Understanding the physiology and agronomic performance of four perennial grass species under different nitrogen and water conditions in New Zealand drylands

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Marcus Vinicius Talamini Junior

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By

M. V. Talamini Junior

The main aim of this thesis was to quantify the effects of nitrogen (N) and moisture on monocultures of brome, cocksfoot, perennial ryegrass and tall fescue over time and understand any species differences in the physiology responses. According to previous studies available in the literature, those species perfom agronomically differently on water and nitrogen lacking environments. Our hypothesis is that, physiologically, the species will show a difference that will justify the different agronomic performance. The pastures were established, at Ladbrooks and Ashley Dene, Canterbury, New Zealand, in the agronomic year of 2014/2015 and this thesis reports assessments for the years 2018/2019 (Year 5) and 2019/2020 (Year 6). The were selected because they have contrasting soil types that leads differences in plant available water content (PAWC). The Wakanui soil at Ladbrooks (LB) had a PAWC, of ~194 mm compared with ~131 mm for the stony Lismore silt at Ashley Dene (AD). The experimental design was a strip-plot design with the four grass species, fertilised (N+) or not (N-) with urea, with four replicates. This research focused on Year 6 and Year 6 after establishment.

Total dry matter production at LB was 18100 kg ha^{-1} in Year 5 and Year 6 for the N+ pastures but 5360 and 6000 kg ha^{-1} for those unfertilised (N-). At AD the pastures yielded 9340 and 6000 kg ha^{-1} for N+ and N- in Year 5 but both yielded ~7395 kg ha^{-1} in Year 6. Cocksfoot yielded the highest (2630 kg ha^{-1}) vegetative dry matter (leaves and swards, VDM) after six years under the driest conditions at Ashley Dene.

The effects of water and nitrogen availability were quantified using a thermal time dry matter accumulation model, with a base temperature of 3 °C for all species. The temperature growth rates ranged from 0.3 kg DM °Cd^{-1} ha^{-1} during the water restricted summer dry periods, for N- plants, at Ashley Dene, up to 13.1 kg DM °Cd^{-1} ha^{-1} for N+ plants during the Spring 2019 at Ladbrooks when water is not restricted.

The highest N+ pasture yields at Ladbrooks were explained by the higher photosynthetically active radiation (PAR) interceptance, and higher radiation use efficiency (RUE) coupled with greater water extraction, water use (WU) and water use efficiency (WUE) than at Ashley Dene.

Plant physiology traits showed that under nitrogen or water deficit, the species had different response strategies. Cocksfoot and brome maintain their leaf area by keeping the cells turgid, with a higher relative water content and osmotic potential than p. ryegrass and tall fescue. These species protected their photosynthetic apparatus and chlorophyll concentration by accumulating higher levels of proline, particulary when nitrogen was provided to enable its synthesis. The strategy of preserving cell turgor and leaf area allowed greater vegetative material for animal production systems at Ashley Dene, which meant cocksfoot provided higher quality forage than the other three species in this dry environment six years after the establishment.

Keywords: Nitrogen, *Festuca arundinacea* Schreb., *Lolium perenne* L., *Dactylis glomerata* L., *Bromus valdivianus* Phil., drylands, pasture, plant physiology, water stress

TABLE OF CONTENTS

ABSTRACT i
Table of Contents iii
List of Tables ix
List of Figures xix
List of Equations xxix
List of Appendices xxx
1 INTRODUCTION 1
  1.1 Farming in summer dry environments. 1
  1.2 Aim, objectives, and thesis structure. 2
2 REVIEW OF THE LITERATURE 5
  2.1 Yield Components 6
    2.1.1 Dry matter accumulation – generating yield 6
    2.1.2 Radiation interceptance (R/Ro) – How much radiation can crops intercept? 7
    2.1.3 Measuring radiation interceptance – Using NDVI and the Green Seeker 8
    2.1.4 Radiation use efficiency (RUE) 9
  2.2 Temperature effects on yield and quantifying it 10
  2.3 Nitrogen effect on crop yield and quality 10
    2.3.1 Nitrogen role in crops 10
    2.3.2 Nitrogen deficiency – limits to dry matter accumulation 11
    2.3.3 Nitrogen effects on forage quality 13
  2.4 Water availability and its effect on pastures 14
    2.4.1 Water availability 14
    2.4.2 Water stress- even more limits to dry matter accumulation 14
  2.5 Physiological response to stress on measurable traits 16
    2.5.1 Chlorophyll concentration 16
    2.5.2 Osmotic potential 17
    2.5.3 Photosynthetic rate and stomatal conductance 18
    2.5.4 Proline 19
    2.5.5 Relative water content 19
  2.6 Species used in this project 20

2.6.1 Pasture brome (*Bromus valdivianus* Phil.) 21
2.6.2 Cocksfoot (*Dactylis glomerata* L.) 21
2.6.3 Perennial ryegrass (*Lolium perenne* L.) 22
2.6.4 Tall fescue (*Festuca arundinacea* Schreb.) 22
2.7 Management of pastures 23
2.8 Persistence 23
2.9 Conclusions 25
3 General materials and methods 26
3.1 Introduction 26
3.2 Materials and Methods 26
3.2.1 Experimental sites 26
3.2.2 Experimental design 26
3.2.3 Soil Characteristics 27
3.2.3.1 Experimental Site 1 – Ladbrooks 27
3.2.3.2 Experimental Site 2 – Ashley Dene 27
3.2.4 Land Use History 28
3.2.4.1 Experimental Site 1 – Ladbrooks 28
3.2.4.2 Experimental Site 2 – Ashley Dene 28
3.2.5 Environmental variables: Temperature and Rainfall 29
3.2.5.1 Historical Data 29
3.2.5.2 Meteorological Data during the experiment 30
3.2.5.3 Potential Soil Moisture Deficit (PMSD) 33
3.2.5.4 Radiation Incidence and Photosynthetic Active Radiation (PAR) 34
3.2.6 Fertility Management 37
3.2.7 Weed Management 39
3.2.7.1 Experiment 1 at Ladbrooks 39
3.2.7.2 Experiment 2 at Ashley Dene 40
3.2.8 Harvest process 41
3.2.9 Dry Matter Yield 42
3.2.9.1 Destructive Measurements 42
3.2.9.2 Non-Destructive Measurements 42
3.2.9.3 Botanical composition 42
3.2.9.4 Dry Matter Quality 42

3.2.9.5 Nitrogen recovery 43
3.3 Statistical Analysis 44
4 Agronomic performance of brome, cocksfoot, perennial ryegrass and tall fescue monocultures under two different nitrogen regimes 46
4.1 Introduction 46
4.2 Materials and methods 47
4.3 RESULTS 47
4.3.1 Total Dry Matter (TDM) 47
4.3.2 Sown Species Dry Matter (SSDM) 53
4.3.3 Vegetative Dry Matter (VDM) 60
4.3.4 Reproductive Dry Matter (RDM) 67
4.3.5 Weed Fraction (WF) 73
4.3.6 Nitrogen Recovery (NR) 79
4.3.7 Protein yield (PY) 85
4.3.8 Metabolisable Energy (ME) 91
4.3.9 Fibres - ADF and NDF 97
4.3.9.1 ADF - Ladbrooks 97
4.3.9.2 NDF – Ladbrooks 100
4.3.9.3 ADF – Ashley Dene 103
4.3.9.4 NDF – Ashley Dene 106
4.3.10 Dry matter accumulation over time 109
4.4 DISCUSSION 120
4.4.1 Pasture yield and persistence 120
4.4.1.1 Total dry matter accumulation per unit of thermal time 120
4.4.1.2 Different botanical components 124
4.4.2 Nitrogen recovery 126
4.4.3 Pasture quality 128
4.5 CONCLUSIONS 130
5 Water use and water use efficiency of pastures under dryland conditions 131
5.1 Introduction 131
5.2 Materials and Methods 131
5.2.1 Soil water content (SWC) 132
5.2.2 Soil moisture content (SWC) for the soil profile 132

5.2.3 Drained upper limit (DUL) and lower limit (LL) 132
5.2.4 Plant available water content (PAWC) 133
5.2.5 Actual soil moisture deficit (ASMD) 133
5.2.6 Water Use (WU) 135
5.2.7 Water use efficiency (WUE)....135
5.3 RESULTS 137
5.3.1 Plant Available Water Content (PAWC)....137
5.3.2 Actual Soil Moisture Deficit (ASMD)....140
5.3.3 Water Use (WU) per year and regrowth cycle 146
5.3.4 Yearly Water Use Efficiency (WUE) 153
5.3.5 Water use efficiency (WUE) per regrowth cycle 160
5.4 DISCUSSION....166
5.4.1 Plant available water content and soil moisture deficit 166
5.4.2 Water use and water use efficiency 169
5.4.2.1 Yield differences within sites and between sites 172
5.5 CONCLUSION....174
6 Light interception and radiation use efficiency of grasses under dryland conditions........175
6.1 Introduction 175
6.2 Materials and Methods....175
6.2.1 Leaf area index (LAI) 175
6.2.2 Radiation Interceptance (R/Ro)....177
6.2.3 Estimation of intercepted PAR in each regrowth cycle....179
6.2.4 Radiation Use Efficiency (RUE) 180
6.3 RESULTS 181
6.3.1 Leaf area index through seasons.....181
6.3.2 Intercepted PAR per regrowth cycle and year 187
6.3.3 Radiation Use Efficiency (RUE) per year....193
6.3.4 Radiation Use Efficiency (RUE) per regrowth cycle....201
6.4 DISCUSSION....206
6.4.1 The effect of nitrogen fertilisation and water availability on the LAI, intercepted PAR and RUE 206
6.4.2 Impact of PAR interceptance and RUE changes in relation to species. 208

6.4.2.1 Yield differences within sites and between sites 209
6.5 CONCLUSION 212
7 Physiological traits 213
7.1 Introduction 213
7.2 Materials and Methods 213
7.2.1 Osmotic Potential 213
7.2.2 Proline Accumulation 214
7.2.3 Photosynthetic Rate and Conductance 215
7.2.4 Chlorophyll Concentration 216
7.2.5 Relative Water Content (RWC) 218
7.2.6 Statistical Analyses 219
7.3 RESULTS 220
7.3.1 Osmotic Potential 220
7.3.2 Proline accumulation 224
7.3.3 Photosynthetic Rate and Conductance 228
7.3.4 Chlorophyll concentration 238
7.3.5 Relative water content (RWC) 242
7.3.6 Principal Component Analysis (PCA) 246
7.3.6.1 General Data 246
7.3.6.2 Ladbrooks 250
7.3.6.3 Ashley Dene 253
7.4 DISCUSSION 256
7.4.1 How do grasses react to changes in the environment? 256
7.4.2 The role of nitrogen 260
7.4.3 Any differences amongst the species ? 261
7.5 CONCLUSIONS 263
8 GENERAL DISCUSSION AND CONCLUSIONS 264
8.1 Pasture production, quality and persistence 264
8.1.1 Temperature 264
8.1.2 Quality 266
8.1.3 Persistence 268
8.2 Soil water availability 268
8.3 Yield reduction mechanisms and their physiology 270

8.4 Implications for farming systems....................272
8.5 Recommendations for future research....................274
8.6 Conclusions....................275
Acknowledgements....................276
References....................277
Appendices....................294

LIST OF TABLES

Table 3-1 Cultivars, sowing rate (kg ha^{-1}) and germination percentage of four grass species at Ladbrooks and Ashley Dene (Sharifiamina, 2018)..28

Table 3-2 Long term monthly means from 1975 to 2012 for total solar radiation (Ro), maximum (Tmax), minimum (Tmin) and mean (Tmean) air temperatures (°C), rainfall (mm), Penman potential evapotranspiration (EP), wind run (km d^{-1}), and vapour pressure deficit (VPD)..30

Table 3-3 Soil test results from Ladbrooks and Ashley Dene from samples taken in 2018 and 2019. 38

Table 3-4 Nitrogen application on each site according to date and rate..39

Table 4-1 Mean accumulated total dry matter (kg DM ha^{-1}), harvested from July 2018 to July 2019 from monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury, New Zealand. 49

Table 4-2 Mean accumulated total dry matter (kg DM ha^{-1}), harvested from July 2019 to July 2020 from monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury, New Zealand. 50

Table 4-3 Total Accumulated Dry Matter (kg ha^{-1}), from July 2018 to July 2019, of brome, cocksfoot, perennial ryegrass and tall fescue sown in monoculture, grown with (N+) and without (N-) nitrogen, at Ashley Dene, Canterbury. 52

Table 4-4 Total Accumulated Dry Matter (kg ha^{-1}), from July 2019 to July 2020, of brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) and without (-N) nitrogen fertilisation, at Ashley Dene, Canterbury..53

Table 4-5 Mean sown species dry matter (kg DM ha^{-1}), harvested from July 2018 to July 2019 from monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury, New Zeland. 55

Table 4-6 Mean sown species dry matter (kg DM ha^{-1}), harvested from July 2019 to July 2020 from monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury, New Zealand. 56

Table 4-7 Mean Sown Species Dry Matter (kg ha^{-1}), harvested from July 2018 to July 2019, of brome, cocksfoot, perennial ryegrass and tall fescue sown in monoculture, grown with (N+) and without (N-) at Ashley Dene, Canterbury..58

Table 4-8 Mean sown species dry matter (kg ha^{-1}), harvested from July 2019 to July 2020, of brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without (N-) at Ashley Dene, Canterbury. 59

Table 4-9 Mean Vegetative Dry Matter (kg DM ha^{-1}) of brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019 grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury. 61

Table 4-10 Mean vegetative dry matter (kg DM ha^{-1}),from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury. 62

Table 4-11 Mean vegetative dry matter (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, grown with (N+) and without (N-) nitrogen, harvested from July 2018 to July 2019 at Ashley Dene, Canterbury. 65

Table 4-12 Mean vegetative dry matter (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, grown with (N+) and without (N-) nitrogen, harvested from July 2019 to July 2020 at Ashley Dene, Canterbury. 66

Table 4-13 Mean reproductive dry matter (kg DM ha^{-1}), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019, grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury, New Zealand. 68

Table 4-14 Mean reproductive dry matter (kg DM ha^{-1}), from significant interactions, for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019 from monocultures grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury, New Zealand..........69

Table 4-15 Mean reproductive dry matter (kg DM ha^{-1}), from four grasses, brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury, New Zealand. 69

Table 4-16 Mean reproductive dry matter (kg DM ha^{-1}), from significant interactions, for four grasses, brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury, New Zealand. 70

Table 4-17 Mean reproductive dry matter (kg^{-1} ha), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, grown with (N+) and without (N-) nitrogen, harvested from July 2018 to July 2019 at Ashley Dene, Canterbury. 72

Table 4-18 Mean reproductive dry matter (kg^{-1} ha), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, grown with (N+) and without (N-) nitrogen, harvested from July 2019 to July 2020 at Ashley Dene, Canterbury. 73

Table 4-19 Mean weed fraction, from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019 from

monocultures grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury. 75
Table 4-20 Mean weed fraction, from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury. 76
Table 4-21 Mean weed fraction, from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen fertilisation, harvested from July 2018 to July 2019 at Ashley Dene, Canterbury. 78
Table 4-22 Mean weed fraction, from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen fertilisation, harvested from July 2019 to July 2020 at Ashley Dene, Canterbury. 79
Table 4-23 Mean nitrogen recovery (NR) (kg N kg N^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2018 to July 2019 at Ladbrooks, Canterbury. 81
Table 4-24 Mean nitrogen recovery (NR) (kg N kg N^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2019 to July 2020 at Ladbrooks, Canterbury. 82
Table 4-25 Mean nitrogen recovery (kg N kg N^{-1})for brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2018 to July 2019 in Ashley Dene, Canterbury, New Zealand. 84
Table 4-26 Mean nitrogen recovery (kg N kg N^{-1}) brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2019 to July 2020 in Ashley Dene, Canterbury, New Zealand. 84
Table 4-27 Mean protein yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019 from monocultures grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury, New Zealand. 87
Table 4-28 Mean protein yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury, New Zealand. 88
Table 4-29 Mean protein yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) and without (N-) nitrogen fertiliser, harvested from July 2018 to July 2019 at Ashley Dene, Canterbury, New Zealand. 90
Table 4-30 Mean protein yield (kg ha^{-1}), for significant interactions, from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) and without (N-) nitrogen fertilisation, harvested from July 2018 to July 2019 at Ashley Dene, Canterbury, New Zealand. 90

Table 4-31 Mean protein yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) and without (N-) nitrogen fertilisation, harvested from July 2019 to July 2020 at Ashley Dene, Canterbury, New Zealand. 91

Table 4-32 Mean metabolisable energy (GJ ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019 from monocultures grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury. 93

Table 4-33 Mean metabolisable energy yield (GJ ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury. 94

Table 4-34 Mean metabolisable energy (GJ ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019 from monocultures grown with (N+) and without (N-) nitrogen at Ashley Dene, Canterbury, New Zealand. 96

Table 4-35 Mean metabolisable energy (GJ ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ashley Dene, Canterbury, New Zealand. 97

Table 4-36 Mean ADF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue grown with (N+) and without nitrogen (N-), harvested from July 2018 to July 2019 from monocultures at Ladbrooks, Canterbury, New Zealand. 99

Table 4-37 Mean ADF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue with (N+) and without nitrogen (N-), harvested from July 2018 to July 2019 from monocultures grown at Ladbrooks, Canterbury, New Zealand. 99

Table 4-38 Mean ADF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue with (N+) and without nitrogen (N-) harvested from July 2019 to July 2020 from monocultures grown at Ladbrooks, Canterbury, New Zealand. ..100

Table 4-39 Mean NDF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue with (N+) and without nitrogen (N-), harvested from July 2018 to July 2019 from monocultures grown at Ladbrooks, Canterbury..........................102

Table 4-40 Mean NDF yield (kg ha^{-1}), in significant interactions, from brome, cocksfoot, perennial ryegrass and tall fescue with (N+) and without nitrogen (N-), harvested from July 2018 to July 2019 from monocultures grown at Ladbrooks, Canterbury. 102

Table 4-41 Mean NDF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury, New Zealand......103

Table 4-42 Mean ADF yield (kg ha^{-1}), from brome, cocksfoot, p. ryegrass and tall fescue harvested from July 2018 to July 2019 from monocultures grown with (N+) and without (N-) nitrogen at Ashley Dene, Canterbury, New Zealand. 105

Table 4-43 Mean ADF yield (kg ha^{-1}) from brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ashley Dene, Canterbury, New Zealand. .106

Table 4-44 Mean NDF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2018 to July 2019 from monocultures grown with (N+) and without (N-) nitrogen at Ashley Dene, Canterbury, New Zealand. .108

Table 4-45 Mean NDF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ashley Dene, Canterbury, New Zealand. .109

Table 4-46 Regression equations for accumulated yield (kg DM ha^{-1}) per unit of accumulated Thermal Time (°Cd, Tb= 3°C) by fertilised and non-fertilised monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand..........................113

Table 4-47 Regression equations for accumulated yield (kg DM ha^{-1}) per unit of accumulated Thermal Time (°Cd, Tb= 3°C) by fertilised and non-fertilised monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand..........................114

Table 4-48 Regression equations for accumulated yield (kg DM ha^{-1}) per unit of accumulated Thermal Time (°Cd, Tb= 3 °C) by fertilised and non-fertilised monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2018 to July 2019 at Ashley Dene, Canterbury, New Zealand. 118

Table 4-49: Regression equations for accumulated yield (kg DM ha^{-1}) per unit of accumulated Thermal Time (°Cd, Tb= 3°C) by fertilised and non-fertilised monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2019 to July 2020 at Ashley Dene, Canterbury, New Zealand. 119

Table 5-1 Maximum water extraction depth (m), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2018 to July 2020 at Ladbrooks, Canterbury, New Zealand...................137

Table 5-2 Calculated maximum plant available water content (mm), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2018 to July 2020 at Ladbrooks, Canterbury, New Zealand. 139

Table 5-3 Maximum water extraction depth (m), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2018 to July 2020 at Ashley Dene, Canterbury, New Zealand. 139

Table 5-4 Calculated maximum plant available water content (mm), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2018 to July 2020 at Ashley Dene, Canterbury, New Zealand. 140

Table 5-5 Estimated drainage (mm), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand...................................143

Table 5-6 Estimated drainage (mm), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand. 144

Table 5-7 Estimated drainage (mm), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2018 to July 2019 at Ashley Dene, Canterbury, New Zealand. 145

Table 5-8 Estimated drainage (mm), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2019 to July 2020 at Ashley Dene, Canterbury, New Zealand. 146

Table 5-9 Water use (mm) for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, fertilised with nitrogen (N+) or not (N-), at Ladbrooks, Canterbury, New Zealand from July 2018 to July 2019. 149

Table 5-10 Water use (mm) by brome, cocksfoot, perennial ryegrass, tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen fertilisation at Ladbrooks, Canterbury, New Zealand, from July 2019 to July 2020. 150

Table 5-11 Water Use (mm) for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, fertilised with nitrogen (N+) or not (N-), at Ashley Dene, Canterbury, New Zealand from July 2018 to July 2019. 152

Table 5-12 Water Use (mm) for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, fertilised with nitrogen (N+) or not (N-), at Ashley Dene, Canterbury, New Zealand from July 2019 to July 2020. 152

Table 5-13 Regression equations for accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand. 154

Table 5-14 Regression equations for accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand. 156

Table 5-15 Regression equations for accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2018 to July 2019 at Ashley Dene, Canterbury, New Zealand. 158

Table 5-16 Regression equations for accumulated yield (kg ha^{-1}) against accumulated water use (mm) by fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue in from July 2019 to July 2020 at Ashley Dene, Canterbury, New Zealand. 160

Table 5-17 Water Use Efficiency (kg DM mm^{-1} ha^{-1}) for brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) or without (N-) nitrogen application, from July 2018 to July 2019 at Ladbrooks,Canterbury, New Zealand. 162

Table 5-18 Water Use Efficiency (kg DM mm^{-1} ha^{-1}) for brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without nitrogen (N-), from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand. 163

Table 5-19 Water use efficiency (kg DM mm^{-1} ha^{-1}) of monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) or without (N-) nitrogen, from July-2018 to July 2019 at Ashley Dene, Canterbury, New Zealand. 165

Table 5-20 Water use efficiency (kg DM mm^{-1} ha^{-1}) of brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) or without (N-) nitrogen, from July-2019 to July 2020 at Ashley Dene, Canterbury, New Zealand..165

Table 6-1 Mean leaf area index (LAI), from monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without nitrogen (N-), harvested from July 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand...........182

Table 6-2 Mean leaf area index, for the interaction of brome, cocksfoot, perennial ryegrass and tall fescue, when grown in monoculture, with (N+) or without nitrogen (N-) measured from July 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand. 183

Table 6-3 Mean leaf area index (LAI) from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, from July 2019 to July 2020 grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury...183

Table 6-4 Mean leaf area index, for the interaction of brome, cocksfoot, perennial ryegrass and tall fescue, when grown in monoculture, with (N+) or without nitrogen (N-) measured from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand. 184

Table 6-5 Mean leaf area index (LAI), of brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) and without (-N) nitrogen fertiliser measured from monocultures from July 2019 to July 2020, at Ashley Dene, Canterbury......186

Table 6-6 Mean leaf area index (LAI), of brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) and without (-N) nitrogen fertilisation harvested from July 2019 to July 2020, at Ashley Dene, Canterbury. 187

Table 6-7 Intercepted PAR per regrowth cycle (MJ m^{-2}) measured from December 2018 to July 2019, for brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) or without (N-) nitrogen, at Ladbrooks, Canterbury, New Zealand...................189

Table 6-8 Intercepted PAR per regrowth cycle (MJ m^{-2}) measured from July 2019 to July 2020, for brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) or without (N-) nitrogen, at Ladbrooks, Canterbury, New Zealand...................190

Table 6-9 Intercepted PAR per regrowth cycle (MJ m^{-2}) for brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) or without (N-) nitrogen fertilisation, measured from December 2018 to July 2019 at Ashley Dene, Canterbury, New Zealand. 192

Table 6-10 Intercepted PAR per regrowth cycle (MJ m^{-2}) for brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without (N-) nitrogen measured from July-2019 to July-2020 at Ashley Dene, Canterbury, New Zealand. 193

Table 6-11 Regression equations for accumulated yield (g DM m^{-2}) against accumulated intercepted photosynthetically active radiation (MJ m^{-2}) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from November 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand. 196

Table 6-12 Regression equations for accumulated yield (g DM m^{-2}) against accumulated intercepted photosynthetically active radiation (MJ m^{-2}) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand. 197

Table 6-13 Regression equations for accumulated yield (g DM m^{-2}) against accumulated intercepted photosynthetically active radiation (MJ m^{-2}) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, from December 2018 to July 2019, at Ashley Dene, Canterbury, New Zealand. 200

Table 6-14 Regression equations for accumulated yield (g DM m^{-2}) against accumulated intercepted photosynthetically active radiation (MJ m^{-2}) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, from July 2019 to July 2020, at Ashley Dene, Canterbury, New Zealand. 201

Table 6-15 Radiation Use Efficiency (g DM MJ^{-1} PAR) for brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) or without (-N) nitrogen fertilisation, from November 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand. 203

Table 6-16 Radiation Use Efficiency (g DM MJ^{-1} PAR) for brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) and without (-N) nitrogen fertilisation, sown in monoculture, from July 2019 to July 2020 in Ladbrooks, Canterbury, New Zealand. 203

Table 6-17 Radiation Use Efficiency (g DM MJ^{-1} PAR m^{-2}) for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen fertilisation, from December 2018 to July 2019, at Ashley Dene, Canterbury, New Zealand. 205

Table 6-18 Radiation Use Efficiency (g DM MJ^{-1} PAR m^{-2}) for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen fertilisation, from July 2019 to July 2020, at Ashley Dene, Canterbury, New Zealand. 205

Table 7-1 Date, Site, Light Intensity and Average Leaf Temperature (Ave. Leaf Temp.) from taken LICOR measurements for brome, cocksfoot, perennial ryegrass and tall fescue, grown with or without nitrogen, from December 2018 to March 2020 in Ladbrooks (LB) and Ashley Dene (ASH), Canterbury, New Zealand. PPFD represents the photosynthetic photon flux density......................................216

Table 7-2 Regressions used to obtain the Chlorophyll content (mg m-2) from the SPAD reading (X), for brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen..218

Table 7-3 Mean osmotic potential (MPa) from December 2018 to March 2020, from Ladbrooks, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D)..222

Table 7-4 Mean osmotic potential (MPa) from December 2018 to March 2020, from Ashley Dene, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D). 224

Table 7-5 Mean proline accumulation (mg g^{-1} DM) for brome, cocksfoot, p. ryegrass, cocksfoot and tall fescue, grown with (N+) and without (N-) nitrogen, from December 2018 to January 2021, at Ladbrooks, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D). 226

Table 7-6 Mean proline accumulation (mg g^{-1} DM), for brome, cocksfoot, perennial ryegrass, cocksfoot and tall fescue, grown with (N+) and without (N-) nitrogen, in Ladbrooks, Canterbury, New Zealand, from, for the interaction Species x Nitrogen. 226

Table 7-7 Mean proline accumulation (mg g^{-1} DM) for brome, cocksfoot, perennial ryegrass, cocksfoot and tall fescue, grown with (N+) and without (N-) nitrogen, from December 2018 to January 2021, in Ashley Dene, Canterbury, New Zealand according to species (S), nitrogen fertilisation (N) and date (D). 228

Table 7-8 Mean proline Concentration (mg g^{-1} DM), for brome, cocksfoot, perennial ryegrass, cocksfoot and tall fescue, grown with (N+) and without (N-) nitrogen, in Ashley Dene, Canterbury, New Zealand, from, for the interaction Species x Nitrogen. 228

Table 7-9 Phostosynthetic rate (µmol CO_2 m^{-2} s^{-1}) of brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen from December 2018 to March 2020 at Ladbrooks, Canterbury, New Zealand...................................231

Table 7-10 Net phostosynthetic rate (µmol CO_2 m^{-2} s^{-1}) of brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen from December 2018 to March 2020 at and Ashley Dene, Canterbury, New Zealand. 233

Table 7-11 Mean stomatal conductance (mol H_20 m^{-2} s^{-1}) of brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen from December 2018 to March 2020, at Ladbrooks, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D)...235

Table 7-12 Mean stomatal conductance (mol H_20 m^{-2} s^{-1}) of brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen from December 2018 to March 2020, at Ashley Dene, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D)...237

Table 7-13 Mean chlorophyll concentration (mg m^{-2}) for brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen, from December 2018 to February 2020 at Ladbrooks, Canterbury, New Zealand with (N+) or without (N-) nitrogen fertilisation. (S), nitrogen fertilisation (N) and date (D). 239

Table 7-14 Mean chlorophyll concentration (mg m^{-2}), for brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen, at Ladbrooks, Canterbury, New Zealand for the interaction of Nitrogen and Species. 240

Table 7-15 Mean chlorophyll concentration (mg m^{-2}) for brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen, from December 2018 to February 2020 at Ashley Dene, Canterbury, New Zealand with (N+) or without (N-) nitrogen fertilisation. (S), nitrogen fertilisation (N) and date (D). 242

Table 7-16 Mean chlorophyll concentration (mg m^{-2}), for brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen, at Ashley Dene, Canterbury, New Zealand for the interaction of Nitrogen and Species. 242

Table 7-17 Mean relative water content (%) for brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen from December 2018 to March 2020, at Ladbrooks, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D). 244

Table 7-18 Mean relative water content (%) for brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen from December 2018 to March 2020, at Ashley Dene, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D). 246

Table 7-19 Physiological variables Principal Components Analysis (PCA) Correlation Matrix for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown, with and without nitrogen fertilisation, at Ladbrooks and Ashley Dene, Canterbury, New Zealand, from December 2018 to March 2020. 249

Table 7-20 Physiological variables Principal Components Analysis (PCA) Correlation Matrix for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown, with and without nitrogen, in Ladbrooks, Canterbury, New Zealand, from December 2018 to March 2020. 251

Table 7-21 Physiological variables Principal Components Analysis (PCA) Correlation Matrix for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown, with and without nitrogen, in Ashley Dene, Canterbury, New Zealand, from December 2018 to March 2020. 255

LIST OF FIGURES

Figure 1-1 Fluxogram explaining thesis structure and chapter organization.......................4

Figure 3-1 Field plan of two experiments with four replicates of four species. Cocksfoot (cf),tall fescue (tf), perennial ryegrass (pr) and brome (br) grown with (+N) or without (-N). “REP#” represent the block. 27

Figure 3-2 Monthly rainfall (black bars) and average air temperature (solid line) for the 2018/2020 period in Ladbrooks (top) and Ashley Dene (bottom). The historical series for rainfall is in grey and for average air temperature, the dashed line. 32

Figure 3-3 Potential Soil Moisture Deficit in Ladbrooks (A) and Ashley Dene (B) from July 2018 to July 2020. Lines represent the Potential Soil Moisture Deficit (–) and Soil Moisture Deficit with PET reduction (–) when SMD reaches half the available plant available water content (225 mm for Ladbrooks, 1.5 m deep and 100 mm for Ashley Dene, 0.8 m deep)..34

Figure 3-4 Mean incident solar radiation for Ladbrooks (black) and Ashley Dene (gray) from 01/07/2018 until 30/08/2020. Historical series is in white.............................35

Figure 3-5 Total accumulated incident PAR (MJ m^{-2}) at Ladbrooks (A and B) and Ashley Dene (C and D), for each regrowth cycle as indicated by harvest date....................36

Figure 3-6 Brome as a weed in other plots. Both images were taken in Ashley Dene on 11/02/2020, to the left, there is plot 7 (tall fescue), to the right, plot 12 (perennial ryegrass). 41

Figure 4-1 Dry matter accumulation (kg ha^{-1}) for Ladbrooks from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for brome (●,○), cocksfoot (■,□),perennial ryegrass (▲,△) and tall fescue (▼,▽). with (closed) or without nitrogen fertilisation (open). Standard Errors of the Mean are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” show when differences were observed. 48

Figure 4-2 Dry matter accumulation (kg ha^{-1}) for Ashley Dene from July 2018 to July 2019 (A,C) and from July-2019 to July 2020 (B,D), for brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△), tall fescue (▼,▽),with (closed) or without nitrogen fertilisation (open). Standard Errors of the Mean are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” show when differences were observed. 51

Figure 4-3 Sown species dry matter (kg ha^{-1}) for Ladbrooks from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△), tall fescue(▼,▽) with (closed) or without nitrogen fertiliser (open). Standard Errors of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” show when differences among species were observed. 54

Figure 4-4 Sown species dry matter yield (kg ha^{-1}) for Ashley Dene from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△), tall fescue(▼,▽) with (closed) or without nitrogen fertiliser (open). Standard errors of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” show when differences among species were observed. 57

Figure 4-5 Vegetative dry matter (kg ha^{-1}) for Ladbrooks from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△), tall fescue(▼,▽) with (closed) or without nitrogen fertilisation (open). Standard Errors of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” show when differences among species were observed. 60

Figure 4-6 Vegetative dry matter yield (kg ha^{-1}) for Ashley Dene from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△), tall fescue(▼,▽) with (closed) or without nitrogen fertilisation (open). Standard errors of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” show when differences among species were observed. 63

Figure 4-7 Reproductive dry matter yield (kg ha^{-1}) for Ladbrooks from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△), tall fescue(▼,▽) with (closed) or without nitrogen fertiliser (open). Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” shows when differences among species were observed. 67

Figure 4-8 Reproductive dry matter yield (kg ha^{-1}) for Ashley Dene from July 2018 to July 19 (A,C) and from July 2019 to July 2020 (B,D), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△), tall fescue(▼,▽) with (closed) or without nitrogen fertiliser (open). Standard Error of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” show when differences were observed. 71

Figure 4-9 Weed fraction for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△) and tall fescue(▼,▽) harvested from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) at Ladbrooks, Canterbury, per harvest, with (closed) or without nitrogen fertilisation (open).Standard errors of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” indicate when differences were observed for species. 74

Figure 4-10 Weed fraction for brome(●,○), cocksfoot (■,□), p.ryegrass(▲,△) and tall fescue(▼,▽) harvested from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) at Ashley Dene, per harvest, for all the species with (closed) or without nitrogen fertiliser (open). Standard error of means are represented for (a) nitrogen (b) species and (c) nitrogen*species. "*" indicate when differences among species were observed. 77

Figure 4-11: Nitrogen yield (kg ha^{-1}) for brome(●,○), cocksfoot (■,□), p.ryegrass(▲,△) and tall fescue(▼,▽) harvested from July 2018 to July 2019 (A,C) and from July 2019 July 2020 (B,D) at Ladbrooks, per harvest, with (closed) or without nitrogen fertilisation (open). .Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represents when differences among species were observed. 80

Figure 4-12 Nitrogen yield (kg ha^{-1}) for Ashley Dene, for brome(●,○), cocksfoot (■,□), p.ryegrass(▲,△) and tall fescue(▼,▽) with (closed) or without nitrogen fertilisation (open), from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D). Standard Error of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. 83

Figure 4-13 Protein yield (kg ha^{-1}) for Ladbrooks, from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) for cocksfoot (■,□), tall fescue(▼,▽) brome(●,○), p.ryegrass(▲,△) with (closed) or without nitrogen fertilisation (open). Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represents when differences among species were observed. 85

Figure 4-14 Protein yield (kg ha^{-1}) at Ashley Dene from July 2019 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), per harvest, for brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽),with (closed) or without nitrogen fertilisation (open). Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. 89

Figure 4-15 Mean metabolisable energy (GJ ha^{-1}), for Ladbrooks from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for all regrowth cycles, for brome(●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽), with (closed) or without nitrogen fertilisation (open). Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represents when differences among species were observed. 92

Figure 4-16 Mean metabolisable energy (GJ ha^{-1}), for each harvest of brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽), from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) at Ashley Dene, with (closed) or without nitrogen fertilisation (open). Standard Errors of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represents when differences among species were observed. 95

Figure 4-17 Mean acid detergent fibre (kg ha^{-1}), from brome (●,○), cocksfoot (■,□),perennial ryegrass (▲,△) and tall fescue (▼,▽), harvested from July 2018 to July 2019 and from July 2019 to July 2020 from monocultures grown with (closed) and without (open) nitrogen at Ladbrooks, Canterbury, New Zealand. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” represents when differences among species were observed. 98

Figure 4-18 Mean neutral detergent fibre yield (kg ha^{-1}), from brome(●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue(▼,▽), harvested from July 2018 to July 2019 and from July 2019 to July 2020 from monocultures grown with (closed) and without (open) nitrogen at Ladbrooks, Canterbury, New Zealand. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” represents when differences among species were observed. 101

Figure 4-19 Mean ADF yield (kg ha^{-1}), for Ashley Dene from July 2018 to July 2019, per harvest, from brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽), with (closed) or without nitrogen fertilisation (open). Standard Error of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” represent when differences among species were observed. 104

Figure 4-20 Mean NDF yield (kg ha^{-1}), for Ashley Dene from July 2018 to July 2019, per harvest, from brome (●,○), cocksfoot (■,□) ,perennial ryegrass(▲,△) and tall fescue(▼,▽), with (closed) or without nitrogen fertilisation (open). Standard Error of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” represent when differences among species were observed. 107

Figure 4-21: Accumulated dry matter (kg ha^{-1}) per unit of accumulated Thermal Time (Cd°, Tb = 3°C) for Ladbrooks, from July 2018 to July 2019 for brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) tall fescue (D) (▼,▽).with (closed) or without nitrogen fertilisation (open). Standard Error is represented for (a) nitrogen. “*” represents the harvests. 111

Figure 4-22 Accumulated dry matter (kg ha^{-1}) per unit of accumulated Thermal Time (Cd°, Tb = 3 °C) for Ladbrooks, from July 2019 to July 2020 for brome (A) (●,○), cocksfoot (B) (■,□), ,perennial ryegrass (C) (▲,△),tall fescue (D) (▼,▽) with (closed) or without nitrogen fertilisation (open). Standard errors are represented for (a) nitrogen. “*” represents the harvests. 112

Figure 4-23 Accumulated dry matter (kg ha^{-1}) per unit of accumulated Thermal Time (Cd°, Tb = 3 °C) for Ashley Dene, from July 2018 to July 2019 for brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) and tall fescue (D) (▼,▽). with (closed) or without nitrogen fertilisation (open). Standard errors are represented for (a) nitrogen. “*” indicate when harvests happened. 115

Figure 4-24 Accumulated dry matter (kg ha^{-1}) per unit of accumulated Thermal Time (Cd°, Tb = 3 °C) for Ashley Dene, from July 2019 to July 2020 for brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) and tall fescue (D) (▼,▽) with (closed) or without nitrogen fertilisation (open). Standard errors are represented for (a) nitrogen. “*” indicate when harvests happened. 116

Figure 5-1 Drained Upper limit (●) and Lower Limit (○), in Ladbrooks (A,B) and Ashley Dene (C,D) for Plots CF 26 N+ (A,C) and CF 5 N-(B,D). The Measured Plant Available Water Content (mm^3 mm^{-3}) is represented by the shaded area. The text indicates the Plant Available Water content ± SEM Depth is Ladbrooks reached 1.65 m and in Ashley Dene, 1.05 m. 138

Figure 5-2 Mean actual soil moisture deficit (mm) for monocultures of brome fertilised with nitrogen (closed symbols; A,C) or not (open symbols; B,D), from July 2018 to July 2019 (A,B) and from July 2019 to July 2020 (C,D) grown at Ladbrooks, Canterbury New Zealand from July 2018 to July 2020. ● represents measurement days done with the neutron probe. Black bars indicate accumulated weekly rainfall (mm). The average plant available water content is 196 ± 6.8 mm for the N+ grasses and 138 ± 6.8 mm for the N- ones. Total rainfall from July 2018 to July 2019 was 623 mm, and from July 2019 to July 2020 was 786 mm...141

Figure 5-3 Mean actual soil moisture deficit (mm) for monocultures of brome fertilised with nitrogen (closed symbols; A,C) or not (open symbols; B,D), from July 2018 to July 2019 (A,B) and from July 2019 to July 2020 (C,D) grown at Ashley Dene, Canterbury New Zealand from July 2018 to July 2020. ● represents measurement days done with the neutron probe. Black bars indicate accumulated weekly rainfall (mm). The average plant available water content is 124 ± 2.7 mm for the N+ grasses and 111 ± 2.7 mm for the N- ones. Total rainfall from July 2018 to July 2019 was 566 mm, and from July 2019 to July 2020 was 529 mm...142

