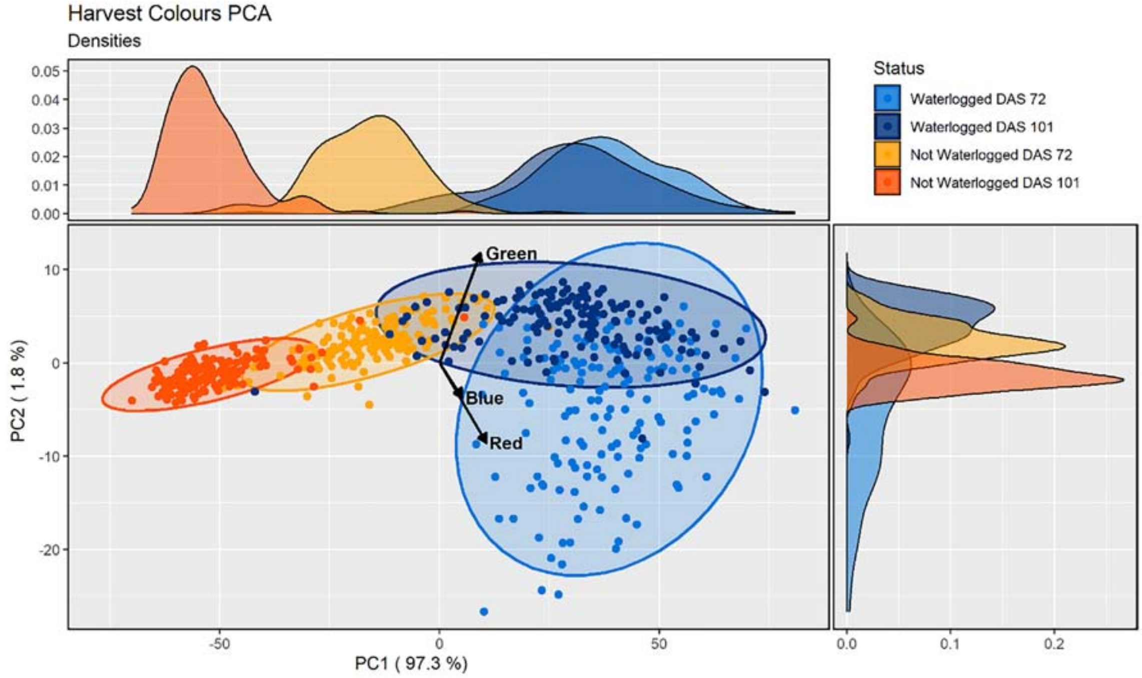


Figure 4.6. PCA of the three colours (RGB) of the harvested material for all plants, water status and harvest (June and July) as identified by image analysis. Each ellipse represents the 95% confidence region for each treatment group.

likely to have caused the lighter intensities seen in our plants, which in turn will reduce photosynthesis and overall energy and growth regulation.

The PCA modelling also showed that the other two principal components (PC2 and PC3) captured 1.8% and 0.9% of the colour variation, respectively. These axes describe pure colour gradients rather than colour intensities. PC2 describes a green to purple gradient, while PC3 describes an orange to blue gradient. PC2 is of particular importance since it indicates the presence of purple pigments, likely to be anthocyanins, in waterlogged plants. These were especially prevalent in waterlogged plants in June but mostly dissipated as the plants started to recover in July. Anthocyanins in plants are created as a response to environmental or nutritional stressors, and they have previously been attributed to excessive light intensity and nutrient imbalances caused by a lack of phosphorus and/or nitrogen absorption (Chalker-Scott, 1999). An imbalance of phosphorus or nitrogen absorption is the likely cause of anthocyanins in our case, as waterlogging can reduce root proliferation and facilitate leaching and transport of nutrients in the soil column, which is likely to hinder the overall absorption of nutrients in young waterlogged plants.

4.4 Effects of Waterlogging on the Plant Phenotype

Overall leaf colour intensity, as quantified by the first principal component from the PCA, was further modelled using a linear model with leaf weight, leaf height, soil moisture, SPAD meter reading, waterlogging treatment, ryegrass variety and month as explanatory variables. The linear model was used to identify which variables contributed most to the variation in colour intensity, and, by association, photosynthetic ability and function.

Higher soil moisture was strongly associated with lighter colour intensity, illustrating that soil moisture is directly proportional and likely to be a causal agent of the lighter colour intensities (Table 4.1). Lighter colour intensities were also associated with lower SPAD measurements, further indicating that waterlogging contributes to reduced photosynthetic ability and functioning in perennial ryegrass. Waterlogging also contributed to lower maximum height and lower dried biomass, showing that the overall growth of the plants was reduced, probably from reduced photosynthetic ability but also from reduced nutrient absorption from the waterlogged soil.

We tested how the colour intensity differed among the four varieties (Aberchoice, Abergain, Carraig and Dunluce) and found that the diploid variety (Aberchoice) had the lightest leaf colour intensity. The three tetraploid varieties were darker overall, with Carraig having the darkest leaf colour intensity. This suggests that tetraploid varieties of perennial ryegrass have, on average, higher amounts of plant pigments.

Colour intensities increased from June to July as plants started to recover from waterlogging and acquired additional resources as the growing season progressed. We hypothesised that the climate change treatment would increase colour intensities. Increased temperatures and elevated CO_2 concentrations have

Table 4.1. Model statistics and significance levels for the linear model for the first PCA axis (colour intensity) isolated from the colour analysis of the harvested perennial ryegrass material

	Model statistics							
Variable	df	Sum of squares	RSS	AIC	Δ AIC	*F*-value	*p*-value	Significance
None			79,595	3107				
Weight	1	29,367	108,962	3306	–199	232	$< 10^{-10}$	***
Height	1	7318	86,914	3161	–54	58	$< 10^{-10}$	***
SPAD	1	31,587	111,182	3319	–212	250	$< 10^{-10}$	***
Soil moisture	1	22,068	101,663	3262	–155	170	$< 10^{-10}$	***
Treatment	1	2426	82,021	3124	–17	19	1.4×10^{-5}	***
Variety	3	12,664	92,259	3195	–89	33	$< 10^{-10}$	***
Month	1	539	80,134	3109	–2.3	4.3	0.039	*

Significance: ******p* **< 0.001; *****p* **< 0.01; ****p* **< 0.05; NS, non-significant (*p* **> 0.05).**

AIC, Akaike information criterion; df, degrees of freedom; RSS, residual sum of squares.

been shown to increase growth in many plants, mostly by mediating photosynthetic ability and plant respiration (Chen *et al*., 1996; Dusenge *et al*., 2019; Yiotis *et al*., 2021). However, we found that the climate change treatment reduced overall colour intensity, causing lighter hues. This suggests, at least in our case, that, overall, waterlogging has stronger effects on the plant phenotype than the climate change treatment (Table 4.1).

4.5 Colour Darkening and Waterlogging Recovery

During the harvest in September (the last month of the experiment), a 10% subsample of the plants was again photographed and analysed as above to determine the extent to which the plants had regained their colouration as a function of recovery from the waterlogging. The colouration of the corresponding plants from the previous months was compared with the colouration in September (Figure 4.7). The waterlogged plants had become significantly darker as their recovery progressed. This could be due to compensatory growth as unfavourable conditions subsided and soil nutrients again became available for absorption and proper pigment synthesis. The leaves of the non-waterlogged plants, however, had become significantly lighter, possibly because the plants had adapted to the growth conditions outside the favourable peak season.

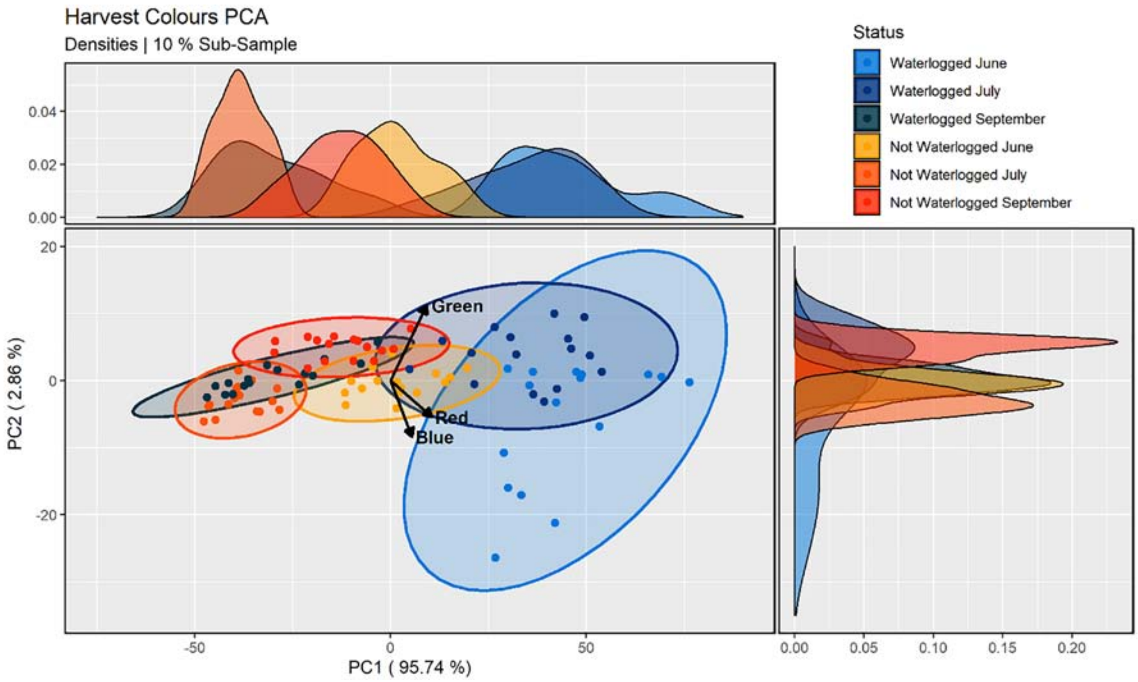


Figure 4.7. PCA of the three colours (RGB) of the harvested material for the 10% subsample, water status and harvest (June, July and September) as identified by image analysis. Each ellipse represents the 95% confidence region for each group.