Figure 5-4 Mean measured water use (WU, mm) (●) and potential evapotranspiration (PET_p,mm) (solid line) from monocultures of brome, cocksfoot, p. ryegrass and tall fescue at Ladbrooks (A,B) and Ashley Dene (C,D), from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D). "*" represents regrowth cycles that when water use was estimated from PET_p. 147

Figure 5-5 Water Use (mm) per regrowth cycle for cocksfoot (■,□), tall fescue(▼,▽) brome (●,○), perennial ryegrass(▲,△).sown in monoculture for Ladbrooks, from July-2018 to July-2019 (A,C) and from July-2019 to July-2020 (B,D) with (closed) or without nitrogen fertilisation (open). Standard Error of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species..................................148

Figure 5-6 Water use (mm) per regrowth cycle for cocksfoot (■,□), tall fescue (▼,▽) brome (●,○), perennial ryegrass(▲,△) sown in monoculture at Ashley Dene from July-2018 to July-2019 (A,C) and from July-2019 to July-2020 (B,D) for all the species with (closed) or without nitrogen fertilisation (open). 151

Figure 5-7 Accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by N+ (closed) and N- (open) monocultures of Brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) in 2018/2019 in Ladbrooks, Canterbury, New Zealand. Regression equation details for N+ and N- pastures are reported in Table 5-13. 153

Figure 5-8 Accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by N+ (closed) and N- (open) monocultures of Brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) in 2019/2020 in Ladbrooks, Canterbury, New Zealand. Regression equation details for N+ and N- pastures are reported in Table 5-14. 155

Figure 5-9 Accumulated yield (kg ha^{-1}) against accumulated water use (mm) by nitrogen fertilised N+ (closed) and non-fertilised N-(open) monocultures of Brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) in 2018/2019 in Ashley Dene, Canterbury, New Zealand. Regression equation details for N+ and N- pastures are reported in Table 5-15. 157

Figure 5-10 Accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by nitrogen fertilised N+ (closed) and non-fertilised N- (open) monocultures of Brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) from July 2019 to July 2020 in Ashley Dene, Canterbury, New Zealand. Regression equation details for N+ and N- pastures are reported in Table 5-16. 159

Figure 5-11 Water use efficiency (kg ha^{-1} mm^{-1}) per regrowth cycle for cocksfoot (■,□), tall fescue(▼,▽), brome(●,○), perennial ryegrass(▲,△) sown in monoculture at Ladbrooks from July-2018 to July-2019 (A,C) and from July-2019 to July-2020 (B,D) with (closed) or without nitrogen fertilisation (open). Standard Errors of Means are represented by (a) for Nitrogen, (b) for Species and (c) for Nitrogen*Species. 161

Figure 5-12 Water Use Efficiency (kg ha^{-1} mm^{-1}) per regrowth cycle for cocksfoot (■,□), tall fescue(▼,▽), brome (●,○), perennial ryegrass (▲,△) with (closed) or without nitrogen fertilisation (open), from July-2018 to July-2019 (A,C) and from July-2019 to July-2020 (B,D), sown in monoculture at Ashley Dene, Canterbury, New Zealand Standard Errors of Means are represented by (a) for Nitrogen, (b) for Species and (c) for Nitrogen*Species. 164

Figure 6-1 Leaf area index (LAI) against compressed pasture height (CPH, cm). Measurements were taken for nitrogen fertilised (closed) and non-fertilised (open) monocultures of brome (●,○), cocksfoot (■,□), perennial ryegrass(▲,△) and tall fescue (▼,▽) at both Ladbrooks and Ashley Dene, Canterbury, New Zealand, from September 2018 to July 2020. 177

Figure 6-2 Leaf area index (LAI) from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) at Ladbrooks, Canterbury, New Zealand, for every regrowth cycle, for brome (●,○), cocksfoot (■,□), perennial ryegrass(▲,△) and tall fescue (▼,▽) with (closed) (A,B) or without nitrogen fertilisation (open) (B,D).”*” represents when differences were observed for the species. SEMs are represented for (a) nitrogen, (b) species and (c) nitrogen*species. LAI at 95% light interception is 5.07. 181

Figure 6-3 Leaf area index (LAI) from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) at Ashley Dene, Canterbury, New Zealand. For every regrowth cycle, for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△) and tall fescue(▼,▽)

with (closed) (A,B) or without nitrogen fertilisation (open) (B,D). ”*” represents when differences where observed for the species. SEMs are represented for (a) nitrogen, (b) species and (c) nitrogen*species. LAI at 95% light interception is 5.07. Data are absent for regrowth cycles harvested previous to 03/12/2018. 185

Figure 6-4 Intercepted PAR (MJ m^{-2}) from November 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) at Ladbrooks, Canterbury, New Zealand, for every regrowth cycle, for brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△), tall fescue (▼,▽) with (closed) (A,B) or without nitrogen fertilisation (open) (B,D). “X” symbols represent incident PAR. ”*” represents when differences were observed for the species. SEMs are represented for (a) nitrogen, (b) species and (c) nitrogen*species......188

Figure 6-5 Intercepted PAR (MJ m^{-2}) at Ashley Dene, Canterbury, New Zealand for every regrowth cycle, for brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△), tall fescue (▼,▽) with (closed) or without nitrogen fertilisation (open) from December 2018 to July 2019 and from July 2019 to July 2020. “X” symbols represent incident PAR. Standard errors of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” represents observed differences among species. 191

Figure 6-6 Accumulated yield (g m^{-2}) against accumulated photosynthetically active radiation (PAR; MJ m^{-2}) by N+ (closed) and N- (open) monocultures of brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) and tall fescue (D) (▼,▽) from November 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand. Regression equation details for N+ and N- pastures are reported in Table 6-11. Error bars are shown for (a) species, (b) nitrogen and (c) nitrogen*species......194

Figure 6-7 Accumulated yield (g DM m^{-2}) against accumulated photosynthetically active radiation (PAR) (MJ m^{-2}) for nitrogen fertilised N+ (closed) and unfertilised N- (open) monocultures of brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) and tall fescue (D) (▼,▽) from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand. Symbols are represented for harvests. Regression equation details for N+ and N- pastures are reported in Table 6-12. Error bars are shown for (a) species, (b) nitrogen and (c) nitrogen*species. 195

Figure 6-8 Accumulated yield (g DM m^{-2}) against accumulated photosynthetically active radiation (PAR) (MJ m^{-2}) by N+ (closed) and N- (open) monocultures of brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) and tall fescue (D) (▼,▽) from December-2018 to July-2019 at Ashley Dene, Canterbury, New Zealand. Symbols are represented for harvests. Regression equation details for N+ and N- pastures are reported in Table 6-13. Error bars are shown for (a) species, (b) nitrogen and (c) nitrogen*species......198

Figure 6-9 Accumulated yield (kg ha^{-1}) against accumulated photosynthetically active radiation (PAR) (MJ m^{-2}) by N+ (closed) and N- (open) monocultures of brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) and tall fescue (D)

(▼,▽) from July-2019 to July 2020, at Ashley Dene, Canterbury, New Zealand. Symbols are represented for harvests. Regression equation details for N+ and N- pastures are reported in Table 6-14. Error bars are shown for (a) species, (b) nitrogen and (c) nitrogen*species. 199

Figure 6-10 Radiation use efficiency (g DM MJ^{-1} PAR m^{-2}), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△) and tall fescue(▼,▽) with (closed) or without nitrogen fertilisation (open) at Ladbrooks, from July 2018 to July 2019 and from July 2019 to July 2020. Standard errors of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represents when differences were observed among species. 202

Figure 6-11 Radiation Use Efficiency (g DM MJ^{-1} PAR m^{-2}) for brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) with (closed) or without nitrogen fertilisation (open). From July 2018 to July 2019 (A;C) and from July 2019 to July 2020 (B,D) at Ashley Dene, Standard errors of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. 204

Figure 7-1 Osmotic Potential of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△)and tall fescue (▼,▽) from December 2018 to February 2020 at Ladbrooks, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. "*" represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. 220

Figure 7-2 Osmotic Potential of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ashley Dene, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. "*" represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. 223

Figure 7-3 Proline accumulation (mg g^{-1} DM) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ladbrooks and Ladbrooks, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation."*" represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. 225

Figure 7-4 Proline accumulation (mg g^{-1} DM) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ladbrooks and Ashley Dene, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. "*" represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. 227

Figure 7-5 Net photosynthetic rate (µmol of CO_2 m^{-2} s^{-1}) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ladbrooks, Canterbury, New Zealand with (closed) or without (open) nitrogen fertilisation.”*” represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. 230

Figure 7-6 Photosynthetic rate (μmol of CO$_2$ m^{-2} s^{-1}) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ashley Dene, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. ”*” represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. 232

Figure 7-7 Stomatal conductance (mol H$_2$O m^{-2} s^{-1}) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ladbrooks, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. “*” represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. 234

Figure 7-8 Stomatal conductance (mol H$_2$O m^{-2} s^{-1}) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ashley Dene, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. “*” represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. 236

Figure 7-9 Chlorophyll concentration (mg m^{-2} of leaf area) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to April 2020 at Ladbrooks, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. “*” represents when differces amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. 238

Figure 7-10 Chlorophyll concentration (mg m^{-2} of leaf area) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to April 2020 at Ashley Dene, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. “*” represents when differces amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. 241

Figure 7-11 Relative water content (RWC) (%) of brome (●,○),cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to April 2020 in Ladbrooks, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. “*” symbols represents when differences amongst the species were observed. Standard error of means is represented for (a) nitrogen, (b) species and (c) nitrogen*species. 243

Figure 7-12 Relative water content (RWC) (%) of brome (●,○),cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to April 2020 at Ashley Dene, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation.“*” symbols represents when differences amongst the species were observed. Standard error of means is represented for (a) nitrogen, (b) species and (c) nitrogen*species. 245

Figure 7-13 Component Loadings with individual scores for monocultures of brome (BR), cocksfoot (CF), perennial ryegrass (PR) and tall fescue (TF) grown, with (+N) and without (-N) nitrogen, at Ladbrooks and Ashley Dene, Canterbury, New Zealand, from December 2018 to March 2020. “RWC” is the relative water content, “Condu” is the stomatal conductivity, “Pn” is the net photosynthetic rate, “Chlorophyll” is the chlorophyll concentration, “Proline” is the proline accumulation, “OsmPotential” is the osmotic potential. 247

Figure 7-14 Hierarchical Clustering from Individuals according to the PCA analysis for monocultures of brome (BR), cocksfoot (CF), perennial ryegrass (PR) and tall fescue (TF) grown, with (N+) and without (N-) nitrogen, at Ladbrooks (LB) and Ashley Dene (AD), Canterbury, New Zealand, from December 2018 to March 2020. 250

Figure 7-15 Component Loadings with individual scores for monocultures of brome (BR), cocksfoot (CF), perennial ryegrass (PR) and tall fescue (TF) grown, with (+N) and without (-N) nitrogen, in Ladbrooks, Canterbury, New Zealand, from December 2018 to March 2020. “RWC” is the relative water content, “Condu” is the stomatal conductivity, “Pn” is the net photosynthetic rate, “Chlorophyll” is the chlorophyll concentration, “Proline” is the proline accumulation, “OsmPotential” is the osmotic potential. 252

Figure 7-16 Component Loadings with individual scores for monocultures of brome (BR), cocksfoot (CF), perennial ryegrass (PR) and tall fescue (TF) grown, with (+N) and without (-N) nitrogen, in Ashley Dene, Canterbury, New Zealand, from December 2018 to March 2020. “RWC” is the relative water content, “Condu” is the stomatal conductivity, “Pn” is the net photosynthetic rate, “Chlorophyll” is the chlorophyll concentration, “Proline” is the proline accumulation, “OsmPotential” is the osmotic potential. 254

LIST OF EQUATIONS

Equation 1 $TDM = Ro * RRo * RUE * HI$ 6

Equation 2 $Tdaily = (Th - Tb)$ 31

Equation 3 $CP\% = N\%/6.25$ 43

Equation 4 $ME = DOMD * 0.16$ 43

Equation 5 $NR = TNH/(TNA + NNON)$ 43

Equation 6 $TNH = VegDM * Nveg\% + RepDM * Nrep\%$ 44

Equation 7 $SWC = Top\ LayerBottom\ Layer\theta * d$ 132

Equation 8 $PAWC = Top\ LayerBottom\ Layer(DUL - LL)$ 133

Equation 9 $ASMDn + 1 = SMDn + PETan + 1 - Rn + 1$ 133

Equation 10 $PETa = PETp * (PAWC - ASMD)/(0.5 * PAWC)$ 134

Equation 11 $PSMD(n)$ = SMD1 + $1nPETp$ - $1nR$ 134

Equation 12 $WU = PR - SMCd - SMCp - D$ 135

Equation 13 $WUE = TDM/WU$ 135

Equation 14 $NDVI = NIRED - VISI(NIRED + VISI)$ 178

Equation 15 $NDVI\ R/Ro = C * NDVIr - NDVIs(NDVImax - NDVIs)$ 178

Equation 16 $INTn = n * INTk - INTk - 1DDays$ 179

Equation 17 $PAR = n = 1kDIPARn * INTn$ 179

Equation 18 $RUE = TDM/PAR$ 180

Equation 19 $\Psi s = RT.Cj$ 214

Equation 20 $Chloro = 17.9 * A647 + 8.08 * A664.5$ 217

Equation 21 $RWC = (FW - DW) / (TW - DW)$ 218

LIST OF APPENDICES

Appendix A Linear regressions Compressed Plate Height x Dry Matter Production used for different seasons. 294

Appendix B Protein content (% of dry matter) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ladbrooks. 295

Appendix C Protein content (% of dry matter) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ashley Dene 296

Appendix D Metabolisable energy (MJ kg^{-1}) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ladbrooks. 297

Appendix E Metabolisable energy (MJ kg^{-1}) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ashley Dene 298

Appendix F Acid Detergent Fiber (% of Dry Matter) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ladbrooks. 299

Appendix G Neutral Detergent Fiber (% of Dry Matter) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ladbrooks. 300

Appendix H Acid Detergent Fiber (% of dry matter) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ashley Dene 301

Appendix I Neutral Detergent Fiber (% of dry matter) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ashley Dene 302

Appendix J Potential soil moisture deficit (mm) when NIWA's proposed PET$_a$ reduction is not considered at Ladbrooks, from July 2018 to July 2019 and from July 2019 to July 2020 for fertilised (closed) and non-fertilised (open) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue. "●" symbols represent days when neutron probe measurements were done.................................303

Appendix K Potential soil moisture deficit (mm), when NIWA's proposed PET_a reduction is not considered at Ashley Dene, from July 2018 to July 2019 and from July 2019 to July 2020 for fertilised (closed) and non-fertilised (open) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue. "●" symbols represent days when neutron probe measurements were done.................................304

Appendix L Mean soil moisture deficit (mm) in relation to WU/PET_p for mono cultures of for brome(●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽), with (closed) or without nitrogen fertilisation (open) at Ladbrooks (A,B) and Ashley Dene (C,D) from Jully 2018 to July 2020. 305

Appendix M Accumulated PET_p (black, mm), PET_a for N+ grasses (red, mm), PET_a for N- grasses (green, mm) and rainfall (blue, mm) for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen fertilisation at Ladbrooks (A,B) and Ashley Dene (C,D) from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D). 306

Appendix N Scree Plot from Components in the PCA for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown, with and without nitrogen, in Ladbrooks and Ashley Dene, Canterbury, New Zealand, from December 2018 to March 2020. 307

Appendix O Scree Plot from Components in the PCA for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown, with and without nitrogen, in Ladbrooks, Canterbury, New Zealand, from December 2018 to March 2020. 308

Appendix P Scree Plot from Components in the PCA for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown, with and without nitrogen, in Ashley Dene, Canterbury, New Zealand, from December 2018 to March 2020. 309

Appendix Q Variables' correlations with each Principal Components and Wilk's Lambda Value from PCAs from monocultures of brome (BR), cocksfoot (CF), perennial ryegrass (PR) and tall fescue (TF) grown, with (+N) and without (-N) nitrogen, at Ashley Dene and Ladbrooks and Combined (General Data), Canterbury, New Zealand, from December 2018 to March 2020. "RWC" is the relative water content, "Sto.Condu." is the stomatal conductivity, "Pn" is the net photosynthetic rate, "Chlorophyll" is the chlorophyll concentration, "Proline" is the proline accumulation, "Osm.Pot." is the osmotic potential................310

1 INTRODUCTION

1.1 Farming in summer dry environments.

Livestock farming in the summer dry, east coast regions of New Zealand presents unique challenges. These include the inability to irrigate or easily renew pastures in most areas of hill country. Therefore there is a need to maximise production and persistence of pastures in areas that can be cultivated. In addition, the topography of hill country areas means fertiliser application has predominantly been in the form of superphosphate. This increases phosphorous (P) and sulphur (S) availability to encourage the legume content , particularly white clover (*Trifolium repens* L.). However, in summer dry environments white clover is frequently a minor contributor to pastures due to drought and its loss of a tap root after the first year. The emphasis on legumes is to provide high quality feed for animals and a source of nitrogen to overcome the major N deficit that develops in these grass dominant pastures (Mills *et al.* 2006). The direct application of nitrogen is less common where aerial application is required but large responses to the strategic use of N have been reported (Fasi *et al.* 2008).

The main grass species used in New Zealand is perennial ryegrass. However, it may not always be suitable for summer dry environments due to lack of persistence, particularly when it is exposed to multiple stresses (water, nitrogen, ravagers). Furthermore, climate change predictions indicate mean temperatures are predicted to rise by 0.7 to 5.1°C, depending upon the model parameters, by 2090 (Reisinger *et al.*, 2014), with east coast areas expected to have longer summer-dry periods (Salinger, 2003). Higher temperatures and less water is expected to reduce the available growing time and restrict rates of photosynthesis. Therefore there is a need to identify pasture grasses that may provide greater production and persistence in summer dry environments, and quantify there response to water stress and the availability of nitrogen fertiliser.

The research in this thesis focuses on the production, persistence and quality of four perennial pasture grasses grown under two levels of moisture availability in summer dry conditions in New Zealand. The results are presented for Years 5 and 6 after establishment, and builds on results presented by Sharifiamina (2018) one and two years after being sown. Differences in yield, forage quality and multiple physiological responses are quantified with an emphasis on explaining the underlying physiological responses in the field to examine whether species employed different strategies to cope with water and nitrogen stress. In this thesis water stress refers soil moisture deficit rather than an excess water which can also cause water stress.

1.2 Aim, objectives, and thesis structure.

The main aim of this thesis was to evaluate and to quantify the effects of nitrogen and moisture over time on monocultures of brome, cocksfoot, perennial ryegrass and tall fescue. From this recommendations can be made on species and nitrogen fertiliser strategies to maximize pasture production and persistence in summer dry regions. To do this the thesis had nine objectives based on measurements from two experiments established in 2014 by Sharifiamina who reported on the first two years of results in her PhD thesis in 2018. Using the same sites from these previous experiments on soils with two different levels of water holding capacity enabled responses to be examined over a longer time period (up to six years after sowing). This adds relevance to sheep and beef farmers who do not regularly renew pastures. Therefore, Objective 1 of this research is to quantify the pasture production, persistence and quality of herbage produced in Years 5 and 6 of the established experiments., Objective 2 is to summarise these pasture growth responses using thermal time to enable comparisons across environments. Objective 3 is to quantify the water extraction of the different species in the different environments and determine if this was affected by nitrogen fertiliser treatments. This analysis goes along with quantifying soil moisture deficit (Objective 4) and also enables a comparison of water use and water use efficiency of the pastures which is Objective 5. Objective 6 is to explain the effects of water and nitrogen on the canopy expansion and therefore light interception which enables comparisons of radiation use efficiency. Objective 7, examines the plant physiological parameters to determine any species differences.

To meet these objectives measurements were taken over two years at the experiments sown in 2014, in two different sites with similar climates but different soil water holding capacity. The deeper soil at Ladbrooks has a higher water holding capacity than the stony soil at Ashley Dene. These pastures were able to be established, following commercial fertiliser and pesticide recommendations, and then in the second year, 2015/2016, nitrogen was spread on the experiment to establish two different nitrogen regimes, fertilised or not. The unfertilised regime was therefore exclusively reliant on N from mineralisation.

The thesis is organized in eight chapters (Figure 1-1): Chapter 1 is the general introduction. Chapter 2 is the literature review that describes the main role of nitrogen and water in crop growth as well the effect of their deficiencies, which physiological parameters are affected by those deficiencies and how they can affect pasture persistence along with some management techniques.

Chapter 3 describes the materials and methods used for measurements to obtain the data for each experimental chapter. Chapter 4 relates to Objectives 1 and 2 and is where yield and quality aspects of the pasture are discussed. Chapter 5 deal with moisture stress and water use efficiency (Objectives 3; 4 and 5).

Chapter 6 relates to the photosynthetically active radiation interceptance and how it relates to dry matter accumulation (Objective 6). Chapter 7 is the last result chapter, where measurable physiology traits are discussed for every species and nitrogen regimes, to explain differences observed in the field (Objective 7).

Finally, the last chapter, Chapter 8, is a general discussion from all previous results and which conclusions we can have from this study, as well as suggestions for future research topics and projects in dryland pastures.

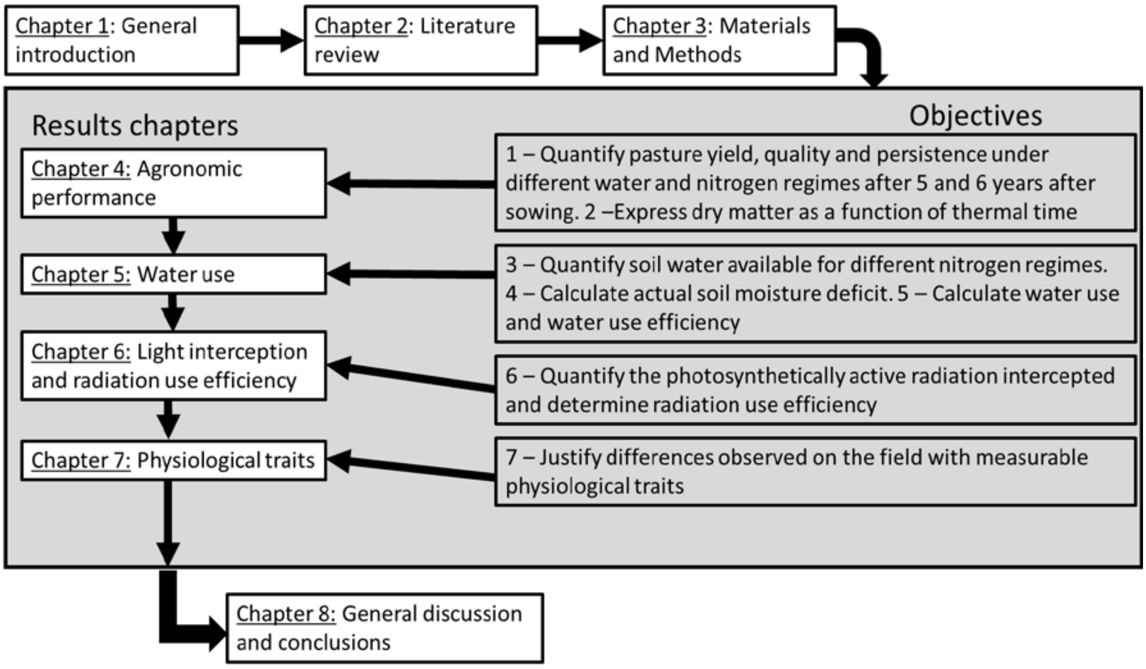


Figure 1-1 Fluxogram explaining thesis structure and chapter organization.

2 REVIEW OF THE LITERATURE

Climate change poses new challenges for agricultural production systems. New constraints will defy traditional systems and which makes adaptation imperative to feed the growing human population, reduce poverty and avoid starvation (Hertel and Rosch, 2010). Worldwide, these changes imply spatial displacements, lower yields, reduction in available water for irrigation and loss of land through increased sea levels and salinization (Aydinalp and Cresser, 2008). For New Zealand, and specifically the summer dry east coast regions from Central Otago to Gisborne, predictions from climate change models include temperatures rises (Howden *et al.* 2007), which shortens crop lifecycles, and changes in rainfall that will result in increased frequency and intensity of droughts (Salinger, 2003).

These changes are particularly important for areas where irrigation is non-viable, such as the hill country. To provide information appropriate management strategies to cope with the changing conditions, and improve New Zealand farm resilience, the Dryland Pastures Group at Lincoln University was developed. Their current research involves a hill country focus as part of the Hill Country Futures research programme supported by Beef + Lamb NZ. The overall aim of the programme is to understand the environmental, agronomic, and social constraints on summer dry sheep and cattle systems. As part of this research an experiment was set up in 2014 in two different locations in Lincoln, Canterbury, New Zealand, to quantify the yield of four common grass species: brome (*Bromus valdivianus* Phil.), cocksfoot (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.; syn. *Schedonorus phoenix* (Scop.)), under high and low nitrogen conditions.

The chosen locations have contrasting soil water holding capacity. In Ladbrooks, the finer and deeper soil structure allows a much higher water volume to be retained and represents the moist slopes or valley floor regions of hill country while the Ashley Dene Dryland Experimental Research Farm is a site that represents extreme dry north facing hill country.

In the first two years of this experiment, the low soil water site did not show a strong nitrogen dry matter yield response, except in Spring, when temperature and moisture were not limiting factors to plant growth (Sharifiamina, 2018). Cocksfoot under nitrogen fertilisation was the species that had the highest yield attributed to its higher radiation interception from larger leaf area. The other species had lower yields due to less water and less nitrogen, with the decrease being most pronounced for perennial ryegrass and tall fescue.

The two years (2014/15 and 2015/16) reported on Sharifiamina (2018) were insufficient to make accurate long term recommendations on pasture persistence so further monitoring was recommended. In addition to continuing the yield and pasture physiology measurements started by Sharifiamina this study investigates the physiological traits of species to explain differences in their response over the entire 6 years. Consequently, experiments were maintained until July 2020 with the final two years (2018/19 and 2019/20) reported in detail in this thesis.

This literature describes previous results related to yield and persistence of the four species used and describes the grass and plant physiological measurements that are required to identify any species differences.

2.1 Yield Components

2.1.1 Dry matter accumulation – generating yield

The accumulation of dry matter can be represented by Equation 1, whereby total dry matter accumulation in any given year is the result of light interception and the efficiency of its utilization (Monteith, 1965):

Equation 1

$$\boldsymbol{TDM = Ro * \left(\frac{R}{Ro}\right) * RUE * HI}$$

Where "TDM" is total dry matter on an annual basis, "Ro" is the incident photosynthetically active radiation (PAR), "(R/Ro)" is the intercepted fraction from the incident radiation, "RUE" is the radiation use efficiency. For annual crops the equations includes the "HI" which is the harvest index which represents economic component of crop yield but for forages this term can be ignored or considered as 1.0 because all of the above ground dry matter harvested in measurements is potentially consumed by the animals.

For pastures, “R/Ro” and RUE can be affected by the plant nutritional status, management, temperature and resources availability, which are discussed in this review.

2.1.2 Radiation interceptance (R/Ro) – How much radiation can crops intercept?

Crops need to intercept photosynthetically active radiation (PAR) to be able to photosynthesize to produce sugars as the basis of accumulation of dry matter. Lower radiation interceptance, for example, coupled with low temperatures, can decrease leaf expansion rate and cause lower dry matter accumulation during colder seasons (Martini *et al.* 2009).

The amount of intercepted radiation by the canopy is influenced by plant population, canopy architecture, water and nutrient status. The initial plant population of crops and pastures affects the total PAR intercepted per unit area, with more light intercepted at higher plant population. However, pastures are defoliated at regularly intervals and this has a major impact on the total amount of light intercepted. Overgrazing a pasture will have a negative impact on how fast the canopy can recover, particularly from set-stocking (Fedrigo *et al.* 2018). Low residual leaf area results in a low leaf area expansion and therefore, low dry matter accumulation rate. This means intense defoliation results in longer regrowth intervals, which reduce total forage production (Lemaire and Chapman, 1996).

Furthermore, canopy architecture also plays an important role in light interception. The two most important characteristics of a canopy to allow higher interceptance are (a) the area covered by leaves and (b) the angle of leaves related to the incoming radiation (Trenbath and Angus, 1975), plants with an erect canopy (higher leaf angle) capture more radiation (Sheehy and Cooper, 1973) than a flat canopy. For example, perennial ryegrass has been shown to have an erectophile leaf arrangement compared with the more planiophile white clover. Thus, in most cases grass based pastures have a higher potential growth rate than legumes because they intercept more total radiation and have a higher critical leaf area index (LAI) for canopy closure.

Water and nutrient status also have a major impact on light interception (Sharifiamina, 2018). These effects are explained in Sections 2.4 and 2.5. How we measure the interceptance is described in Section 2.1.3.

2.1.3 Measuring radiation interceptance – Using NDVI and the Green Seeker

NDVI (Normalized Difference Vegetation Index) is an index commonly used worldwide to determine evapotranspiration coefficients (Alam *et al.*2018), pasture biomass assessments (Flynn *et al.* 2018; Schaefer, 2016) and canopy radiation interception and cover in grasses and other crops. Measuring vegetable cover with NDVI has been used in many different scales, such as satellite images from large areas (Trout *et al.* 2008, Johnson & Trout, 2012) and farm-scale level from small areas with dimensions less than a meter (Chakwizira, 2015a).

Improvements in devices that measure NDVI make them financially accessible and easier to use in agriculture. In this study a portable, rapid assessment Green Seeker® (Trimble Agriculture, Sunnyvale, California) was used. This handheld sensor generates light centred at two wavelengths bands (Martin *et al.* 2012), red and near-infrared (NIR). The device estimates radiation interceptance based on the contrast of reflectance between the visible (red) and near-infrared wavebands (Gamon *et al.*, 1995). Reflectance at these two wavelengths is then combined to the Normalized Difference Vegetation Index (NDVI) to predict crop radiation interception (Tucker and Sellers, 1986).

There are a range of other techniques available to measure radiation interceptance, such as tube solarimeters, the SunScan Canopy Analysis System and digital imaging (Chakwizira *et al.*, 2015b). Each has its advantages and disadvantages, but overall, intercepted radiation measurements are inherently difficult for pastures (Mills, 2009). The SunScan, which is a bar sensitive to light, is placed under the canopy, to measure direct and diffuse components of solar radiation. This avoids the need for shade ring adjustments, but does not discriminate between green and senesced leaves, which therefore overestimates the radiation interceptance (Chakwizira, 2015a) particularly, when dead material is present, as would be expected in a summer dry pasture. The SunScan also poses problems for pastures with a low canopy structure because the sensor head is difficult to place at ground level, particularly in stony soils (Sharifiamina, 2018). Thus, the Green Seeker, which has the advantage of doing above-canopy measurements, that can differentiate between green leaves and yellow-brown senesced was preferred. Nonetheless, doing above-canopy measurements implies that the Green Seeker is vulnerable to soil spots and do not differentiate between the pasture species and weed.

2.1.4 Radiation use efficiency (RUE)

Radiation use efficiency quantifies how much dry matter is accumulated per unit of intercepted PAR (g MJ^{-1} m^{-2}) (Sinclair and Muchow, 1999). RUE can differ according to species, nitrogen, management and environmental conditions. In the literature RUE is reported per unit of total radiation or PAR.

To maximize RUE plants must rapidly develop their photosynthetic apparatus particularly after grazing/cutting. The rate of photosynthetic recovery post-grazing is dependent on the level of nitrogen and carbon reserves available to expand leaves and photosynthesize.

Low values of RUE occur when plants are subjected to frequent grazing management (Schneider *et al.* 2019) due to delayed re-establishment of the canopy and photosynthetic apparatus. RUE can also be affected by phenological development, the older the leaf, the lower the RUE, and seasonally in response to changes in partitioning, as shown for lucerne, which influenced yield by about 5 t DM ha^{-1} (Brown *et al.* 2006).

An appropriate foliar nitrogen level is required for a high RUE (Fletcher *et al.* 2013), in forages, values below 3.5% N can penalize RUE already. A meta-analysis that considered different species, resource availability and grazing strategy identified nitrogen as the second most influential factor for RUE, behind species, but ahead of water and light availability (Druille *et al.* 2019).

In the first two years of the current investigation a RUE increase of up to 100% was observed when nitrogen was applied and when water was available in the system (Sharifiamina, 2018). The importance of nitrogen on RUE and maintaining a high radiation interceptance is described in Section 2.3.2. In pastures, RUE is assessed by dividing the accumulated yield for each regrowth cycle by the amount of intercepted PAR.

2.2 Temperature effects on yield and quantifying it

Air temperature affects a wide range of crop physiological processes, such as water and nutrient absorption, germination, respiration, transpiration, photosynthesis, enzyme activities and permeability of walls and membranes. Temperature can be a limiting factor on pasture growth (Coleman, 1964) and survival (Jones, 1969). Plants need heat over and above their base temperature to accumulate dry matter, since, at lower temperatures metabolic processes cease or occur at a rate so low that they can be disregarded for the development of plants.

Provided there are no limitating factors, there is a linear relationship between temperature and plant development. This includes the rate of leaf appearance or phyllochron, which influences leaf area expansion rate (Davidson and Milthorpe, 1965) and therefore light interception and yield. For cocksfoot, grown with no restrictions on Canterbury flat lands, in the east of the South Island (NZ), Mills (2006) reported a growth rate of 3.3 kg DM ha^{-1} °Cd^{-1} for nitrogen restricted grasses and 7.0 kg DM ha^{-1} °Cd^{-1} for grasses without restrictions. For the experiments this thesis is based on, in 2015/16, cocksfoot, under no water or nitrogen restrictions, had a similar growth rate of 6.94 kg DM ha^{-1} °Cd^{-1}, but was as low as 1.19 kg DM ha^{-1} °Cd^{-1} when water was restricted (Sharifiamina, 2018). In this investigation, a base temperature for growth was set at 3 °C for cocksfoot (Mills, 2007), and used by Sharifiamina (2018) for the other grasses too.

2.3 Nitrogen effect on crop yield and quality

2.3.1 Nitrogen role in crops

Nitrogen is the fourth most abundant element in plants, in mass, after carbon, hydrogen and oxygen, but the most abundant one that comes from the soil in mineral forms. The former are derived from atmospheric gases and water. Nitrogen is present in many structural compounds, amino acids and enzymes, DNA and RNA, ATP, NAD, NADP, vitamins, hormones and is interal to many biohemical processes in plants.

In the chlorophyll molecule, each central magnesium cation is connected to four nitrogen atoms. More than half of the plant soluble nitrogen is utilized by the photosynthetic system (Horst, 1979), which means the net photosynthetic rate has a close relationship to the nitrogen status of the plants, for each crop species (Sinclair, 1989).

Nitrogen is a building block in the protein synthesis as well as the Rubisco enzyme (Novoa and Loomis, 1981), though between 60-80% is used in proteins, which is why nitrogen availability often limits crude protein content in grasses. In C3 plants (the species in this project), Rubisco corresponds to more than 50% of all the soluble protein (Schmitt and Edwards, 1981).

This macronutrient also promotes changes in canopy structure, including the increase in leaf bulk density, and Leaf Area Index (LAI) (Silva *et al.*, 2016). As a structural compound, it is required for leaf extension and canopy expansion which affects light interception. Because nitrogen has such a central role in plants, understanding how its deficiency affects different traits is important.

2.3.2 Nitrogen deficiency – limits to dry matter accumulation

Nitrogen and water are the two main determinants of pasture growth in the majority of productive systems (Sinclair and Rufty, 2012). Their deficiencies are the most frequent worldwide. Both factors have complex interactions spatially and temporally (Whitehead, 1995; Akmal and Janssens, 2004; Gonzalez-Dugo *et al.*, 2005).

Nitrogen deficiency causes low productivity in pastures (Braga, 1998; Costa *et al.*, 2011a). Grass-based pastures need nitrogen to maintain their forage yield and prevent degradation, therefore, increasing persistence (Gianluppi *et al.*, 2001; Costa *et al.*, 2011b).

In Canterbury, nitrogen deficient grasses (N% in dry matter varying from 1.8 – 2.5 %) produced 50% less dry matter than grasses with non-limiting nitrogen supply in grass monocultures (Mills *et al.* 2006). However, nitrogen supply can also be provided by mixing a grass with a legume, to maintain a high yield for grasses, such as cocksfoot (Mills *et al.* 2014).

Nitrogen fertilisation can double (Peri *et al.* 2002a) or even triple the dry matter production from rangelands depending on the annual rainfall and moisture in the region (Elliott, 2003). Nonetheless, when the annual rainfall is less than 300 – 400 mm, fertilising pastures becomes less profitable (Guevara, 2000).

Though nitrogen deficiency is generally associated with the lack of mineral nitrogen in the soil, it can be induced by droughts (Lemaire and Meynard, 1997; Haddad *et al.*, 2002; Kunrath *et al.* 2018) or accentuaded by them (Greenwood, 1976), for the response of the nitrogen supply depends on the availability of water (Akmal, 2004). This is one reason that fertilisers have different efficiencies related to water availability as observed in the first two years of Sharifiamina's (2018) experiment. Fertiliser efficiency is important economically as its costs represent a significant proportion of the production costs (Singh, 2006). Other factors that affect nitrogen fertilisation are species, grazing management and phenological stage.

In summary, nitrogen stress can reduce LAI (Colnenne *et al.* 2002) which affects light interception, by reducing leaf appearance rate (Korte *et al.* 1985), leaf expansion rate (Lemaire and Chapman, 1996) and longevity. It also affects photosynthetic apparatus (Lemaire, 2008), as shown by changes in RUE (Section 2.1.4) and chlorophyll levels (Section 2.5.1). An adequate supply of N is essential for the expansion and photosynthetic functioning of plant canopies (Grindlay, 1997). Nitrogen fertilisation can increase both leaf canopy expansion and photosynthetic capacity per unit of water used (Novoa and Loomis, 1981; Peri *et al.* 2002;). Net photosynthesis rate affects how intensely a plant consumes the intercepted light and transforms it into dry matter (Monteith, 1972, 1977; Sinclair, 1999) and it is affected by a decrease in nitrogen as well as stomatal conductance (Radin, 1981; Peri et al, 2002b), limiting access, at the cellular level, to photosynthetic raw materials.

Plants also respond to nitrogen deficiency either by reducing resource capture and/or reducing use efficiency, each crop having its own strategy (Lemaire *et al.*, 2008), and a decrease in the resource use efficiency may occur before decreasing leaf area expansion (Lemaire *et al.* 1997). However, the most important factor, for yield reduction, due to nitrogen deficiency, is the limitation on leaf area increase (Watson, 1952). When plants have maximum leaf area when nitrogen deficiency occurs, RUE declines rather than leaf area expansion. However, when N deficiency occurs in early stages of regrowth, a reduction in leaf expansion and therefore light interception is the mechanism that most affects growth rate (Insua *et al.*, 2019).

Though nitrogen fertilisation may overcome part of this problem (Fasi *et al.* 2008), by allowing leaves to expand and increasing tiller production, which contributes to an increase in the area covered by the crops (Baker, 1987), it may not be viable in summer dry farming systems.

2.3.3 Nitrogen effects on forage quality

When nitrogen levels in plants are low, nitrogen fertilisation can increase the nitrogen concentration (Diouf *et al.* 2004) and crude protein content of forage (Delevatti *et al.* 2019, Silva *et al.* 2015). This may affect the digestibility of forage as well, for there is an increase in hay digestibility with an increase in crude protein (Van Soest, 1973). Forages are the prime source of protein for ruminants, so it is important to ensure the pasture meets animal needs.

Neutral detergent fibres and acid detergent fibres content can increase when nitrogen levels are below optimum. Fertiliser can reduce their content but the level of reduction is dependent on species and the amount of nitrogen (Costa *et al.* 2010, Paulino *et al.* 2010, Minski da Motta *et al.* 2020).

Forages have a response in quality to a nitrogen supply that exceeds the maximum required for dry matter production. However, when the N supply is low, it doesn´t necessarily improve quality, and in this case the increase in the dry matter yield is more important and more affected (Silva, 2015). Supplying nitrogen can increase whole plant quality but different parts have different quality and can respond differently to fertiliser. Leaves are usually the highest quality part of forage plants (Hides, 1983), mainly the blade part, while the sheath quality is slightly lower (Cherney, 1983). In a mixed pasture legumes have high crude protein content from nitrogen fixation which increased pasture quality directly through the forage (Licitra *et al.* 1997) and indirectly when that forage is consumed and returns from dung and urine contribute to the nitrogen available to the associated grasses.

The effect of nitrogen fertilisation at different times of the year, with different levels of soil water availability, was evaluated in this study, to understand how quality traits were affected for each .

2.4 Water availability and its effect on pastures

2.4.1 Water availability

Climate change scenarios suggest an increasingly dry environment in New Zealand east coast every summer (Salinger, 2003). This means plants will be water-restricted environments for longer. This has prompted irrigation development on flat land in eastern coast districts with consequent negative environmental impacts (Fitzharris, 2007). However there are many hill country areas where irrigation is not technically feasible such as the hill country. Understanding how each forage species respond to water restrictions is essential to define strategies to allow farmers to use all of their available area in an economically and environmentally sustainable way under different climate scenarios.

2.4.2 Water stress- even more limits to dry matter accumulation

Water stress is characterized by a reduction in plant and soil water content that reduces leaf water potential and causes loss of turgor, decreased cell growth and enlargement (Jaleel *et al.* 2009) and eventually closure of stomata which stops photosynthesis. The response of plants to water stress is complex and depends on the intensity, duration, and rate of progress of imposed stress (Pinheiro, 2011), the plant species and its growth stage (Jaleel *et al.* 2008).

Water stress effects on photosynthesis can be classed as direct, such as decreased CO_2 availability from diffusion limitations (Flexas *et al.,* 2007) or variation in photosynthetic metabolism (Lawlor and Cornic, 2002) or indirect/secondary, such as oxidative stress.

Oxidative stress is most apparent under multiple stress conditions and can significantly affect the photosynthetic apparatus (Ort, 2001). The final outcomes of these effects are reduced growth, yield and plant death in more severe situations. For example, as water deficit increases the overall production of Reactive Oxygen Species (ROS), such as superoxide ($\bullet O^{2-}$) and hydrogen peroxide (H_2O_2) increases. Superoxide, hydrogen peroxide and other ROS compounds can damage the cell and nucleus membranes by lipid oxidation, damage the nucleic acids, cause protein oxidation and chlorophyll bleaching.

Plants can produce compounds to reduce or prevent damage from ROS compounds. To maintain enzyme activity that detoxifies ROS, or to increase it, has a significant role in the tolerance to water stress (Terzi & Kadioglu, 2006). Plants can increase “sacrifice-compounds” that are produced so ROS will attack them instead of cell components. The most common example is the amino acid proline, and therefore it is measured in this research.

Leaf expansion is the first and most important parameter to be affected by drought (Chaves, 1991). At higher levels of water stress, photosynthesis is affected. Rubisco is affected, but the extent of response depends on plant species and the intensity of stress (Parry *et al.* 2002). Cell growth and expansion reduce due to low turgor pressure (Jaleel *et al.* 2008) and photosynthesis decrease occurs due to lower CO_2 diffusion from the atmosphere to the site of carboxylation (Grassi and Magnani, 2005).

The reduced leaf expansion results in the formation of smaller leaves (Hsiao, 1973), as was discussed for nitrogen, and thus also reduces LAI. Developing optimal levels of leaf area is the essential factor for radiation interception, and consequently dry matter yield accumulation through photosynthesis.

With reduced water availability, nutrient uptake is also reduced by the roots, as well as transport from roots to the shoots, due to lowered transpiration rates and the impaired active transport and membrane permeability (Yuncai and Schmidhalter, 2005). As a consequence, the amount of dry matter produced for every millimetre of water used in evapotranspiration, known as water use efficiency (WUE), is also reduced when water stress gets severe (de Carvalho *et al.* 2009).

In the end, stresses from a lack of nitrogen and/or water, impact on dry matter yield by lowering it. This is because nitrogen is a main component in biochemical units that drive yield producing processes and water is the universal media where biochemical reactions take place inside the cells.

2.5 Physiological response to stress on measurable traits

Some traits that are readily affected by insufficient nitrogen or water can be measured in the field others are analysed in a laboratory, as briefly described in Sections 2.3.2and 2.4.2. It is important to understand the relevance of these traits and how they are undertaken to justify the time and energy required in their collection.

2.5.1 Chlorophyll concentration

The chlorophyll content of leaves indicates their physiological condition and photosynthetic capacity. It is influenced by nitrogen nutrition, water status, temperature and biological factors such as cultivar, growth stage, leaf thickness and leaf position (Ebrahimiyan *et al.* 2013).

Chlorophyll can be degraded when water stress is severe (Hendry *et al.* 1987), which reduces both chlorophyll a and b (Huand and Wang, 1998; Jiang and Huang, 2001). This causes irreversible damage to the photosynthetic apparatus (Clarke *et al.* 1996). The main cause is ROS damage, (Section 2.5.2), which bleaches the chlorophyll molecule (Scandalios, 1993) and makes it non-functional (Loggini *et al.*, 1999). The reduction in chlorophyll concentration implies in a reduction in the photosynthetic rate, diminishing the transfer of photo assimilates to other parts of the plants, thus, reducing their dry weight (Khalid, 2006).

The chlorophyll content in a leaf is correlated with leaf nitrogen concentration (Lohry and Schepers, 1988). This along with chlorophyll degradation due to water stress is an interesting trait to analyse differences among forage grasses under different nitrogen regimes.

An efficient way to measure chlorophyll on leaves is the Chlorophyll Meter SPAD-502 Plus (Soil Plant Analysis Meter, Konica Minolta). This device performs quick measurements in the field that can be calibrated with chlorophyll extraction in the laboratory (Bullock, 1998) when certain precautions are taken, such as the measurement point on the leaf (Ata-Ul-Karim *et al.* 2014; Yuan *et al.* 2016).

2.5.2 Osmotic potential

The osmotic adjustment is considered an important physiological response to water stress that enables plants to tolerate water deficits (Begg and Turner, 1970). A decrease in the osmotic potential is considered a potential cell mechanism of drought resistance as it enables turgor and volume maintenance and growth continuation (Munns, 1988). This adjustment is done mostly in younger leaves, because older leaves lose their osmotic adjustment ability and senesce under water stress (O'Neill, 1983). The compounds that account for this adjustment are be diverted from essential processes like protein and cell wall synthesis (Munns, 1988). Different grasses have different strategies of osmotic adjustment during a stress period as observed for *F. arundinacea* and *L. multiflorum* (Abdolzadeh *et al.* 1998). Some authors proposed that C_4 grasses have a greater osmotic adjustment than C_3 grasses (Barker *et al.* 1993). The strategies may not even the same across the genotypes of a species, as observed for cocksfoot (Abdolzadeh *et al.* 2008). Osmotic potential was measured for all species to understand their strategies when water and nitrogen were restricted.

2.5.3 Photosynthetic rate and stomatal conductance

The photosynthetic rate (Pn) describes the amount of carbon assimilated by the plant per unit of leaf area per second. Stomatal conductance indicates how much of gases involved in the photosynthetic process, water vapour and CO_2, can be exchanged via the stomata. High stomatal conductance is vital to a high Pn. Stomatal movements can be affected by the accumulation of photosynthesis-linked metabolites, like sugars and organic acids (Fujita *et al.* 2013), and restrictions in available water may increase the amount of these compounds. In C_3 species, stomatal limitations have a principal role in the drought-induced reduction in photosynthesis (Ghannoum, 2009), whilst in C_4 species, the reduction is more linked to biochemical processes inside the cells. Other factors also affect Pn, some of non-biological nature. Shading limits forage photosynthesis, though some grasses, such as cocksfoot, show tolerance to it (Peri *et al.* 2001). Shading also diminishes Pn by reducing the number of millimoles of photons arriving at the leaf surface.

Factors such as leaf nitrogen, chlorophyll content and stomatal conductance are affected also by leaf age, as analysed for Cocksfoot (Peri *et al.* 2003). The negative effects of the lack of nitrogen, water and low temperatures have previously been discussed, and a multiplicative model was used to explain their collective effects for cocksfoot (Peri *et al.* 2002b). A regrowth component that accounts for leaf age has not been added to this model, but was accounted for (Peri *et al.* 2003), with older a leaves, or leaves that emerged first, having a lower Pn.

The net photosynthetic rate has a negative association with leaf age and leaf area (Reich *et al.* 1999), because plants have to invest materials in building more leaf surface by sacrificing an optimal Pn. Photosynthetic rate (Pn) and conductance are measured by the LICOR 6400 Portable Photosynthesis System, using gas exchanges as the main parameter to calculate them under controlled light conditions. Pn requires an adjustment to the leaf area from the leaf used to measure. We measured Pn and stomatal conductance to compare the strategy of each species, either preferring to expand leaves sacrificing Pn levels or preserving Pn, under the two different nitrogen regimes.

2.5.4 Proline

Proline is an amino acid originated from glutamate. It differs from the other 19 protein-forming amino acids because its molecule has a secondary amine group instead of a primary one. It also does not form ionic or hydrogen bonds, and so its structure is non-polar. The accumulation of proline, and other osmolytes, is a typical response to stress of many different origins, such as cold (Hekneby *et al.* 2001), high salinity (Delauney and Verma, 1993) and drought (Yancey *et al.* 1982; Csonka, 1989; Delauney and Verma, 1993, Yoshiba *et al.* 1997). However, the majority of studies that relate stress and proline accumulation are related to water stress (Kemble *et al.* 1954, Singh *et al.* 1973, Chu *et al.* 1976, Barker *et al.* 1993). The rate of proline accumulated in response to water stress differs among species, intensity and duration, with more pronounced effects as stress is prolonged (Bandurska and Jozwiak, 2010).

Proline, with conformation rigidity, has a cardinal role in adjusting the osmotic potential, detoxifying ROS (Seki *et al.* 2007) and protecting the membrane of many plants. Khoshkholghsima and Rohollahi (2015) found that the concentration of proline in the leaves, increased as soil water stress increased, in non-irrigated treatments could reach almost double those in treatments at 75% Field Capacity, for three grasses, including *F. arundinacea.* Other studies have confirmed, for *F.arundinacea*, proline increased as water stress intensity increased, but showed no relationship between its increase and yield loss (Ebrahimiyan *et al.* 2013).

Proline laboratory analyses from field samples were performed in this study to understand how the plants invested nitrogen under different water situations, to protect their cell apparatuses.

2.5.5 Relative water content

Leaf water status is related to many leaf physiological variables, including turgor, growth, stomatal conductance, transpiration, photosynthesis and respiration (Kramer & Boyer, 1995).

The relative water content is easily measurable and also a useful indicator of plant water balance, because it expresses the relative amount of water present in the plant tissues (Yamasaki and Dillenburg, 1999) related to the maximum amount of water those tissues can hold.

Species and cultivar responses to cell water loss differ (de Carvalho *et al.* 2011), which is reflected in their RWC. However, species known to be resistant to drought manage to maintain a higher RWC (Pirzad *et al.*, 2010) than others so investigation at the species level is appropriate. For most species, a RWC under 40% means a complete pause in the photosynthetic process (de Carvalho *et al.* 2011), though reductions in Pn can already be seen when RWC is below optimum. Reductions in RWC may be caused by factors other than water scarcity, such as excessive salinity (Kapoor and Pande, 2015), but this is not expected to be an issue in this study. Some authors have reported linear (Wilson *et al.*, 1979) or curvilinear (Rascio *et al.*, 1988) relationships between cell turgor and RWC, that indicates higher RWCs contributes to maintaining an expanded leaf area, an essential trait to keep yield (Section 2.2).

To understand if one species had a higher RWC than others and if nitrogen fertilisation influenced it, RWC was measured in this experiment. Fresh leaf samples were taken from each plot to determine RWC, following the method proposed by Turner (1981), and further described in Chapter 7.

2.6 Species used in this project

Pasture brome, cocksfoot, perennial ryegrass and tall fescue are all recommended for dryland regions (Fasi *et al.* 2008),along with subterranean clover and lucerne. The legumes have been the focus of several studies (Brown, 2006; Teixeira, 2005; Ta, 2018) over the last 15 years within the Dryland Pastures Group at Lincoln Univeristy. However, most summer dry environments predominantly grow grass based pastures. Therefore this thesis focuses on the grass component of these swards, with discussion of the role of legumes as companion species where appropriate. The grass species were selected for to assess their potential for dry hill country based on their persistence, agronomic and physiology traits responses to water and nitrogen stress, with results in this thesis for Years 5 and 6.

2.6.1 Pasture brome (*Bromus valdivianus* Phil.)

This species, native to temperate humid regions of Chile, can dominate pastures in soil conditions that are fit for perennial ryegrass (López *et al.* 1997), its accumulated annual herbage mass is similar to perennial ryegrass under those conditions (Balocchi and López, 2007). It has a high Spring-summer growth and a moderate Winter growth and, like other brome species, it does not tolerate waterlogging or pugging, though it tolerates higher rainfall conditions than other brome grasses (Charlton and Stewart, 1999). Brome reportedly tolerates greater water stress than perennial ryegrass, probably due to a deeper root system (López *et al.* 2013), which results in access to more water allowing a higher yield when under moisture stress (Stewart, 1996).

Defoliation in this species should occur after three leaves have fully expanded but before six expanded leaves, because when the seventh leaf appears, the first senesces (Ordoñez *et al.* 2017).

2.6.2 Cocksfoot (*Dactylis glomerata* L.)

Cocksfoot has been recommended as the main grass for summer dryland regions (Mills *et al.* 2014). In these environments, where other grass species have not persisted, cocksfoot mixed with subterranean clover has been shown to be a viable option (Mills *et al.* 2010). One reason proposed from the results of the first two years of this study is that it manages to keep its canopy intercepting a higher amount of radiation than the other grasses (Sharifiamina, 2018).

Cocksfoot is susceptible to water stress and responds with a reduction in the number of tillers (Mills, 2006), which contributes to a lower leaf area thus PAR interception, and consequently lower yield (Sharifiamina, 2018). Recent data suggests farmers should reconsider the intense use of ryegrass-based pastures in their summer dry systems and consider cocksfoot as an option in increasingly dry scenarios (Taylor *et al.* 2021).

2.6.3 Perennial ryegrass (*Lolium perenne* L.)

Perennial ryegrass is a species that admits different endophytes. Endophyte infection, mutualistic in nature, may confer tolerance to some environmental stresses, protection from pests and herbivory to the host species (Clay and Schardl, 2002). Under dry or even sub-optimal irrigation conditions, this species enters a state where growth is suppressed and energy conserved so the plant can survive during the period of water stress (Laude, 1953; Nie and Norton, 2009). It is known to be adapted to mild, moist climates and free draining highly fertile soils (Peeters, 2004; Chapman *et al*., 2011). Perennial ryegrass is commonly used for dairy systems in New Zealand when sown with white clover, and it has shown to be an excellent option for flat land where irrigation and fertiliser are able to be done (Sharifiamina, 2018). In these environments the species can easily have all its demands met and express its full yield and quality potential (Reed, 1996). However, perennial ryegrass is not usually persistent (Mills *et al.* 2014), especially in dry summer conditions that occurs in the eastern regions of the North and South Islands of New Zealand (Tozer, 2011a). Reasons for this lack of persistence includes its low tolerance to desiccation (Volaire *et al.* 1998), and vulnerability to insect pests even when infected with endophyte (Morris *et al.* 2016). This poor persistence is considered a major limitation for pasture productivity (Dodd *et al.* 2018).

2.6.4 Tall fescue (*Festuca arundinacea* Schreb.)

This species is a cool-season grass that has cultivars used for home lawns, recreational surfaces, roadsides but also for pasture. Natural populations can be found from North Africa to Northern Europe, from dry to wet environments. It is now widely spread outside its natural areas, and it is popular in Australia and New Zealand. In Australia, it is used in regions where annual rainfall varies from 650-700 mm or even the waterlogged soils in Victoria where annual rainfall reaches 900 mm. It does not expand into temperate coast where perennial ryegrass is adapted or into the sub-tropical coast where C_4 species are present. In New Zealand, it is seen as an option to regions where perennial ryegrass has its yield and persistence limited by summer moisture stress (Easton *et al.* 1994). Like perennial ryegrass, tall fescue is a species that admits endophyte infection. Though this infection is facultative, the endophyte infection in this species is documented to improve its persistence (Joost and Coombs, 1988).

However, the presence of endophytes may cause a variety of toxic effects on ruminants (Stuedemann and Hoveland, 1988), though, nowadays, endophytes that protect the plants from insects such as the Argentine stem weevil (*Listronotus bonariensis* (Kuchel, 1955)) or black beetle (*Heteronychus arator* (Fabricius, 1775)) are available. Those endophytes produce the alkaloids peramine and loline, which are safe for the cattle and sheep. As for cocksfoot, different tall fescue cultivars can be tolerant or susceptible to drought conditions (Man *et al.* 2011), deep-rooted cultivars being more tolerant to drought, though summer conditions of Canterbury region, where annual rainfall is about 600 mm, were considered limiting to establishment and growth of this species in the first two years of this experiment (Sharifiamina, 2018). Slow establishment also affects is competitive ability with weeds (Black, 2015; Sharifiamina, 2018), further limiting its persistence.

2.7 Management of pastures

Management practices have a major impact on yield accumulating components (Section 2.1), and pasture persistence which is a focus of this study of Year 5 and Year 6.

2.8 Persistence

Pasture persistence can be defined as the physical survival of sown cultivars (Chapman, 2011) or the decline in the expression of a trait within the sown species (Parsons *et al.* 2010). Poor persistence happens when the desired species are replaced by weedy, undesirable species (Tozer, 2011b). Persistence can be affected by hot summers with water stress, when the soil moisture is limited (Waller, 2001), inappropriate fertiliser input and mismanaging weeds and pests (Lee *et al.* 1993, Brouwer *et al.* 1994), endophyte presence (Reed *et al.* 1985, 2000; Cunningham *et al.* 1993), the mixture of pasture species (Nie *et al.* 2004), grazing management (Tozer, 2011b) as well as characteristics in the year of pasture establishment (Maxwell *et al.* 2018).

Poor persistence has been identified as a major problem for New Zealand farmers, mostly due to costs implied in the operations required for pasture renewal (Malcolm *et al.* 2014) and the lack of grazing during forage establishment. Therefore, it is imperative to understand how persistence is being affected in a farm-scale level to improve farm economic profitability and resilience. When the pasture starts to lose its persistence, that means it is already losing the yield and quality levels required by the animals grazing it. The longer a pasture can persist on the field, the higher is the probability it can be profitable (Scott *et al.* 2000). This is most important in dryland hill regions where pasture renewal is difficult. Some species are seen by farmers as more persistent than others and therefore should always be considered in a grazing system. Plaintain and cocksfoot are species reported by New Zealand farmers to be the most persistent amongst all used species (Tozer, 2011b). Cocksfoot has been reported to have a higher persistence than other grasses and can remain productive for at least 10 years, provided appropriate management is used (Mills, 2014).

Considering the scenario of climate change (Salinger, 2003) and the already dry hill country on the east coast of New Zealand, the choice of pasture must be considered along with climate and irrigation strategy (when possible) with regard to the extent of the water stress the plants will be enduring during summer. This later point was the reason for two separate experiments in this study which allowed the impact on species to be assessed under similar climates but different levels of water stress, created by the different water holding capacity of the soils.

Some persistence measurements are costly or time consuming on a large scale, such as plant and tiller populations and basal covers. The easiest way to measure persistence is by measuring long-term dry matter yield and botanical composition data (Dodd *et al.*, 2018). In these experiments, persistence is quantified after six years in these contrasting sites.

2.9 Conclusions

The following conclusions can be drawn from the literature:

- Yield is a function highly dependent on intercepting solar energy and being able to convert it into carbon chemical bounds. How much energy is intercepted and how well that energy is transformed depends on many biotic and abiotic factors, such as available water and mineral nutrients, species and temperature.
- The effect of nitrogen and water stress on the intercepted PAR and RUE differs with species and cultivars.
- To date cocksfoot has shown to be the forage grass that had the highest yield under the greaterst water stress due to increased PAR interception.
- Physiological traits can induce different strategies, to cope with water stress amongst species, cultivars, and nitrogen deficient pastures. Those traits must be measurable on field conditions and relate to the mechanisms of light interception and radiation use efficiency.
- Management practices can interfere with intercepted PAR, RUE and pasture persistence. Allowing the plants on the field for too long for seeding purposes may come at a cost to forage quality and this may differ amongst species.
- The choice of species, as part of pasture management, must be provided with sufficient information for the future conditions of New Zealand hill country drylands so appropriate measures can be taken.

To answer the main questions posed in this study, as described in the objectives (Section 1.2) the experiments started in 2014 were examined until July 2020. The fact the experiments had started allowed longer term differences amongst species and the effect of nitrogen fertiliser to be investigated. The methods used to do this are outlined in Chapter 3.

3 GENERAL MATERIALS AND METHODS

3.1 Introduction

This chapter provides an overview of the materials and methods used for both experiments. Further details specific to each experiment are given in the results (Chapters 4-7).

3.2 Materials and Methods

3.2.1 Experimental sites

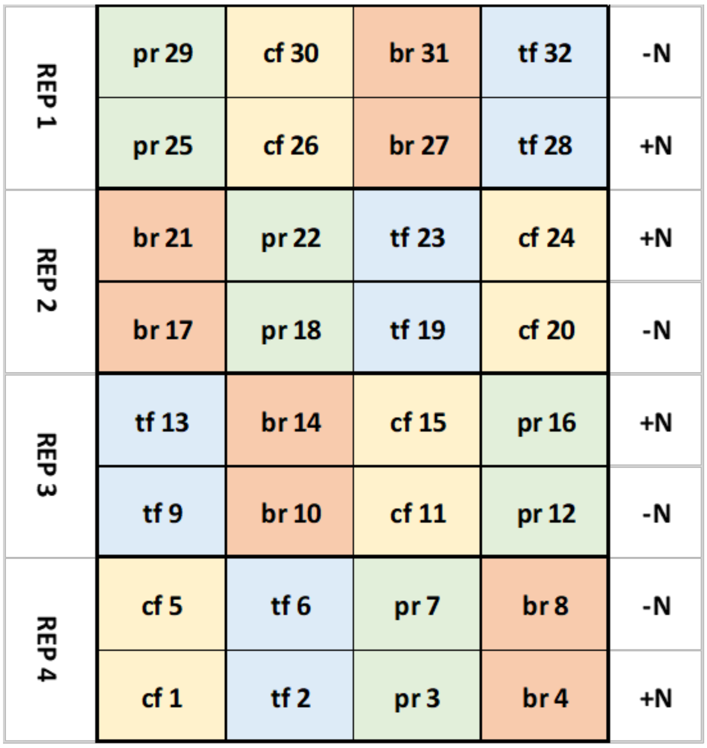
For this study identical experiments were established at two sites:, Experiment 1 at Ladbrooks (43° 37′ S; 172° 30′ E; 12 m a.s.l, datum WGS 84 used for all coordinates in this thesis unless mentioned otherwise) was on 0.357 ha of flat land. This experiment was established in 2014 and monitored by PhD students and FRC technical staff until July 2020.

Experiment 2 was established on 0.28 ha block of Paddock C8 at Ashley Dene Dryland Research Farm (43° 39′ S, 172° 20′ E, 30 m a.s.l.), belonging to Lincoln University. This site is located about 22.5 km south-west of Experiment 1.

3.2.2 Experimental design

Both sites had a strip-plot design with two nitrogen levels as the main plot and four species as sub-plot, in four replicates (Figure 3-1).

Plot dimension was of 6.3 x 9 m in Ladbrooks and 6.3 x 10 m at Ashley Dene. In Ashley Dene, 3 m boundary space was left between each replicate, but this could not be done in Ladbrooks because of space restrictions.



REP 1	pr 29	cf 30	br 31	tf 32	-N
	pr 25	cf 26	br 27	tf 28	+N
REP 2	br 21	pr 22	tf 23	cf 24	+N
	br 17	pr 18	tf 19	cf 20	-N
REP 3	tf 13	br 14	cf 15	pr 16	+N
	tf 9	br 10	cf 11	pr 12	-N
REP 4	cf 5	tf 6	pr 7	br 8	-N
	cf 1	tf 2	pr 3	br 4	+N

Figure 3-1 Field plan of two experiments with four replicates of four species. Cocksfoot (cf),tall fescue (tf), perennial ryegrass (pr) and brome (br) grown with (+N) or without (-N). "REP#" represent the block.

3.2.3 Soil Characteristics

3.2.3.1 Experimental Site 1 – Ladbrooks

This area is flat and soil depth here is 2.3 m with few stones in the profile. The northeast end of this site consists of an imperfectly drained Wakanui Silt Loam (Mottled Immature Pallic or Aquic Haplustepts, USDA Soil Taxonomy) and a poorly drained Temuka clay (Typic Orthic Gley, USDA Soil Taxonomy) on the southeast end. The whole site has poor drainage and limited aeration to the root zones. The soil is vulnerable to water logging with a plant available water content (PAWC) estimated of 125 mm/m (Sharifiamina, 2018).

3.2.3.2 Experimental Site 2 – Ashley Dene

This site is flat, with a soil depth of 1.5 m but with stones through the profile. The paddock is on a Lismore Stony Silt Loam (Typic Dystrustept, USDA Soil Taxonomy) on the west side with a deep fine sandy loam on the east side. The soil is well drained with a PAWC is 60 mm/m and 450 – 750 mm of overlaying horizons of coarse gravels in firmly packed sandy loam.

3.2.4 Land Use History

3.2.4.1 Experimental Site 1 – Ladbrooks

Sharifiamina (2018) outlined the land use in Ladbrooks prior to the establishment was kale (*Brassica oleracea* L.) and rapeseed (*Brassica napus* L.). The area had no crops cultivated on it from January 2014 to September 2014. Common weeds were fathen (*Chenopodium album* L.), wireweed (*Polygonum arenastrum* L.), stinging nettle (*Urtica dioica* L.), shepherd´s purse (*Capsella bursa-pastoris* L.) and white clover (*Trifolium repens* L.). On 4/8/14 MCPB (4-(4-chloro-o-tolyloxy) butyric acid; 1.5 kg a.i. ha^{-1}) was used to control the weeds, and by the end of September 2014, the herbicide glyphosate at 1.4 kg a.i/ha was sprayed. Seeds were sown, in line, on 16/10/2014 at rates shown in Table 3-1.

Table 3-1 Cultivars, sowing rate (kg ha^{-1}) and germination percentage of four grass species at Ladbrooks and Ashley Dene (Sharifiamina, 2018).

Species	Cultivars	Sowing rate (kg ha^{-1})	Germination (%)
Brome	Bareno (9045D)	35	98
Cocksfoot	Sfr36-009	10	88
Perennial ryegrass	Stellar AR1	20	96
Tall fescue	Finesse Q	25	95

From October 2014 to the all rest of the experiment at this site was ungrazed with all the cuts performed by grass-mowers and other machinery.

3.2.4.2 Experimental Site 2 – Ashley Dene

The site in Paddock C8, had not been cultivated for more than 60 years. Resident vegetation included brown-top (*Agrostis capillaris* L.), cocksfoot, white clover and twitch (*Elymus repens* (L.) Gould). On 29/4/14, Buster (glufosinate-ammonium; 1 kg a.i. ha^{-1}) at 5 L^{-1} was applied to control the weeds. On 1/10/14 the residuals were mown and eliminated from the site, then the area was ploughed. Seeds were sown on 15/10/2014.

From establishment until 2015-2016, the plots on Experiment 2 were grazed by sheep (with ewes being removed when residual height was about 30 mm to avoid overgrazing). This was done because no suitable equipment was available to cut and carry the herbage. However, for this research, from November 2018, the plots were mown by a grass-mower, consistent with Experiment site 1. In order to make data reliable, urine patches were avoided for measurements.

3.2.5 Environmental variables: Temperature and Rainfall

Weather stations were installed at both Ashley Dene and Ladbrooks. Thermistors set at 1.5 m above soil level measured air temperature at hourly intervals using a 'Hobo 4-channel logger' (Onset Computer Corporation, Bourne, Maryland, USA). Windrun (km d^{-1}), solar radiation (MJ m^{-2} d^{-1}), wet and dry bulb temperatures were all measured at Lincoln Broadfields Meteorological Station (NIWA, National Institute of Water and Atmosphere Research, New Zealand, agent number 17603), which is 3.5 km west of Ladbrooks site and 12 km north of Ashley Dene. This station records hourly measurements, daily means were used in this thesis.

3.2.5.1 Historical Data

Historical weather data from 1975 to 2012 are summarized in Table 3-2 to provide long-term mean values. Rainfall is slightly higher from June to August, but over time averages ~50 mm $month^{-1}$.

Table 3-2 **Long term monthly means from 1975 to 2012 for total solar radiation (Ro), maximum (Tmax), minimum (Tmin) and mean (Tmean) air temperatures (°C), rainfall (mm), Penman potential evapotranspiration (EP), wind run (km d^{-1}), and vapour pressure deficit (VPD).**

Month	Ro (MJ m^{-2} d^{-1})	Tmax (°C)	Tmin (°C)	Tmean (°C)	Rainfall (mm)	EP (mm)	Windrun (km d^{-1})	VPD (kPa)
January	22.7	21.1	11.6	17.1	45.2	140	398	1.4
February	19.5	22	11.9	17	43	112	387	1.4
March	14.9	20.6	9.6	15.1	51.3	89.3	359	1.3
April	9.4	17.2	6.7	12	48.2	46.3	318	1.1
May	61	14.4	4.3	9.4	51.5	27.1	296	0.9
June	4.6	11.8	2	6.9	61.6	16.4	271	0.8
July	5.4	11	1.5	6.3	69	18.4	274	0.7
August	8	12.5	3	7.9	63	34.5	321	0.8
September	12.7	14.8	4.8	9.9	42	59.7	353	1
October	17.7	17.3	6.6	12.1	50.5	97.8	385	1.1
November	22.3	18.7	8.3	13.6	54.4	126	391	1.2
December	23.1	21	10.4	15.7	54.1	144	389	1.3
Annual	221	16.9	6.7	11.9	634	912	345	1.1

Note: From 1975-2000 measurements were taken at EDL Broadfields Meteorological Station (Open paddock, 2 km northwest of Lincoln township), Canterbury, New Zealand. From 2000 to 2012, measurements were taken from EWS Broadfields Meteorological Station (Open paddock, 200 m northeast of EDL site), Lincoln, Canterbury, New Zealand (Sharifiamina, 2018).

Mean Potential Evapotranspiration (PET) is higher than rainfall for Canterbury by about 280 mm, from late September until April. This creates a soil moisture deficit at the time when temperatures are the closest to the species optimum temperatures in late Spring and summer. Thus, stored soil water has an impact on potential production in these dryland environments.

3.2.5.2 Meteorological Data during the experiment

Daily weather variables were measured on a 2 min base from the installed weather stations. Data were recorded by dataloggers (model CR211x, Campbell Scientific, Logan, UT, USA).

Temperature was used to calculate thermal time accumulation, as follows:

Equation 2 $$\boldsymbol{Tdaily} = \sum \ (\boldsymbol{Th} - \boldsymbol{Tb})$$

Where Tdaily (°C) is the daily thermal time accumulation, Th is the hourly temperature and Tb is the species´ base temperature, which was considered 3 °C for all species (Sharifiamina, 2018). The optimal growth temperature for all species was considered to be 23 °C. On site records for rainfall followed the long-term means except for an atypical rainfall event of 185 mm in October 2018 at both sites. This was followed by higher than average evapotranspiration (270 mm) in the following summer (Figure 3-2) so a summer moisture deficit still occurred, particularly at Ashley Dene.

The temperatures recorded at both experimental sites were 2.2 °C lower than the historical series at both sites. The biggest differences occurring in April/19 and April/20, which were, respectively, about 5.2 °C and 4.3 °C cooler. The highest monthly mean temperature recorded was in January 2019, at 15.6 °C and the lowest average in June 2019, at 4.6 °C, both at Ladbrooks (Figure 3-2).

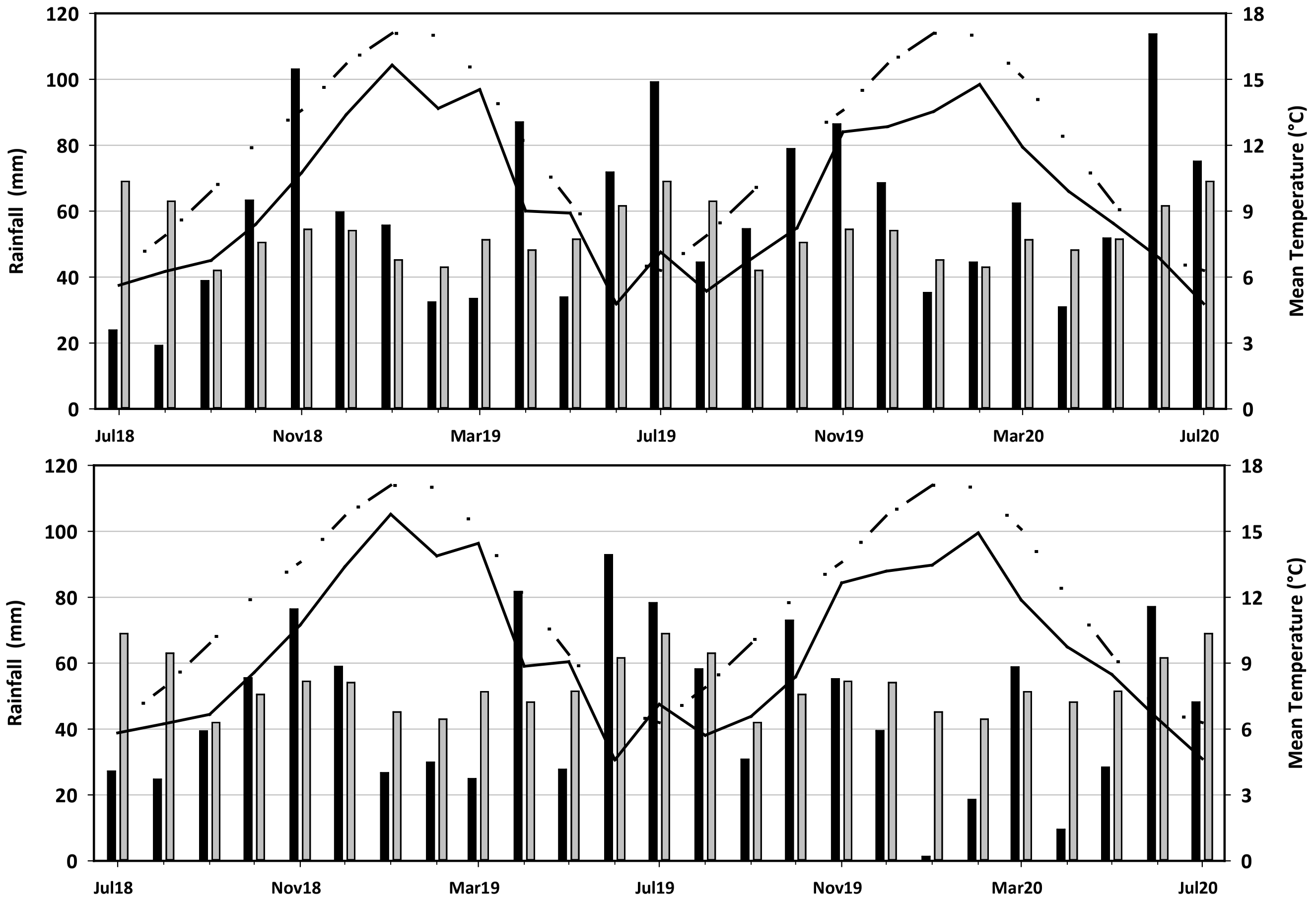


Figure 3-2 Monthly rainfall (black bars) and average air temperature (solid line) for the 2018/2020 period in Ladbrooks (top) and Ashley Dene (bottom). The historical series for rainfall is in grey and for average air temperature, the dashed line.

3.2.5.3 Potential Soil Moisture Deficit (PMSD)

The potential soil moisture deficit was calcauted from 1st July each year. This increased after July in 2018/2019 and 2019/2020 for both sites. In Ladbrooks, the PSMD reached 100 mm in January 2019, in Year 5, and early December 2019, in Year 6. It then increases to 250 mm by mid-February in both years, and to a maximum of 305 mm in early April 2019 in Year 5 and 320 m by late March 2020, and this level reoccurred in mid-May 2019, in Year 6 (Figure 3-3). After late May, the PMSD is gradually reduced as rainfall increased, in both years.

When considering a reduction in soil water based on PET, NIWA suggests plants are stressed when PET reduces the soil moisture to half of the plant available water content. Based on this the calculated soil moisture deficit (SMD) in Ladbrooks reached a maximum of 115 mm by late February 2019 in Year 5 and 170 mm by late March 2020 in Year 6.

In Ashley Dene, the PSMD reached 100 m in early November 2018, Year 5, and early December 2019 in Year 6. It reached 250 mm in early February 2019, in Year 5 and mid-January 2020 in Year 6. The maximum PSMD was 380 mm at the start of April 2019 in Year 5. In Year 6, the maximum PMSD was 495 mm by the end of March 2020 but a rainfall event of more than 50 mm during a whole week reduced the PMSD to 450 mm at the start of April 2020. However, after this the maximum PSMD increased further to reach 510 mm by late May 2020. After this date, the PSMD was gradually reduced by rainfall. The SMD based on PET reduction never reached higher than 80 mm, in both years, at Ashley Dene. The maximum SMD, in this case, was 115 mm by late February 2019, in Year 5 and 113 mm by early February 2020, in Year 6 (Figure 3-3).

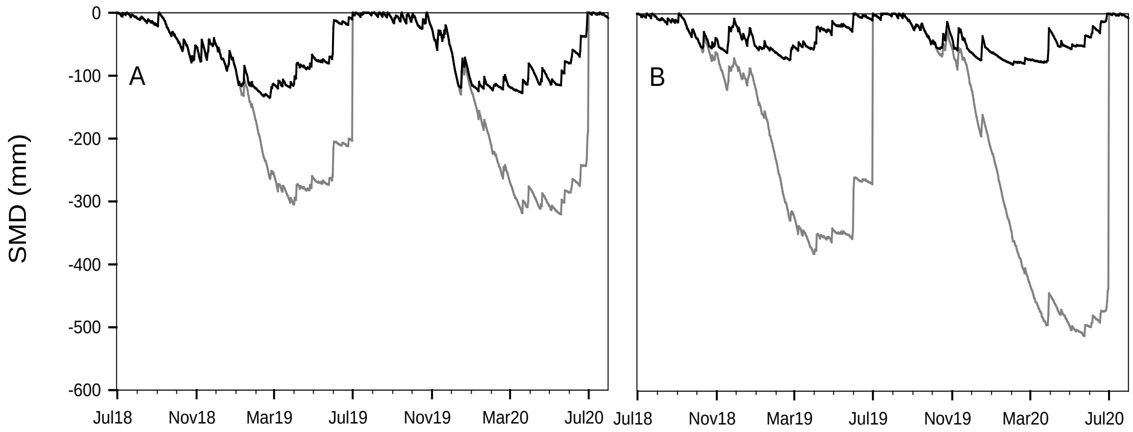


Figure 3-3 Potential Soil Moisture Deficit in Ladbrooks (A) and Ashley Dene (B) from July 2018 to July 2020. Lines represent the Potential Soil Moisture Deficit (–) and Soil Moisture Deficit with PET reduction (–) when SMD reaches half the available plant available water content (225 mm for Ladbrooks, 1.5 m deep and 100 mm for Ashley Dene, 0.8 m deep)

3.2.5.4 Radiation Incidence and Photosynthetic Active Radiation (PAR)

Total incident solar radiation was measured at Ashley Dene and Ladbrooks by sensors 1 m above the ground, installed at the on-site meteorological stations. From 01/07/2018 until 30/08/2020, the total amount of radiation was 941 MJ m^{-2} higher at Ladbrooks than at Ashley Dene, or about 4%. The photosynthetic active radiation (PAR) was obtained by multiplying the total incident solar radiation by 0.5 (Meek *et al.* 1984), so the difference in total PAR received between sites was 470 MJ m^{-2}.