5 Effects of Climate Change and Waterlogging on Perennial Ryegrass Phenology

5.1 Phyllochron and Phenological Modelling

The phenological progression of all perennial ryegrass plants was monitored throughout the first and second chamber experiments. Multiple distinct phenological phases were accounted for: germination, leaf appearance (phyllochron), stem elongation and heading (flower or seed head emergence). Germination was monitored in the simulated month of May only, with random emergence being unsuitable for further modelling. Stem elongation and heading were only infrequently observed because of the continual harvesting of the plants, leaving leaf appearance as the main phenological phase observed.

The timing of leaf appearance is important for practical applications, since it is closely associated with overall plant development and optimal grazing timing. Grazing after the appearance of the third leaf is generally encouraged since it allows proper plant establishment and ensures plant survival. The first experiment (A) monitored phyllochron twice weekly, continuously every third or fourth day, while the second experiment (B) monitored it continuously every weekday, yielding higher resolution data. We modelled the phyllochron for both experimental datasets to investigate how the climate change treatment, waterlogging and progression of the season affected it. Because the second experiment was of higher resolution, this was used as the primary data source, while the first experiment was used to validate the findings. The simulated month of May was excluded from the modelling, as it was directly connected with the random germination rate and the phyllochron resolution was initially overall lower. The modelling results include the four months (June to September), all main modelling variables and their first-order interaction terms separated into two tables, one per experiment (Tables 5.1 and 5.2). Experiment A does not include waterlogging and waterlogging interactions (Table 5.1), while experiment B contains all interaction terms (Table 5.2).

Table 5.1. Model statistics and significance levels for the linear mixed model for the phyllochron progression of perennial ryegrass

Variable		Model statistics						
Main effect	Interaction effect	Sum of squares	Mean square	Num. df	Den. df	*F*-value	*p*-value	Significance
Climate treatment		0.15	0.15	1	4.1	1.1	0.350	NS
	Variety	6.5	0.50	13	10,995	3.6	9.9×10^{-6}	***
	Month	11	3.8	3	10,995	28	$< 10^{-10}$	***
	DSH	3.1	3.1	1	10,995	22	2.2×10^{-6}	***
Variety		2.9	0.2	13	10,995	1.6	0.079	NS
	Month	16	0.4	39	10,995	2.9	3.3×10^{-9}	***
	DSH	0.6	0.04	13	10,995	0.3	0.989	NS
Month		250	82	3	10,995	590	$< 10^{-10}$	***
	DSH	350	120	3	10,995	850	$< 10^{-10}$	***
DSH		6500	6500	1	10,995	47,000	$< 10^{-10}$	***

Significance: ******p*$<$0.001; *****p*$<$**0.01; ****p*$<$**0.05; NS, non-significant (***p*$>$**0.05).**

Model statistics: ANOVA (type III) using Satterthwaite's method. Random error: chamber. Data source: experiment A. Marginal $R^2 = 0.831$.

DSH, days since harvest; Num. df, numerator degrees of freedom; Den. df, denominator degrees of freedom.

Table 5.2. Model statistics and significance levels for the linear mixed model for the phyllochron progression of perennial ryegrass

Variable		Model statistics						
Main effect	**Interaction effect**	**Sum of squares**	**Mean square**	**Num. df**	**Den. df**	***F*-value**	***p*-value**	**Significance**
Water status		0.26	0.26	1	22,998.0	1.8	0.19	NS
	Climate treatment	3.8	3.8	1	22,998.0	25	5.0×10^{-7}	***
	Variety	1.2	0.41	3	22,998.0	2.7	0.043	*
	Month	720	240	3	22,998.0	1590	$< 10^{-10}$	***
	DSH	34	34	1	22,998.0	230	$< 10^{-10}$	***
Climate treatment		0.24	0.24	1	3.1	1.6	0.29	NS
	Variety	1.1	0.38	3	22,998.0	2.5	0.055	NS
	Month	25	8.2	3	22,998.0	54	$< 10^{-10}$	***
	DSH	9.5	9.5	1	22,998.0	63	$< 10^{-10}$	***
Variety		4.695	1.6	3	22,998.0	10	7.7×10^{-7}	***
	Month	19	2.1	9	22,998.0	14	$< 10^{-10}$	***
	DSH	2.9	0.96	3	22,998.0	6.4	2.5×10^{-4}	***
Month		46	15	3	22,998.0	100	$< 10^{-10}$	***
	DSH	64	21	3	22,998.0	140	$< 10^{-10}$	***
DSH		5900	5900	1	22,998.0	39,000	$< 10^{-10}$	***

Significance: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; NS, non-significant ($p > 0.05$).

Model statistics: ANOVA (type III) using Satterthwaite's method. Random error: chamber. Data source: experiment B. Marginal $R^2 = 0.831$.

DSH, days since harvest; Num. df, numerator degrees of freedom; Den. df, denominator degrees of freedom.

The effects of the main variables cannot readily be interpreted if evidence of an interaction is found. Therefore, we have chosen to focus the following sections on the three main interactions in the analysis: interactions between (i) climate treatment and progression of the season, (ii) climate treatment and waterlogging and (iii) progression of the season and waterlogging. The reason for including these three main interactions and not others is that days since harvest is naturally going to lead to an increase in phyllochron, and no strong general and direct effect was found from ploidy (diploid vs tetraploid) or between varieties. Although the experiment B model found significant differences between the varieties, there is no clearly discernible pattern. That said, in the comparison of Aberchoice, Abergain, Carraig and Dunluce, the data suggest that Dunluce seems to be the slowest-growing variety and Carraig the fastest-growing one, which is in agreement with previously published data (Yiotis *et al.*, 2021).

5.2 Climate Change Might Extend the Growing Season

Modelling the interaction between the progression of the season (month) and the climate change treatment revealed that the plants' quickest leaf developmental period is in July and August, coinciding with the peak of the growing season (Figure 5.1). Growth was on average better in September than in June. It is interesting that the difference between the climate change treatment and the ambient treatment is bigger in June and September than in July and August, with it being largest in September (Figure 5.2). This suggests that climate change might extend the growing season by prolonging optimal growth conditions rather than by improving growth during the already optimal peak season. This is also consistent with no evidence of any difference between growth in August under the ambient treatment and growth in September under the climate change treatment. This implies that climate change will extend the period of optimal growth

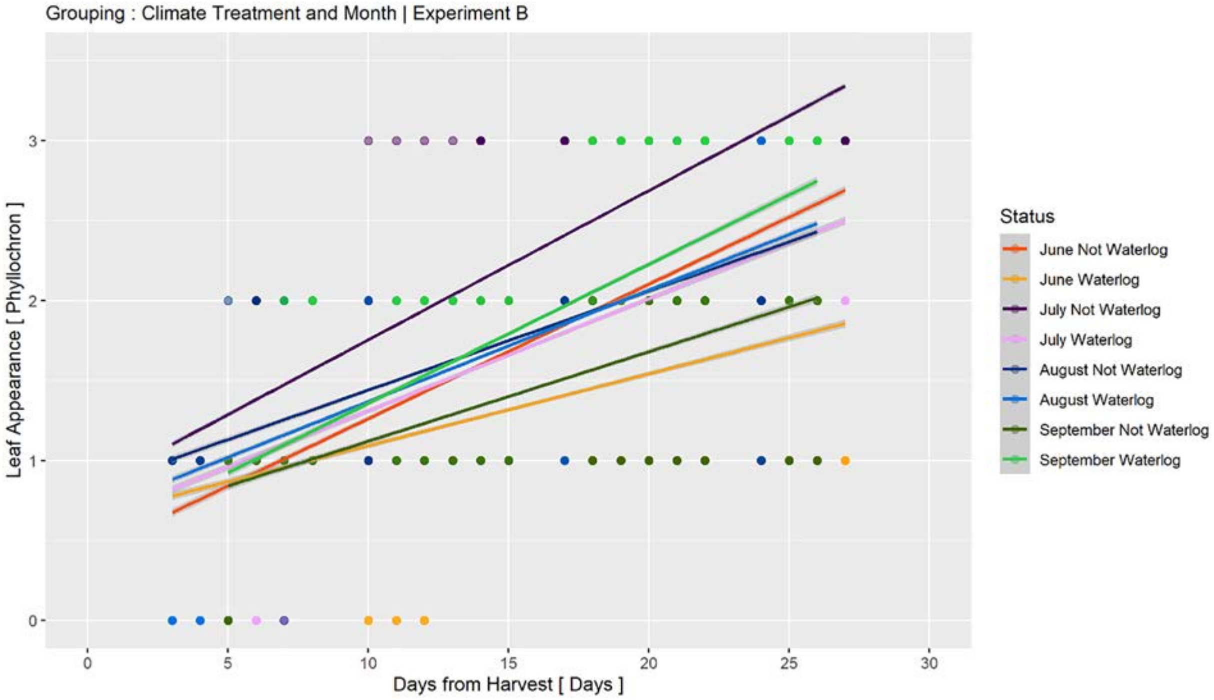


Figure 5.1. Linear phyllochron progression as each month progresses in experiment B. The graph shows the interaction between climate treatment and month. For each month, the climate treatment is shown in the darker shade and the ambient treatment in the lighter shade of the same colour range. Many data points overlap, as the graph contains approximately 23,000 data points.