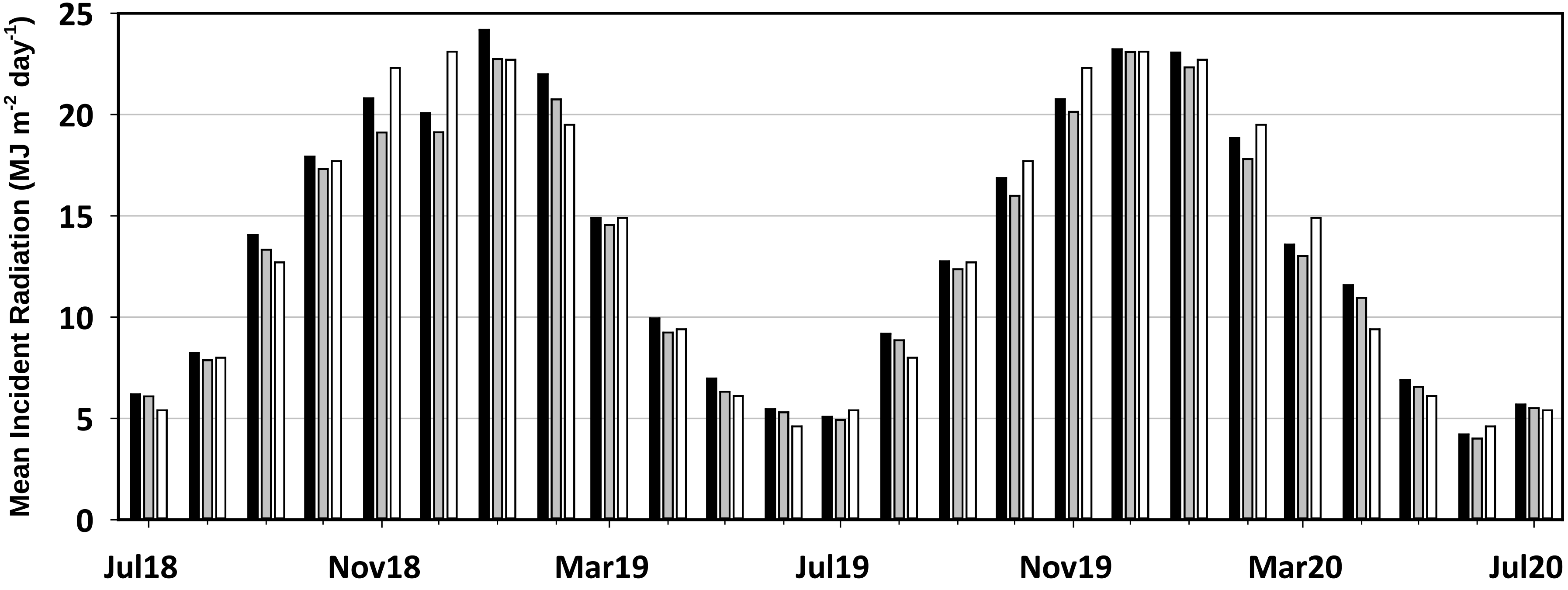


Figure 3-4 Mean incident solar radiation for Ladbrooks (black) and Ashley Dene (gray) from 01/07/2018 until 30/08/2020. Historical series is in white.

In Ashley Dene, the day with the highest total solar radiation was 17/01/2020, with 30.4 MJ m^{-2}, and the day with the lowest solar incidence was 19/06/2020, at 2.0 MJ m^{-2}. In Ladbrooks, the highest daily incidence, 31.0 MJ m^{-2}, took place on 05/01/2019, and the lowest, 1.6 MJ m^{-2}, on 19/06/2020.

Mean PAR accumulation per regrowth cycle (Figure 3-5) was lower at Ladbrooks than in Ashley Dene because regrowth cycles were shorter in duration, but more frequent.

The longest regrowth cycle (interval between two consecutive defoliation events) in Ladbrooks was 97 days (12/02/2020 to 19/05/2020), with a mean of 58 days and a standard deviance of 25 days, for Ashley Dene, the longest regrowth cycle lasted 167 days (30/11/2019 to 14/05/2020), with an average of 73 days with a standard deviance of 43 days.

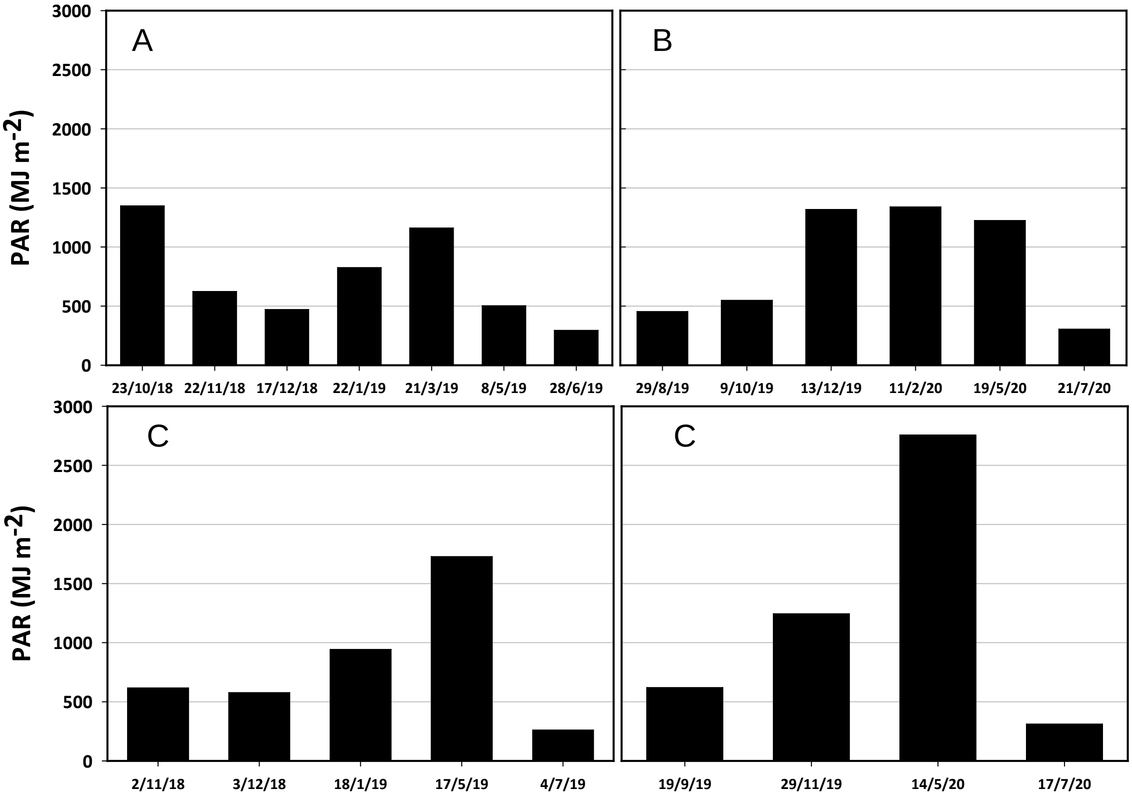


Figure 3-5 Total accumulated incident PAR (MJ m^{-2}) at Ladbrooks (A and B) and Ashley Dene (C and D), for each regrowth cycle as indicated by harvest date.

3.2.6 Fertility Management

Soil fertility was measured from both sites in 2019 and from Ladbrooks only in 2018. One sub-sample from a random nitrogen fertilised plot for each species was taken to represent the N+ treatments (four sub-samples making one sample), and one sub-sample from a random unfertilised plot for each species was taken to represent the N- treatment. Samples were taken 7.5 or 20 cm. Soil analyses were performed at Hill Laboratories in the Christchurch unit. The differences in the sampling depth because of different sampling material used.

Table 3-3 Soil test results from Ladbrooks and Ashley Dene from samples taken in 2018 and 2019**.**

	PARAMETER	pH	Olsen P	K	Ca	Mg	Na	CEC	Total Base Saturation	Volume Weight	Sulphate	Potentially Available Nitrogen	Anaerobically Mineralisable N	Soil Sample Depth
	UNIT	pH Units	mg/L			me/ 100g			%	g/mL	mg/kg	kg/ha	µg/g	cm
SITE, TREATMENTS AND DATE	LB +N 14/12/2018	5.8	31	0.31	15.5	2.38	0.39	26	73	0.78	3	137	118	7.5
	LB -N 14/12/2018	6.2	40	0.33	17.3	2.73	0.46	26	79	0.8	4	145	122	7.5
	LB +N 05/11/2019	5.9	24	0.21	13.6	2.11	0.4	23	72	0.84	4	169*	101	20
	LB -N 05/11/2019	6.4	38	0.28	14.7	2.42	0.42	23	79	0.83	6	169*	102	20
	AD +N 05/11/2019	5.2	17	0.27	4.7	0.57	0.08	4	41	1	1	176*	88	20
	AD -N 05/11/2019	5.7	9	0.24	5.7	0.8	0.14	13	51	0.99	2	188*	95	20

Note: Values represented with "*" were corrected from a 20 cm depth sample to a 15 cm depth sample, which is the standard representation for those numbers.

The values found in Ashley Dene were low for phosphorus and pH but no lime or fertiliser was applied in the last two years.

To ensure that pastures in the N+ treatment had sufficient N to maximize growth urea was applied after each harvest (Table 3-4). The amount was changed throughout the year with less applied in periods of low growth. The difference in the application rates in both sites is to to the slight difference in the plot size. Urea was applied when the weather forecast indicated rain following day.

Table 3-4 Nitrogen application on each site according to date and rate.

Site	Date	Amount of Nitrogen (kg ha^{-1})
Ladbrooks	17/07/18	91
Ladbrooks	03/09/19	91
Ladbrooks	4/11/18	91
Ladbrooks	18/12/18	91
Ladbrooks	23/01/19	91
Ladbrooks	18/04/19	91
Ladbrooks	14/05/19	45
Ladbrooks	13/09/19	91
Ladbrooks	17/10/19	91
Ladbrooks	06/01/20	91
Ladbrooks	19/02/20	91
Ladbrooks	03/06/20	41
Ashley Dene	08/07/18	90
Ashley Dene	03/09/18	82
Ashley Dene	12/11/18	82
Ashley Dene	05/12/18	82
Ashley Dene	23/01/19	82
Ashley Dene	23/05/19	41
Ashley Dene	24/09/19	82
Ashley Dene	16/12/19	82
Ashley Dene	03/06/20	41

3.2.7 Weed Management

3.2.7.1 Experiment 1 at Ladbrooks

Trimec was used at Ladbrooks (600 g L^{-1} mecoprop, 150 g L^{-1} MCPA, 18.7 g L^{-1} dicamba). It was applied on 31/01/2019 (3 L of Trimec / 200L of water ha^{-1}), on 16/5/2019 (3L / 150L of Water ha^{-1}) during morning, but there was rain in the afternoon, so a second application was made on 18/06/2019 (3L of Trimec/200L of water ha^{-1}). Common weeds targeted for control were white clover, wire weed (*Polygonum aviculare* L.) and shepherd´s purse (*Capsella bursa-pastoris* (L.) Medik.). Brome from the sown plots invaded other grass plots but no herbicide was used for control. Individual plants were rogued from around the area of water measurements.

3.2.7.2 Experiment 2 at Ashley Dene

No herbicide was applied on Ashley Dene for the main weed species were grasses and no selective herbicide was available. Brome spp. was also a major weed in other grass treatments. However, discriminating between these and the sown brome was difficult in all plots so they were classified as “Brome” weeds in on tall fescue, cocksfoot and perennial ryegrass plots.

Figure 3-6 illustrates how brome was very invasive on the other plots.

Figure 3-6 Brome as a weed in other plots. Both images were taken in Ashley Dene on 11/02/2020, to the left, there is plot 7 (tall fescue), to the right, plot 12 (perennial ryegrass).

3.2.8 Harvest process

At both sites, on each harvest day, destructive pasture samples were taken as described in Section 3.2.9. The objective for the experimental period was to start a harvest was to maximize vegetative growth and minimize seedhead production. This meant harvests usually occurred to minimize the appearance of seedheads on the Brome plants, which were the most prolific at flowering. However, for one harvest on 29/11/2019 at Ashley Dene and 13/12/2019 in Ladbrooks plots were deliberately left to produce seedheads to the yield and quality of different components could be assessed for a the reproductive phase. After samples were taken (Section 3.2.9), plots at Ladbrooks were mown with a Walker T25i Lawn Mower and a Field Master Forage Harvester with a cage. A 1.5 m radius around the neutron probe tubes was avoided and mown with a garden lawn mower. Residual height after mowing aimed to be 30 mm.

3.2.9 Dry Matter Yield

3.2.9.1 Destructive Measurements

Dry matter at the end of each rotation was sampled using a rectangular 0.2 m² quadrat, with a set of electric shears to the residual height of 30 mm. The harvested material was sorted as described in Section 3.2.9.3. After sorting, the material was dried in a forced air oven at 60 °C to constant weight then weighed. Those measurements served to determine: Total Dry Matter (TDM), Vegetative Dry Matter (VDM), Reproductive Dry Matter (RDM), Sown Species Dry Matter (SSDM; equals VDM + RDM), Weed Fraction (WF), Nitrogen Recovery (NR), Protein Yield (PY), Metabolisable Energy (ME) and Fibres (ADF and NDF).

3.2.9.2 Non-Destructive Measurements

Non-destructive measurements of dry matter were taken every 7-10 days. This was done with a rising pasture plate meter (RPM; Filips Ltd, Feilding, New Zealand). This was calibrated with a 0.2 m² quadrat cuts on 15 occasions through the year. A least squares linear regression between RPM and dry matter yield was performed for each grass at two nitrogen levels and shown on Appendix A. Those measurements were used to determine the dry matter accumulation over time.

3.2.9.3 Botanical composition

Harvested samples, for every defoliation event, were sorted into vegetative (leaves and tillers of sown species), reproductive (stems with flowers or seedheads), dead (material that was, at least, 50% yellowing) and weed (unsown species). The sorting was done for each plot individually at every harvest, for a total of 704 samples. This was done using a sub-sample of about 50 g fresh weight, and using the quartering technique (Cayley and Bird, 1996). The sum of the mass of all components was considered to be the Total Dry Matter.

3.2.9.4 Dry Matter Quality

Vegetative and reproductive components to be analysed for quality were ground separately in a Cyclotec Mill (USA) to pass through a 1 mm sieve. In Year 5 (2018/2019) on both sites, only DM harvested from 23/10/2018 onwards was analysed. The ground material was placed in a Foss NIR Systems 5000 Rapid Content Analyser (Welltech Scientific Inc. 14600 Flint Lee Road, Unit-A, Chantilly, VA 20151 USA), in the Animal and Food Sciences Group facilities, at Lincoln University Riddols Laboratory. This machine uses Near Infrared Spectroscopy (NIRS) to determine nitrogen (N%), acid detergent fibre (ADF), neutral detergent fibre (NDF), organic matter (OM) and dry organic matter digestibility (DOMD).

Equation 3 was used to calculate N% from Crude Protein (CP%) (Kyriazakis and Oldham, 1993):

Equation 3 $$\boldsymbol{CP\% = N\%/6.25}$$

Metabolisable energy (ME) is calculated from DOMD as:

Equation 4 $$\boldsymbol{ME = DOMD * 0.16}$$

The total protein (kg), ADF (kg), NDF (kg), digestibility (%) and organic matter (%) were calculated for the sown species as the sum of vegetative and reproductive dry matter.

3.2.9.5 Nitrogen recovery

Nitrogen recovery (NR) was calculated as the amount of nitrogen present in fertilised pastures relative to unfertilised pastures as:

Equation 5 $$\boldsymbol{NR = TNH/(TNA + NNON)}$$

Where the TNH = Total nitrogen (kg) in herbage at harvest in N+ components, TNA is the total nitrogen applied via fertiliser (kg), and NNON is the nitrogen (kg) in the components of the paired N-plots in each replicate. This gives an indication of nitrogen mineralized and taken up by the plants.

TNH is determined as:

Equation 6

$$\boldsymbol{TNH} = \boldsymbol{VegDM} * \boldsymbol{Nveg}\% + \boldsymbol{RepDM} * \boldsymbol{Nrep}\%$$

Where "VegDM" is the vegetative components dry matter (kg), Nveg% is the percentage of nitrogen in the vegetative dry matter, "RepDM" is the reproductive components dry matter (Section 3.2.9.3) and "Nrep%" is the percentage of nitrogen in the reproductive matter.

3.3 Statistical Analysis

For both sites and for the whole duration of the experiment, the effects of nitrogen and moisture were investigated for each species, under the strip-plot design, unless the NR, which was analysed under the latin square design. A total of 12 harvests were taken in Ladbrooks and 9 in Ashley Dene, over two years. Analysis of variance for the strip-plot design were run on the annual yield and quality components.

A repeated measures ANOVA was used in each regrowth cycle to investigate the effect of time on DM and pasture quality components.

For calculations of thermal time, the same base (3 °C) and optimum temperatures (23 °C) were used for all species (Mills, 2007). Least squares linear regression of accumulated DM against accumulated thermal time was used to obtain temperature adjusted growth rates (kg DM $°C^{-1}$ ha^{-1}). When the linear regression was multi-staged, breaking points were determined where they would minimize the errors.

Unless stated otherwise, the significance level tested was $\alpha = 0.05$. Means were separated using Fishers' protected least significant difference (LSD). Interactions are only reported when significant, and their means were separated by the appropriate interaction LSD.

For the “weed fraction”, arcsin transformation was required to normalise the data, to fit the ANOVA assumptions.

GenStat 16.1 (VSN International) was used to run all analysis except for thermal time x dry matter regressions, which were run on SigmaPlot 14.0 (Systat Software Inc.). SigmaPlot 14.0 was also used for all the figures.

4 AGRONOMIC PERFORMANCE OF BROME, COCKSFOOT, PERENNIAL RYEGRASS AND TALL FESCUE MONOCULTURES UNDER TWO DIFFERENT NITROGEN REGIMES

4.1 Introduction

Nitrogen and water are the two main determinants of pasture growth in most productive systems (Sinclair and Rufty, 2012). Their deficiency results in severe yield reductions (Section 2.3). Insufficient soil nitrogen in New Zealand grass-dominant pastures is very frequently the primary limiting factor of production. To overcome this plants need to be well supplied with nitrogen, with inorganic fertiliser the most common form.

Species differ in their behavior under water and/or nitrogen deficiency (Section 2.3.2and 2.4.2). For the first two years of this experiment, Sharifiamina (2018) showed “Wana” cocksfoot had a higher dry matter yield at Ashley Dene under summer dry conditions compared with tall fescue, perennial ryegrass and brome. Under higher soil water conditions at Ladbrooks, the total dry matter yield for those species did not differ and averaged 19800 ± 520 kg ha^{-1}. In addition, when water was available, plants fertilised with nitrogen yielded more than double those unfertilised. In contrast, the increase in dry matter from N was only ~2000 kg ha^{-1} year^{-1} in the summer dry environment on a soil of low water holding capacity at Ashley Dene.

This chapter deals with Objective 1 – To characterize pasture quantity and quality for these four speices grown under different water and nitrogen regimes. The analysis of the field experiment also meets Objective 2 – To relate dry matter production to the accumulation of thermal time to account for differences in temperature that affects daily growth rates. Thus, thisis chapter quantifies these agronomic results which are explained through pasture and plant physiology principles in Chapters 567, 6 and 7.

4.2 Materials and methods

Materials and methods for this chapter were fully described in Chapter 3.

4.3 RESULTS

4.3.1 Total Dry Matter (TDM)

Experiment 1 – Ladbrooks

The accumulated Total Dry Matter was calculated by the sum of all biomass from all harvests. For the Year 5 (2018/2019), TDM was not different ($P=0.267$) amongst species (Table 4-1), and averaged 11720 ± 568.9 kg ha^{-1}. However, the TDM of the N+ treatments was 18075 ± 362 kg ha^{-1} which was more than three times ($P<0.001$) the 5360 ± 362 kg ha^{-1} from N- treatments. This yield difference was evident from September 2019 onwards (Figure 4-1).

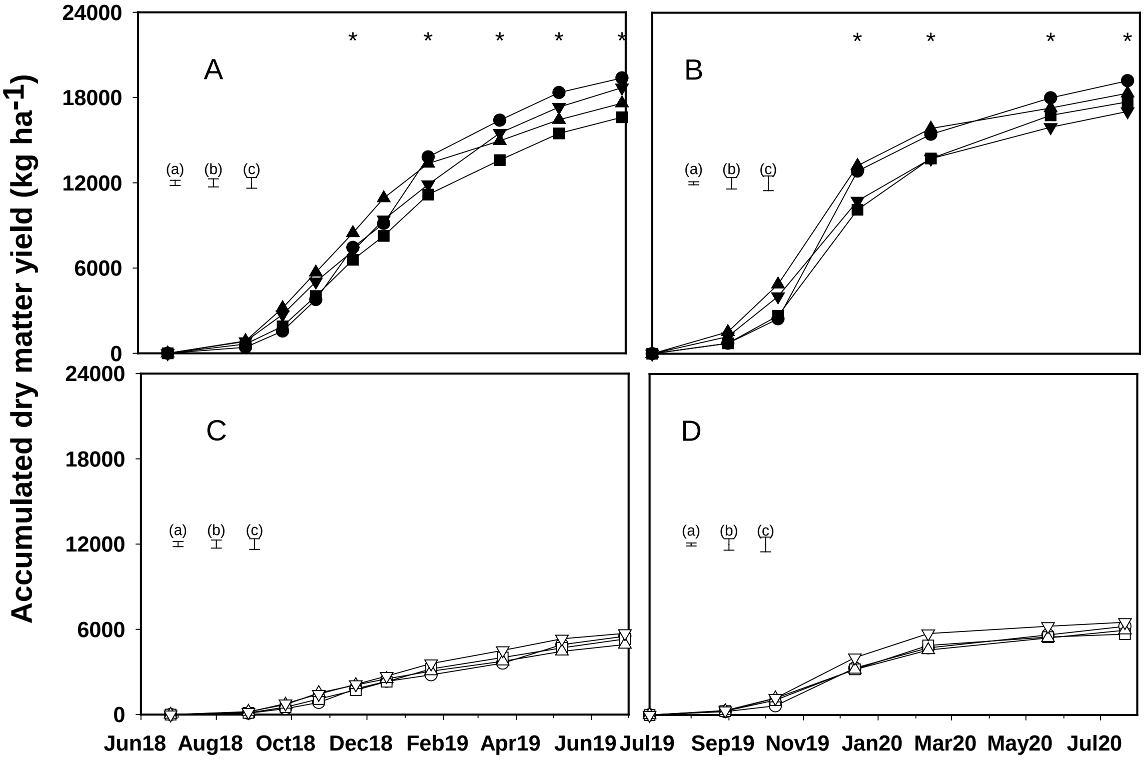


Figure 4-1 Dry matter accumulation (kg ha^{-1}) for Ladbrooks from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for brome (●,○), cocksfoot (■,□),perennial ryegrass (▲,△) and tall fescue (▼,▽). with (closed) or without nitrogen fertilisation (open). Standard Errors of the Mean are represented for (a) nitrogen, (b) species and (c) nitrogen*species. “*” show when differences were observed.

There were yield differences for some individual harvests. For example, by the end of Spring, in late November, brome had accumulated 2310 ± 70 kg DM ha^{-1} and the other species 1560 ± 70 t DM ha^{-1} (P<0.001). The Spring period, until mid-December produced ~50% of all the dry matter.

In Year 6 (2019/2020), there was also no difference among species (P=0.598), and mean production was 12100 ± 1110 kg DM ha^{-1}. TDM of N+ pastures was again 18100 ± 207.2 kg ha^{-1} or three times the 6100 ± 207.2 t ha^{-1} from N- treatments (Table 4-2). This difference was significant in the December harvest when the fertilised plots accumulated ~8200 ± 155 kg DM ha^{-1} compared with 2400 ± 155 kg DM ha^{-1} from unfertilised plots. As expected regrowth cycles that occurred in Spring when water was available (Figure 4-1) had higher yields than those in summer and Autumn.

Year 5 (2018/2019)

Table 4-1 Mean accumulated total dry matter (kg DM ha^{-1}), harvested from July 2018 to July 2019 from monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
27/08/18	420	650	870	830	90	120	200	170	420
26/09/18	1560	1890	3190	2730	420	520	710	770	1470
23/10/18	3770	4030	5700	5040	850	1090	1520	1440	2930
22/11/18	7440	6580	8470	7200	1820	1720	2080	2120	4680
17/12/18	9140	8260	10930	9410	2330	2320	2510	2710	5950
22/01/19	13820	11170	13360	11890	2790	3210	3050	3600	7860
21/03/19	16400	13600	14960	15510	3610	4010	3750	4520	9550
09/05/19	18355	15480	16440	17330	4920	4690	4450	5330	10870
28/06/19	19380	16620	17600	18700	5510	5320	4920	5710	11720
Mean Yearly N	18075				5360				

	BR	CF	PR	TF	Mean
Mean Yearly S	12400	11000	11300	12200	11720

	P-value	SEM	LSD
S	0.267	574.7	-
N	<0.001	362.0	1629.3
S*N	0.275	745.2	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "S" is for species, "N" for nitrogen and "N*S" for the interaction nitrogen*species. "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%.

Year 6 (2019/2020)

Table 4-2 Mean accumulated total dry matter (kg DM ha^{-1}), harvested from July 2019 to July 2020 from monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
29/08/19	730	730	1530	1200	230	270	280	310	660
09/10/19	2450	2670	4880	4030	640	1040	1180	1180	2260
13/12/19	12850	10140	13230	10760	3300	3210	3200	4030	7590
11/02/20	15450	13750	15860	13730	4720	4890	4570	5730	9840
19/05/20	18010	16790	17300	15940	5640	5485	5420	6250	11350
21/07/20	19220	17730	18340	17060	6230	5690	5960	6520	12090
Mean Yearly N		18100					6100		
	BR		CF		PR		TF		Mean
Mean Yearly S	12700		11700		12150		11800		12100
			P-value		SEM			LSD	
S			0.598		806.0			-	
N			<0.001		207.2			932.4	
S*N			0.528		1038.4			-	

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "S" is for species, "N" for nitrogen and "N*S" for the interaction nitrogen*species."SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%.

Experiment 2 – Ashley Dene

For the Year 5, TDM was not different (P=0.484) amongst species, with a mean yield of 7670 ± 440 kg ha^{-1} (Figure 4-2). However, N+ pastures produced 9340 ± 439.7 kg ha^{-1} which was 50% more (P<0.001) than the 6000 ± 215 kg ha^{-1} from the N- pastures (Table 4-3). More than 75% of all TDM was produced from 01/10/2018 until the harvest of 18/01/2019 (Figure 4-2).

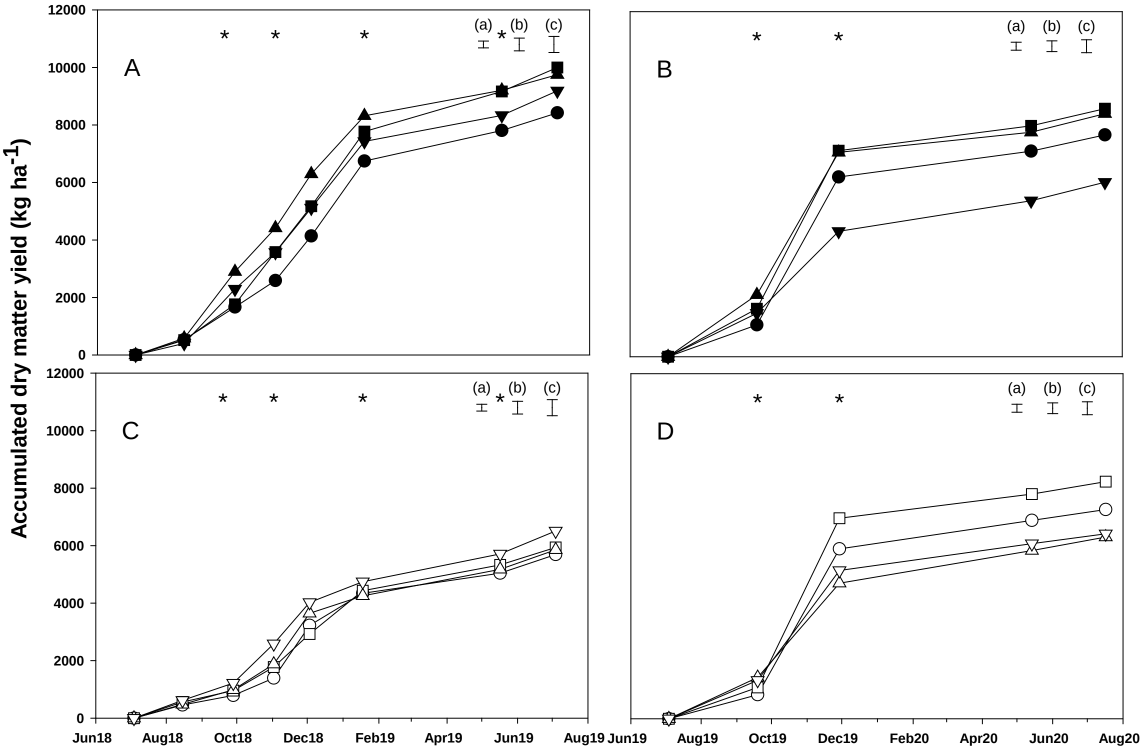


Figure 4-2 Dry matter accumulation (kg ha^{-1}) for Ashley Dene from July 2018 to July 2019 (A,C) and from July-2019 to July 2020 (B,D), for brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△), tall fescue (▼,▽),with (closed) or without nitrogen fertilisation (open). Standard Errors of the Mean are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" show when differences were observed.

In Year 6, there were no differences among species (P=0.060) or nitrogen treatments (P=0.211) for total dry matter (Table 4-4). More than 80% of all dry matter was grown from 04/07 to 29/11/19 (Figure 4-2). The regrowth cycle harvested on 29/11/2019 produced ~54% of all TDM accumulated through this year.

For the second regrowth cycle, from 20/9 to 29/11/2019, plants were left unharvested to reach the reproductive stage when they start to produce seeds, or about the time they would be cut for hay. This determined how 'deferred grazing' affected the botanical composition and quality of vegetative and reproductive components of the pastures.

Year 5 (2018/2019)

Table 4-3 Total Accumulated Dry Matter (kg ha^{-1}), from July 2018 to July 2019, of brome, cocksfoot, perennial ryegrass and tall fescue sown in monoculture, grown with (N+) and without (N-) nitrogen, at Ashley Dene, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean AccH
15/08/18	535	520	580	405	460	560	460	620	520
26/09/18	1670	1770	2900	2300	790	960	985	1220	1580
02/11/18	2590	3580	4420	3570	1395	1780	1880	2590	2730
03/12/18	4140	5175	6300	5115	3225	2930	3650	4030	4320
18/01/19	6750	7770	8320	7430	4340	4430	4250	4750	6005
17/05/19	7810	9170	9210	8330	5050	5340	5180	5720	6975
04/07/19	8420	10000	9750	9190	5695	5940	5860	6510	7670
Mean Yearly N		9340					6000		

	BR	CF	PR	TF	Mean
Mean Yearly S	7060	7970	7805	7850	7670

	P-value	SEM	LSD
S	0.484	439.7	-
N	0.002	214.6	966
S*N	0.457	558.0	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "S" is for species, "N" for nitrogen and "N*S" for the interaction nitrogen*species, "Mean AccH" represents the accumulated dry matter until that harvest."SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%.

Year 6 (2019/2020)

Table 4-4 Total Accumulated Dry Matter (kg ha^{-1}), from July 2019 to July 2020, of brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) and without (-N) nitrogen fertilisation, at Ashley Dene, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean AccH
19/09/19	1110	1675	2155	1515	835	1090	1435	1335	1395
29/11/19	6250	7170	7110	4360	5910	6980	4715	5160	5960
14/05/20	7150	8030	7810	5430	6900	7820	5850	6090	6890
17/07/20	7720	8630	8450	6070	7280	8250	6320	6430	7400
Mean Yearly N		7715					7070		
	BR		CF		PR		TF		Mean
Mean Yearly S	7500		8440		7390		6250		7395

	P-value	SEM	LSD
S	0.060	473.0	-
N	0.211	288.5	-
S*N	0.498	684.9	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “S” is for species, “N” for nitrogen and “N*S” for the interaction nitrogen*species, “Mean AccH” represents the accumulated dry matter until that harvest.“SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%.

4.3.2 Sown Species Dry Matter (SSDM)

Experiment 1 – Ladbrooks

In Year 5, SSDM production was not different for species (P=0.259), with an annual yield of 10610 ± 1140 kg ha^{-1}. The N- pastures produced 4360 kg SSDM/ha^{-1} compared with (P<0.001) 16870 ± 360 kg ha^{-1} for N+ (Table 4-5). For this year, the production profile for the N- plots was low and stable across harvests (Figure 4-3), with no difference amongst species. However, there were differences amongst species on four occasions for the N+ plots.

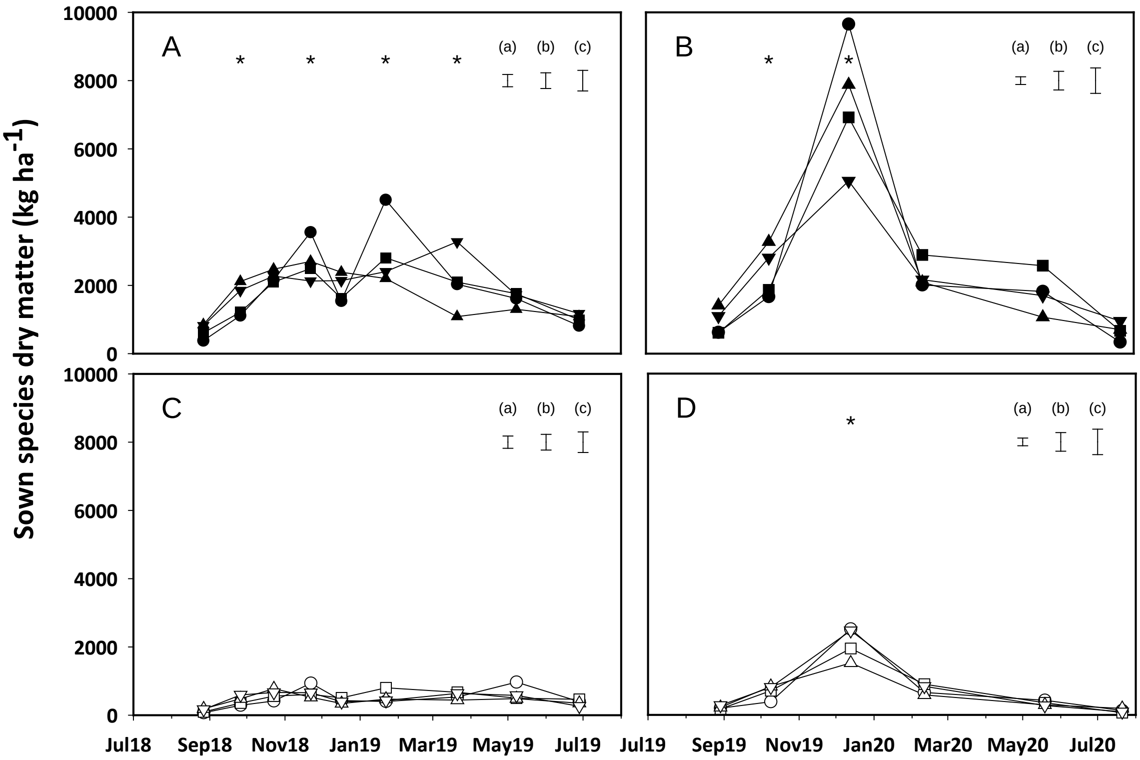


Figure 4-3 Sown species dry matter (kg ha^{-1}) for Ladbrooks from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△), tall fescue(▼,▽) with (closed) or without nitrogen fertiliser (open). Standard Errors of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" show when differences among species were observed.

In Year 6, SSDM was not different (P=0.687) amongst species and averaged 9845 ± 1050 kg DM ha^{-1}. The added nitrogen (N+) increased (P<0.001) the mean yield from 4210 to 15480 ± 226.9 kg ha^{-1} (Table 4-5). The yearly production profile showed the delayed "hay" harvest in mid December 2019 had a higher (P<0.001) SSDM yield than the other rotations. For this harvest differences (P<0.05) among species were observed. For the non-fertilised plots, brome and tall fescue had a higher yield of 2500 ± 305 kg ha^{-1} than ryegrass, 1520 ± 305 kg ha^{-1}. For the N+ plots, brome had the highest yield 9660 ± 1010 kg ha^{-1}, and cocksfoot and ryegrass were not different at 7400 ± 1010 kg ha^{-1} (Table 4-6).

Table 4-5 Mean sown species dry matter (kg DM ha^{-1}), harvested from July 2018 to July 2019 from monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury, New Zeland.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
27/08/18	380	610	850	820	70	100	190	170	400 g
26/09/18	1120	1220	2115	1850	290	355	480	590	1000 e
23/10/18	2160	2100	2475	2280	420	560	775	660	1430 b
22/11/18	3560	2500	2700	2130	940	610	520	660	1700 a
17/12/18	1540	1620	2400	2140	430	510	340	390	1170 cd
22/01/19	4510	2810	2200	2410	400	801	470	430	1750 a
21/03/19	2040	2100	1090	3280	530	670	440	640	1350 bc
09/05/19	1620	1760	1300	1720	970	500	480	580	1120 de
28/06/19	820	990	1085	1180	400	465	350	260	690 f

	N+	N-
Mean Yearly N	16870	4360

	BR	CF	PR	TF	Mean
Mean Yearly S	11090	10130	10130	11090	10165

	P-value	SEM	LSD
S	0.259	439.2	-
N	<0.001	360.0	1620.2
S*N	0.066	603.6	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “S” is for species, “N” for nitrogen and “N*S” for the interaction nitrogen*species.”H” represents the harvest. “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Table 4-6 Mean sown species dry matter (kg DM ha^{-1}), harvested from July 2019 to July 2020 from monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
29/08/19	620	610	1420	1100	200	170	220	280	580 d
09/10/19	1670	1870	3280	2810	380	710	840	810	1550 b
13/12/19	9660	6920	7880	5060	2520	1940	1520	2470	4750 a
11/02/20	2010	2890	2100	2170	660	900	580	840	1520 b
19/05/20	1820	2580	1070	1700	430	350	290	270	1070 c
21/07/20	340	670	700	950	130	60	190	90	390 e
Mean Yearly N	15480				4210				
	BR		CF		PR		TF		Mean
Mean Yearly S	10220		9840		10050		9270		9845
			P-value		SEM			LSD	
S			0.687		574.6			-	
N			<0.001		226.9			1021.1	
S*N			0.213		746.2			-	

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “S” is for species, “N” for nitrogen and “N*S” for the interaction nitrogen*species, “H” represents the harvest. “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Experiment 2 – Ashley Dene

In Year 5, SSDM was not different amongst species at Ashley Dene with a mean of 6.2 ± 0.35 t ha^{-1}. The N+ plots increased (P=0.001) mean yield from 4.5 to 7.9 ± 0.21 t ha^{-1} (Table 4-7). During the year, differences among species were observed for some regrowth cycles (P<0.001) until late January 2019 (Figure 4-4), after which yield was not affected by N.

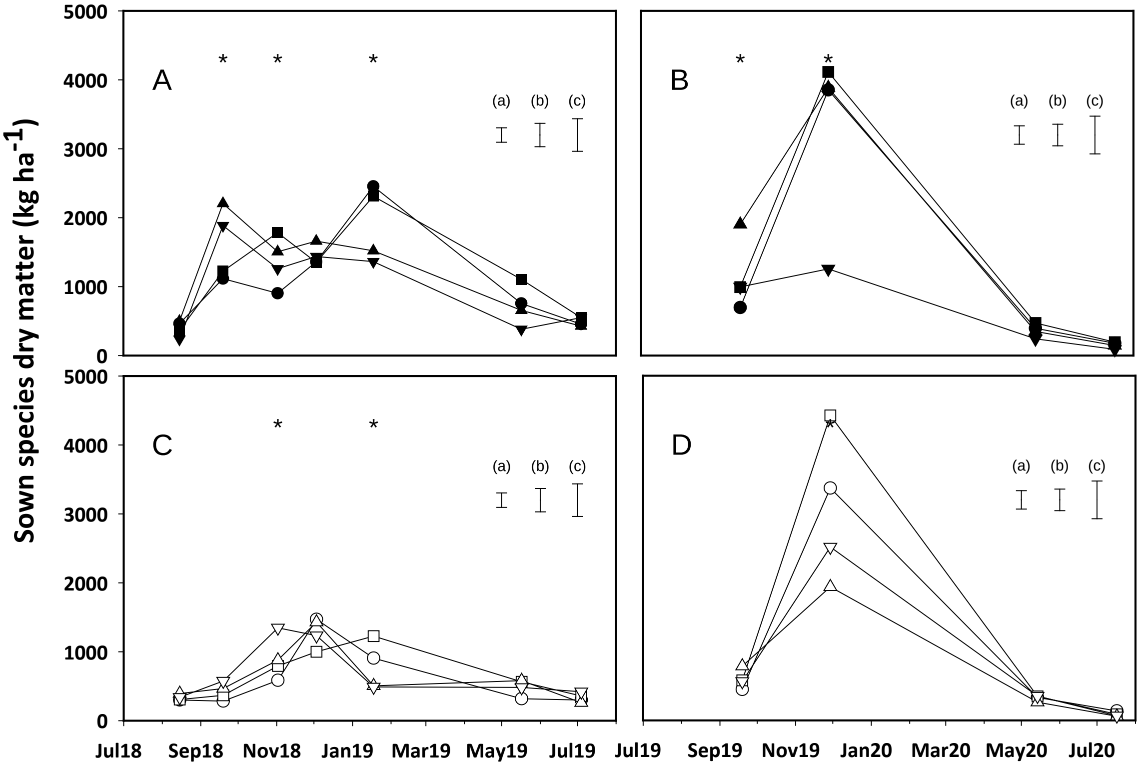


Figure 4-4 Sown species dry matter yield (kg ha^{-1}) for Ashley Dene from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△), tall fescue(▼,▽) with (closed) or without nitrogen fertiliser (open). Standard errors of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" show when differences among species were observed.

In Year 6, cocksfoot had the highest (P=0.002) annual SSDM yield of 5.6 ± 0.34 t ha^{-1}, followed by brome and p. ryegrass, 4.9 ± 0.34 t ha^{-1} and tall fescue was lowest at 3.0 t ha^{-1}

(Figure 4-8). N fertiliser did not (P=0.103) change the SSDM yield which averaged 4.5 ± 0.31 t ha^{-1}. About 90% of all SSDM was produced up to late Spring (Figure 4.4).

Year 5 (2018/2019)

Table 4-7 Mean Sown Species Dry Matter (kg ha^{-1}), harvested from July 2018 to July 2019, of brome, cocksfoot, perennial ryegrass and tall fescue sown in monoculture, grown with (N+) and without (N-) at Ashley Dene, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
15/08/18	460	360	500	240	300	310	390	340	360 d
26/09/18	1120	1230	2210	1890	280	370	470	570	1020 b
2/11/18	900	1780	1510	1260	580	790	880	1350	1130 b
3/12/18	1360	1350	1660	1440	1470	1000	1430	1230	1370 a
18/01/19	2460	2310	1520	1360	900	1230	500	490	1350 a
17/05/19	760	1110	660	380	320	560	580	480	600 c
4/07/19	460	550	430	550	300	360	260	420	420 cd
Mean Yearly N		7950					4550		
	BR		CF		PR		TF		Mean
Mean Yearly S	5840		6650		6500		6000		6250

	P-value	SEM	LSD
S	0.409	337.5	-
N	0.001	210.5	947.3
S*N	0.136	473.2	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "S" is for species, "N" for nitrogen and "N*S" for the interaction nitrogen*species, "H" represents the harvest."SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 4-8 Mean sown species dry matter (kg ha^{-1}), harvested from July 2019 to July 2020, of brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without (N-) at Ashley Dene, Canterbury.

		N+					N-		
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
19/09/19	700	990	1900	1000	450	580	790	570	870 b
29/11/19	3850	4110	3890	1260	3370	4420	1940	2510	3170 a
14/05/20	400	470	350	240	330	350	260	350	350 c
17/07/20	180	190	150	90	140	90	60	70	120 c
Mean Yearly N		4950					4070		

	BR	CF	PR	TF	Mean
Mean Yearly S	4710	5610	4670	3040	4510

	P-value	SEM	LSD
S	0.002	313.1	442.7
N	0.103	266.7	-
S*N	0.111	557.6	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "S" is for species, "N" for nitrogen and "N*S" for the interaction nitrogen*species, "H" represents the that harvest."SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

4.3.3 Vegetative Dry Matter (VDM)

Experiment 1 – Ladbrooks

In Year 5, the vegetative dry matter (VDM) yield changed over time (Figure 4-5) and increased (P<0.001) from 4000 to 15200 ± 292 kg ha^{-1} for N+ (Figure 4-9). Amongst species, cocksfoot, p. ryegrass and tall fescue produced more (P=0.013) VDM (10135 ± 481 kg ha^{-1}) than brome at 8000 ± 481 kg ha^{-1}.

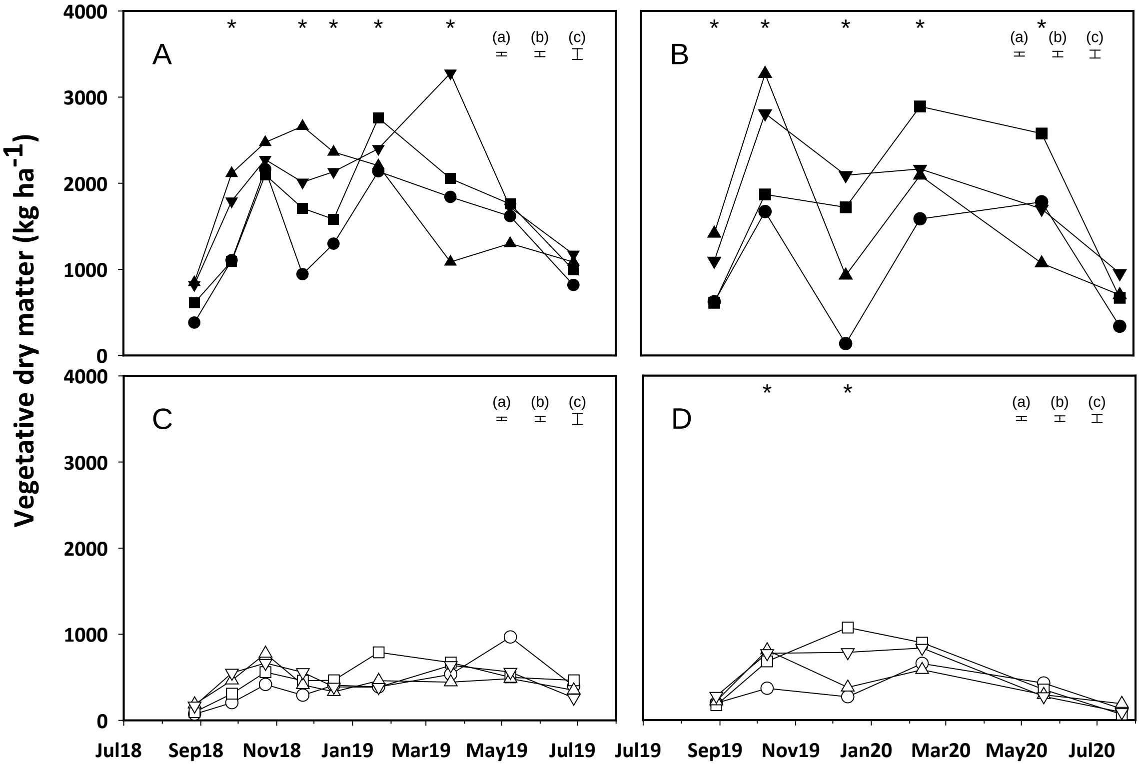


Figure 4-5 Vegetative dry matter (kg ha^{-1}) for Ladbrooks from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△), tall fescue(▼,▽) with (closed) or without nitrogen fertilisation (open). Standard Errors of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" show when differences among species were observed.

Year 5 (2018/2019)

Table 4-9 Mean Vegetative Dry Matter (kg DM ha^{-1}) of brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019 grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
27/08/18	380	610	850	820	70	95	185	170	400 e
26/09/18	1105	1095	2115	1795	205	310	465	550	955 c
23/10/18	2160	2100	2475	2280	420	560	775	665	1430 a
22/11/18	940	1710	2660	2015	290	460	415	560	1130 b
17/12/18	1300	1580	2365	2135	410	465	330	390	1120 b
22/01/19	2140	2760	2205	2400	390	790	460	385	1440 a
21/03/19	1840	2055	1085	3280	535	670	445	640	1320 a
09/05/19	1620	1760	1300	1725	970	500	485	560	1115 b
28/06/19	820	995	1085	1180	400	465	350	260	695 d
Mean Yearly N	15200				4000				

	BR	CF	PR	TF	Mean
Mean Yearly S	7950	9500	10000	10900	9600

	P-value	SEM	LSD
S	0.013	480.8	1538.2
N	<0.001	292.2	1318.4
S*N	0.015	619.4	1851.8

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "S" is for species, "N" for nitrogen and "N*S" for the interaction nitrogen*species, "H" represents the harvest."SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 4-10 Mean vegetative dry matter (kg DM ha^{-1}),from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
28/08/19	625	610	1420	1100	170	280	220	200	580 c
09/10/19	1670	1870	3270	2810	370	680	810	775	1530 a
13/12/19	140	1720	930	2095	270	1070	380	790	925 b
11/02/20	1585	2890	2090	2110	660	900	585	840	1460 a
19/05/20	1780	2580	1070	1700	430	350	290	270	1060 b
21/07/20	340	670	700	950	130	65	190	90	390 c
Mean Yearly N	9200				2700				

	BR	CF	PR	TF
Mean Yearly S	4090	6800	6000	6900
Yearly Mean		5950		

	P-value	SEM	LSD
S	0.004	318.1	1017.5
N	<0.001	160.6	722.9
S*N	0.554	493.5	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "S" is for species, "N" for nitrogen and "N*S" for the interaction nitrogen*species, "H" represents the harvest."SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Experiment 2 - Ashley Dene

Figure 4-6 shows that VDM production at Ashley Dene had a similar profile to Ladbrooks but at a different yield scale. Regrowth cycles in Spring produced three times or more the amount of VDM of regrowth cycles Summer and Winter.

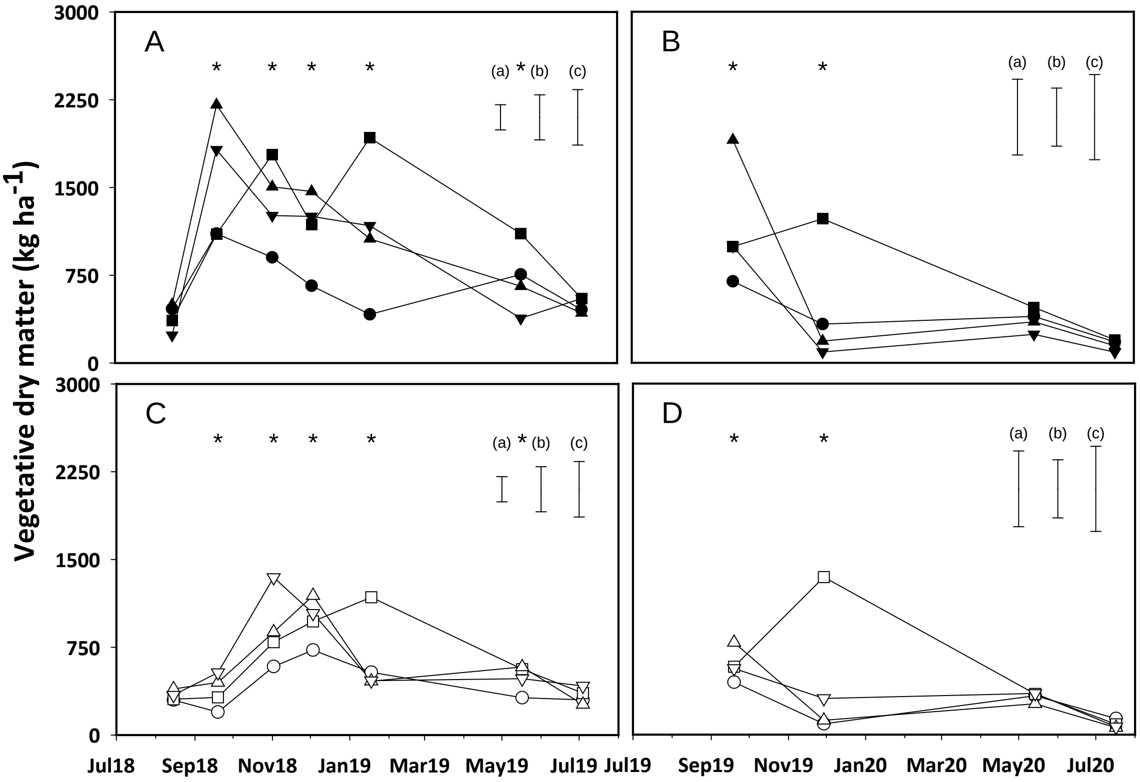


Figure 4-6 Vegetative dry matter yield (kg ha^{-1}) for Ashley Dene from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△), tall fescue(▼,▽) with (closed) or without nitrogen fertilisation (open). Standard errors of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" show when differences among species were observed.

For Year 5, VDM was highest for cocksfoot (P=0.007) at 6250 ± 385.3 kg ha^{-1} and lowest for brome at 3860 ± 385 kg ha^{-1} regardless of N regime (Figure 4-11). Cocksfoot produced at least 40% more VDM than the other species at the 18/01/2019 and 17/05/2019 harvests (Figure 4-6). Nitrogen increased the VDM of all species (P=0.003) by ~45%.

For Year 6, VDM was the highest for cocksfoot (P<0.001), regardless of nitrogen regime with a mean of 2630 ± 155.3 kg ha^{-1} (Table 4-12). This occurred because it produced and maintained the highest amount of VDM in the summer harvest, when all the species were reproductive and seeding. Nitrogen increased the yield (P<0.001) for all species, and this effect was most apparent in Spring.

VDM was predominantly produced in the first two harvests. Perennial ryegrass N+ produced the highest VDM of 1905 ± 130 kg ha^{-1} at the first harvest but it did not contain as much green material as cocksfoot in the second harvest, for either nitrogen treatments. Perennial ryegrass only produced 9% and 15% of cocksfoot VDM, for N- and N+ treatments, respectively. For the late Autumn (14/05/2020) and Winter (17/07/2020) harvest all species had VDM yields of <500 kg DM/ha (Figure 4-6).

Year 5 (2018/2019)

Table 4-11 Mean vegetative dry matter (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, grown with (N+) and without (N-) nitrogen, harvested from July 2018 to July 2019 at Ashley Dene, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
15/08/18	465	365	500	240	300	305	395	340	365 d
26/09/18	1105	1100	2205	1825	195	320	450	535	970 ab
2/11/18	905	1785	1510	1260	585	795	880	1345	1130 a
3/12/18	660	1185	1465	1190	725	970	1255	1040	1060 a
18/01/19	415	1925	1060	1175	535	1180	460	465	900 b
17/05/19	760	1105	655	380	320	565	580	480	605 c
04/07/19	455	550	430	550	300	360	260	420	415 d
Mean Yearly N	6820				4075				
	BR		CF		PR		TF		Mean
Mean Yearly S	3860		6250		6020		5655		5450

	P-value	SEM	LSD
S	0.007	385.3	1232.7
N	0.003	214.7	966.4
S*N	0.055	474.4	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “S” is for species, “N” for nitrogen and “N*S” for the interaction nitrogen*species, “H” represents the harvest.“SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 4-12 Mean vegetative dry matter (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, grown with (N+) and without (N-) nitrogen, harvested from July 2019 to July 2020 at Ashley Dene, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
19/09/19	700	995	1905	1000	450	580	790	565	870 a
29/11/19	330	1230	190	95	90	1345	120	310	465 b
14/05/20	400	475	350	145	330	345	265	350	345 b
17/07/20	180	195	145	90	140	85	60	65	120 c
Mean Yearly N	2130				1475				

	BR	CF	PR	TF	Mean
Mean Yearly S	1310	2630	1910	1360	1805

	P-value	SEM	LSD
S	<0.001	155.3	496.8
N	0.049	143.9	647.7
S*N	0.131	241.2	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “S” is for species, “N” for nitrogen and “N*S” for the interaction nitrogen*species, “H” represents the harvest.“SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

4.3.4 Reproductive Dry Matter (RDM)

Experiment 1 – Ladbrooks

All reproductive dry matter (RDM) was produced in regrowth cycles harvested by the end of Spring until the end of summer in both years (Figure 4-7) with highest levels from brome.

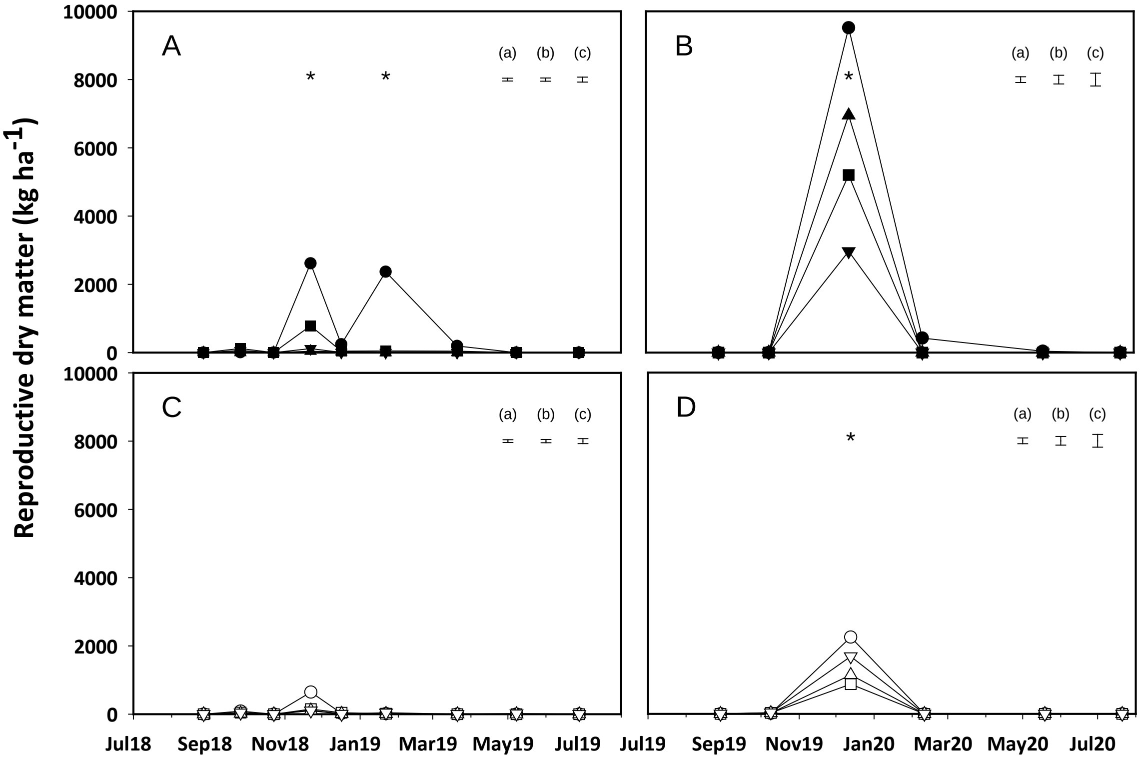


Figure 4-7 Reproductive dry matter yield (kg ha^{-1}) for Ladbrooks from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△), tall fescue(▼,▽) with (closed) or without nitrogen fertiliser (open). Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" shows when differences among species were observed.

In Year 5, brome was the species that produced the most (P<0.001) RDM. The start of flowering stages in those plots was used as one criteria for harvest to minimize the contamination of other plots (Figure 3-6). By the end of this year, brome had an RDM yield that was five times higher than cocksfoot, 30 times higher than perennial ryegrass and 20 times higher than tall fescue. The N+ plots had a higher (P=0.014) RDM production than the N- plots, by about five times or 1340 kg ha ^{1} (Table 4-13). Higher RDM was produced in cycles harvested by late November and late January which influenced those quality results.

In Year 6, all species produced high RDM with 6125 ± 255.6 kg ha^{-1} (P<0.003) for N+ brome, due to this regrowth cycle that allowed plants to reach an advanced reproductive stage (Table 4-15). N+ plots produced four times more (P<0.001) RDM than N- at 6280 and 1510 ± 170 kg ha^{-1}, respectively.

Year 5 (2018/2019)

Table 4-13 Mean reproductive dry matter (kg DM ha^{-1}), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019, grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
27/08/18	0	0	0	0	0	0	0	0	0 d
26/09/18	10	120	0	60	90	45	20	40	50 c
23/10/18	0	0	0	0	0	0	0	0	0 d
22/11/18	2615	790	45	120	645	145	110	100	570 a
17/12/18	245	45	25	5	20	40	10	5	50 c
22/01/19	2370	50	0	5	15	10	10	45	310 b
21/03/19	195	45	0	0	0	0	0	0	30 c
09/05/19	0	0	0	0	0	0	0	20	2 d
28/06/19	0	0	0	0	0	0	0	0	0 d
Mean Yearly N	1685				340				
	BR		CF		PR		TF		Mean
Mean Yearly S	3100		645		105		200		1015

	P-value	SEM	LSD
S	<0.001	92.7	547.2
N	0.014	85.5	834.8
S*N	<0.001	143.1	876.2

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “S” is for species, “N” for nitrogen and “N*S” for the interaction nitrogen*species, “H” represents the harvest. “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Table 4-14 Mean reproductive dry matter (kg DM ha^{-1}), from significant interactions, for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019 from monocultures grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury, New Zealand.

Nitrogen	BR	CF	PR	TF
N+	5435	1045	65	190
N-	765	245	150	205

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue. The P-value for the interaction in <0.001, the standard error of means is 143.1 kg ha^{-1} and the Least Significant Difference at 5% is 876.2 kg ha^{-1}.

Year 6 (2019/2020)

Table 4-15 Mean reproductive dry matter (kg DM ha^{-1}), from four grasses, brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
28/08/19	0	0	0	0	0	0	0	0	0 b
09/10/19	0	0	10	0	15	30	25	35	15 b
13/12/19	9520	5205	6950	2970	2245	870	1140	1680	3830 a
11/02/20	430	0	5	0	0	0	0	0	55 b
19/05/20	40	0	0	0	0	0	0	0	5 b
21/07/20	0	0	0	0	0	0	0	0	0 b
Mean Yearly N	6280				1510				

	BR	CF	PR	TF	Mean
Mean Yearly S	6125	3050	4065	2345	3895

	P-value	SEM	LSD
S	0.003	255.6	1635.3
N	0.002	168.8	1520.1
S*N	0.027	376.1	2218.4

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "S" is for species, "N" for nitrogen and "N*S" for the interaction nitrogen*species, "H" represents the harvest, "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Table 4-16 Mean reproductive dry matter (kg DM ha^{-1}), from significant interactions, for four grasses, brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury, New

Nitrogen	BR	CF	PR	TF
N+	9990	5200	6970	2970
N-	2270	900	1170	1720

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue. The P-value for the interaction in 0.027, the standard error of means is 376.1 kg ha^{-1} and the Least Significant Difference at 5% is 2218.4 kg ha^{-1}.

Experiment 2 – Ashley Dene

In Ashley Dene, for both years, RDM was produced only in regrowth cycles in late Spring and Summer (Figure 4-8). Brome had more ($P<0.001$) RDM than other species in Year 5 (Table 4-17). Nitrogen fertilisation had no ($P=0.059$) effect on RDM yield.

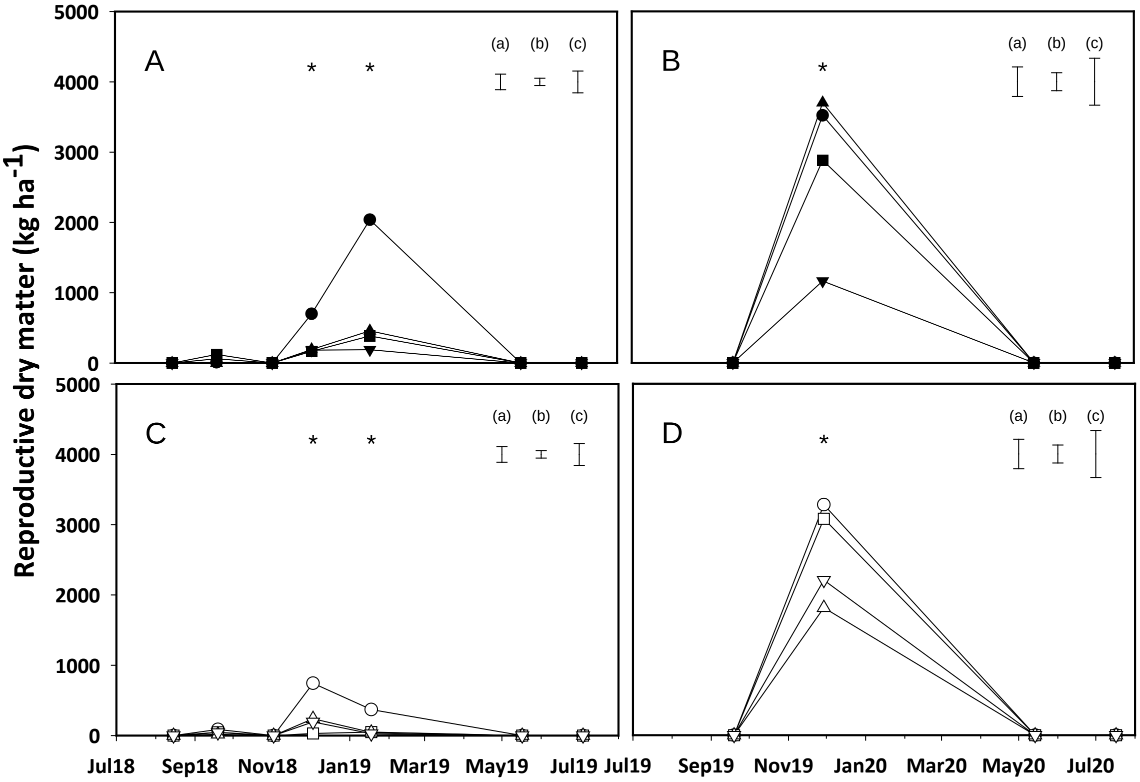


Figure 4-8 Reproductive dry matter yield (kg ha^{-1}) for Ashley Dene from July 2018 to July 19 (A,C) and from July 2019 to July 2020 (B,D), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△), tall fescue(▼,▽) with (closed) or without nitrogen fertiliser (open). Standard Error of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" show when differences were observed.

In Year 6, RDM for brome, cocksfoot and ryegrass averaged 3045 ± 297.1 kg ha^{-1} which was more ($P<0.015$) than the 1685 ± 297.1 kg ha^{-1} of tall fescue. Again nitrogen fertiliser did not ($P=0.447$) affect RDM yield. As expected, the greatest amount of RDM was produced in the late Spring harvest that was deliberately left to grow to an advanced reproductive stage (Table 4-18).

Year 5 (2019/2020)

Table 4-17 Mean reproductive dry matter (kg^{-1} ha), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, grown with (N+) and without (N-) nitrogen, harvested from July 2018 to July 2019 at Ashley Dene, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
15/08/18	0	0	0	0	0	0	0	0	0 c
26/09/18	10	125	0	60	85	45	20	40	50 c
2/11/18	0	0	0	0	0	0	0	0	0 c
3/12/18	700	170	195	185	745	30	240	195	305 b
18/01/19	2040	390	460	190	370	50	45	25	445 a
17/05/19	0	0	0	0	0	0	0	0	0 c
04/07/19	0	0	0	0	0	0	0	0	0 c
Mean Yearly N		1130				470			
	BR		CF		PR		TF		Mean
Mean Yearly S	1975		400		475		350		805

	P-value	SEM	LSD
S	<0.001	73.6	235.3
N	0.059	155.9	-
S*N	0.114	218.3	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “S” is for species, “N” for nitrogen and “N*S” for the interaction nitrogen*species, “H” represents the harvest, “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 4-18 Mean reproductive dry matter (kg^{-1} ha), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, grown with (N+) and without (N-) nitrogen, harvested from July 2019 to July 2020 at Ashley Dene, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
19/09/19	0	0	0	0	0	0	0	0	0 b
29/11/19	3520	2885	3700	1165	3280	3080	1815	2205	2705 a
14/05/20	0	0	0	0	0	0	0	0	0 b
17/07/20	0	0	0	0	0	0	0	0	0 b
Mean Yearly N	2820				2595				
	BR		CF		PR		TF		Mean
Mean Yearly S	3400		2980		2755		1685		2710

	P-value	SEM	LSD
S	0.015	297.1	950.6
N	0.447	180.6	-
S*N	0.207	472.0	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “S” is for species, “N” for nitrogen and “N*S” for the interaction nitrogen*species, “H” represents the harvest, “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

4.3.5 Weed Fraction (WF)

Experiment 1 – Ladbrooks

Figure 4-9 shows that the weed fraction was minimal in Year 5 and most of Year 6 in the fertilised plots. In Year 5 at Ladbrooks, weed content never reached >10% and there was no difference (P=0.469) among species (Table 4-19) or nitrogen treatments (P=0.139).

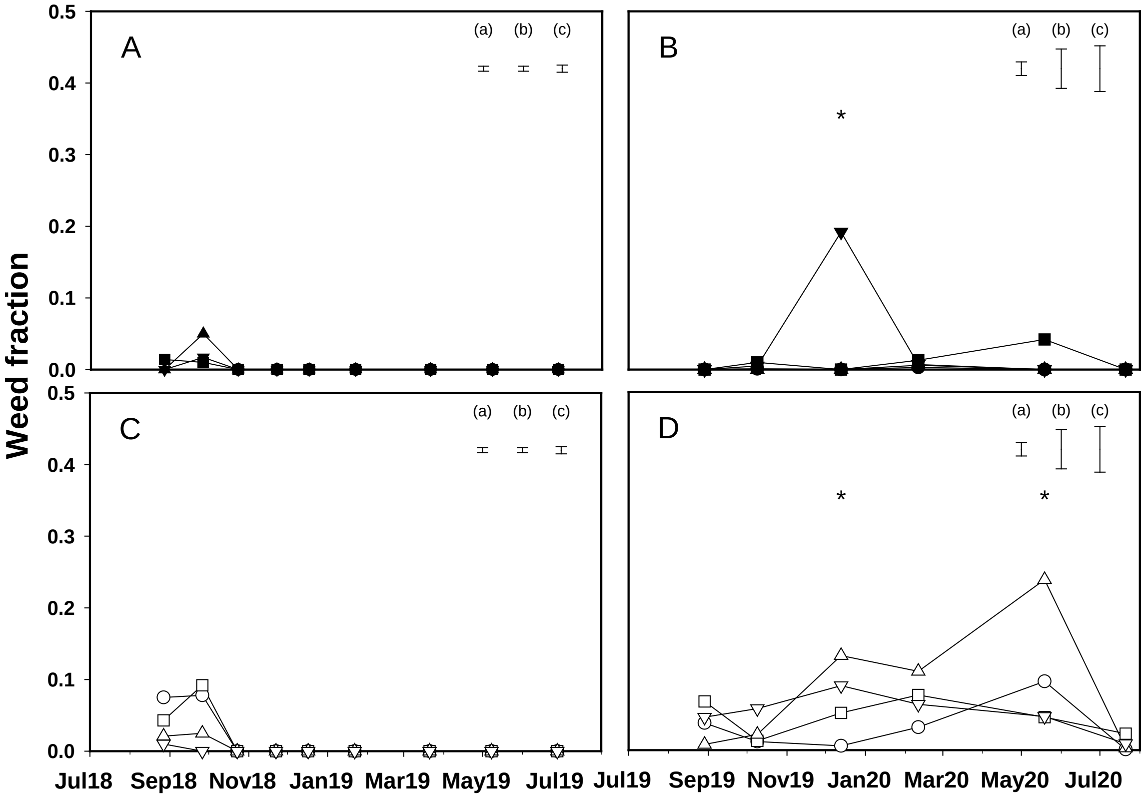


Figure 4-9 Weed fraction for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△) and tall fescue(▼,▽) harvested from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) at Ladbrooks, Canterbury, per harvest, with (closed) or without nitrogen fertilisation (open).Standard errors of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" indicate when differences were observed for species.

In Year 6, no species differences (P=0.428) were found (Table 4-20), but the weed proportion in the N+ plots was lower (P=0.026) than the N- plots.

Year 5 (2018/2019)

Table 4-19 Mean weed fraction, from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019 from monocultures grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
27/08/18	0.00	0.01	0.00	0.00	0.08	0.04	0.02	0.01	0.02 ab
26/09/18	0.00	0.01	0.05	0.02	0.08	0.09	0.03	0.00	0.03 a
23/10/18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 c
22/11/18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00 c
17/12/18	0.00	0.00	0.00	0.00	0.01	0.02	0.02	0.07	0.01 b
22/01/19	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.06	0.01 b
21/03/19	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.03	0.01 b
09/05/19	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.01	0.01 b
28/06/19	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.03	0.01 b
Mean Yearly N	0.00				0.02				

	BR	CF	PR	TF	Mean
Mean Yearly S	0.01	0.01	0.01	0.01	0.01

	P-value	SEM	LSD
S	0.469	0.007	-
N	0.139	0.007	-
S*N	0.277	0.010	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “S” is for species, “N” for nitrogen and “N*S” for the interaction nitrogen*species, “H” represents the harvest, “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. P-Values, SEM and LSD are shown for data transformed used ArcSin. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 4-20 Mean weed fraction, from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
28/08/19	0.00	0.00	0.00	0.00	0.04	0.07	0.01	0.05	0.02 bc
09/10/19	0.00	0.01	0.00	0.00	0.01	0.01	0.02	0.06	0.02 bc
13/12/19	0.00	0.00	0.00	0.19	0.01	0.05	0.13	0.09	0.06 a
11/02/20	0.00	0.01	0.01	0.01	0.03	0.08	0.11	0.06	0.04 ab
19/05/20	0.00	0.04	0.00	0.00	0.10	0.05	0.24	0.05	0.06 a
21/07/20	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.00 c
Mean Yearly N	0.01				0.05				

	BR	CF	PR	TF	Mean
Mean Yearly S	0.02	0.03	0.04	0.04	0.03

	P-value	SEM	LSD
S	0.428	0.055	-
N	0.026	0.019	0.084
S*N	0.093	0.064	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "S" is for species, "N" for nitrogen and "N*S" for the interaction nitrogen*species, "H" represents the harvest, "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. P-Values, SEM and LSD are shown for data transformed used ArcSin. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Experiment 2 - Ashley Dene

Weed ingress in Ashley Dene was much higher than in Ladbrooks, sometimes reaching 60% of all TDM production, such as in the harvest in July 2020 (Figure 4-10). The profile of weed fraction show that weed were always present in significant amounts except for the harvest at the beginning of November 2018.

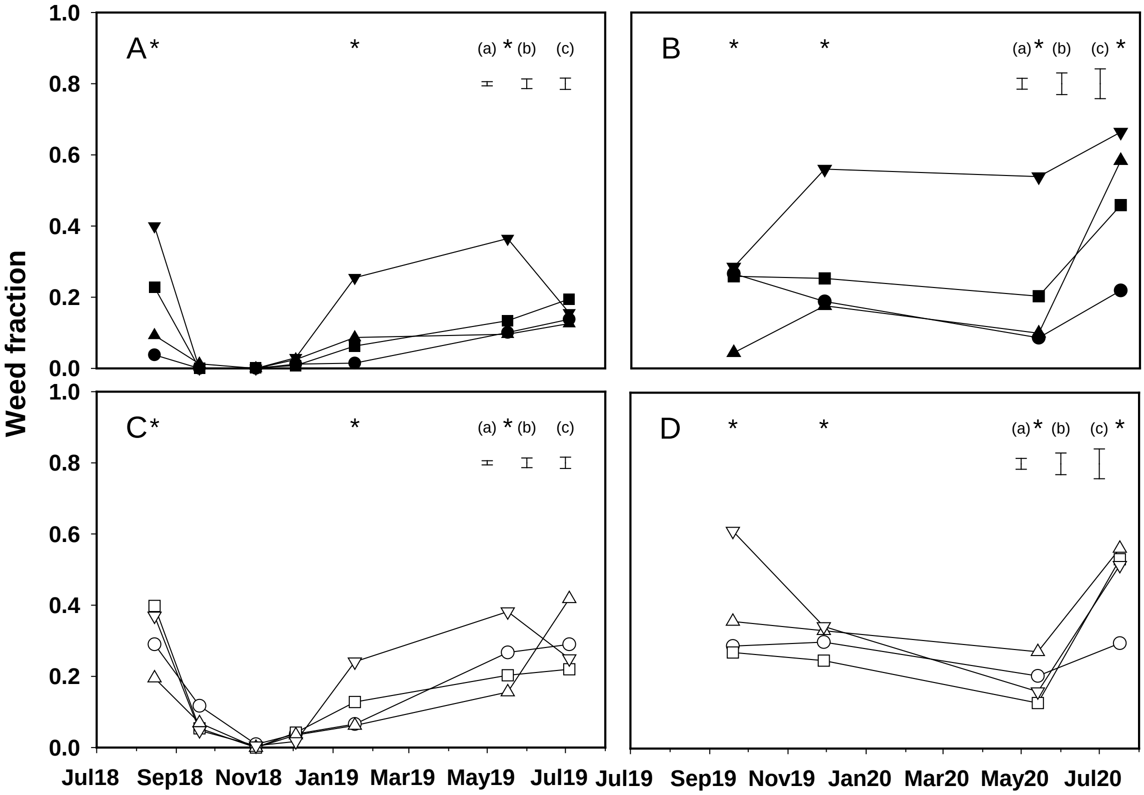


Figure 4-10 Weed fraction for brome(●,○), cocksfoot (■,□), p.ryegrass(▲,△) and tall fescue(▼,▽) harvested from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) at Ashley Dene, per harvest, for all the species with (closed) or without nitrogen fertiliser (open). Standard error of means are represented for (a) nitrogen (b) species and (c) nitrogen*species. "*" indicate when differences among species were observed.

In Year 5, Weed Fraction (WF) differed (P=0.337) for nitrogen levels (Table 4-21) and for the different harvests (P<0.001). Nitrogen fertilised plots had, an average of 45% less weed mass than the fertilised plots.

In Year 6, WF was the highest for tall fescue (P=0.030) and not different amongst other species (Table 4-22). Tall fescue had, on average, at least a 40% higher WF than the other species.

WF amount differed (P<0.001) across harvests. The last harvest had the highest weed fraction and the most present weeds here were the annual grasses, *Poa annua* L. and barley grass (*Critesion* spp.). The third harvest had the lowest weed proportion. No difference (P=0.337) was observed for different nitrogen levels in WF in this year.

Year 5 (2018/2019)

Table 4-21 Mean weed fraction, from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen fertilisation, harvested from July 2018 to July 2019 at Ashley Dene, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
15/8/18	0.04	0.23	0.09	0.40	0.29	0.40	0.19	0.37	0.25 a
19/9/18	0.00	0.00	0.01	0.00	0.12	0.05	0.07	0.05	0.04 bc
2/11/18	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00 c
3/12/18	0.01	0.01	0.03	0.03	0.04	0.04	0.03	0.02	0.03 c
18/01/19	0.01	0.06	0.09	0.25	0.07	0.13	0.06	0.24	0.11 b
17/05/19	0.10	0.13	0.10	0.36	0.27	0.20	0.16	0.38	0.21 a
04/07/19	0.14	0.19	0.13	0.16	0.29	0.22	0.42	0.25	0.22 a
Mean Yearly N	0.09				0.16				
	BR		CF		PR		TF		Mean
Mean Yearly S	0.10		0.12		0.10		0.18		0.13

	P-value	SEM	LSD
S	0.250	0.028	-
N	0.034	0.012	0.052
S*N	0.270	0.032	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “S” is for species, “N” for nitrogen and “N*S” for the interaction nitrogen*species, “H” represents the harvest, “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. P-Values, SEM and LSD are shown for data transformed used ArcSin. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 4-22 Mean weed fraction, from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen fertilisation, harvested from July 2019 to July 2020 at Ashley Dene, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
19/09/19	0.27	0.26	0.04	0.28	0.29	0.27	0.36	0.61	0.31 b
29/11/19	0.19	0.25	0.18	0.56	0.30	0.25	0.33	0.34	0.30 b
14/05/20	0.09	0.20	0.10	0.54	0.20	0.13	0.27	0.16	0.21 c
17/07/20	0.22	0.46	0.58	0.66	0.30	0.53	0.56	0.52	0.51 a
Mean Yearly N		0.31				0.34			

	BR	CF	PR	TF	Mean
Mean Yearly S	0.23	0.30	0.30	0.46	0.33

	P-value	SEM	LSD
S	0.030	0.043	0.138
N	0.337	0.022	-
S*N	0.170	0.059	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "S" is for species, "N" for nitrogen and "N*S" for the interaction nitrogen*species, "H" represents the harvest, "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. P-Values, SEM and LSD are shown for data transformed used ArcSin. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

4.3.6 Nitrogen Recovery (NR)

As described in Section 3.2.9.4, only material harvested from 23/10/18, for Ladbrooks and Ashley Dene were analysed for NR and quality traits.