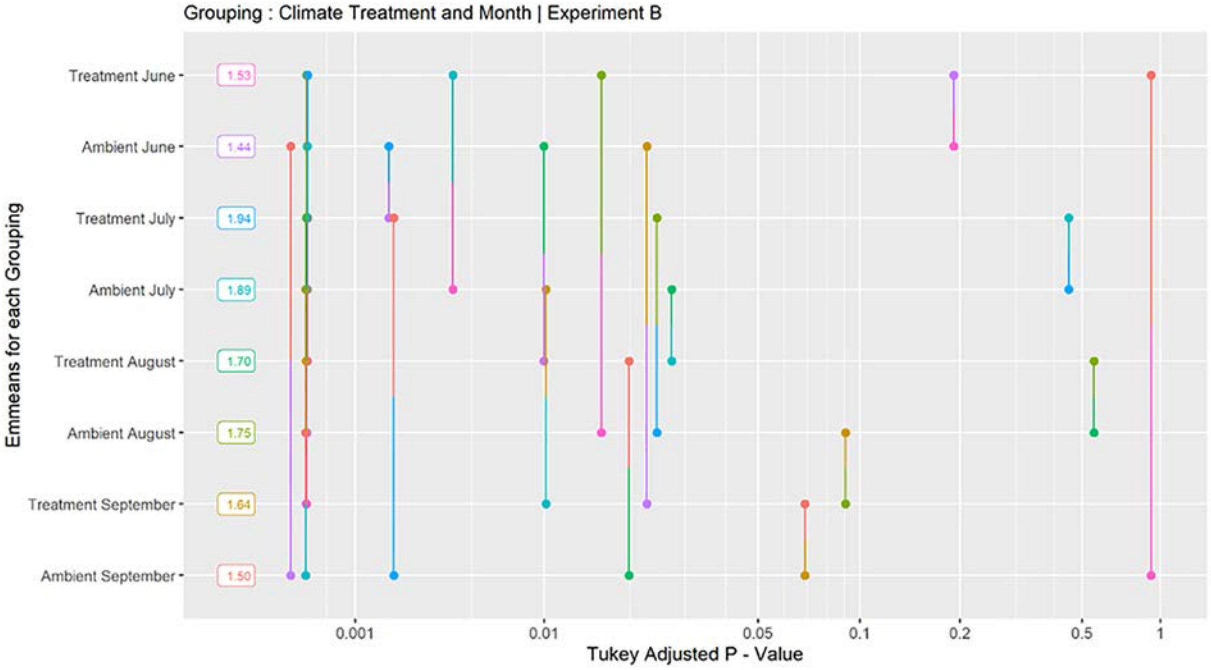


Figure 5.2. Pairwise comparison of the interaction between climate treatment and month from the model in Table 5.2 and experiment B.

conditions from August to August and September. The first experiment (A) confirms this general pattern using different varieties and excluding any waterlogging effect.

5.3 Climate Change Reduces the Negative Effects of Waterlogging

Modelling the interaction between the climate change treatment and waterlogging revealed that waterlogging has a strong negative effect on leaf appearance, while climate change has no direct effect (Figures 5.3 and 5.4). The pairwise comparison shows that there is a difference between ambient waterlogging and treatment non-waterlogging but not between ambient non-waterlogging and treatment waterlogging. This implies that plants waterlogged during future climate change conditions will grow as well as plants not waterlogged during current conditions. The climate change treatment is therefore able to reduce some of the negative growth-reducing effects from long-term waterlogging but not fully compensate for them. The larger effect sizes between the climate change and ambient treatment in the waterlogged plants compared with the non-waterlogged plants also point to this indirect effect of climate change on waterlogging. It is unclear if it is the temperature or the CO_2 concentration component that influences the waterlogging effects individually or together. It is possible that the components increase leaf appearance to offset some of the negative effects of the waterlogging, or that they influence the negative effects directly by reducing how waterlogging influences the plant physiologically.

5.4 Compensatory Recovery After Waterlogging

Modelling the interaction between the progression of the season (month) and waterlogging revealed that waterlogging has a strong negative effect on the plant phyllochron in the short term but that the negative growth effects are eventually reversed (Figure 5.5). The negative effects on growth were strong in June and July, negligible in August and reversed to become positive in September. We observed that there was no difference in the effect sizes on phyllochron between non-waterlogged plants in August and waterlogged plants in September (Figure 5.6) and that the effect of waterlogging gradually decreased from June to September as the plants started to recover. Not only

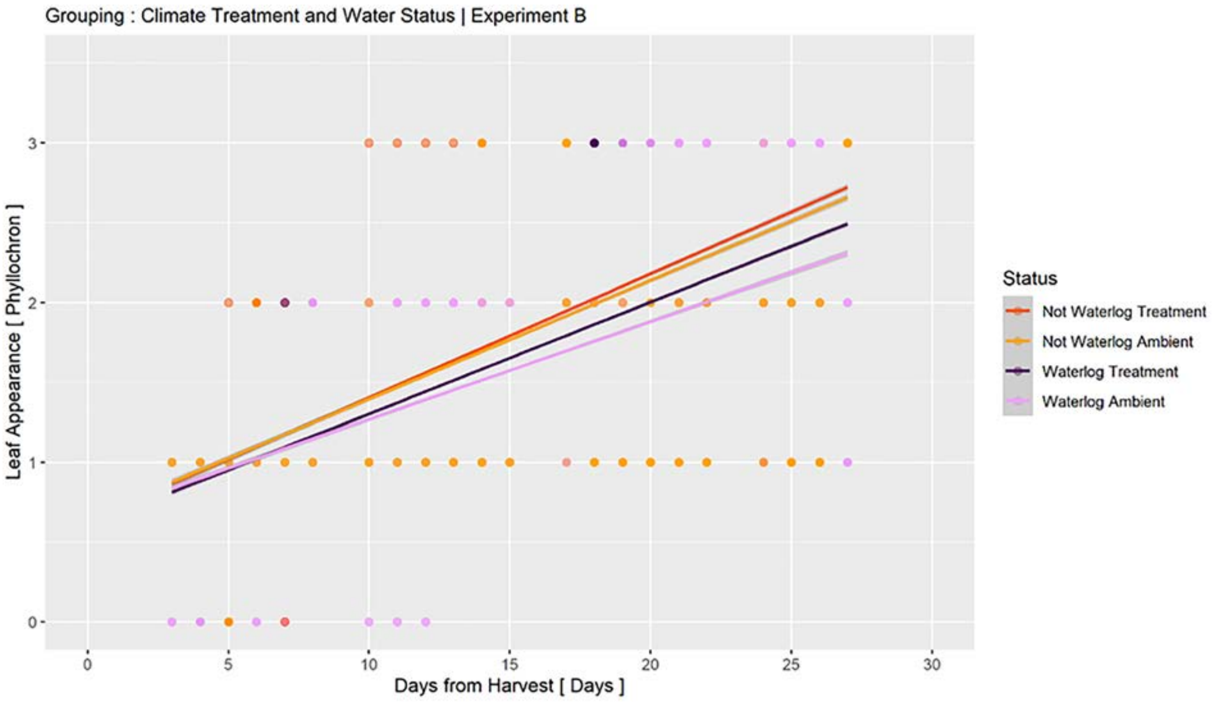


Figure 5.3. Linear phyllochron progression as each month progresses in experiment B. The graph shows the interaction between climate treatment and water status. For each water status, the climate treatment is shown in the darker shade and the ambient treatment in the lighter shade of the same colour range. Many data points overlap, as the graph contains approximately 23,000 data points.

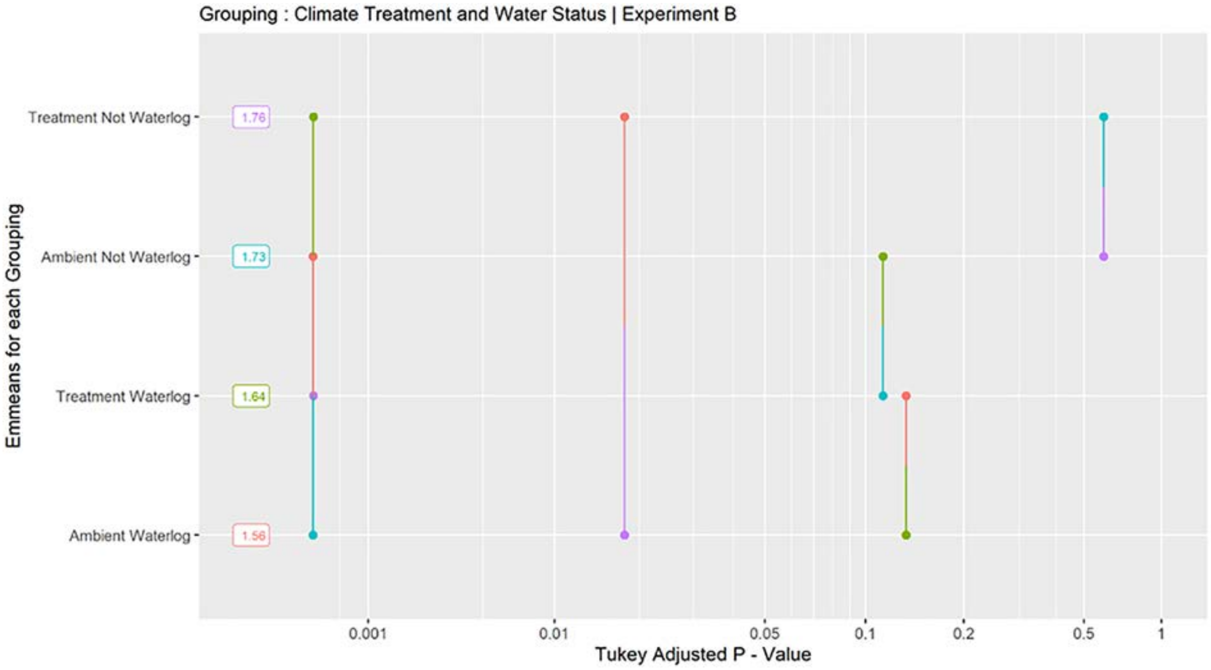


Figure 5.4. Pairwise comparison of the interaction between climate treatment and water status from the model in Table 5.2 and experiment B.