Experiment 1 – Ladbrooks

Nitrogen yields from each harvest, used to calculate NR, in this year are shown in Figure 4-11 and Figure 4-12. The nitrogen yield was determined as shown in Equation 5 in Section 3.2.9.5.

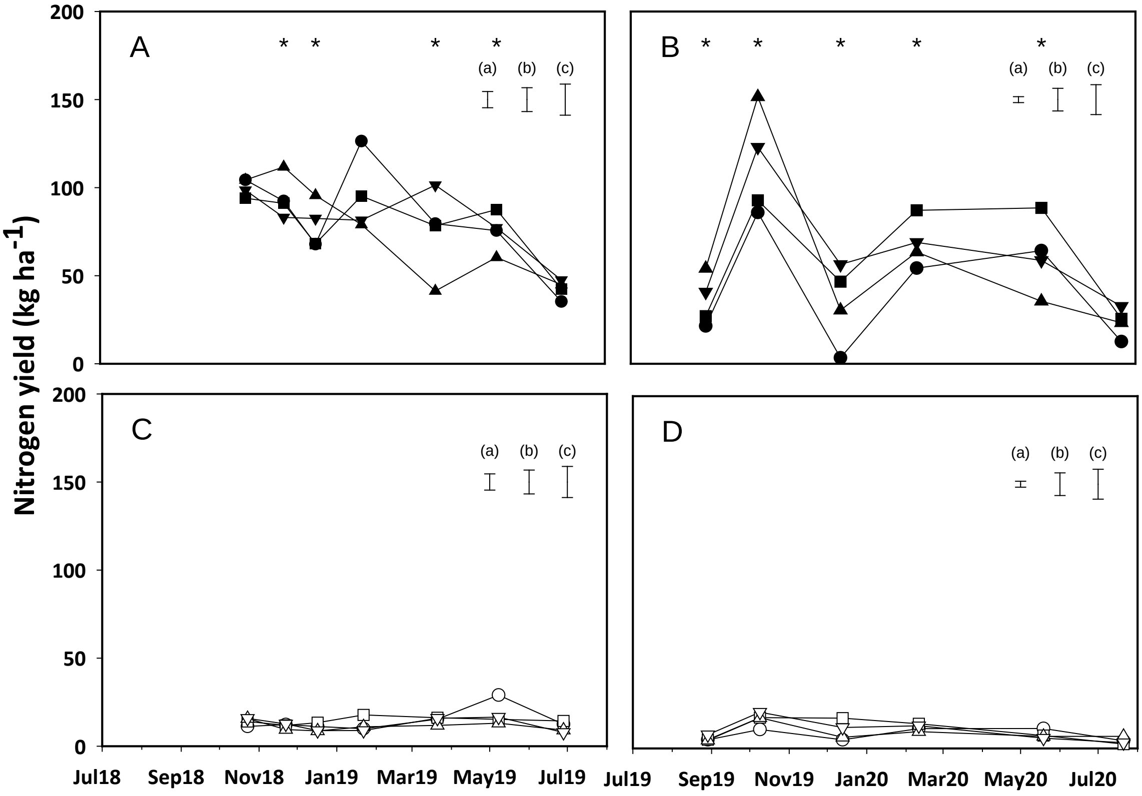


Figure 4-11: Nitrogen yield (kg ha^{-1}) for brome(●,○), cocksfoot (■,□), p.ryegrass(▲,△) and tall fescue(▼,▽) harvested from July 2018 to July 2019 (A,C) and from July 2019 July 2020 (B,D) at Ladbrooks, per harvest, with (closed) or without nitrogen fertilisation (open). .Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represents when differences among species were observed.

In Year 5, the NR did not differ (P=0.070) amongst species (Table 4-23). However, through the year, the amount of nitrogen recovered through the harvests differed (P=0.011). The regrowth cycles harvested on 23/10/18 and on 22/01/19 had the highest mean NR of 0.88 ± 0.040 kg N/kg N. The regrowth cycle harvested on 22/01/19 did not differ from all other regrowth cycles with a mean of 0.74 ± 0.040 kg N kg N^{-1}.

In the Year 6, the NR differed (P= 0.029) amongst species. All species recovered less nitrogen than perennial ryegrass, but cocksfoot, brome and tall fescue did not differ from each other (Table 4-24).

The differences between ryegrass and the other species occurred mostly in the three first harvests, when soil water was available. In the drier periods, some species showed higher NR than ryegrass (Table 4-24).

The NR was highest in the first regrowth cycle (P<0.001). This regrowth cycle received no fertiliser and plants relied on the nitrogen in the soil, or from internal tissues to recycle for from other regrowth cycles. The results from this harvest showed that the previously fertilised plots contained an average of 7.43 times more nitrogen (5.56 ± 3.25 kg N ha^{-1} and 35.85 ± 3.25 kg N ha^{-1} on the N- and N+ plots respectively) than the non-fertilised pastures.

Year 5 (2018/2019)

Table 4-23 Mean nitrogen recovery (NR) (kg N kg N^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2018 to July 2019 at Ladbrooks, Canterbury.

	Species				
Harvest	BR	CF	PR	TF	Mean H
23/10/18	1.02	0.89	0.98	0.92	0.95 a
22/11/18	0.41	0.71	1.11	0.78	0.75 b
17/12/18	0.61	0.65	0.95	0.82	0.76 b
22/01/19	0.82	0.86	0.78	0.82	0.82 ab
21/03/19	0.70	0.72	0.40	0.94	0.69 b
09/05/19	0.63	0.82	0.58	0.72	0.69 b
28/06/19	0.61	0.71	0.82	0.89	0.76 b
Mean	0.69	0.77	0.80	0.84	0.78

	P-value	SEM	LSD
S	0.070	0.037	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “H” represents the harvest, “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 4-24 Mean nitrogen recovery (NR) (kg N kg N^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2019 to July 2020 at Ladbrooks, Canterbury.

	Species				
Harvest	BR	CF	PR	TF	Mean
28/08/19	4.36	5.55	13.0	6.80	7.43 a
09/10/19	0.84	0.85	1.40	1.10	1.05 b
13/12/19	0.94	1.11	1.76	0.84	1.16 b
11/02/20	0.54	0.83	0.63	0.66	0.67 b
19/05/20	0.63	0.91	0.36	0.61	0.63 b
21/07/20	0.25	0.53	0.45	0.67	0.47 b
Mean	1.26	1.63	2.93	1.78	1.90

	P-value	SEM	LSD
S	0.029	0.329	1.050

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "H" represents the harvest , "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Experiment 2 – Ashley Dene

Figure 4-12shows that nitrogen yield was related to the VDM yield through the year. On December 2020, N yield was low because of the low VDM values and high RDM values, which contain less nitrogen per kilogram of DM.

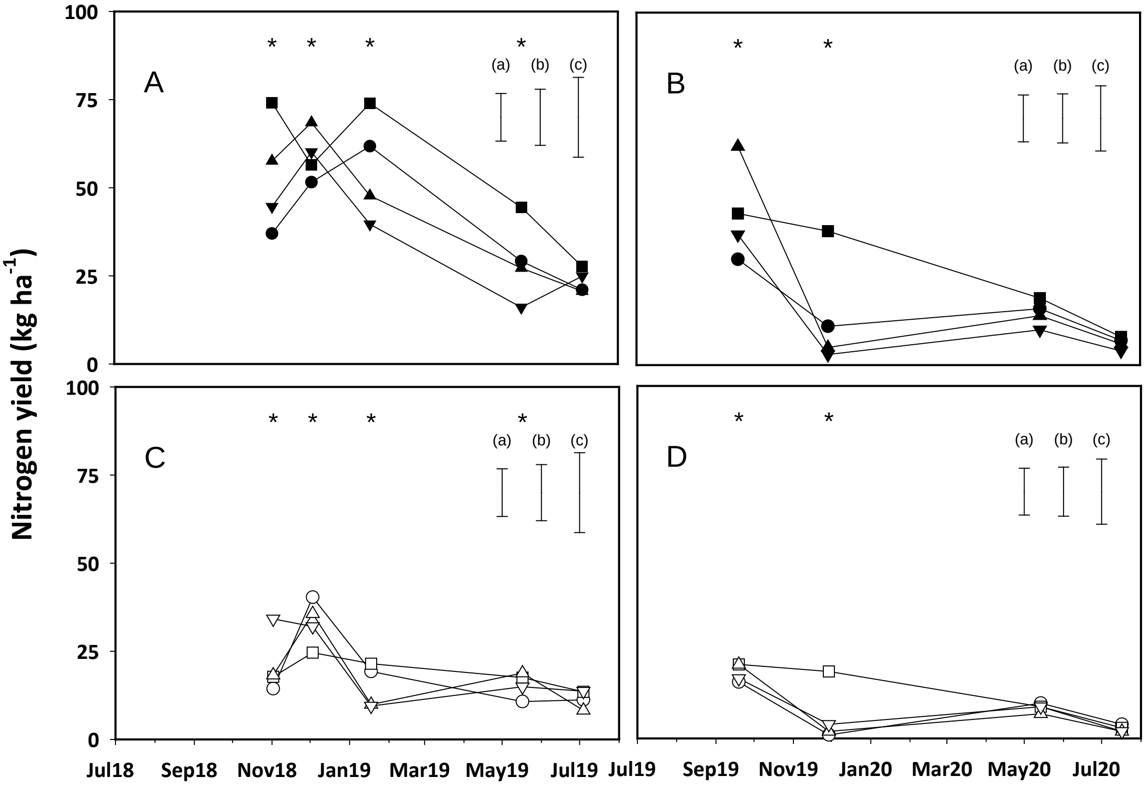


Figure 4-12 Nitrogen yield (kg ha^{-1}) for Ashley Dene, for brome(●,○), cocksfoot (■,□), p.ryegrass(▲,△) and tall fescue(▼,▽) with (closed) or without nitrogen fertilisation (open), from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D). Standard Error of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

For Year 5, NR was highest (P = 0.012) from cocksfoot (Table 4-25), 0.59 ± 0.032 kg N kg N^{-1}. Brome, p. ryegrass and tall fescue were lower at 0.43 ± 0.033 kg N/kg N. The NR from last two harvests (17/05/2019 and 04/07/2019) was at least 30% lower (P=0.002) than the other harvests.

Unlike Year 5, in Year 6 NR was not different (P=0.492) for species, but differed with regrowth cycles (Table 4-26). The first regrowth cycle of this year (19/09/2019) had no nitrogen fertiliser applied, but plants in the N+ plots yielded more dry matter. In the last two harvests (14/05/2020 and 17/07/2020), NR was only 0.16 and 0.14 ± 0.319 kg N/kg N, 25% or less than the harvest on 29/11/2019.

Year 5 (2018/2019)

Table 4-25 Mean nitrogen recovery (kg N kg N^{-1})for brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2018 to July 2019 in Ashley Dene, Canterbury, New Zealand.

	Species				
Harvest	BR	CF	PR	TF	Mean
2/11/2018	0.39	0.75	0.59	0.40	0.53 a
3/12/2018	0.42	0.53	0.59	0.53	0.52 a
18/01/2019	0.61	0.71	0.52	0.43	0.57 a
17/05/2019	0.31	0.44	0.27	0.17	0.30 b
04/07/2019	0.40	0.51	0.42	0.45	0.45 a
Mean	0.43	0.59	0.48	0.40	0.48

	P-value	SEM	LSD
S	0.012	0.032	0.105

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "H" represents the harvest, "S" represents species "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2018/2019)

Table 4-26 Mean nitrogen recovery (kg N kg N^{-1}) brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2019 to July 2020 in Ashley Dene, Canterbury, New Zealand.

Harvest	BR	CF	PR	TF	Mean
19/09/2019	2.18	2.00	3.50	5.18	3.22 a
29/11/2019	0.66	0.82	0.82	0.26	0.64 b
14/05/2020	0.18	0.22	0.16	0.11	0.16 b
17/07/2020	0.16	0.19	0.13	0.09	0.14 b
Mean	0.79	0.81	1.15	1.41	1.04

	P-value	SEM	LSD
S	0.492	0.319	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "H" represents the harvest. "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

4.3.7 Protein yield (PY)

The protein content (% of dry matter) for Ladbrooks, from July 2018 until July 2020 is shown at Appendix B. In this section the product of dry matter yield and crude protein content is used to compare protein yield across treatments and time.

Experiment 1 – Ladbrooks

The amount of protein produced for non-fertilised plants, was almost constant through Years 5 and 6. The only difference was among species (P<0.001) in the harvest of May 2019 (Figure 4-13), where brome had a higher protein yield than the other grasses.

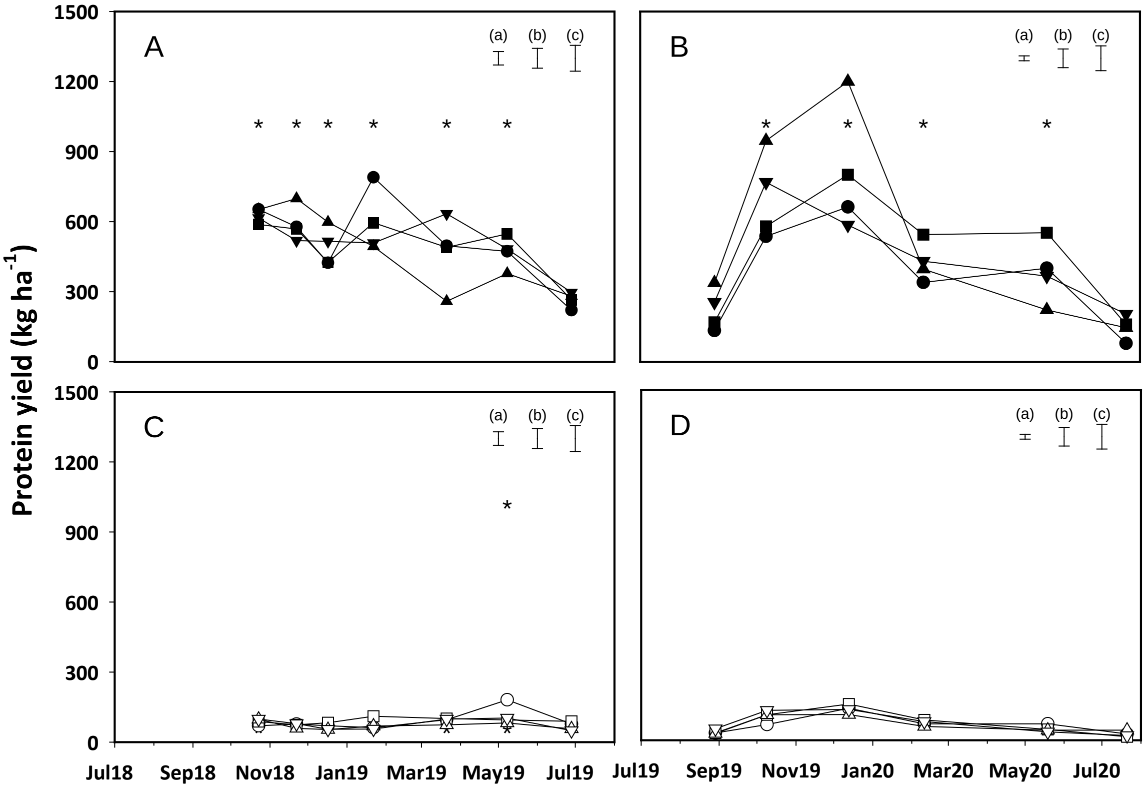


Figure 4-13 Protein yield (kg ha^{-1}) for Ladbrooks, from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) for cocksfoot (■,□), tall fescue(▼,▽) brome(●,○), p.ryegrass(▲,△) with (closed) or without nitrogen fertilisation (open). Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represents when differences among species were observed.

For Year 5, protein content was not different ($P=0.416$) among species (Table 4-27). Nitrogen fertiliser increased the protein content ($P<0.001$) by ~3500 kg ha^{-1} in this year compared with the non-fertilised plots. The last regrowth cycle harvested on 28/06/19 had a lower protein yield than the other regrowth cycles.

In Year 6, protein yields of ryegrass and cocksfoot were higher ($P<0.007$) at 1700 ± 80 kg ha^{-1} than brome at 1280 ± 80 kg ha^{-1}. For the harvest on 13/12/19, the protein content of all species was mainly in their reproductive structures due to the high amount of RDM (Figure 4-7) compared with VDM (Figure 4-3). Though the TDM yield in this cycle was much higher than in all other regrowth cycles, it did not necessarily reflect a huge increase in the protein yield, for reproductive material had a lower crude protein than vegetative materials.

This can be observed from increases in protein yield from regrowth cycles harvested on 09/10/19 and 13/12/19, whilst TDM increase was high (Figure 4-1), the protein increases were minimal (Table 4.28).

Nitrogen fertiliser increased the protein content ($P<0.001$) by ~2300 kg ha^{-1}. This year, as in Year 5, longer regrowth cycles with warmer temperatures had a higher ($P<0.001$) protein yield than the others.

Year 5 (2018/2019)

Table 4-27 Mean protein yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019 from monocultures grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
23/10/18	655	590	650	615	70	90	100	100	360 a
22/11/18	580	570	700	520	75	75	60	80	305 bc
17/12/18	425	425	600	515	70	80	55	55	275 c
22/01/19	790	595	495	510	60	110	70	55	320 ab
21/03/19	485	490	260	635	95	100	75	100	280 bc
09/05/19	475	550	380	485	180	95	80	105	295 bc
28/06/19	220	265	280	295	80	90	60	50	165 d
Mean Yearly N	3515				575				
	BR		CF		PR		TF		Mean
Mean Yearly S	2135		2060		1925		2055		2015

	P-value	SEM	LSD
S	0.416	84.7	-
N	<0.001	57.4	258.5
S*N	0.594	110.6	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “N” represents nitrogen, “S” represents species, “N*S” the interaction among nitrogen and species, “H” represents the harvest. “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 4-28 Mean protein yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
28/08/19	135	170	340	255	30	30	30	50	130 d
09/10/19	535	580	945	770	65	110	110	130	405 b
13/12/19	665	800	1200	585	135	155	110	130	475 a
11/02/20	340	545	400	430	70	85	60	80	250 c
19/05/20	400	555	220	370	70	45	45	35	215 c
21/07/20	80	160	145	205	25	15	45	20	90 d
Mean Yearly N	2705				420				
	BR		CF		PR		TF		Mean
Mean Yearly S	1280		1625		1820		1530		1565

	P-value	SEM	LSD
S	0.007	80.1	256.4
N	<0.001	21.0	94.6
S*N	0.016	106.1	314.5

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “N” represents nitrogen, “S” represents species, “N*S” the interaction among nitrogen and species, “H” represents the harvest. “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Experiment 2 – Ashley Dene

The protein content (% of dry matter) for Ashley Dene, from July 2018 until July 2020 is shown at Appendix C.

In Year 5, the total protein yield was 990 ± 81 kg ha^{-1} which was not different (P=0.106) among species. Nitrogen fertiliser increased (P=0.003) protein yield by more than double (Figure 4-14), from 605 to 1380 ± 0.06 kg ha^{-1} (Table 4-29).

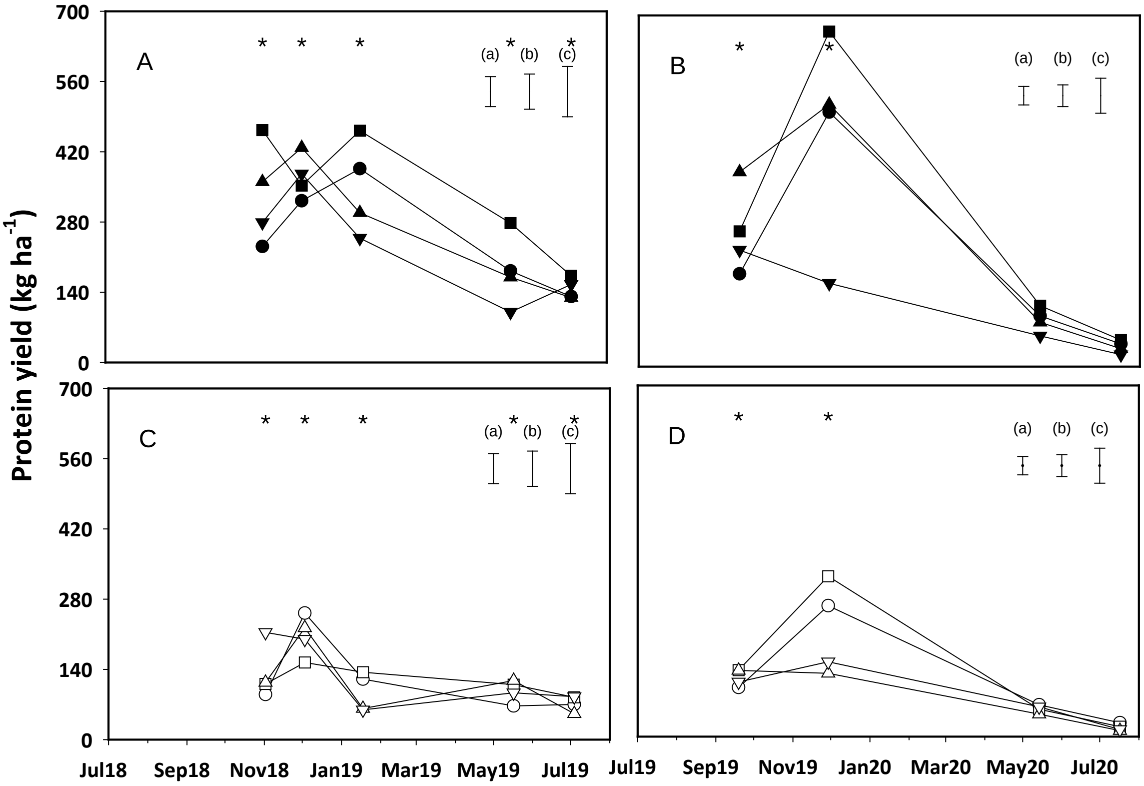


Figure 4-14 Protein yield (kg ha^{-1}) at Ashley Dene from July 2019 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), per harvest, for brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽),with (closed) or without nitrogen fertilisation (open). Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

The protein yield also differed among harvests (P<0.001). The crude protein yield was highest when more SSDM was produced in the first three regrowth cycles.

Nitrogen fertiliser increased protein yield in all species, but more for cocksfoot (P=0.015), with an increase of ~1140 ± 100 kg ha^{-1}. The increase in protein yield for the other species was never higher than 800 ± 100 kg ha^{-1}.

In Year 6, the protein yield was the highest (P<0.001) for cocksfoot, with its yield double that of tall fescue (Table 4-31). Perennial ryegrass and brome did not differ, with a mean of

660 ± 21.7 kg ha^{-1}. Nitrogen fertiliser more than doubled (P=0.003) protein yield from 405 to 870 ± 18.4 kg ha^{-1}.

Year 5 (2018/2019)

Table 4-29 Mean protein yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) and without (N-) nitrogen fertiliser, harvested from July 2018 to July 2019 at Ashley Dene, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
2/11/18	230	465	360	280	90	110	115	215	235 b
3/12/18	320	355	430	375	250	155	225	215	290 a
18/01/19	385	465	300	250	120	135	60	60	220 b
17/05/19	180	280	170	100	65	110	120	95	140 c
04/07/19	130	175	130	155	70	85	50	85	110 c
Mean Yearly N	1380				605				
	BR		CF		PR		TF		Mean
Mean Yearly S	930		1160		980		910		995
	P-value		SEM		LSD				
S	0.106		70.3		-				
N	0.003		59.8		269.1				
S*N	0.015		100.1		303.7				

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue. "S" is for species, "N" is for nitrogen and "N*S" for the interaction nitrogen*species, "H" represents the harvest. "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Table 4-30 Mean protein yield (kg ha^{-1}), for significant interactions, from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) and without (N-) nitrogen fertilisation, harvested from July 2018 to July 2019 at Ashley Dene, Canterbury, New Zealand.

	Species			
Nitrogen	BR	CF	PR	TF
N+	1255	1730	1385	1160
N-	600	595	570	650

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue. The P-value for the interaction Species*Nitrogen is 0.015, the standard error of means is 100.1 kg ha^{-1} and the Least Significant Difference at 5%. is 303.7 kg ha^{-1}.

Year 6 (2019/2020)

Table 4-31 Mean protein yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) and without (N-) nitrogen fertilisation, harvested from July 2019 to July 2020 at Ashley Dene, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
19/09/19	185	270	390	230	100	130	130	110	195 b
29/11/19	510	670	525	165	260	320	125	150	340 a
14/05/20	100	120	90	60	65	55	45	60	75 c
17/07/20	45	55	35	25	30	20	10	15	30 c
Mean Yearly N	870				405				

	BR	CF	PR	TF	Mean
Mean Yearly S	645	820	675	410	640

	P-value	SEM	LSD
S	<0.001	21.7	139.2
N	0.003	18.4	165.6
S*N	0.030	34.85	207.0

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "N" represents nitrogen, "S" represents species, "N*S" the interaction among nitrogen and species, "H" represents the harvest. "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

4.3.8 Metabolisable Energy (ME)

The metabolisable energy (MJ kg^{-1} of dry matter) for Ladbrooks, from July 2018 until July 2020 is shown at Appendix D. The analysis here is of the product of DM and ME at each harvest to quantify total ME yield from each treatment.

Experiment 1 – Ladbrooks

The amount of ME (GJ/ha) produced in each regrowth cycle was low and constant, for non-fertilised grasses, in Year 5 (Figure 4-15) but higher in Year 6, due to the TDM accumulation in December 2019. No difference was observed for species in the N- treatment.

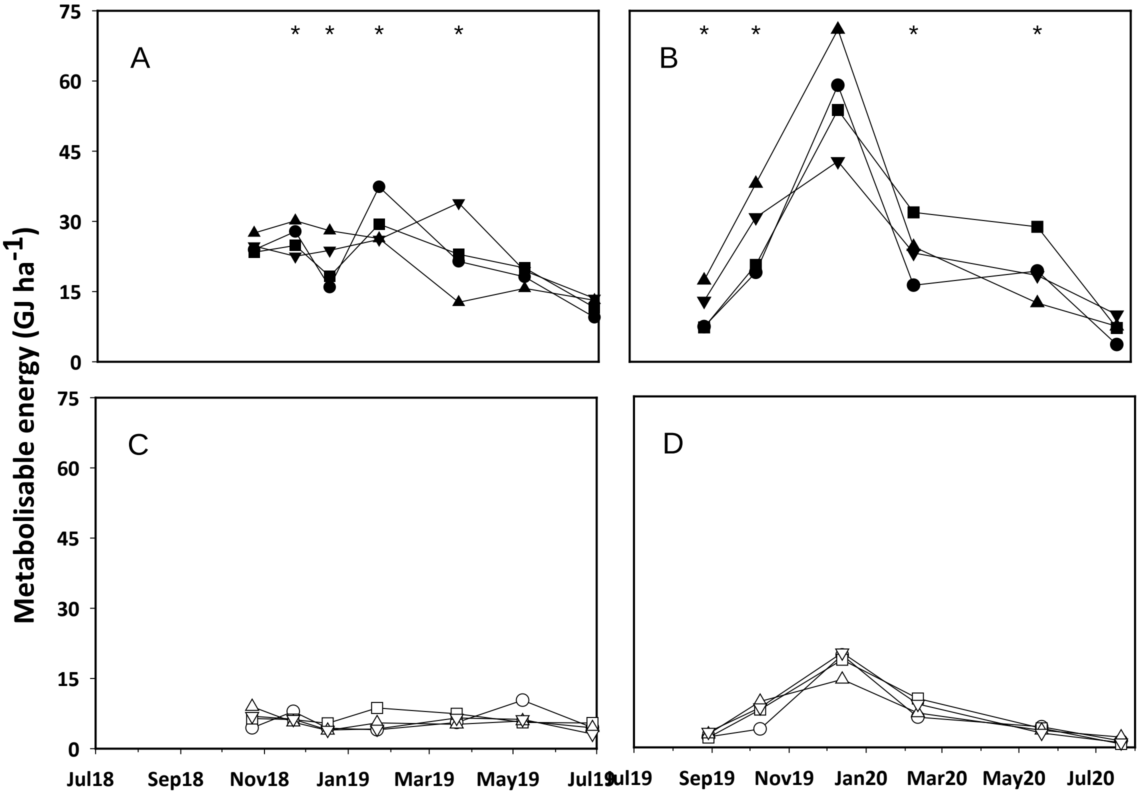


Figure 4-15 Mean metabolisable energy (GJ ha^{-1}), for Ladbrooks from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D), for all regrowth cycles, for brome(●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽), with (closed) or without nitrogen fertilisation (open). Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represents when differences among species were observed.

In Year 5, no difference (P=0.918) was observed among species (Table 4-32), with a total ME yield of 98.2 ± 10.50 GJ ha^{-1}. Nitrogen increased (P<0.001) the metabolisable energy by ~115 ± 0.18 GJ ha^{-1}.

In Year 6, ryegrass and cocksfoot had the highest ME yield at 101 ± 13.6 GJ ha^{-1} and brome and tall fescue the lowest, 87.7 GJ ha^{-1}. N+ plots had an increase (P<0.001) of ~105 GJ ha^{-1} (Table 4-33). Different regrowth cycles had different (P<0.001) ME yields. The mid-December harvest had the highest value, 37.5 ± 0.64 GJ ha^{-1}, or about double from the second regrowth cycle this year.

Year 5 (2018/2019)

Table 4-32 Mean metabolisable energy (GJ ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019 from monocultures grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
23/10/18	23.9	23.4	27.5	24.7	4.4	6.5	9.0	6.9	15.8 ab
22/11/18	27.8	24.9	30.1	22.6	7.9	6.2	5.6	6.3	16.4 ab
17/12/18	15.9	18.2	28.0	23.8	4.4	5.4	3.9	4.0	13.0 c
22/01/19	37.4	29.4	26.4	26.2	4.0	8.7	5.5	4.2	17.7 a
21/03/19	21.5	23.0	12.7	34.0	5.6	7.5	5.2	6.5	14.5 bc
09/05/19	18.2	20.1	15.7	19.4	10.3	5.6	5.9	6.3	12.7 c
28/06/19	9.5	11.6	13.1	13.6	4.5	5.5	4.4	3.1	8.2 d
Mean Yearly N	155.7				40.8				

	BR	CF	PR	TF	Mean
Mean Yearly S	97.7	98.0	96.6	100.8	98.3

	P-value	SEM	LSD
S	0.918	4.43	-
N	<0.001	3.84	17.28
S*N	0.118	6.18	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue,”S” represents species, “N” nitrogen and “S*N” the interaction nitrogen*species, “H” represents the harvest. “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 4-33 Mean metabolisable energy yield (GJ ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
28/08/19	7.5	7.4	17.4	13.0	2.2	2.1	2.8	3.3	7.0 d
09/10/19	19.1	20.7	38.1	30.9	3.9	8.1	9.8	8.5	17.4 b
13/12/19	59.1	53.8	71.0	42.9	19.7	18.7	14.5	20.2	37.5 a
11/02/20	16.4	31.9	24.6	23.3	6.4	10.4	7.3	9.2	16.2 b
19/05/20	19.4	28.9	12.6	18.5	4.4	4.1	3.6	3.1	11.8 c
21/07/20	3.7	7.2	7.6	10.1	1.4	0.7	2.2	1.0	4.2 d
Mean Yearly N	146.2				41.9				

	BR	CF	PR	TF	Mean
Mean Yearly S	81.6	97.0	105.8	91.9	94.1

	P-value	SEM	LSD
S	0.019	4.25	13.61
N	<0.001	1.63	7.34
S*N	0.051	5.75	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue. "S" represents species, "N" represents nitrogen and "S*N" represents the interaction nitrogen*species, "H" represents the harvest. "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Experiment 2 – Ashley Dene

The metabolisable energy (MJ kg^{-1} of dry matter) for Ashley Dene, from July 2018 until July 2020 is shown at Appendix E.

In Year 5, no differences (P=0.095) were found among species for total ME produced (Table 4-34) and they yielded 52.1 ± 2.81 GJ ha^{-1}. Nitrogen increased the ME yield from 40.5 to 63.8 ± 2.48 GJ ha^{-1}. Differences (P<0.001) were observed among the regrowth cycles in Year 5 (Figure 4-16). The last regrowth cycles produced at least 50% less ME than the other three cycles.

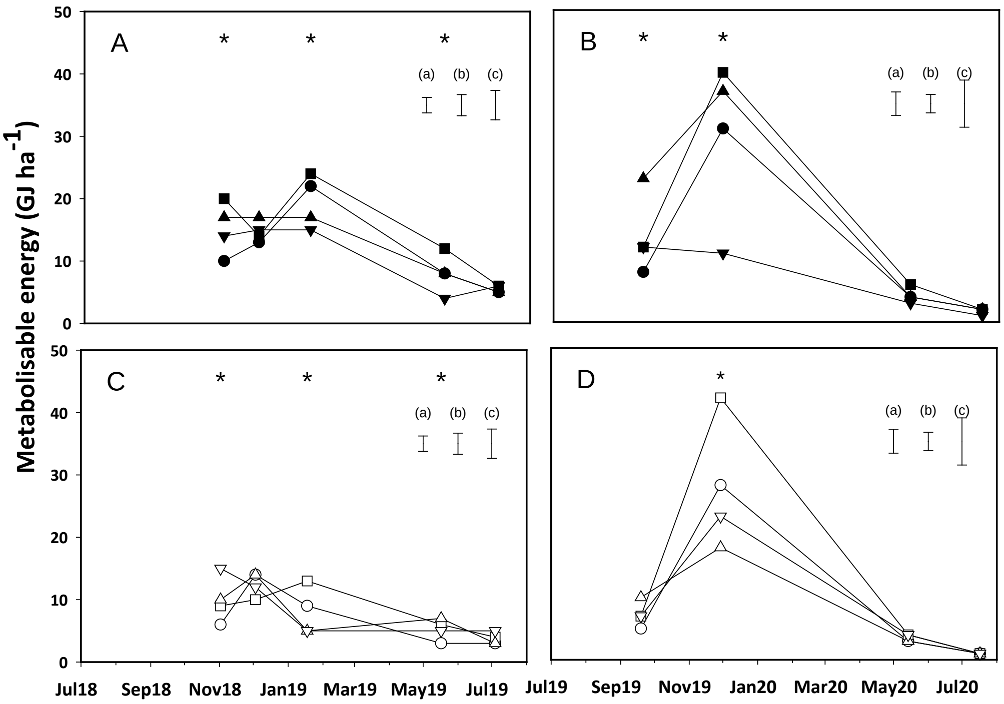


Figure 4-16 Mean metabolisable energy (GJ ha^{-1}), for each harvest of brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽), from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) at Ashley Dene, with (closed) or without nitrogen fertilisation (open). Standard Errors of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represents when differences among species were observed.

For Year 6, p. ryegrass and cocksfoot had the highest mean ($P < 0.001$) ME yield at 52.8 ± 2.96 GJ ha^{-1}, and brome and tall fescue the lowest one, 36 ± 2.96 GJ ha^{-1} (Table 4-35). The harvests from regrowth cycles in Winter had higher ME values ($P<0.001$) than the mature late Spring harvest. As for Ladbrooks, the harvest with the advanced reproductive stage had the highest ($P<0.001$) total ME yield.

Year 5 (2018/2019)

Table 4-34 Mean metabolisable energy (GJ ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2018 to July 2019 from monocultures grown with (N+) and without (N-) nitrogen at Ashley Dene, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean
2/11/18	10.0	19.9	17.4	14.2	6.4	9.0	10.5	14.7	12.7 a
3/12/18	13.0	14.4	17.4	15.0	14.0	10.1	14.2	12.2	13.8 a
18/01/19	22.1	24.0	17.3	14.6	9.1	13.5	5.4	5.3	13.9 a
17/05/19	8.5	12.4	7.6	4.3	3.5	6.3	6.7	5.2	6.8 b
04/07/19	5.2	6.5	5.2	6.4	3.4	4.1	3.3	4.9	4.9 b
Mean Yearly	63.8				40.5				
	BR		CF		PR		TF		
Mean Yearly	47.6		60.1		52.6		48.4		
Yearly Mean	52.2								

	P-value	SEM	LSD
S	0.095	3.38	-
N	0.007	2.48	11.18
S*N	0.152	4.71	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue. “S” represents species, “N” represents nitrogen and “N*S” the interaction nitrogen*species, “H” represents the harvest. “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the $\alpha = 0.05$ level).

Year 6 (2019/2020)

Table 4-35 Mean metabolisable energy (GJ ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ashley Dene, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
19/09/19	8.1	11.8	23.2	11.8	5.1	6.7	9.7	6.5	10.4 b
29/11/19	31.4	40.4	37.5	10.9	28.2	41.6	18.2	22.5	28.8 a
14/05/20	4.3	5.6	3.9	2.7	3.5	4.1	3.1	3.9	3.9 c
17/07/20	2.0	2.2	1.7	1.0	1.5	1.0	0.7	0.7	1.4 c
Mean Yearly N	49.7				39.2				
	BR		CF		PR		TF		Mean
Mean Yearly S	42.0		56.7		49.0		30.1		44.5
			P-value		SEM			LSD	
S			<0.001		2.96			9.48	
N			0.068		3.75			-	
S*N			0.087		7.56			-	

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue. "S" represents species, "N" represents nitrogen and "N*S" the interaction nitrogen*species, "H" represents the harvest. "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

4.3.9 Fibres - ADF and NDF

Experiment 1 – Ladbrooks

The acid detergent fibre (% of dry matter) for Ladbrooks, from July 2018 until July 2020 is shown at Appendix F.

4.3.9.1 ADF - Ladbrooks

For Year 5, ADF differed ($P<0.004$) among species (Figure 4-17). Brome produced more ADF than all the other species, at 3270 ± 481.1 kg ha^{-1}, the other species had a mean ADF yield of 2450 ± 481.1 kg ha^{-1} (Table 4-36).