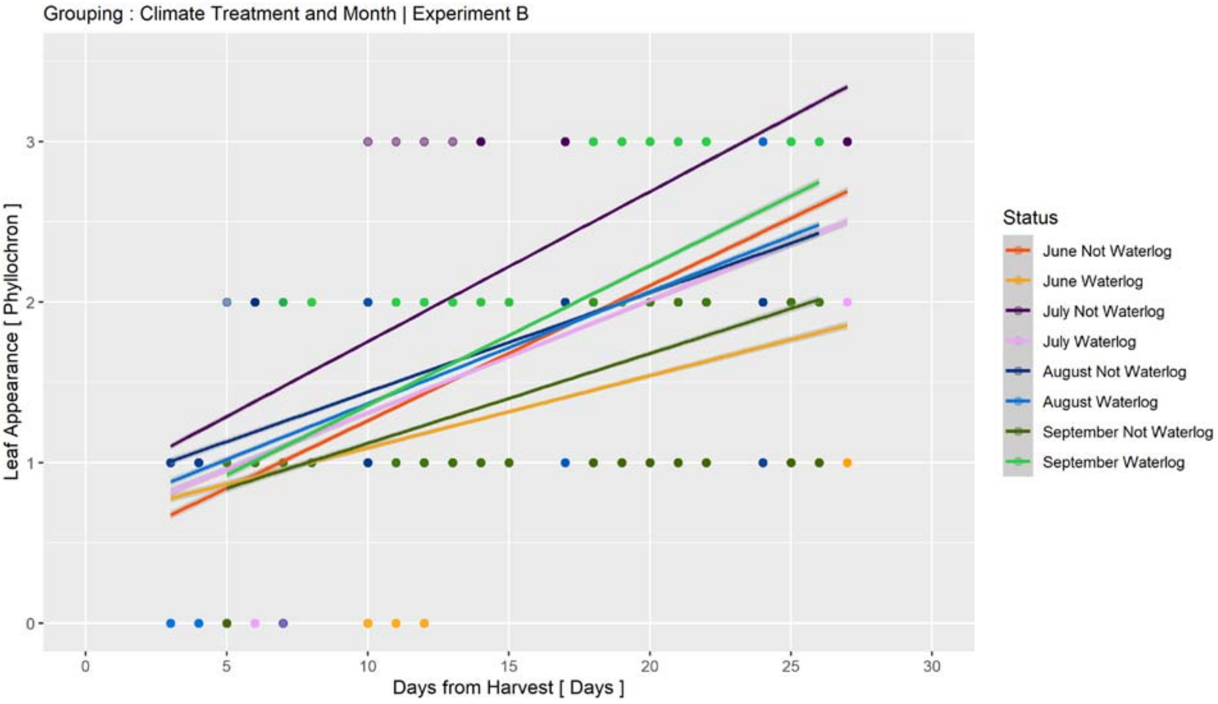


Figure 5.5. Linear phyllochron progression as each month progresses in experiment B. The graph shows the interaction between month and water status. For each month, the non-waterlogging treatment is shown in the darker shade and the waterlogging treatment in the lighter shade of the same colour range. Many data points overlap, as the graph contains approximately 23,000 data points.

did the plants recover, but in September they also far outperformed the non-waterlogged plants. How far this effect stretches beyond September is uncertain, as is how far waterlogging in other months would influence this dynamic. The effects of waterlogging in our case are likely to have been reversed because of

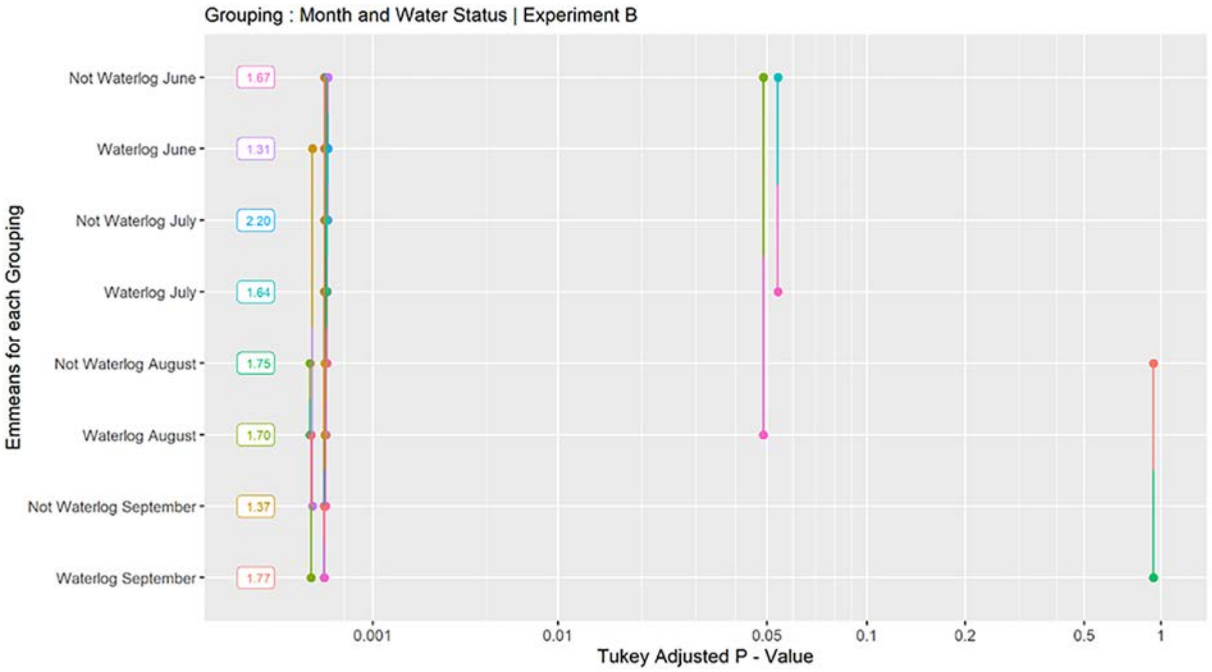


Figure 5.6. Pairwise comparison of the interaction between month and water status from the model in Table 5.2 and experiment B.

accelerated root proliferation and increased nutrient absorption by previously waterlogged plants as the water receded. Waterlogging has previously been shown to reduce nutrient uptake by plants and even cause nutrient migration in the soil column, potentially making nutrients temporarily unavailable. While it can be argued that the nutrients in non-waterlogged plants might have been used up over the course of 5 months, it is not the case here, as dissolved fertiliser was added to all plants at the beginning of September, reducing this particular difference.

6 Contribution of Environmental and Developmental Drivers to Overall Growth in Perennial Ryegrass

6.1 Overall Plant Growth Modelling

The overall growth of the perennial ryegrass was quantified using three metrics of the harvested plant material: total dried biomass, maximum height and number of tillers. These metrics were measured for all plants during the monthly harvests of both experiments. To understand how the overall growth was influenced primarily by waterlogging and climate change but also between varieties and months as the season progressed, we created two multiple response (i.e. multivariate) linear mixed models, one for each experiment. Using these advanced models, we could investigate how the three growth metrics co-vary within a single model. To do this, the metrics were first standardised to facilitate comparison on the same scale. A multiple response correlation structure was then created, which allowed the model to constrain the variation based on individual plants. Lastly, we accounted for the random variation found between chambers to properly evaluate the environmental factors using replicate chambers. The models included both of the main variables mentioned above but also first-degree interactions (e.g. how the effects of climate change might be influenced by waterlogging). The full model was then compared with models from which either a main variable (and all associated interactions) or one single interaction had been removed. This allowed us to properly evaluate the importance of single variables and interactions to the overall performance of the model. This was done by removing the predicted values of a reduced model from the predicted values of the full model, which gives us only the predicted importance of single variables and interactions. These predicted values were then modelled using PCA to visualise how these single variables contribute to the overall growth (the three growth metrics). The predictive variables were the same for the two models except for waterlogging, which was present only in the model from experiment B. The month of May was excluded on the grounds discussed in Chapter 5.

The two models were highly accurate in predicting the overall growth of the perennial ryegrass, with marginal R^2 being 71% and 73% for the models of experiment A and B, respectively (Table 6.1). This shows that the current climatic conditions, the progression of the season, the variety used and whether there are adverse soil water conditions are very good indicators of the overall dried biomass, maximum height and number of tillers in perennial ryegrass. In general, there seems to be a trade-off between height and tiller count in all variables, showing that ryegrass can to some extent prioritise available energy to either of these functions but not to both at the same time. All variables and interactions (except a climate–variety interaction in experiment B) in the two experiments had significant effects on the overall growth. Below we discuss how the four main environmental and developmental conditions influenced the overall growth individually.

6.2 Waterlogging Causes Decreased Growth

Waterlogging of half of the plants during experiment B was conducted at the beginning of June for 1 month and then the water was allowed to subside naturally. The waterlogging was found to have a strong negative effect on the overall growth of perennial ryegrass, accounting for approximately 40% of the overall variation. The interaction between month and waterlogging accounted for approximately 15% of the model variation, caused by the time-limited extent of the waterlogging during June and the recovery process that followed as the waterlogging dissipated. There were significant interactions with variety and climate change for waterlogging, but the effects were minor ($R^2 \sim 1\%$), showing that these interactions have only limited practical implications and that varieties respond similarly to the negative effects of waterlogging. The waterlogging affected all three growth metrics but primarily dried biomass (Figure 6.1). The difference in biomass between the waterlogged and non-waterlogged plants increased as the season progressed, until September, when the plants started to properly recover. The recovery was primarily seen in maximum height, as the waterlogged plants had grown

Table 6.1. Model statistics and significance levels for comparing the full multiple response linear mixed model including first-degree interaction terms with removing one main variable and all associated interaction terms one at a time or one interaction term alone

Model			Model statistics							
	Main effect	Interaction effect	df	AIC	Δ AIC	Test	Likelihood ratio	*p*-value	Significance	R^2m
A	<full>		231	6756		1				0.70
	Climate treatment		180	6800	–44.0	1 vs 2	150	$< 10^{-10}$	***	0.67
		Climate treatment: variety	192	6764	–8.1	1 vs 3	86	2.1×10^{-5}	***	0.70
		Climate treatment: month	222	6784	–28	1 vs 4	46	5.5×10^{-7}	***	0.71
	Variety		36	7718	–960	1 vs 5	1400	$< 10^{-10}$	***	0.58
		Variety: month	114	6892	–140	1 vs 6	370	$< 10^{-10}$	***	0.69
	Month		96	10,086	–3300	1 vs 7	3600	$< 10^{-10}$	***	0.13
B	<full>		105	5483		8				0.73
	Water status		81	7840	–2400	8 vs 9	2400	$< 10^{-10}$	***	0.34
		Water status: variety	96	5550	–67	8 vs 10	85	$< 10^{-10}$	***	0.72
		Water status: climate treatment	102	5496	–13	8 vs 11	19	3.1×10^{-4}	***	0.72
		Water status: month	96	7061	–1600	8 vs 12	1600	$< 10^{-10}$	***	0.56
	Climate treatment		81	5496	–13	8 vs 13	61	4.1×10^{-5}	***	0.72
		Climate treatment: variety	96	5470	13	8 vs 14	5.3	0.81	NS	0.73
		Climate treatment: month	96	5497	–14	8 vs 15	32	2.1×10^{-4}	***	0.72
	Variety		51	5748	–270	8 vs 16	370	$< 10^{-10}$	***	0.69
		Variety: month	78	5557	–74	8 vs 17	130	$< 10^{-10}$	***	0.72
	Month		51	8730	–3200	8 vs 18	3400	$< 10^{-10}$	***	0.21

Significance: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; NS, non-significant ($p > 0.05$).

Random error: chamber. Function: ANOVA. Multiple responses: biomass growth rate, height growth rate, tiller numbers. Multiple response correlation structure: response | chamber/month and ID. Data source: experiments A and B.

AIC, Akaike information criterion; df, degrees of freedom; R^2m, marginal R^2.

taller than the non-waterlogged plants by the harvest in September. Tiller count was the growth metric that recovered the least from the waterlogging, suggesting that this has the longest recuperation period.

6.3 Climate Change Resilience

Climate change was found to have a significant but small positive effect on the overall growth of perennial ryegrass. The main variable accounted for approximately 1–4% of overall model understanding. This shows that, by 2050, a 2°C increase in temperature and a CO_2 concentration of 550 ppm will have a small positive effect on overall growth. The interaction with varieties varied between the two experiments, with experiment A involving a larger range of varieties, indicating that some varieties respond differently to climate change, causing larger overall variation (Figure 6.2). This is probably because experiment A included varieties of semi-natural and wild origin rather than only cultivars, and the semi-natural and wild varieties responded to climate change in relation to their origin location. Meanwhile, experiment B, which included four common cultivars, suggested that these four varieties (Aberchoice, Abergain, Carraig and Dunluce) respond to climate

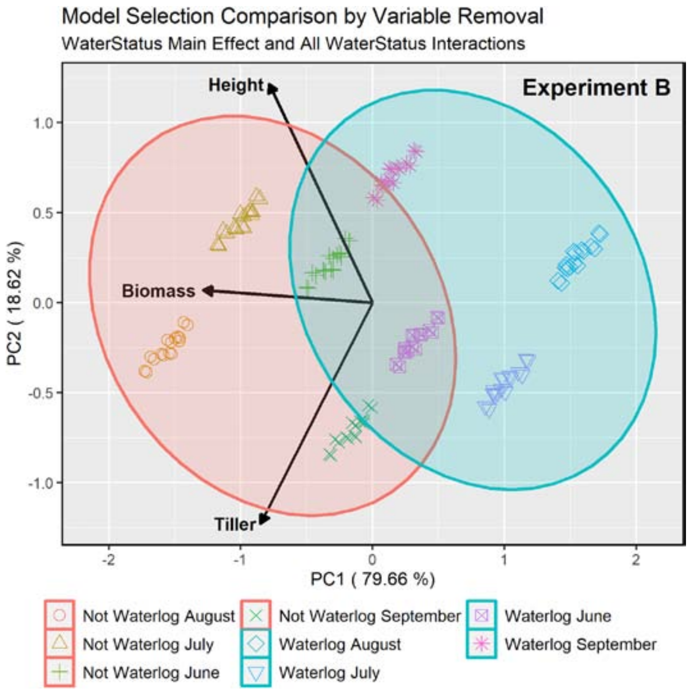


Figure 6.1. PCA of the model variation contributed by waterlogging and all waterlogging interactions to the modelling of perennial ryegrass biomass, height and tiller count. Only experiment B is included. Each ellipse represents the 95% confidence region for each group. See Table 6.1 for the full model and all interaction terms.

change in the same way, with small positive effects seen on overall growth. We observed significant but small effects of the waterlogging and month interactions ($R^2 < 1\%$), suggesting that these have few practical implications.

6.4 Genetic Variation and Response to Environmental Stimuli

We found significant differences in overall plant growth between perennial ryegrass varieties. Variety corresponds to approximately 5–13% of model understanding, with the larger number being because experiment A contained more varieties. This makes sense, as we would expect greater variation with the inclusion of more varieties (Figure 6.3). The genetic background is likely to be relevant here, with the cultivars generally showing higher overall growth than the semi-natural and wild varieties, having been bred for increased performance and yield. The four tetraploid varieties (Abergain, Aspect, Carraig and Dunluce) generally have higher maximum height than the diploid varieties, with Carraig also having the highest dried biomass of all varieties in both experiments. While the response of many varieties overlaps, some have distinctly different responses, suggesting that there is genetic variation between the varieties that causes their growth responses to environmental stimuli to diverge. This is partly confirmed by the significant but small effect of the interactions with waterlogging, with climate change and between months ($R^2 < 2\%$). This suggests that varieties respond differently to environmental stimuli, but that the effects are generally small.

6.5 Peak Growth Months

The progression of the season is fundamental to plant growth. This is because of not only increased light hours and temperatures, but also accelerated growth, as plants grow to acquire more resources from their environment. This can be observed in our models as well, as the progression of the season (months) corresponds to approximately 50–55% of the model understanding, making it the most important variable in predicting perennial ryegrass growth. August is the month of optimal growth, and June is the month of the least increase in growth (Figure 6.4). This is likely to be influenced partly by the sowing and germination being in May (only 1 month before),

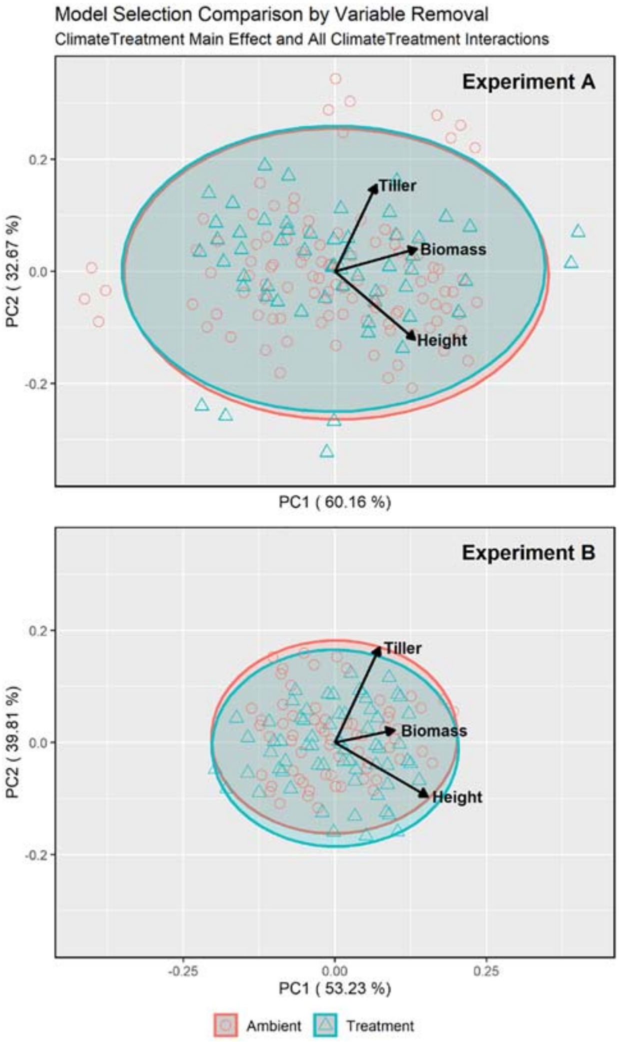


Figure 6.2. PCA of the model variation contributed by climate treatment and all climate treatment interactions to the modelling of perennial ryegrass biomass, height and tiller count. Both experiments A and B are included. Each ellipse represents the 95% confidence region for each group. See Table 6.1 for the full model and all interaction terms.