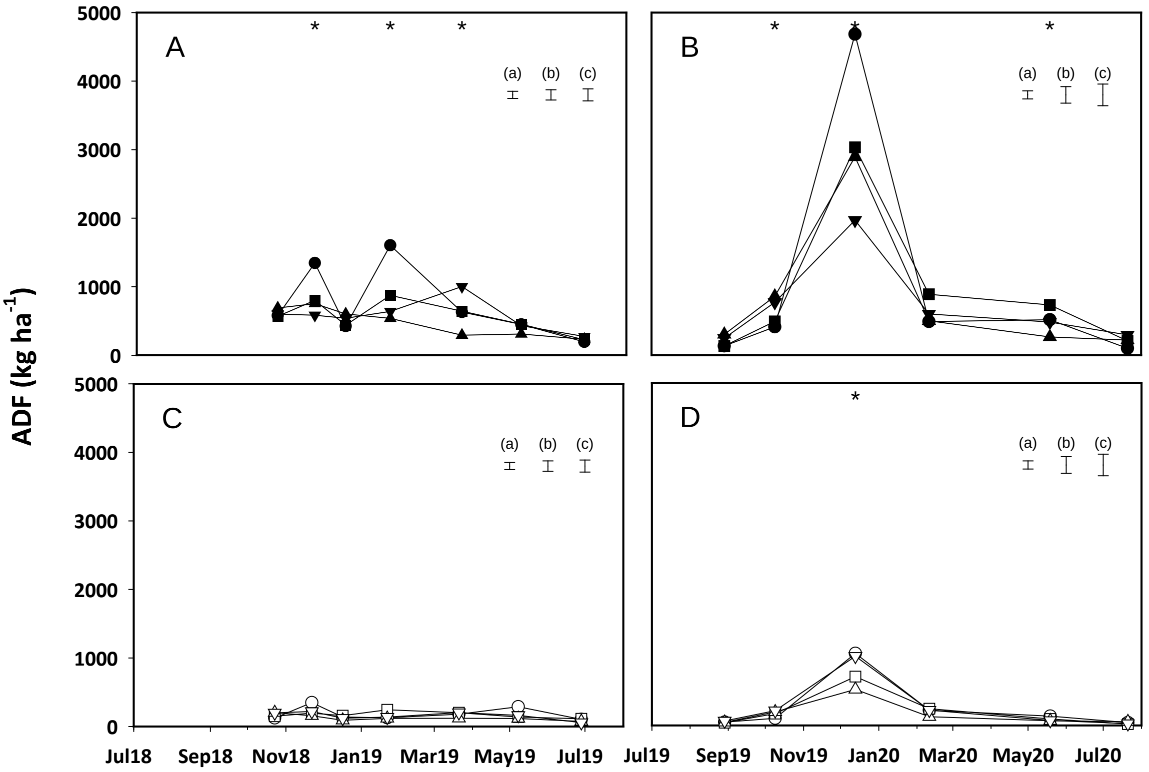


Figure 4-17 Mean acid detergent fibre (kg ha^{-1}), from brome (●,○), cocksfoot (■,□),perennial ryegrass (▲,△) and tall fescue (▼,▽), harvested from July 2018 to July 2019 and from July 2019 to July 2020 from monocultures grown with (closed) and without (open) nitrogen at Ladbrooks, Canterbury, New Zealand. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represents when differences among species were observed.

The total ADF increase was affected by the interaction of nitrogen and species (P=0.005). Ryegrass had the lowest increase of 2550 ± 592.9 kg ha^{-1} and brome the highest of 3985 kg ha^{-1} (Table 4-37).

For Year 6, there were no differences (P<0.061) among species (Table 4-38), with a mean ADF yield of 3350 ± 291.2 t ha^{-1}. N+ plots increased (P<0.001) total ADF yield from 1375 to 5320 kg ha^{-1}. The harvest on 13/12/19 showed higher (P<0.001) mean ADF values of 1985 kg ha^{-1}, due to the greater proportion of RDM.

Year 5 (2018/2019)

Table 4-36 Mean ADF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue grown with (N+) and without nitrogen (N-), harvested from July 2018 to July 2019 from monocultures at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
23/10/18	580	570	690	600	125	150	200	200	390 b
22/11/18	1345	800	755	585	350	195	160	220	550 a
17/12/18	425	440	605	540	140	160	90	120	315 c
22/01/19	1605	875	540	640	130	245	120	140	535 a
21/03/19	630	645	295	1005	180	200	120	200	410 b
09/05/19	450	455	310	435	290	140	115	165	295 c
28/06/19	195	230	235	275	105	110	75	60	160 d
Mean Yearly N	4185				1120				

	BR	CF	PR	TF	Mean
Mean Yearly S	3270	2605	2150	2585	2655

	P-value	SEM	LSD
S	0.004	150.6	481.8
N	<0.001	104.2	469.1
S*N	0.005	196.8	592.9

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue. “S” represents species, “N” represents nitrogen and “N*S” representes the interaction species with nitrogen, “H” represents the harvest, “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Table 4-37 Mean ADF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue with (N+) and without nitrogen (N-), harvested from July 2018 to July 2019 from monocultures grown at Ladbrooks, Canterbury, New Zealand.

	Species			
Nitrogen	BR	CF	PR	TF
N+	5230	4010	3425	4080
N-	1306	1200	875	1095

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue. The P-value for the interaction Nitrogen*Species is 0.005, the standard error of means is 196.8 kg ha^{-1} and the Least Significant Difference at 5%. is 592.9 kg ha^{-1}.

Year 6 (2019/2020)

Table 4-38 Mean ADF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue with (N+) and without nitrogen (N-) harvested from July 2019 to July 2020 from monocultures grown at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
28/08/19	135	135	305	250	50	40	45	65	130 c
09/10/19	415	495	860	775	105	170	195	215	405 b
13/12/19	4685	3035	2895	1970	1050	715	525	1010	1985 a
11/02/20	490	890	505	605	220	245	125	220	415 b
19/05/20	520	735	270	485	135	95	65	75	300 b
21/07/20	100	210	220	300	40	20	55	25	120 c
Mean Yearly N	5320				1375				

	BR	CF	PR	TF	Mean
Mean Yearly S	3975	3390	3035	2995	3350

	P-value	SEM	LSD
S	0.061	240.9	-
N	<0.001	119.5	537.8
S*N	0.076	315.9	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue. "S" represents species, "N" represents nitrogen and "S*N" represents the interaction nitrogen*species, "H" represents the harvest. "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

4.3.9.2 NDF – Ladbrooks

The neutral detergent fibre (% of dry matter) for Ladbrooks, from July 2018 until July 2020 is shown at Appendix G.

For the Neutral Detergent Fibre (NDF) yield in Year 5, brome produced 6270 ± 810 kg ha^{-1} which was more ($P<0.001$) than cocksfoot and tall fescue (4995 ± 810 kg ha^{-1}) while p. ryegrass had the lowest of 4025 ± 810 kg ha^{-1} (Table 4-39). Figure 4-18 shows that the production profile of NDF through the year for non-fertilised grasses followed the same pattern as ADF values.

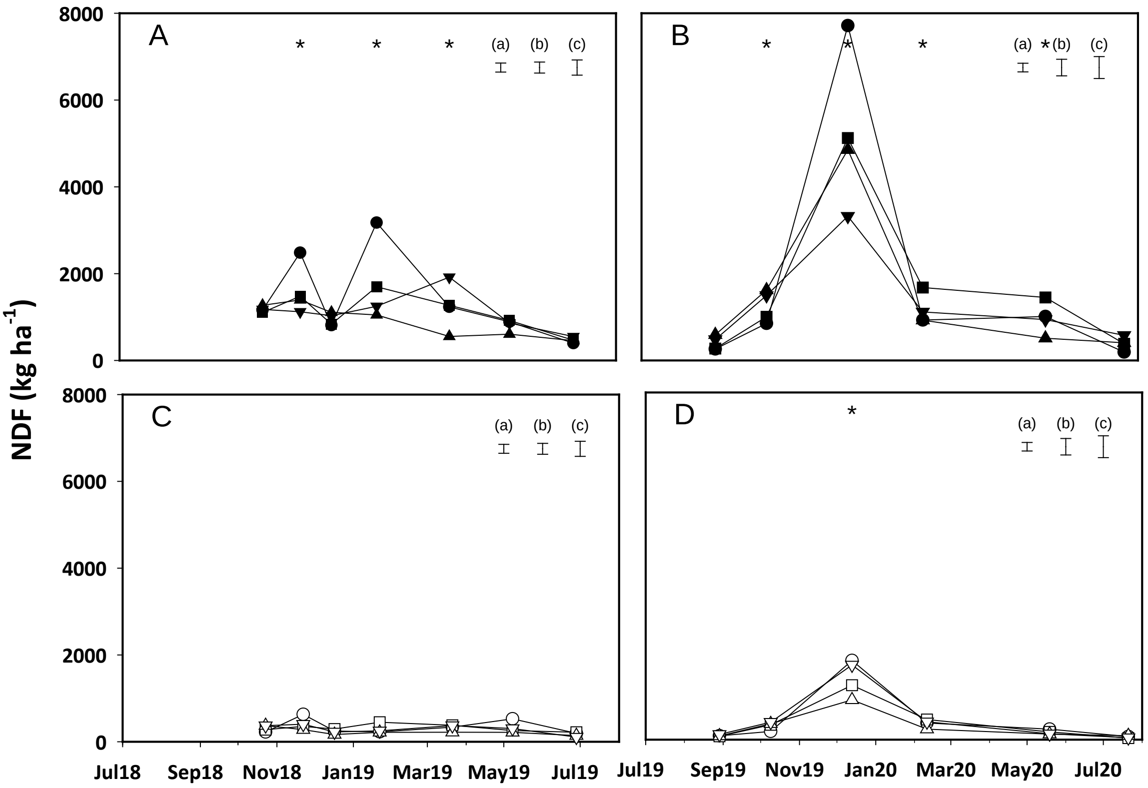


Figure 4-18 Mean neutral detergent fibre yield (kg ha^{-1}), from brome(●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue(▼,▽), harvested from July 2018 to July 2019 and from July 2019 to July 2020 from monocultures grown with (closed) and without (open) nitrogen at Ladbrooks, Canterbury, New Zealand. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represents when differences among species were observed.

Nitrogen increased (P<0.001) the NDF yield by 6020 kg ha^{-1}, or three times the yield of the non-fertilised plots. However the N effect differed with species (N*S P<0.001). The highest increase in the NDF yield was for brome (7780 kg ha^{-1}) and the lowest for ryegrass (4845 kg ha^{-1}) which reflects their differences in RDM (Section 4.3.4).

In Year 6, the mean NDF yield all species (P<0.077) was 5945 ± 672.3 kg ha^{-1} (Table 4-41). The N+ plots yielded more (P<0.001) NDF than N- plots , (2450 to 9440 ± 899.8 kg ha^{-1}) due to the increased TDM production.

Year 5 (2018/2019)

Table 4-39 Mean NDF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue with (N+) and without nitrogen (N-), harvested from July 2018 to July 2019 from monocultures grown at Ladbrooks, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
23/10/18	1160	1105	1270	1180	225	280	370	370	745 b
22/11/18	2485	1485	1400	1125	630	355	280	405	1020 a
17/12/18	810	840	1110	1030	245	290	165	225	590 bc
22/01/19	3175	1700	1050	1250	225	450	215	250	1040 a
21/03/19	1235	1270	555	1920	335	380	220	370	785 b
09/05/19	890	920	605	865	530	260	215	305	575 c
28/06/19	400	470	460	550	195	220	140	105	320 c
Mean Yearly N	8080				2060				
	BR		CF		PR		TF		Mean
Mean Yearly S	6270		5010		4030		4975		5070

	P-value	SEM	LSD
S	0.001	253.7	811.7
N	<0.001	208.8	939.9
S*N	0.001	351.4	1068.9

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue. "S" represents species, "N" represents nitrogen and "N*S" the interaction between nitrogen and species, "H" represents the harvest. "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the $\alpha = 0.05$ level).

Table 4-40 Mean NDF yield (kg ha^{-1}), in significant interactions, from brome, cocksfoot, perennial ryegrass and tall fescue with (N+) and without nitrogen (N-), harvested from July 2018 to July 2019 from monocultures grown at Ladbrooks, Canterbury.

	Species			
Nitrogen	BR	CF	PR	TF
N+	10160	7790	6450	7920
N-	2380	2230	1605	2030

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue. The P-value for the interaction is 0.001, the standard error of means is 351.4 kg ha^{-1} and the Least Significant Difference at 5% is 1068.9 kg ha^{-1}.

Year 6 (2019/2020)

Table 4-41 Mean NDF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
28/08/19	265	275	590	480	90	75	85	120	250 c
09/10/19	855	1010	1625	1500	190	340	365	400	785 b
13/12/19	7715	5125	4855	3325	1825	1255	915	1720	3340 a
11/02/20	930	1680	930	1120	385	460	240	410	770 b
19/05/20	1015	1450	515	940	240	175	125	135	575 b
21/07/20	190	385	410	580	70	35	90	45	225 c
Mean Yearly N	9440				2450				

	BR	CF	PR	TF	Mean
Mean Yearly S	6880	6140	5375	5390	5945

	P-value	SEM	LSD
S	0.077	381.7	1221.0
N	<0.001	199.9	899.8
S*N	0.086	504.1	1492.6

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue. "S" represents species, "N" represents nitrogen and "N*S" the interaction among species and nitrogen, "H" represents the harvest. "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Experiment 2 – Ashley Dene

The acid detergent fibre (% of dry matter) for Ashley Dene, from July 2018 until July 2020 is shown at Appendix H.

4.3.9.3 ADF – Ashley Dene

In Year 5, there was no difference in total ADF (P=0.194) amongst species (Table 4-42) with a mean annual yield of 1460 ± 82.5 t ha^{-1}. Nitrogen increased (P=0.008) ADF yield from 1150 to 1770 t ± 307.1 kg ha^{-1}, or by >70% (Figure 4-19), due to greater VDM (Table 4-7).

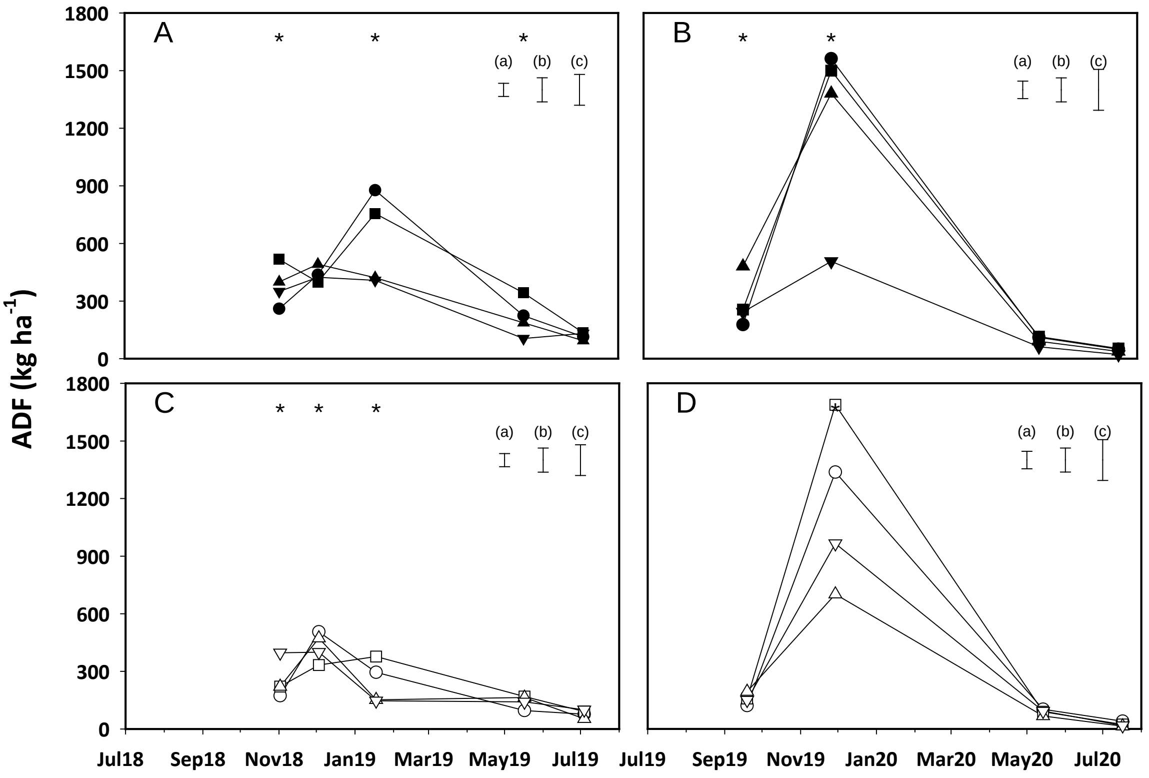


Figure 4-19 Mean ADF yield (kg ha^{-1}), for Ashley Dene from July 2018 to July 2019, per harvest, from brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽), with (closed) or without nitrogen fertilisation (open). Standard Error of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represent when differences among species were observed.

Total ADF yield differed (P<0.001) with regrowth rotation predominantly due to the high reproductive material for the regrowth cycle harvested on 18/01/19.

In Year 6, the species had different (P=0.005) total ADF yields with cocksfoot (1940 ± 135.2 kg ha^{-1}) being higher than p. ryegrass (1485 ± 135.2 kg ha^{-1}) (Table 4-43). In this year, N fertiliser did not increase ADF yield (P=0.183). The regrowth cycle harvested on 29/11/19 had the highest (P<0.001) ADF yield (1205 ± 43.9 kg ha^{-1}), due to high TDM and RDM.

Year 5 (2018/2019)

Table 4-42 Mean ADF yield (kg ha^{-1}), from brome, cocksfoot, p. ryegrass and tall fescue harvested from July 2018 to July 2019 from monocultures grown with (N+) and without (N-) nitrogen at Ashley Dene, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
02/11/18	260	515	400	350	175	220	220	400	315 b
03/12/18	195	345	435	360	245	320	395	325	430 a
18/01/19	875	755	420	410	295	375	150	145	430 a
17/05/19	225	345	185	105	95	170	165	140	180 c
04/07/19	115	135	95	130	75	90	55	100	100 d
Mean Yearly N	1770				1150				

	BR	CF	PR	TF	Mean
Mean Yearly S	1530	1670	1330	1300	1460

	P-value	SEM	LSD
S	0.194	125.4	-
N	0.008	68.2	307.1
S*N	0.147	160.4	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue. “S” represents species, “N” represent nitrogen and “N*S” represent the interaction among nitrogen and species, “H” represents the harvest. “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 4-43 Mean ADF yield (kg ha^{-1}) from brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ashley Dene, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
19/09/19	175	255	480	245	120	160	195	150	225 b
29/11/19	1560	1500	1380	505	1335	1690	700	965	1205 a
14/05/20	110	115	90	60	100	90	65	95	90 b
14/05/20	50	55	40	20	40	25	15	15	30 b
Mean Yearly N	1660				1440				

	BR	CF	PR	TF	Mean
Mean Yearly S	1750	1940	1485	1030	1550

	P-value	SEM	LSD
S	0.005	135.2	432.6
N	0.183	91.1	-
S*N	0.137	213.0	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue. “S” represents species, “N” represent nitrogen and “N*S” represent the interaction among nitrogen and species, “H” represents the harvest. “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

4.3.9.4 NDF – Ashley Dene

The neutral detergent fibre (% of dry matter) for Ashley Dene, from July 2018 until July 2020 is shown at Appendix I.

For the NDF, in Year 5, the species had no difference (P=0.167) in yield (Table 4-44). Their mean was 2705 ± 150 kg ha^{-1}. Nitrogen fertilisation increased (P=0.010) NDF yield by 50% from 2170 to 3240 ± 127.7 kg ha^{-1} (Figure 4-20).

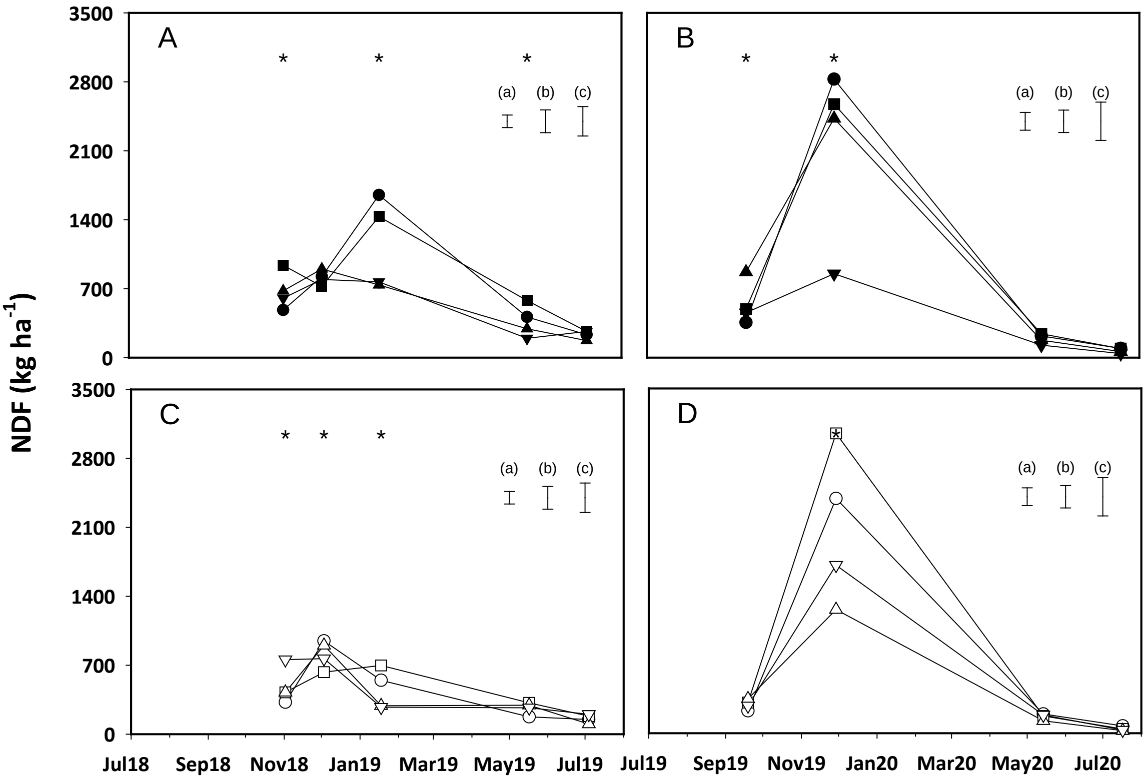


Figure 4-20 Mean NDF yield (kg ha^{-1}), for Ashley Dene from July 2018 to July 2019, per harvest, from brome (●,○), cocksfoot (■,□) ,perennial ryegrass(▲,△) and tall fescue(▼,▽), with (closed) or without nitrogen fertilisation (open). Standard Error of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represent when differences among species were observed.

In Year 6, mean NDF yield differed (P=0.003) among species (Table 4-45). Cocksfoot and brome had a higher mean (3340 ± 227 kg ha^{-1}) than tall fescue (1840 ± 227 kg ha^{-1}) with perennial ryegrass intermediate (2645 ± 227 kg ha^{-1}). NDF yield also differed (P<0.001) among regrowth cycles being highest for the 29/11/19 harvest.

Year 5 (2018/2019)

Table 4-44 Mean NDF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2018 to July 2019 from monocultures grown with (N+) and without (N-) nitrogen at Ashley Dene, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
2/11/18	485	940	680	605	325	425	425	755	580 b
3/12/18	825	725	900	795	945	630	900	765	810 a
18/01/19	1650	1430	740	770	545	700	290	275	800 a
17/05/19	415	580	295	195	175	320	295	270	315 c
04/07/19	235	265	175	265	150	185	105	200	195 c
Mean Yearly N	3240				2170				

	BR	CF	PR	TF	Mean
Mean Yearly S	2875	3100	2400	2450	2705

	P-value	SEM	LSD
S	0.167	231.8	-
N	0.010	127.7	180.6
S*N	0.136	299.4	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue. “S” represents species, “N” represent nitrogen and “N*S” represent the interaction among nitrogen and species, “H” represents the harvest. “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 4-45 Mean NDF yield (kg ha^{-1}), from brome, cocksfoot, perennial ryegrass and tall fescue harvested from July 2019 to July 2020 from monocultures grown with (N+) and without (N-) nitrogen at Ashley Dene, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
19/09/19	355	495	870	455	225	310	350	280	420 b
29/11/19	2825	2575	2430	850	2385	3045	1255	1705	2135 a
14/05/20	220	240	175	130	195	175	125	185	180 b
14/05/20	95	90	60	40	75	45	25	30	60 b
Mean Yearly N	2980				2605				
	BR		CF		PR		TF		Mean
Mean Yearly S	3190		3490		2645		1840		2795
	P-value			SEM			LSD		
S	0.003			227.0			726.2		
N	0.238			180.3			-		
S*N	0.155			388.0			-		

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue. "S" represents species, "N" represent nitrogen and "N*S" represent the interaction among nitrogen and species, "H" represents the harvest. "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

4.3.10 Dry matter accumulation over time

Pasture yields and growth rates were impacted by differences in temperature and water availability. Therefore, yield was related to thermal time by linear regressions to determine the temperature adjusted growth rate, which enables separation of temperature and moisture effects.

Ladbrooks

Year 5 (2018/2019)

In Year 5, thermal time accumulation was started on 25/06/18 with the first regrowth cycle. The rate of dry matter accumulation was constant ($P=0.498$) at 5.3 ± 0.54 kg DM °Cd^{-1} ha^{-1} for all species. However, N fertilised pastures grew 8.2 ± 0.27 kg DM °Cd^{-1} ha^{-1} compared with ($P<0.001$) 2.5 ± 0.27 kg DM °Cd^{-1} ha^{-1} for N- pastures. Figure 4-21 shows the temperature adjusted growth rates for each species at each N level.

The regression shows the first two data points are below the line which suggests a systematic error which may be related to a low LAI, which resulted in a lag phase in recovery which is discussed on Section 6.4.

The duration of this linear growth phase was 1634 ± 23.4 °Cd for N- plots and longer (P=0.013) at 1700 ± 23.4 °Cd for N+ plots. The rate of accumulation of dry matter decreased at the end of January 2019. From then on there was a difference (P=0.017) in the growth rates among species, nitrogen fertiliser (P=0.002) and an interaction (P=0.016). The interaction was because N- ryegrass grew at the lowest rate of 1.2 ± 0.33 kg DM ha^{-1} °Cd^{-1} and only increased to 2.1 ± 0.33 kg DM °Cd^{-1} ha^{-1} in the N+ treatment (Figure 4-21). The other species, growing at similar levels to p. ryegrass when not fertilised with nitrogen, had their adjusted growth rates increased to levels from 3.7 to 4.4 ± 0.33 kg DM °Cd^{-1} ha^{-1}.

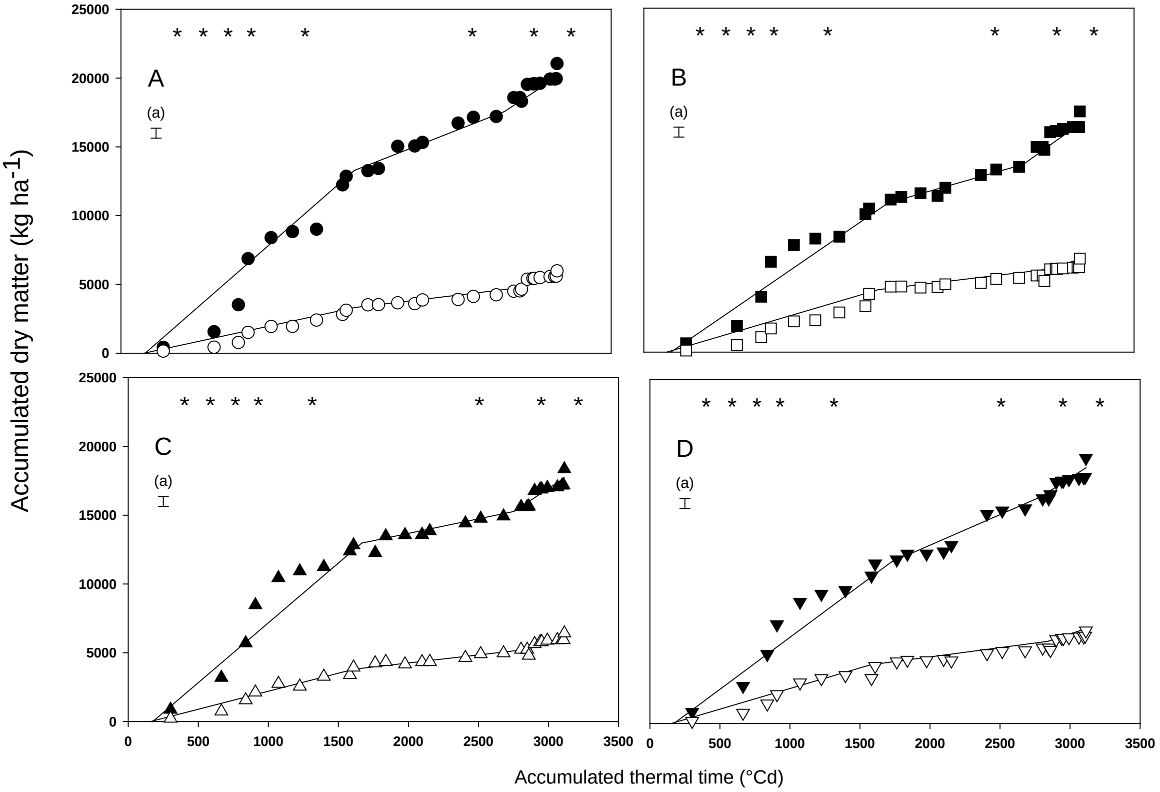


Figure 4-21: Accumulated dry matter (kg ha^{-1}) per unit of accumulated Thermal Time (Cd°, Tb = 3°C) for Ladbrooks, from July 2018 to July 2019 for brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) tall fescue (D) (▼,▽).with (closed) or without nitrogen fertilisation (open). Standard Error is represented for (a) nitrogen. "*" represents the harvests.

After the breaking point, the fertilised plots had a higher (P=0.009) growth of 6.2 ± 0.37 kg DM °Cd^{-1} ha^{-1} than the non-fertilised ones at 3.6 ± 0.37 kg DM °Cd^{-1} ha^{-1}.· This period ended when the agronomic year 2018/2019 ended after 3114 °Cd. Regression details for this year are shown on Table 4-46.

Year 6 (2019/2020)

In Year 6, there were three growth phases (Figure 4-22). There was an interaction (P=0.038) between species and N for the first phase of growth. Specifically, p. ryegrass and tall fescue grew at 11.1 ± 0.97 kg DM °Cd^{-1} ha^{-1} which was higher than the 9.5 ± 0.97 kg DM °Cd^{-1} ha^{-1}

for cocksfoot and brome. In addition the average increase in growth rate with N was from 7.4 to 13.2 ± 1.00 kg DM °Cd^{-1} ha^{-1} but p. ryegrass was more responsive with the increase, from 7.3 to 14.2 ± 0.41 kg DM °Cd^{-1} ha^{-1} .

This phase ended at 885 ± 26.3 °Cd (20/11/19) for N- and 985 ± 26.3 °Cd (30/11/2019) for N+ ($P<0.001$) plots. For Phase 2, the interaction between species and nitrogen ($P<0.001$) showed N+ brome grew at the highest rate of 3.23 ± 0.40 kg DM °Cd^{-1} ha^{-1}, while N- tall fescue was the slowest at just 0.48 ± 0.40 kg DM °Cd^{-1} ha^{-1}.

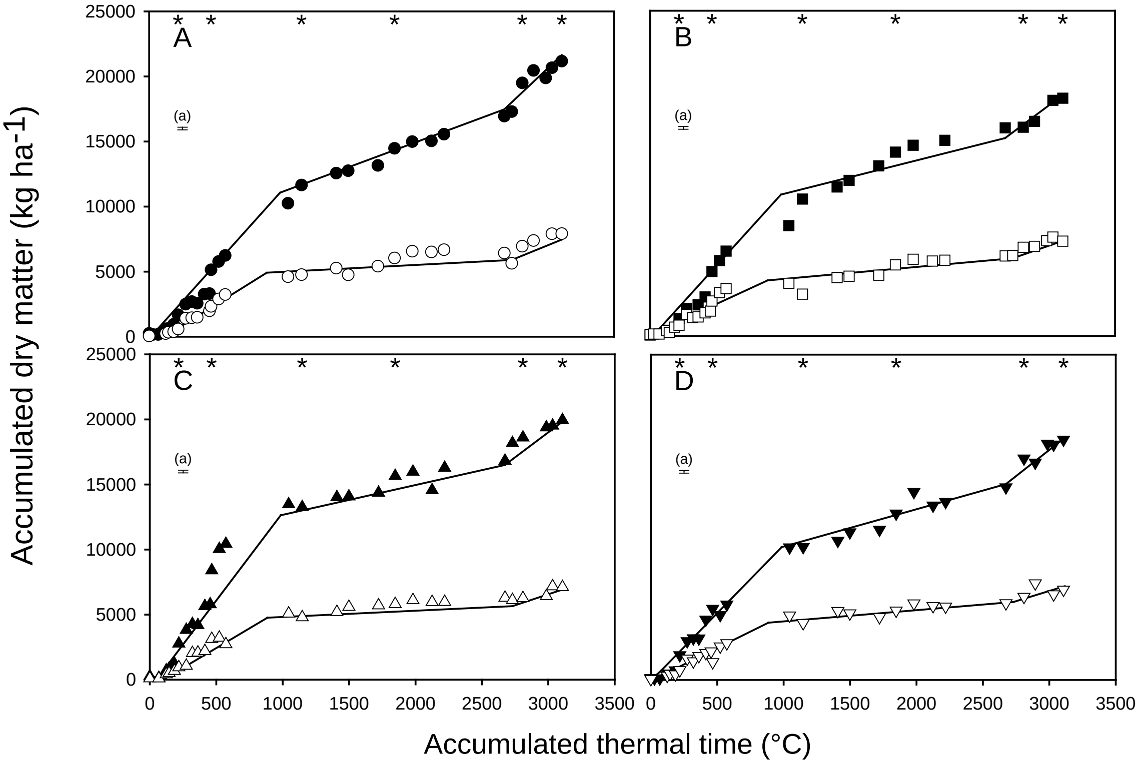


Figure 4-22 Accumulated dry matter (kg ha^{-1}) per unit of accumulated Thermal Time (Cd°, Tb = 3 °C) for Ladbrooks, from July 2019 to July 2020 for brome (A) (●,○), cocksfoot (B) (■,□), ,perennial ryegrass (C) (▲,△),tall fescue (D) (▼,▽) with (closed) or without nitrogen fertilisation (open). Standard errors are represented for (a) nitrogen. "*" represents the harvests.

The final phase was marked by an earlier recovery of the N+ plots. It started at 2701 ± 9.3 and 2730 ± 9.3 °Cd, respectively, for both N treatments. N+ plots grew at 8.8 ± 0.52 kg DM

°Cd^{-1} ha^{-1} compared with (P=0.005) the 3.2 ± 0.52 kg DM °Cd^{-1} ha^{-1} for N-. This phase ended at 3107 °Cd (21/07/20). The regression details for this year are shown in Table 4-47.

Table 4-46 Regression equations for accumulated yield (kg DM ha^{-1}) per unit of accumulated Thermal Time (°Cd, Tb= 3°C) by fertilised and non-fertilised monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand.

	Species	Equation (Y=)	SE Coef (x)	SE Coef	R^2
Phase	Nitrogen level		N+		
1	Brome	8.9x-1538	0.55	85	0.96
	Cocksfoot	7.8x-1354	0.27	48	0.96
	P. ryegrass	8.8x-1519	0.45	78	0.96
	Tall fescue	7.5x-1295	0.30	51	0.96
	Nitrogen level		N-		
	Brome	2.1x-362	0.41	72	0.96
	Cocksfoot	2.8x-494	0.10	18	0.95
	P. ryegrass	2.4x-414	0.30	52	0.96
	Tall fescue	2.6x-455	0.33	58	0.95
2	Nitrogen level		N+		
	Brome	4.3x+13410	0.42	841	-
	Cocksfoot	3.7x+12955	0.30	437	-
	P. ryegrass	2.1x+14896	0.45	584	-
	Tall fescue	4.4x+13168	0.31	315	-
	Nitrogen level		N-		
	Brome	1.2x+3182	0.21	489	-
	Cocksfoot	1.2x+4160	0.27	155	-
	P. ryegrass	1.2x+3794	0.02	357	-
	Tall fescue	1.3x+3939	0.37	371	-
3	Nitrogen level		N+		
	Brome	6.7x+20434	0.46	882	-
	Cocksfoot	7.3x+19450	0.72	423	-
	P. ryegrass	6.8x+19695	0.32	767	-
	Tall fescue	6.5x+20071	0.44	737	-
	Nitrogen level		N-		
	Brome	4.4x+5874	1.43	1080	-
	Cocksfoot	3.0x+6423	0.24	347	-
	P. ryegrass	3.7x+6191	0.21	373	-
	Tall fescue	3.4x+6564	0.73	1041	-

Note: "SE Coef" stands for Standard Error of the Coefficient. Displayed R^2 values are for the whole multi-stage model.

Table 4-47 Regression equations for accumulated yield (kg DM ha^{-1}) per unit of accumulated Thermal Time (°Cd, Tb= 3°C) by fertilised and non-fertilised monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand.

	Species	Equation (Y=)	SE Coef (x)	SE Coef	R^2
Phase	Nitrogen level		N+		
1	Brome	11.8x-192	0.39	151	0.88
	Cocksfoot	12.2x-469	0.06	99	0.90
	P. ryegrass	14.2x-1061	0.30	83	0.89
	Tall fescue	14.4x+34	0.46	179	0.89
	Nitrogen level		N-		
	Brome	6.8x+74	0.18	82	0.91
	Cocksfoot	7.2x+238	0.43	182	0.92
	P. ryegrass	7.3x+471	0.29	74	0.92
	Tall fescue	8.2x+114	0.32	56	0.91
2	Nitrogen level		N+		
	Brome	3.9x+10689	0.38	258	
	Cocksfoot	5.3x+10710	0.21	143	
	P. ryegrass	1.6x+14117	0.30	316	
	Tall fescue	2.7x+13290	0.27	507	
	Nitrogen level		N-		
	Brome	1.7x+5267	0.19	216	
	Cocksfoot	1.5x+5764	0.20	355	
	P. ryegrass	1.4x+6032	0.10	163	
	Tall fescue	1.6x+6399	0.21	269	
3	Nitrogen level		N+		
	Brome	9.6x+17295	0.97	675	
	Cocksfoot	6.1x+19655	1.13	476	
	P. ryegrass	5.8x+16899	0.71	681	
	Tall fescue	5.4x+17936	0.65	710	
	Nitrogen level		N-		
	Brome	4.0x+8487	0.48	494	
	Cocksfoot	2.6x+8597	0.25	193	
	P. ryegrass	4.2x+8690	0.14	243	
	Tall fescue	1.9x+9416	0.35	562	

Note: “SE Coef” stands for Standard Error of the Coefficient. Displayed R^2 values are for the whole multi-stage model.

Ashley Dene

Year 5 (2018/2019)

In Year 5, the agronomic year started on 04/07/18 and there was a difference ($P<0.001$) in growth rates between nitrogen treatments. N+ pastures grew 6.4 ± 0.10 kg DM °Cd^{-1}, while N- pastures grew 3.7 ± 0.10 kg DM °Cd^{-1}. This initial phase ended after 1589 ± 19.7 °Cd for all species on 19/01/19. In the second phase, all pastures showed little growth at 0.4 ± 0.07 kg DM °Cd^{-1} (Figure 4-23) regardless of species ($P=0.719$) or nitrogen fertilisation ($P=0.407$).

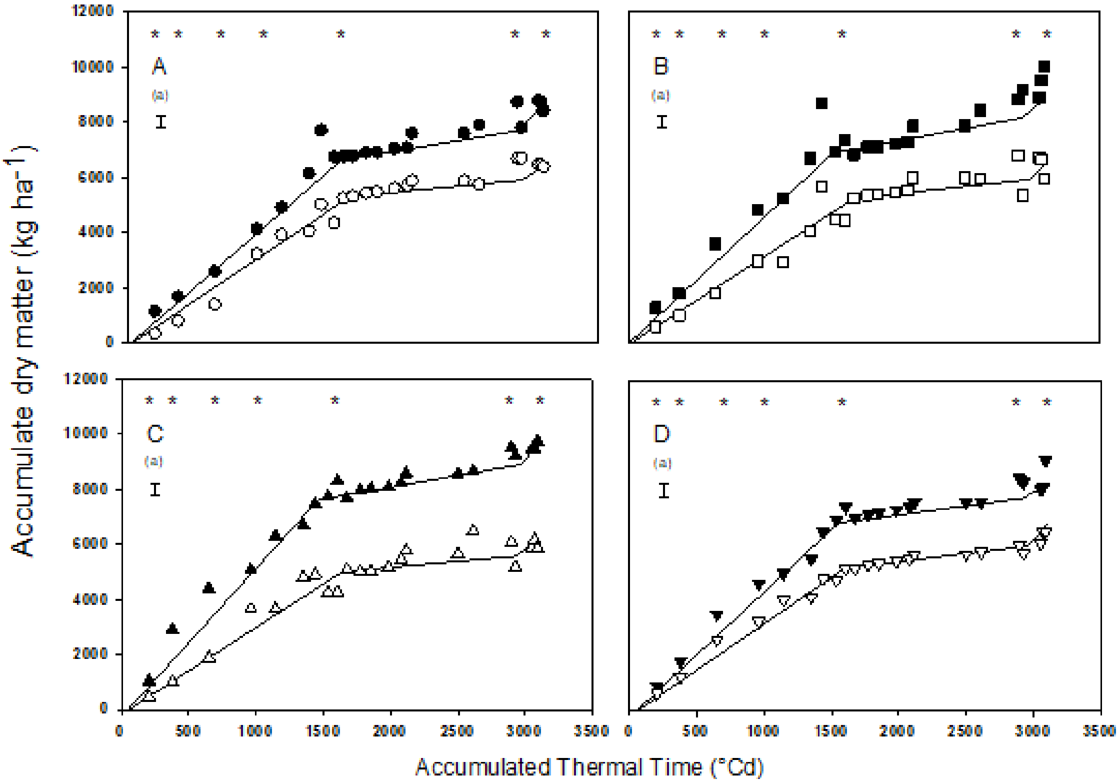


Figure 4-23 Accumulated dry matter (kg ha^{-1}) per unit of accumulated Thermal Time (Cd°, Tb = 3 °C) for Ashley Dene, from July 2018 to July 2019 for brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) and tall fescue (D) (▼,▽). with (closed) or without nitrogen fertilisation (open). Standard errors are represented for (a) nitrogen. "*" indicate when harvests happened.