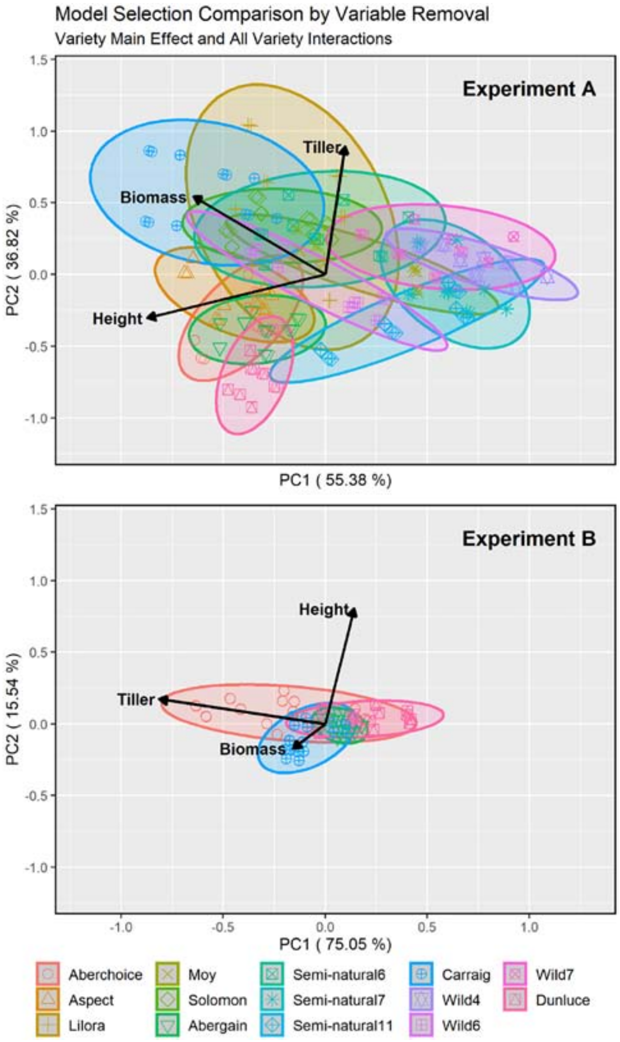


Figure 6.3. PCA of the model variation contributed by variety and all variety interactions to the modelling of perennial ryegrass biomass, height and tiller count. Both experiments A and B are included. Each ellipse represents the 95% confidence region for each group. See Table 6.1 for the full model and all interaction terms.

limiting the overall growth compared with already established plants from previous years in field conditions. The months are naturally separated (as in experiment A), and the overlap in growth in experiment B is caused by the inclusion of waterlogging, which causes greater within-month variation of plant growth. As mentioned previously, this is visible in the strong interaction between waterlogging and month. There is a significant interaction with climate change, but the effects are very small (R^2 ~ 0.1%), suggesting that climate change is likely to affect the growth in every month equally.

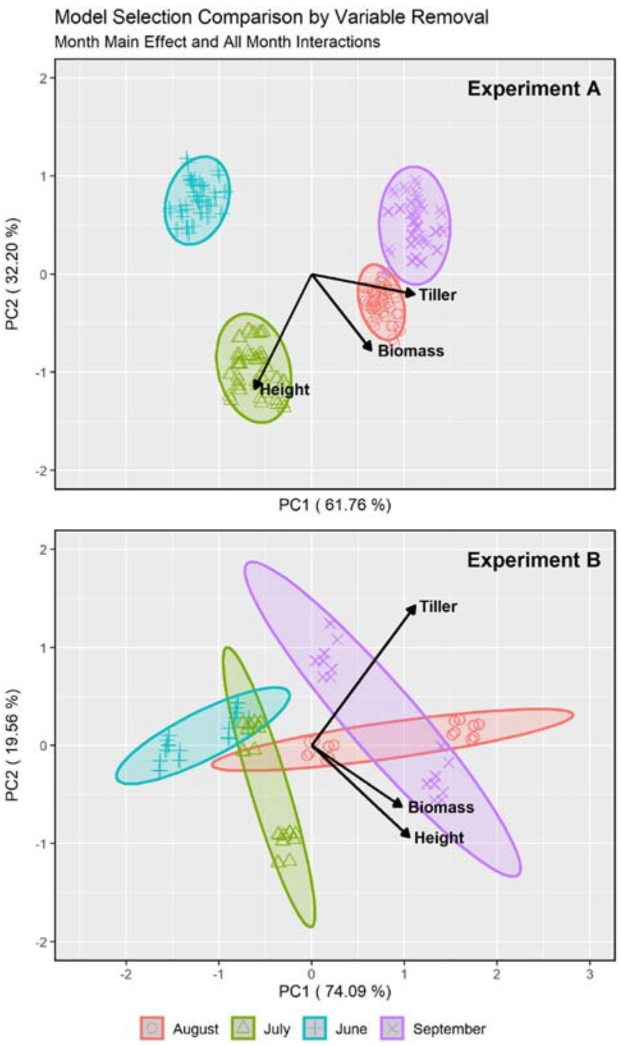


Figure 6.4. PCA of the model variation contributed by month and all month interactions to the modelling of perennial ryegrass biomass, height and tiller count. Includes both experiment A and B. Each ellipse represents a 95% confidence region for each group. See Table 6.1 for the full model and all interaction terms.

7 Summary and Recommendations

7.1 Monitoring of Grassland Phenology Using Remote Sensing

Despite the potential issues of frequent cloud cover over Ireland, we found that satellite data with a high temporal resolution (e.g. daily) can provide sufficiently resolved year-round time-series to quantify the major phenophases of grassland growth across the entire island of Ireland. While we have used satellite data with a medium spatial resolution (250 m), a finer spatial resolution combined with a more accurate land cover map (ideally produced specifically for Ireland) would reduce signal contamination from non-grassland features (e.g. hedges, small groups of trees and buildings) and improve the precision of the phenophase estimates.

We have not been able to associate our phenophase estimates with environmental variables, such as aspect, elevation, land cover and high-resolution meteorological data. Developing these associations would be a valuable way of generating hypotheses about the mechanisms underlying the environmental control of grassland phenology. We have also not been able to perform a ground-based quantitative validation of the remote-sensing phenophase estimates. Our validation has been somewhat subjective, associating exceptional phenophase predictions with exceptional years for grassland growth.

We recommend that remote sensing be incorporated as a new observational approach to complement the existing ground-based phenological recording. We also recommend that ground-based validation data of grassland phenophases be incorporated into the use of remote-sensing data.

At the individual plant level, we have also shown that visual photographs of a plant can be analysed to provide useful information about the plant's condition and potential for growth. This approach is cheap and relatively simple, making it worth developing as an additional approach for monitoring phenological change.

7.2 All-Ireland Trends in Grassland Phenology

Using satellite remote-sensing data we found little temporal trends in start of season, peak of season or end of season. We did find evidence of increased variability in the start of season since 2010, although variation in the other phenophases (peak of season and end of season) is greater than variation in the start of season. For example, variation in the length of the growing season is primarily correlated with the end of season, a result that has been found elsewhere (e.g. Yu *et al.*, 2017).

It is possible that our data do not provide sufficient power to detect phenological change over the two decades that we have studied. However, other published studies have used similar data over similar periods, but for different geographical regions, and found evidence of temporal trends in phenology (e.g. Hua *et al.*, 2021). So, if Ireland has seen a year-on-year change in its grassland phenology, this is likely to be a relatively slow change compared with the results of these other studies.

Since the most evident change in Ireland's grassland phenology is the uncertainty in year-to-year changes in the start of season, we recommend that changes in phenology are quantified using a risk-based approach (e.g. probability that performance will drop below a defined threshold).

7.3 Phenology and Growth of Perennial Ryegrass in Response to Elevated CO_2 Concentration and Temperature

The results of the two growth chamber experiments show that a combination of increased temperature and CO_2 concentration will lead to a small increase in the growth of perennial ryegrass. The effect of this climate change on growth is greatest towards the start and end of the growing season (June and

September), suggesting that climate change will tend to change ryegrass phenology by extending the length of the growing season rather than increasing the peak growth.

The first growth chamber experiment (experiment A), which used 14 ryegrass varieties, had the most power to detect the effects of ryegrass variety on the growth response to climate change. In general, the cultivars showed greater growth than wild and semi-natural varieties. However, we see exceptions. For example, “wild-6” and “semi-natural-6” had biomass production and tiller counts that were greater than or equal to those of several cultivars (Solomon, Aberchoice, Abergain, Aspect, Lilora, Carraig) on the Department of Agriculture, Food and the Marine Irish Recommended List (DAFM, 2022).

We recommend that grassland managers should not expect future increases in temperature and CO_2 concentration to have a strong effect on ryegrass growth and the associated carbon storage in the above-ground plant material that results from plant growth. Some wild and semi-natural ryegrass varieties may have useful traits for breeding varieties that can withstand future climatic conditions with little cost to growth performance.

7.4 Phenology and Growth of Perennial Ryegrass in Response to Waterlogging

Our second growth chamber experiment (experiment B) included the effect of waterlogging on a reduced number of ryegrass varieties. Waterlogging was found to have a much stronger effect on ryegrass growth and the timing of the growth than the climate change treatment. Waterlogging reduced growth, with the effect seen primarily on biomass. The reduced growth also meant that plant development was delayed compared with that of the non-waterlogged plants. This reduction in growth rate was mitigated to some extent by the effect of increased temperature and CO_2 concentration. The different varieties all had very similar responses to the waterlogging.

Once the soil moisture had returned to pre-waterlogging values, the waterlogged plants showed signs of recovery, and even some compensation. Recovery was seen first in the height of the plants.

We recommend that ryegrass breeding programmes include a trait that captures a plant’s resilience to waterlogging, because these extreme weather events are likely to have important impacts on grassland production and animal welfare if the start of season is delayed. Liu *et al*. (2021) come to a similar conclusion, proposing a combination of breeding programmes and management to mitigate some of the effects of waterlogging.

One phenotype that was beyond the scope of the current project but would deserve more attention in the future is root growth and architecture. This may have a direct impact on not only waterlogging resilience but also soil carbon storage, and hence future research and breeding programmes should take this important trait into account.

References

Andrés, F. and Coupland G., 2012. The genetic basis of flowering responses to seasonal cues. *Nature Reviews Genetics* 13: 627–39. https://doi.org/10.1038/nrg3291

Barickman, T.C., Simpson, C.R. and Sams, C.E., 2019. Waterlogging causes early modification in the physiological performance, carotenoids, chlorophylls, proline, and soluble sugars of cucumber plants. *Plants* 160: 1–15. https://doi.org/10.3390/plants8060160

Burke, M., Driscoll, A., Lobell, D.M. and Ermon, S., 2021. Using satellite imagery to understand and promote sustainable development. *Science* 371: eabe8628. https://doi.org/10.1126/science.abe8628

Chalker-Scott, L., 1999. Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology* 70: 1–9. https://doi.org/10.1111/j.1751-1097.1999.tb01944.x

Chen, D.X., Hunt, H.W. and Morgan, J.A., 1996. Responses of a C3 and C4 perennial grass to CO_2 enrichment and climate change: comparison between model predictions and experimental data. *Ecological Modelling* 87: 11–27. https://doi.org/10.1016/0304-3800(94)00199-5

Close, D.C. and Davidson, N.J., 2003. Long-term waterlogging: nutrient, gas exchange, photochemical and pigment characteristics of *Eucalyptus nitens* saplings. *Russian Journal of Plant Physiology* 50: 843–847. https://doi.org/10.1023/B:RUPP.0000003284.25827.95

DAFM, 2022. *Grass and White Clover Varieties: Irish Recommended List 2022*. Department of Agriculture, Fisheries and the Marine, Dublin, Ireland.

Dáil Éireann, 2018. Dáil Éireann debate: the fodder crisis. Available online: https://www.oireachtas.ie/en/debates/question/2018-07-12/15/ (accessed 1 June 2022).

Donnelly, A., 2018. Climate change: potential implications for Ireland's biodiversity. *International Journal of Biometeorology* 62: 1221–1228. https://doi.org/10.1007/s00484-018-1526-2

Donnelly, A., Proctor, H. and O'Connor, B., 2013. *Ireland's National Phenology Network (IE-NPN)*. Climate Change Research Programme (CCRP) 2007–2013, Report Series No. 23. Environmental Protection Agency, Johnstown Castle, Ireland.

Dusenge, M.E., Duarte, A.G. and Way, D.A., 2019. Plant carbon metabolism and climate change: elevated CO_2 and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytologist* 221: 32–49. https://doi.org/10.1111/nph.15283

European Parliament, 2018. Fodder crisis in Ireland. Available online: https://www.europarl.europa.eu/doceo/document/P-8-2018-004316_EN.html (accessed 1 June 2022).

Frisk, C.A., Xistris-Songpanya, G., Osborne, B.A., Biswas, Y., Melzer, R. and Yearsley, J.M., 2022. Phenotypic variation from waterlogging in multiple perennial ryegrass varieties under climate change conditions. *bioRxiv* 1–33. https://doi.org/10.1101/2022.03.07.483244

Green, S., 2013. Fodder crisis mapped from space. Available online: https://www.teagasc.ie/rural-economy/rural-economy/spatial-analysis/fodder-crisis-mapped-from-space/ (accessed 1 June 2022).

Hijmans, R., 2022. terra: Spatial Data Analysis. R package version 1.5–21. Available online: https://CRAN.R-project.org/package=terra

Hu, J., Moore, D.J.P., Burns, S.P. and Monson, R.K., 2010. Longer growing seasons lead to less carbon sequestration by a subalpine forest. *Global Change Biology* 16: 771–83. https://doi.org/10.1111/j.1365-2486.2009.01967.x

Hua, X., Sirguey, P. and Ohlemüller, R., 2021. Recent trends in the timing of the growing season in New Zealand's natural and semi-natural grasslands. *GIScience & Remote Sensing* 58: 1090–1111. https://doi.org/10.1080/15481603.2021.1969629

Huete, A., Didan, K., Miura, T., Rodriguez, E., Gao, X. and Ferreira, L., 2002. Overview of the radiometric and biophysical performance of the MODIS vegetation indices. *Remote Sensing of the Environment* 83: 195–213. https://doi.org/10.1016/S0034-4257(02)00096-2

IPCC, 2014. *Climate Change 2014: Synthesis Report*. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change, Geneva, Switzerland.

Kharouba, H.M., Ehrlén, J., Gelman, A., Bolmgren, K., Allen, J.M., Travers, S.E. and Wolkovich, E.M., 2018. Global shifts in the phenological synchrony of species interactions over recent decades. *Proceedings of the National Academy of Sciences USA* 115: 5211–16. https://doi.org/10.1073/pnas.1714511115

Liu, K., Harrison, M.T., Archontoulis, S.V., Huth, N., Yang, R., Liu, D. L., Yan, H., Meinke, H., Huber, I. and Feng, P., 2021. Climate change shifts forward flowering and reduces crop waterlogging stress. *Environmental Research Letters* 16. https://doi.org/10.1088/1748-9326/ac1b5a

MATLAB, 2021. MATLAB version 9.11.0.1873467 (R2021b). The Mathworks Inc., Natick, MA.

Met Éireann, 2010. *Exceptional Weather Events: Cold Weather, Winter 2009/2010*. Available online: https://www.met.ie/cms/assets/uploads/2017/08/Winter2009-10.pdf (accessed 10 June 2022).

Met Éireann, 2018. *Summer 2018 Analysis: An Analysis of the Heatwaves and Droughts That Affected Ireland and Europe in the Summer of 2018*. Available online: https://www.met.ie/summer-2018-analysis (accessed 10 January 2023).

Muggeo, V.M.R., 2003. Estimating regression models with unknown break-points. *Statistics in Medicine* 22: 3055–3071. https://doi.org/10.1002/sim.1545

O'Neill, F.H., Martin, J.R., Devaney, F.M. and Perrin, P.M., 2013. *The Irish Semi-Natural Grasslands Survey 2007–2012*. Irish Wildlife Manuals No. 78. National Parks and Wildlife Service, Department of Environment, Heritage and Local Government, Dublin, Ireland.

Ou, L.J., Dai, X.Z., Zhang, Z.Q. and Zou, X.X., 2011. Responses of pepper to waterlogging stress. *Photosynthetica* 49: 339–345. https://doi.org/10.1007/s11099-011-0043-x

Pebesma, E.J., 2004. Multivariable geostatistics in S: the gstat package. *Computers & Geosciences* 30: 683–691. https://doi.org/10.1016/j.cageo.2004.03.012

Pebesma, E.J., 2018. Simple features for R: standardized support for spatial vector data. *The R Journal* 10: 439–446. https://doi.org/10.32614/RJ-2018-009

Pinheiro, J.C. and Bates, D.M., 2000. *Mixed-Effects Models in S and S-PLUS*. Springer, New York, NY. https://doi.org/10.1007/b98882

Pinheiro, J., Bates, D. and R Core Team, 2022. nlme: linear and nonlinear mixed effects models. R package version 3.1–157. Available online: https://CRAN.R-project.org/package=nlme (accessed 9 January 2023).

R Core Team, 2022. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: https://www.R-project.org/ (accessed 1 June 2022).

Richardson, A.D., Keenan, T.F., Migliavacca, M., Ryu, Y., Sonnentag, O. and Toomey, M., 2013. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agricultural and Forest Meteorology* 169: 156–73. https://doi.org/10.1016/j.agrformet.2012.09.012

Sandring, S. and Ågren, J., 2009. Pollinator-mediated selection on floral display and flowering time in the perennial herb *Arabidopsis lyrata*. *Evolution* 63: 1292–1300. https://doi.org/10.1111/j.1558-5646.2009.00624.x

Schilling, S., Pan, S., Kennedy, A. and Melzer, R., 2018. MADS-box genes and crop domestication: the jack of all traits. *Journal of Experimental Botany* 69: 1447–69. https://doi.org/10.1093/jxb/erx479

Seanad Éireann, 2013. Seanad Éireann debate: "fodder crisis statements". Available online: https://www.oireachtas.ie/en/debates/debate/seanad/2013-05-09/16/ (accessed 1 June 2022).

Smethurst, C.F. and Shabala, S., 2003. Screening methods for waterlogging tolerance in lucerne: comparative analysis of waterlogging effects on chlorophyll fluorescence, photosynthesis, biomass and chlorophyll content. *Functional Plant Biology* 30: 335–343. https://doi.org/10.1071/FP02192

Weiss, M., Jacob, F. and Duveiller, G., 2020. Remote sensing for agricultural applications: a meta-review. *Remote Sensing of Environment* 236. https://doi.org/10.1016/j.rse.2019.111402

White, H.J., Gaul, W., Sadykova, D., León-Sánchez, L., Caplat, P., Emmerson, M.C. and Yearsley, J.M., 2020. Quantifying large-scale ecosystem stability with remote sensing data. *Remote Sensing in Ecology and Conservation* 6: 354–365. https://doi.org/10.1002/rse2.148

White, H.J., Caplat, P., Emmerson, M.C. and Yearsley, J.M., 2021. Predicting future stability of ecosystem functioning under climate change. *Agriculture, Ecosystems & Environment* 320: 107600. https://doi.org/10.1016/j.agee.2021.107600

White, H.J., Gaul, W., León-Sánchez, L., Sadykova, D., Emmerson, M.C., Caplat, P. and Yearsley, J.M., 2022. Ecosystem stability at the landscape scale is primarily associated with climatic history. *Functional Ecology* 36: 622–634. https://doi.org/10.1111/1365-2435.13957

Wingler, A., Cawkwell, F., Holloway, P., Gourav, M., de la Torre Cerro, R. and Sweeney, C., 2022. *PhenoClimate: Impact of Climate Change on Phenology in Ireland*. Research Report No. 404. Environmental Protection Agency, Johnstown Castle, Ireland.

Wood, S.N., 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society (B)* 73: 3–36. https://doi.org/10.1111/j.1467-9868.2010.00749.x

Wood, S.N., 2017. *Generalised Additive Models: An Introduction with R.* 2nd edition. CRC Press, London.

Yiotis, C., McElwain, J.C. and Osborne, B.A., 2021. Enhancing the productivity of ryegrass at elevated CO_2 is dependent on tillering and leaf area development rather than leaf-level photosynthesis. *Journal of Experimental Botany* 72: 1962–1977. https://doi.org/10.1093/jxb/eraa584

Yu, L., Liu, T., Bu, K., Yan, F., Yang, J., Chang, L. and Zhang, S., 2017. Monitoring the long term vegetation phenology change in Northeast China from 1982 to 2015. *Scientific Reports* 7: 14770. https://doi.org/10.1038/s41598-017-14918-4

Zhu, X., Helmer, E.H., Gwenzi, D., Collin, M., Fleming, S., Tian, J., Marcano-Vega, H., Meléndez-Ackerman, E.J. and Zimmerman, J.K., 2021. Characterisation of dry-season phenology in tropical forests by reconstructing cloud-free Landsat time series. *Remote Sensing* 13: 4736. https://doi.org/10.3390/rs13234736

Abbreviations

AIC	Akaike information criterion
CI	Confidence interval
df	Degrees of freedom
DSH	Days since harvest
EOS	End of season
EVI	Enhanced vegetation index
GAM	Generalised additive model
MODIS	Moderate Resolution Imaging Spectroradiometer
PC1	First principal component
PC2	Second principal component
PC3	Third principal component
PCA	Principal component analysis
POS	Peak of season
RGB	Red, green and blue
SOS	Start of season
SPAD	Soil plant analysis development

An Ghníomhaireacht Um Chaomhnú Comhshaoil

Tá an GCC freagrach as an gcomhshaol a chosaint agus a fheabhsú, mar shócmhainn luachmhar do mhuintir na hÉireann. Táimid tiomanta do dhaoine agus don chomhshaol a chosaint ar thionchar díobhálach na radaíochta agus an truaillithe.

Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

Rialáil: Rialáil agus córais chomhlíonta comhshaoil éifeachtacha a chur i bhfeidhm, chun dea-thorthaí comhshaoil a bhaint amach agus díriú orthu siúd nach mbíonn ag cloí leo.

Eolas: Sonraí, eolas agus measúnú ardchaighdeáin, spriocdhírithe agus tráthúil a chur ar fáil i leith an chomhshaoil chun bonn eolais a chur faoin gcinnteoireacht.

Abhcóideacht: Ag obair le daoine eile ar son timpeallachta glaine, táirgiúla agus dea-chosanta agus ar son cleachtas inbhuanaithe i dtaobh an chomhshaoil.

I measc ár gcuid freagrachtaí tá:

Ceadúnú

- Gníomhaíochtaí tionscail, dramhaíola agus stórála peitril ar scála mór;
- Sceitheadh fuíolluisce uirbigh;
- Úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe;
- Foinsí radaíochta ianúcháin;
- Astaíochtaí gás ceaptha teasa ó thionscal agus ón eitlíocht trí Scéim an AE um Thrádáil Astaíochtaí.

Forfheidhmiú Náisiúnta i leith Cúrsaí Comhshaoil

- Iniúchadh agus cigireacht ar shaoráidí a bhfuil ceadúnas acu ón GCC;
- Cur i bhfeidhm an dea-chleachtais a stiúradh i ngníomhaíochtaí agus i saoráidí rialáilte;
- Maoirseacht a dhéanamh ar fhreagrachtaí an údaráis áitiúil as cosaint an chomhshaoil;
- Caighdeán an uisce óil phoiblí a rialáil agus údaruithe um sceitheadh fuíolluisce uirbigh a fhorfheidhmiú
- Caighdeán an uisce óil phoiblí agus phríobháidigh a mheasúnú agus tuairisciú air;
- Comhordú a dhéanamh ar líonra d'eagraíochtaí seirbhíse poiblí chun tacú le gníomhú i gcoinne coireachta comhshaoil;
- An dlí a chur orthu siúd a bhriseann dlí an chomhshaoil agus a dhéanann dochar don chomhshaol.

Bainistíocht Dramhaíola agus Ceimiceáin sa Chomhshaol

- Rialacháin dramhaíola a chur i bhfeidhm agus a fhorfheidhmiú lena n-áirítear saincheisteanna forfheidhmithe náisiúnta;
- Staitisticí dramhaíola náisiúnta a ullmhú agus a fhoilsiú chomh maith leis an bPlean Náisiúnta um Bainistíocht Dramhaíola Guaisí;
- An Clár Náisiúnta um Chosc Dramhaíola a fhorbairt agus a chur i bhfeidhm;
- Reachtaíocht ar rialú ceimiceán sa timpeallacht a chur i bhfeidhm agus tuairisciú ar an reachtaíocht sin.

Bainistíocht Uisce

- Plé le struchtúir náisiúnta agus réigiúnacha rialachais agus oibriúcháin chun an Chreat-treoir Uisce a chur i bhfeidhm;
- Monatóireacht, measúnú agus tuairisciú a dhéanamh ar chaighdeán aibhneacha, lochanna, uiscí idirchreasa agus cósta, uiscí snámha agus screamhuisce chomh maith le tomhas ar leibhéil uisce agus sreabhadh abhann.

Eolaíocht Aeráide & Athrú Aeráide

- Fardail agus réamh-mheastacháin a fhoilsiú um astaíochtaí gás ceaptha teasa na hÉireann;
- Rúnaíocht a chur ar fáil don Chomhairle Chomhairleach ar Athrú Aeráide agus tacaíocht a thabhairt don Idirphlé Náisiúnta ar Ghníomhú ar son na hAeráide;
- Tacú le gníomhaíochtaí forbartha Náisiúnta, AE agus NA um Eolaíocht agus Beartas Aeráide.

Monatóireacht & Measúnú ar an gComhshaol

- Córais náisiúnta um monatóireacht an chomhshaoil a cheapadh agus a chur i bhfeidhm: teicneolaíocht, bainistíocht sonraí, anailís agus réamhaisnéisiú;
- Tuairiscí ar Staid Thimpeallacht na hÉireann agus ar Tháscairí a chur ar fáil;
- Monatóireacht a dhéanamh ar chaighdeán an aeir agus Treoir an AE i leith Aeir Ghlain don Eoraip a chur i bhfeidhm chomh maith leis an gCoinbhinsiún ar Aerthruailliú Fadraoin Trasteorann, agus an Treoir i leith na Teorann Náisiúnta Astaíochtaí;
- Maoirseacht a dhéanamh ar chur i bhfeidhm na Treorach i leith Torainn Timpeallachta;
- Measúnú a dhéanamh ar thionchar pleananna agus clár beartaithe ar chomhshaol na hÉireann.

Taighde agus Forbairt Comhshaoil

- Comhordú a dhéanamh ar ghníomhaíochtaí taighde comhshaoil agus iad a mhaoiniú chun brú a aithint, bonn eolais a chur faoin mbeartas agus réitigh a chur ar fáil;
- Comhoibriú le gníomhaíocht náisiúnta agus AE um thaighde comhshaoil.

Cosaint Raideolaíoch

- Monatóireacht a dhéanamh ar leibhéil radaíochta agus nochtadh an phobail do radaíocht ianúcháin agus do réimsí leictreamaighnéadacha a mheas;
- Cabhrú le pleananna náisiúnta a fhorbairt le haghaidh éigeandálaí ag eascairt as taismí núicléacha;
- Monatóireacht a dhéanamh ar fhorbairtí thar lear a bhaineann le saoráidí núicléacha agus leis an tsábháilteacht raideolaíochta;
- Sainseirbhísí um chosaint ar an radaíocht a sholáthar, nó maoirsiú a dhéanamh ar sholáthar na seirbhísí sin.

Treoir, Ardú Feasachta agus Faisnéis Inrochtana

- Tuairisciú, comhairle agus treoir neamhspleách, fianaise-bhunaithe a chur ar fáil don Rialtas, don tionscal agus don phobal ar ábhair maidir le cosaint comhshaoil agus raideolaíoch;
- An nasc idir sláinte agus folláine, an geilleagar agus timpeallacht ghlan a chur chun cinn;
- Feasacht comhshaoil a chur chun cinn lena n-áirítear tacú le hiompraíocht um éifeachtúlacht acmhainní agus aistriú aeráide;
- Tástáil radóin a chur chun cinn i dtithe agus in ionaid oibre agus feabhsúchán a mholadh áit is gá.

Comhpháirtíocht agus Líonrú

- Oibriú le gníomhaireachtaí idirnáisiúnta agus náisiúnta, údaráis réigiúnacha agus áitiúla, eagraíochtaí neamhrialtais, comhlachtaí ionadaíocha agus ranna rialtais chun cosaint chomhshaoil agus raideolaíoch a chur ar fáil, chomh maith le taighde, comhordú agus cinnteoireacht bunaithe ar an eolaíocht.

Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil

Tá an GCC á bainistiú ag Bord lánaimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóir. Déantar an obair ar fud cúig cinn d'Oifigí:

1. An Oifig um Inbhunaitheacht i leith Cúrsaí Comhshaoil
2. An Oifig Forfheidhmithe i leith Cúrsaí Comhshaoil
3. An Oifig um Fhianaise agus Measúnú
4. An Oifig um Chosaint ar Radaíocht agus Monatóireacht Comhshaoil
5. An Oifig Cumarsáide agus Seirbhísí Corparáideacha

Tugann coistí comhairleacha cabhair don Ghníomhaireacht agus tagann siad le chéile go rialta le plé a dhéanamh ar ábhair imní agus le comhairle a chur ar an mBord.

EPA Research

Webpages: www.epa.ie/our-services/research/
LinkedIn: www.linkedin.com/showcase/eparesearch/
Twitter: @EPAResearchNews
Email: research@epa.ie

www.epa.ie