The third, recovery, phase began at the end of May at 2974 ± 11.6 °Cd when pastures grew 4.1 ± 0.43 kg DM °Cd^{-1} ha^{-1}, which was not different for species (P=0.719) or nitrogen levels (P=0.386). This regrowth continued until the end of this agronomic year, on 04/07/19, after 3110 °Cd had accumulated. Details for the regressions are shown in Table 4-48.

Year 6 (2019/2020)

In Year 6, there was a difference in total accumulated DM according to species (Figure 4-24) but not for nitrogen (P=0.218). Thermal time accumulation commenced 04/07/2019.

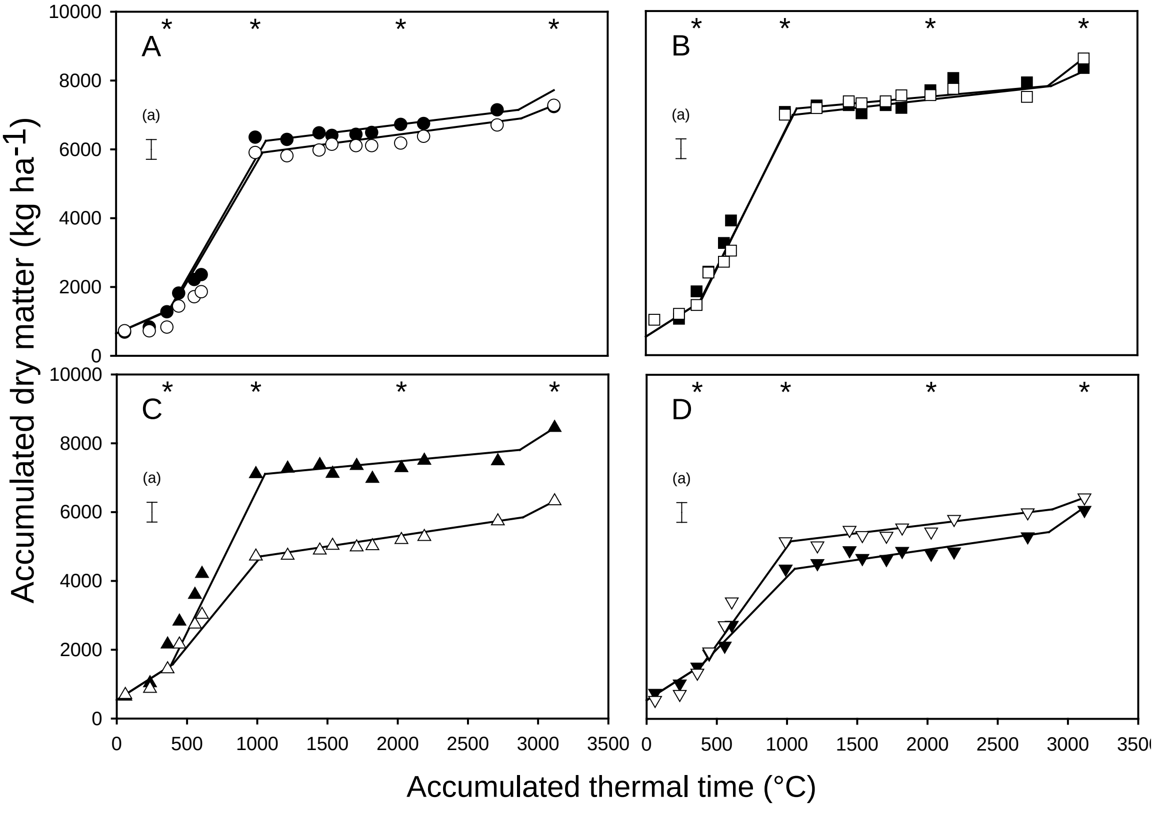


Figure 4-24 Accumulated dry matter (kg ha^{-1}) per unit of accumulated Thermal Time (Cd°, Tb = 3 °C) for Ashley Dene, from July 2019 to July 2020 for brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) and tall fescue (D) (▼,▽) with (closed) or without nitrogen fertilisation (open). Standard errors are represented for (a) nitrogen. "*" indicate when harvests happened.

In the first phase, brome had a lower (P=0.042) growth rate of 1.2 ± 0.52 kg DM °Cd^{-1} ha^{-1} than the other species which were growing at 3.0 ± 0.52 °Cd^{-1} ha$^{-1.}$ but there was no difference between nitrogen treatments (P=0.476).

During Spring, the second growth phase, tall fescue grew at a lower (P=0.008) rate of 4.7 ± 1.8 kg DM °Cd^{-1} ha^{-1}, compared with other species at 7.3 ± 1.8 kg DM °Cd^{-1} ha^{-1}. Those higher growth rates began earlier (P=0.011) at 382 ± 1.5°Cd for N+ plots compared with 393 ± 1.5 °Cd for N-. The growth rate also increased earlier for cocksfoot, brome and perennial ryegrass (P=0.029), at 386 ± 2.4 °Cd, than for tall fescue, 392± 2.4 °Cd, but, in practice, the pastures started at the same time, for this difference in °Cd results in a day of difference in thermal time accumulation.

During summer, the third phase, there was almost no growth (0.3 kg ± 0.13 DM Cd^{-1} ha^{-1}) for any species (P=0.335) or nitrogen level (P=0.835). The reduction in growth started earlier (P<0.001), at 1036 ± 1.2 °Cd for N- compared with N+ plots, at 1062 ± 1.2 °Cd. The final phase began at 2865 ± 2.2 °Cd for the fertilised plots (P=0.005) and 2888 ± 2.2 °Cd for the non-fertilised plots. During this last phase, the same rate of 3.1 ± 1.1 kg DM °Cd^{-1} ha^{-1} was measured for all species and N levels and this finished after 3117 °Cd. Regression details for this year are shown on Table 4-49.

Table 4-48 Regression equations for accumulated yield (kg DM ha^{-1}) per unit of accumulated Thermal Time (°Cd, Tb= 3 °C) by fertilised and non-fertilised monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2018 to July 2019 at Ashley Dene, Canterbury, New Zealand.

	Species	Equation (Y=)	SE Coef (x)	SE Coef	R^2
Phase	Nitrogen level		N+		
1	Brome	5.2x-166	0.20	154	0.82
	Cocksfoot	6.0x-104	0.15	213	0.79
	P. ryegrass	8.0x-400	0.25	189	0.85
	Tall fescue	6.4x-352	0.42	171	0.88
	Nitrogen level		N-		
	Brome	3.4x-240	0.19	175	0.89
	Cocksfoot	3.5x-185	0.10	188	0.82
	P. ryegrass	3.7x-207	0.13	211	0.80
	Tall fescue	4.2x-252	0.62	112	0.89
2	Nitrogen level		N+		
	Brome	0.8x+6622	0.18	423	
	Cocksfoot	0.6x+6875	0.10	248	
	P. ryegrass	0.8x+7617	0.25	861	
	Tall fescue	0.8x+7717	0.09	318	
	Nitrogen level		N-		
	Brome	0.6x+5319	0.14	228	
	Cocksfoot	0.6x+5247	0.07	225	
	P. ryegrass	0.7x+5023	0.07	219	
	Tall fescue	0.6x+5184	0.08	774	
3	Nitrogen level		N+		
	Brome	5.5x+7703	0.27	573	
	Cocksfoot	7.2x+8152	0.06	116	
	P. ryegrass	5.2x+8902	1.58	997	
	Tall fescue	3.2x+7717	0.22	329	
	Nitrogen level		N-		
	Brome	3.4x+5901	0.72	384	
	Cocksfoot	5.0x+5901	0.13	149	
	P. ryegrass	4.5x+5569	0.19	242	
	Tall fescue	3.2x+5984	0.49	837	

Note: “SE Coef” stands for Standard Error of the Coefficient. Displayed R^2 values are for the whole multi-stage model.

Table 4-49: Regression equations for accumulated yield (kg DM ha^{-1}) per unit of accumulated Thermal Time (°Cd, Tb= 3°C) by fertilised and non-fertilised monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2019 to July 2020 at Ashley Dene, Canterbury, New Zealand.

	Species	Equation (Y=)	SE Coef (x)	SE Coef	R^2
Phase	Nitrogen level		N+		
1	Brome	0.9x+538	0.14	58	0.97
	Cocksfoot	2.7x+550	0.90	64	0.95
	P. ryegrass	4.6x+542	1.34	127	0.92
	Tall fescue	2.9x+523	0.43	30	0.96
	Nitrogen level		N-		
	Brome	1.4x+545	0.56	68	0.96
	Cocksfoot	2.7x+539	0.87	73	0.95
	P. ryegrass	2.8x+535	0.41	83	0.93
	Tall fescue	2.6x+539	1.02	74	0.94
2	Nitrogen level		N+		
	Brome	9.3x+1338	0.88	25	
	Cocksfoot	7.8x+1522	0.77	259	
	P. ryegrass	7.6x+1541	0.66	369	
	Tall fescue	3.9x+1502	0.38	156	
	Nitrogen level		N-		
	Brome	7.0x+1334	1.39	164	
	Cocksfoot	7.3x+1528	1.03	245	
	P. ryegrass	4.8x+1576	0.66	85	
	Tall fescue	5.5x+1562	1.06	346	
3	Nitrogen level		N+		
	Brome	0.4x+6250	0.67	593	
	Cocksfoot	1.0x+7172	0.58	454	
	P. ryegrass	0.2x+7111	0.03	695	
	Tall fescue	0.2x+4367	0.03	296	
	Nitrogen level		N-		
	Brome	0.4x+5912	0.04	931	
	Cocksfoot	0.3x+6978	0.03	803	
	P. ryegrass	0.3x+4716	0.05	415	
	Tall fescue	0.2x+5159	0.05	448	
4	Nitrogen level		N+		
	Brome	4.4+7150	0.78	783	
	Cocksfoot	5.6+7824	1.71	487	
	P. ryegrass	3.8+7812	0.16	679	
	Tall fescue	4.3+5438	0.85	237	
	Nitrogen level		N-		
	Brome	4.2+6908	0.78	1011	
	Cocksfoot	4.1+7723	0.82	828	
	P. ryegrass	5.8+5862	0.97	431	
	Tall fescue	5.1+6097	0.55	468	

Note: "SE Coef" stands for Standard Error of the Coefficient. Displayed R^2 values are for the whole multi-stage model.

4.4 DISCUSSION

This chapter summarises the agronomic performance of four grass species grown under two water and nitrogen regimes after six years. Specifically, Objective 1 was to characterize pasture quantity and quality while Objective 2 was to relate dry matter production to the accumulation of thermal time to account for differences in temperature across seasons. These objectives were met and the results provide the basis for species comparison to determine which of these grasses was most productive and persistent in these environments.

4.4.1 Pasture yield and persistence

4.4.1.1 Total dry matter accumulation per unit of thermal time

At Ladbrooks in Year 5, there were no differences amongst species for their annual dry matter production (Table 4-1). However, the addition of N fertiliser showed the productive potential of this site was 18075 ± 362 kg ha^{-1}, or three times higher than that of non-fertilised pastures. The majority (77%) of this TDM was accumulated in the 6 months from October 2018 to March 2019 (Figure 4-1). This resulted from a temperature adjusted growth rate of fertilised grasses of 8.2 ± 0.27 kg DM °C ha^{-1} until thermal time reached 1700 ± 23.4 °Cd (Figure 4-21). This was more than three times the rate at which the non-fertilised grasses were growing (2.5 ± 0.27 kg DM °C ha^{-1}), until they stopped after 1634 ± 23.4 °Cd on 20/01/19. These linear relationships enable the nitrogen and temperature effects to be separated for all species and highlight the impact that nitrogen had on pasture productivity.

The end of the initial linear phase suggests another factor (water) was limiting growth (Table 4-46) and this is explored in Section 5.3.2. The lower growth rate of 1.2 ± 0.33 kg DM °C ha^{-1} for all N- pastures, 2.1 ± 0.33 kg DM °C ha^{-1} for all N+ ryegrass and from 3.7-4.4 ± 0.33 kg DM °C ha^{-1} for all N- pastures to the other N+ plants during the second phase of the regression occurred for 952 thermal units from 1634 to 2586 °Cd . After an Autumn rainfall of >40 mm in one week in April 2019 (Figure 3-2) the growth rates increased to 6.2

± 0.37 kg DM °Cd^{-1} ha^{-1} (Figure 4-21), for the fertilised pastures and compared with 3.6 ± 0.37 kg DM °Cd^{-1} ha^{-1} for the N- pastures. These data highlight the importance of nitrogen to dryland systems and show that all grass species had the potential to grow at similar rates when water and nitrogen were available. The only exception was perennial ryegrass which was slower to recover. It grew, at 1.2 ± 0.33 kg DM °Cd ha^{-1} until the end of April, which was consistent with the other species. However, it only increased to 2.1 ± 0.33 kg DM °Cd ha^{-1} under nitrogen fertilisation, compared with an average of 4.1 ± 0.33 kg DM °Cd ha^{-1} for the other species. The implication is that perennial ryegrass was affected more by the water stress than other species. Rainfall events during this Autumn suggest that there were some periods of growth, but overall, the model linearises to the mentioned growth rates. The physiological basis for species differences is explored in Chapter 7.

At Ashley Dene, for the same year, there was also no difference in species for their annual TDM (Table 4-3), but the pastures that received nitrogen yielded 1.5 times more than those unfertilised (from 6000 to 9340 ± 439.7 kg ha^{-1}). On this soil of low water holding capacity environment, 75% of all TDM was generated from late August 2018 until late January 2019 (Figure 4-2). This highlights the importance of N in the system when water is available to increase dry matter production (Mills *et al.* 2006). The growth rates for period from the beginning of Year 5 until late Spring was 6.4 ± 0.10 kg DM °Cd^{-1} for fertilised plants and 3.7 ± 0.10 kg DM °Cd^{-1} which was lower and occurred for a shorter duration at Ashley Dene compared with Ladbrooks, ending at 1589 ± 19.7 °Cd for all species (19/01/2019). The lower rate in Spring suggests some water stress was occurring during this period with these pastures more reliant on Spring rainfall (Figure 3-2) than stored soil water to grow due to their expected lower soil water storage capacity (Section 3.2.3). When water became restricted, the N+ pastures had their growth rates reduced to 0.4 ± 0.07 kg DM °Cd^{-1} (Table 4-48). The plants show a recovery after a rainfall of >80mm in a week (2958 - 2985 °Cd), when all plants reassumed their growth at 4.1 ± 0.43 kg DM °Cd^{-1} ha^{-1}.

A feature of the Spring from October 2018 to December 2019 was the higher than average rainfall (Section 3.2.5), with <180 mm on both sites. When coupled with average air temperatures from 8 to 11 °C it meant there was little or no restriction to dry matter production on the +N plots. However, the recovery period that began in late April 2019 at Ladbrooks, and mid May 2019 at Ashley Dene, had lower linear growth rates than in Spring, this contradicts the findings of Mills *et al.* (2006) who had Spring and Autumn linear rates for cocksfoot of 7.0 kg DM °Cd^{-1} ha^{-1}. This is explained by the ASMD on Figure 5-2 and Figure 5-3. As the soil moisture deficit was still high at the start of the recovery phase slowly diminishing, the regrowth rates increase after the rainfall events, at both sites, in the first week of June >50 mm, averaging then, 6.2 ± 0.37 kg DM °Cd^{-1} ha^{-1} for N+ plants and 3.6 ± 0.37 kg DM °Cd^{-1} ha^{-1} for the N- grasses at Ladbrooks, and 4.1 ± 0.43 kg DM °Cd^{-1} ha^{-1} at Ashley Dene for all grasses. The frequent rainfall events made some periods of high water availability and others of water scarcity, in a "saw tooth" pattern (Figure 5-2 and Figure 5-3). That averaged the values lower than those found on Spring for both nitrogen treatments.

At Ladbrooks, in Year 6, TDM followed the same temporal yield patterns as Year 5 (Figure 4-1 and Table 4-2) as well as the accumulated TDM for N+ plants, at 18100 ± 207 kg DM ha^{-1}, and N- plants, at 6100 ± 207 kg DM ha^{-1}. The initial phase of growth, that produced hay like grasses, had ryegrass and tall fescue growing at 11.1 ± 0.97 kg DM °Cd^{-1} ha^{-1}, and 9.5 ± 0.97 kg DM °Cd^{-1} ha^{-1} for brome and cocksfoot. Nitrogen fertilisation almost doubled the growth rate (Section 4.3.10) and delayed the end of this initial high growth rate by about 100 °Cd, which suggests it had access to more water or used what it had more efficiently which will be determined in Chapter 5. The lower growth rates in phase 2 (Table 4-47) had the fertilised grasses growing at a rate three times higher than those of the non-fertilised ones (3.6 and 1.2 ± 0.6 kg DM °Cd^{-1} ha^{-1}, respectively) lasted from 885 to 2701 °Cd to N+ plants and from 985 to 2730 °Cd to N- ones. The Autumn recovery phase, was consistent with Year 5. It started at 2701 °Cd for the N+ plants and 2730 for the N-, but the frequent rainfall events made some periods of high water availability and others of water scarcity. The result is periods of fast growth followed by no growth which is then averaged as a lower linear growth rate by the regression analysis. The implication is that there were periods of high growth, which would be equivalent to those found in Spring, followed by periods of no growth which creates a "saw tooth" pattern of growth that requires a greater frequency of measurements to detect (Figure 3-2). The result is a lower temperature adjusted growth rate than in Spring.

In Year 6, at Ashley Dene the accumulated TDM was 7395 ± 90 kg DM ha^{-1}. There was low Winter rainfall (Figure 3-2) at the start of Year 6 which resulted in brome growing at a lower rate at 1.2 ± 0.5 kg DM $°Cd^{-1}$ ha^{-1} and the other grasses at 3.0 ± 0.5 kg DM $°Cd^{-1}$ ha^{-1}, or slower than observed in Year 5. The extended Spring rotation meant all species, except tall fescue, produced similar yields (Table 4-4) and growth rates of 7.3 ± 1.8 kg DM $°Cd^{-1}$ ha^{-1}, whilst for tall fescue it was 4.8 ± 1.8 kg DM $°Cd^{-1}$ ha^{-1}, but there was no difference observed for nitrogen. These growth rates were higher than in Year 5 and inline with those achieved at Ladbrooks in both years. The implication is that nitrogen was not the most limiting factor in this extended rotation where all pastures probably achieved their ceiling yields (Chapter 6). In addition, when pastures recover from hay production there is a delay in regrowth due to the need to remobilise carbon from the roots to aerial plant parts, but below ground growth was not measured in this study. Water becomes restricted at about the same time (1050 °Cd) for all pastures and a period of almost no growth starts, of 0.3 ± 0.13 kg DM $°Cd^{-1}$ ha^{-1}, which will only ended at mid May 2020 (2865 °Cd and 2888 °Cd, for N+ and N- grasses, respectively) when regular rainfall allowed pastures to grow at 3.1 ± 1.1 kg DM $°Cd^{-1}$ ha^{-1}.

In Year 6, the effect of nitrogen differed between Ladbrooks and Ashley Dene probably due to the more frequent and intense rainfall events this year (Figure 3-2). A total of 210 mm of rain fell on Ladbrooks from December 2019 to March 2020, but only 125 mm of rain fell on Ashley Dene, 10 km west, over the same period. This resulted in a difference in annual rainfall (786 mm at Ladbrooks and 529 mm at Ashley Dene) between sites in Year 6 that was not evident in Year 5. This, additional rainfall plus the deeper soil at Ladbrooks resulted in Ladbrooks always having higher water available to the plants, even summer during periods of low rainfall. Thus, as expected the yields at Ladbrooks were higher than at Ashley Dene. This was the reason for using the two contrasting sites, to create pastures that were exposed to different levels of water stress. These differences allow us to examine the strategies each species used to deal with different levels of moisture and nitrogen availaibilty. The ability of plants to access water from each soil profile is covered in Chapter 5 while the more detailed plant physiology resposnes are addressed in Chapter 7.

In summary, these yield results show major impact of N, particularly for pastures grown with higher water availability at Ladbrooks. This allowed the nitrogen fertiliser effect on dry matter accumulation to be maximized and showed throughout the whole year. The temperature adjusted growth rates highlight the differences in linear response and suggest a maximum rate of ~8.0 kg DM/ha/CD was achieveable for all grasses. Chapter 5 describes and quantifies the impacts of water on these growth rates, to determine whether the periods of lower than optimum growth rates align with periods of soil moisture deficit both within and between sites.

4.4.1.2 Different botanical components

As expected the SSDM yields followed temporal patterns as the TDM (Figure 4-3 and Figure 4-4) because sown species dominated the botanical composition, particularly at Ladbrooks (Table 4-5 to Table 4-7). The exception was in Year 6 at Ashley Dene, when cocksfoot had a higher SSDM than any other species (Table 4-8). Of the grasses, brome had greater RDM and the least VDM through all the experiment at both sites (Figure 4-5 and Figure 4-6). A difficulty with experiments that use a common management is that not all species should be harvested at the same time so there is always a compromise. In this research the appearance of brome reproductive structures was frequently used as the criteria for harvest because it was the earliest and most prolific at producing seed heads (Section 4.3.4). In contrast, cocksfoot maintained the highest proportion of green leaves (VDM) (Table 4-11 and Table 4-12), particularly at advanced phenological stages and in the season where high soil moisture deficits (Section 5.3.2) are present. This result suggests that in dryland regions where variable topography and Spring rainfall make controlling pasture supply difficult, cocksfoot is most likely to maintain its quality even when species have gone reproductive.

For all the grasses, the annual yields in Years 5 and 6 were lower at Ladbrooks than those reported for Years 2 and 3 by Sharifiamina (2018). The implication is that there was some drop in productivity over time which is expected (Black and Moir, 2015). For Ashley Dene the TDM results were similar to those reported by Fasi *et al.* (2008) in an experiment in the Lees Valley with the same four grasses. The implication is that the shallow soils at Ashley Dene provide an environment and results that can be used to interpret the response of these grasses in dryland hill country.

The decline in yields over time was also reflected in the weed fraction after five and six years. The weed fraction found in both years at both sites (Figure 4-9 and Figure 4-10), was higher than that found in early years for this experiment by Sharifiamina (2018). The increase in weeds was higher at Ashley Dene, probably due to the fact that species had a slower canopy expansion (Chapter 6) and grew less vigorously under the low soil water supply than at Ladbrooks. The Ashley Dene site showed more incursion of Winter annual grasses (*Poa spp* and barley grass) and tap rooted flat weeds that are suited to the extended summer dry periods.

Mills (2007) reported that cocksfoot monoculture pastures had, 6 years after sowing, 6% of weed in their composition. In this experiment, after six years, cocksfoot had 3% of weed in the composition at Ladbrooks (Table 4-20) and 29% at Ashley Dene (Table 4-21). The higher weed percentage at Ashley Dene was observed for all species, but especially for tall fescue. This higher weed proportion explains why tall fescue yields in Year 5 and 6 in this experiment were, from 5 to 30%, lower than those reported by Jusoh (2013), on a Templeton silt loam at Lincoln, Canterbury New Zealand.

The long period of summer dry has been hypothesized as the reason for low persistence of perennial ryegrass in this environment. Therefore the persistence of the perennial ryegrass at Ashley Dene was unexpected and may have been due to the lax grazing regime employed (Talamini *et al.*, 2021). Overall, these results show that all pastures were equally persistent and productive, and most responsive to N at Ladbrooks on a soil with greater water holding capacity. In contrast, Mills and Moot (2010) observed a decline in ryegrass yield and quality successively up to seven years after sowing in a dryland experiment at Lincoln University. Taylor *et al.* (2021) also reported a decline in sown species proportion for ryegrass based pastures, and this decline was higher than those of cocksfoot based pastures, at Ashley Dene.

The inconsistent response of the N+ treatments at Ashley Dene compared with Ladbrooks (Figure 4-1 to Figure 4-9) highlights why annual sub clover is frequently recommended as the most appropriate companion legume to deliver nitrogen to the system in these dryland environments (Brown *et al.*, 2006; Lucas *et al.*, 2010; Mills *et al.*, 2010). Sub clover is also a Winter annual and its death in summer could provide the nitrogen required to increase pasture production in the Autumn recovery period.

4.4.2 Nitrogen recovery

The nitrogen recovery values were most commonly below 1 (Table 4-23 to Table 4-26). This indicates that not all of the fertiliser N applied was recovered in the following rotation. However, there were also periods when no fertiliser was applied but the N+ treatments produced more TDM, and had greater N yield than the N- treatments. This suggests that N applied in previous rotations was still available for plants use well after the rotation was complete and was not leached. The carry over effects of N application have previsouly been reported for cocksfoot by Peri *et al.* (2002). They showed that a response to urine N could last for up to 133 days in dryland pastures. The carry-over response in production and NR indicates that not all the N applied was not lost from the system via volatilization or leaching. For example, the rotations that ended with harvest on 28/08/2019 at Ladbrooks and the 19/09/2019 at Ashley Dene received no nitrogen fertiliser, but there was still higher nitrogen yield from the N+ treatments (35 compared to 5 ± 1.2 kg N ha^{-1} of the N- treatments at Ladbrooks and 42 compated to 22 ± 2.0 kg N ha^{-1} at Ashley Dene).

Perennial ryegrass, when well supplied with water and nitrogen, manages to properly prospect soil nitrogen, but when water restrictions are present, cocksfoot has a better nitrogen prospection. At Ladbrooks, ryegrass was the species that had the highest NR for Year 6 (2.93 ± 0.33 kg N kg N^{-1}). This was not observed in Ashley Dene, where cocksfoot had, for most regrowth cycles, a higher NR than the other species except for the regrowth cycle where no nitrogen was applied, harvested on 19/09/2019, as shown by Table 4-25 and Table 4-26.

A better use of nitrogen fertiliser is done by pastures that have their demand in water and nitrogen met, which potentially diminishes nitrogen leaching. This is shown by how much nitrogen (kg N ha^{-1}) was yielded comparing to the mass of the applied nitrogen in fertiliser form. The amount of applied nitrogen at Ladbrooks in Years 5 and 6 (Table 3-4) is, respectively, 589 and 405 kg N ha^{-1}, at Ashley Dene, the values for Year 5 and Year 6 were 369 and 205 kg ha^{-1}. In Year 5 (from 23/10/2018),at Ladbrooks, the N+ pastures yielded 562 ± 12 kg N ha^{-1} and the N- ones, 75 ± 12 kg N ha^{-1}, in Year 6, the N+ pastures yielded 433 ± 8.7 kg N ha^{-1} and the N- ones, 67 ± 8.7 kg N ha^{-1}. At Ashley Dene, in Year 5 (from 02/11/2018), the N+ pastures yielded 221 ± 7.7 kg N ha^{-1} , and the N-, 96 ± 7.7 kg N ha^{-1}, in Year 6, the N+ pastures yielded more than the double of the N- ones, 139 ± 6.3 kg N ha^{-1} against 65 ± 6.3 kg N ha^{-1}. Cocksfoot yielded more nitrogen than the other species, 131 ± 8.9 kg N ha^{-1}, ryegrass and brome yielded 105 ± 8.9 kg N ha^{-1} and tall fescue 65 ± 8.9 kg N ha^{-1}. The lower values on Year 6 for both sites are explained by the lower amount of nitrogen applied along with the high low-quality (Appendix B and Appendix C along with Equation 3) RDM yield. If the amount of nitrogen in the N- treatments is considered as a reference of mineralization, this means that, at Ladbrooks, the N+ pastures absorbed 82% of the applied nitrogen in Year 5 and 90% at Year 6. At Ashley Dene, the N+ pastures absorbed 46% of the applied nitrogen in Year 5 and 36% in Year 6, the difference between sites is possibly explained by different temperature in the soil surface, though this was not measured in this experiment.

4.4.3 Pasture quality

Nitrogen fertilisation was important in the quality maintenance though both sites. This is due to a higher VDM. Nitrogen fertilisation increased VDM at both years (Table 4-9 to Table 4-11), even if marginally at Ashley Dene on Year 6 (Table 4-12), but this still resulted in a higher protein production (Table 4-31), though not in a higher ME production (Table 4-35), which differs from what happened in Ladbrooks (Table 4-33). This shows that, even with restrictions to its action, the nitrogen fertilisation still has importance in drylands, for grasses, in providing the correct protein amount to animals. If nitrogen fertilisation is restricted in environments where plants cannot be provided nitrogen from the soil or fertilising, the use of legume species should be considered.

Protein yield six years after sowing was lower than what was observed in the first years (Sharifiamina, 2018), even with the same season patterns (Figure 4-13 and Figure 4-14). This reduction was of 15% at Ladbrooks comparing Year 6 to Year 2 and 10% at Ashley Dene, for the same years. Those differences are explained by the lower VDM yield and higher RDM yield (Table 4-15 and Table 4-18). In Year 2 (2015/2016), VDM yield was, respectively for N+ and N- plants, at Ladbrooks, 18415 and 9300 kg DM ha^{-1},dropping to 9200 and 2700 kg DM ha^{-1}, respectively, in Year 6, at Ashley Dene, the drop from Year 2 to Year 6 was, respectively, from 4000 to 2130 kg DM ha^{-1} for N+ grasses and from 3700 to 1475 in Year 6 for N- grasses.

The quality standards (ME and protein yield) for cocksfoot at Ashley Dene on Year 6 (Table 4-31 and Table 4-35) were higher than the other species. This is due to its higher maintenance of SSDM, especially VDM, even in advanced phenological stages (Table 4-12), as discussed in Section 4.4.1 and seen on the harvest of 29/11/2019, reaching values up to thirteen times the observed for other species. A drop in the protein yield of species from Year 2 (Sharifiamina, 2018) to Year 6 was lower for cocksfoot and p. ryegrass at Ladbrooks, about 600 kg ha^{-1}, than that of brome and tall fescue, 900 kg ha^{-1} (Table 4-28). At Ashley Dene, cocksfoot still had the highest protein yield (820 kg ha^{-1}) even if the protein yield drop was higher (150 kg) from Year 2 to Year 6, higher than the drop of brome and p. ryegrass (30 kg ha^{-1}). Tall fescue had the highest yield drop of 280 kg ha^{-1}, which, along with its highest weed proportion, suggests it is the species with the poorest persistence for this environment (Talamini Junior *et al.*, 2021).

The higher RDM yield infers a higher fibre production. This can be seen on the higher ADF and NDF yield at both sites from Year 5 to Year 6 (Table 4-36 to Table 4-45), the yield increase ranging from 6% to 26% (Table 4-36 to Table 4-38; Table 4-42and Table 4-43) for the ADF and 18% to 20% for NDF (Table 4-39 to Table 4-41; Table 4-44 and Table 4-45), even with a decrease in the TDM yield. Fibrous material (reproductive stems) is slowly and incompletely digested (Buxton, 1997) and has a longer rumen retention that non-fibrous leaves (vegetative components), besides, a higher NDF is negatively correlated with dry matter intake, meaning animals eating less and spending more time to have this forage digested (Schulze *et al.* 2014). This, along with the higher VDM yield, implies that cocksfoot is able to offer animals decent fodder under dryland conditions for longer, especially under nitrogen fertilisation (Table 4-12) (Stevens *et al.*, 1989; Milne, 2011). The physiological behind this are explained on Chapter 7.

Those results show that the main difference in pasture quality within sites, as demonstrated by Sharifiamina (2018) on the same sites, was nitrogen along with species when the management allows the pastures to reach advanced phoenological stages, this comes mostly from the increase in VDM due to the nitrogen fertilisation. Nitrogen fertilisation is more effective with higher water availability (Smika *et al.*, 1965) not only to increase DM yield but also quality. Five and six years of sowing, though all species did not differ in the TDM production on both sites, for drylands, cocksfoot is the species that present the best offer of fodder to animals. Though the TDM reduction affected all the species, cocksfoot maintained a higher amount of green vegetative material, which allowed the species to maintain a higher quality fodder for a longer time.

4.5 CONCLUSIONS

The chapter dealt with differences on yield, quality and growth according to species, nitrogen and moisture levels:

- Temperature adjusted growth rates allowed a visible visualisation of the nitrogen effect on yield as well as the influence of soil moisture in it. Fertilised plants had higher growth rates that lasted longer when dry periods started from summer.
- At Ladbrooks, the results of nitrogen fertilisation are higher than for Ashley Dene, for the water availability at Ladbrooks is higher through the whole year than at Ashley Dene. The pastures were also able to recover more nitrogen from fertiliser at Ladbrooks than at Ashley Dene.
- As about 70-75% of the TDM is generated during Spring and parts of summer, we have indication that irrigation is justifiable in those parts of the year, taking advantage that non-controllable environmental factors (= temperature) is high, and nitrogen fertilisation and water can be controlled.
- Weeds affected Ashley Dene more than Ladbrooks, which is one factor that indicates a lower persistence of that site, along a lower DM production. Tall Fescue was the species that apparently had the worst competing ability with weed.

Next chapter deals with dry matter accumulation in each site as according to the different hydric regimes and nitrogen availability.

5 WATER USE AND WATER USE EFFICIENCY OF PASTURES UNDER DRYLAND CONDITIONS

5.1 Introduction

Chapter 4 quantifed the yield and persistence of the four grass species under two water and nitrogen regimes. Differences in annual dry matter yields were found due to nitrogen, site and species. These were quantified by differences in the temperature adjusted growth rates. These figures showed a higher rate of growth for pastures that had received nitrogen, particularly at Ladbrooks, and that rates were highest in Spring when soil water was expected to be non-limiting. Specifically, the broken line regressions showed a reduction in growth rates in late Spring early summer, and the time of slower growth was later at Ladbrooks than Ashley Dene. After the period of slower growth there was a late summer/Autumn recovery period shown by increased linear growth rates. This chapter relates these changes in temperature adjusted growth rates to the water availability at each site, and examines if they were affected by nitrogen level or species.

Therefore, this chapter relates to Objective 3 to quantify the amount of water available at each site and under each treatment. This involves meeting Objective 4; to determine the actual soil moisture deficit (ASMD) using a soil water balance. This quantifies the amount of water used by each species and when that water was used which allows the water use efficiency to be calculated to meet Objective 5. To meet these objectives this chapter quantifies the total plant available soil water content of each plot by defining the drained upper limit, lower limit, extraction depth and estimates of drainage. Thus, this chapter focuses on the individual plot data from neutron probe measurements which are interpreted to explain treatment effects on the yields reported in Chapter 4.

5.2 Materials and Methods

The description of the experimental design, treatments and site management were given in Chapter 3 (Section 3.2). The main measurements related to this chapter were the 33 soil moisture reading taken in Ladbrooks and the 29 in Ashley Dene, for each of the 32 plots at each site.

5.2.1 Soil water content (SWC)

Volumetric soil water content (SWC) was measured using a neutron probe (CPN International 503DR Hydroprobe, California, USA) at 22 depths in Ladbrooks from 0 to 2.25 m deep and from 0 to 1.45m at Ashley Dene. The top layer was 0.15 m and the others were 0.10 m each, measured in the same aluminium tube inserted in the soil. The variability of these soils (Sharifiamina, 2018), which were formed under alluvial deposits across the Canterbury Plains (Wilson, 1979), requires measurements at this fine granulation, which is uncommon on more uniform soils.

Aluminium access tubes were installed in 2014, when the experiment was sown. The tubes have an external diameter of 47 mm and an internal diameter of 40 mm. The tubes were installed to 2.30 m in Ladbrooks and 1.50 m in Ashley Dene.

Readings were taken, fortnightly, from 31/10/2018 to 17/07/2020 at Ashley Dene and from 23/10/2018 until 21/07/2020 at Ladbrooks. During the New Zealand wide Covid19 Lockdown, readings were taken less frequently from April 2020 until the end of the experiment in July 2020.

5.2.2 Soil moisture content (SWC) for the soil profile

The soil moisture content for every layer of soil in each plot, on each date, was calculated from Equation 7:

Equation 7

$$\boldsymbol{SWC = \sum_{Top\ Layer}^{Bottom\ Layer} \Theta * d}$$

Where Θ is the measured volumetric water content (VMC) (mm^3/mm^3) of each layer and “d” is the depth of each layer, or 0.10 m except the surface 0.15 m.

5.2.3 Drained upper limit (DUL) and lower limit (LL)

The drained upper limit (DUL), measured in millimetres, is the maximum amount of water a soil profile retains after drainage (Raitliff, 1983). The lower limit (LL), also measured in millimetres, is the measured amount of water that remains in the profile after all plant available water has been extracted. This value was obtained in mid-summer after a period of no growth and was validated by comparison with values reported by Sharifiamina (2018).

To determine the DUL, a measurement was taken 5 days after a rainfall of 100 mm when the soil had already recharged from rainfall in Autumn. This happened on 9/5/2017, for both sites, after a period of rainfall of 216 mm over 66 days.

The soil water content for each soil layer was used to determine the actual soil moisture deficit, calculated by the difference between DUL and the soil moisture content measured over time. To obtain the soil moisture deficit for the whole profile, the difference obtained in each layer was summed to 225 cm at Ladbrooks and 145 cm in Ashley Dene.

5.2.4 Plant available water content (PAWC)

The PAWC was defined, as the difference between the DUL and LL and calculated with Equation 8:

Equation 8

$$\boldsymbol{PAWC = \sum_{Top\ Layer}^{Bottom\ Layer}(DUL - LL)}$$

The maximum plant water extraction depth, represented as the “Bottom Layer” in Equation 8, was defined as the deepest soil layer where a difference between DUL and LL in SMC was detected between two sequential neutron probe readings.

5.2.5 Actual soil moisture deficit (ASMD)

Actual soil moisture deficit (ASMD) is defined as the difference (in mm) between PAWC and the SWC on a given day. A model was created to predict the actual soil moisture deficit daily starting from each day a neutron probe measurement was taken. This was reset by each neutron probe measurement as shown in Equation 9:

Equation 9

$$\boldsymbol{ASMD(n+1) = SMD(n) + PETa(n+1) - R(n+1)}$$

Where ASMD(n+1) is the actual soil moisture deficit on day "n+1" (mm), SMD(n) is the soil moisture deficit on day "n" (mm), PET_a(n+1) is the potential evapotranspiration (mm), on day "n+1" proposed by NIWA (2019), which reflects that soil water becomes harder for plants to extract as the soil dries (Equation 10), and R(n+1) is the rainfall on day "n+1" (mm). Drainage (mm) events were considered to have occurred if rain fell when the ASMD(n+1) was already at zero. Effectively, negative values were considered to result in soil water loss from the system as drainage, rather than run-off on these flat sites.

Equation 10 $$\boldsymbol{PETa = PETp * (PAWC - ASMD)/(0.5 * PAWC)}$$

Where PET_a is the potential evapotranspiration (mm) adjusted by the NIWA (2019) method, PET_p is the potential evapotranspiration given by Penman-Monteith and PAWC is the plant available water content (mm) and ASMD(n) is the actual soil moisture on the day.

This model was affected by some periods of water logging caused by a high water table. Waterlogging occurred at Ladbrooks in Spring 2018 and early Winter 2019. Plot 15 (cocksfoot N+), at both sites, had a neutron probe access tube with a hole in the end. At times these tubes would fill with water at times up to 15 cm below the soil surface. This gave an indication that the ground water table was rising but this was not independently monitored. However, this did confound some early Spring measurements of soil water use. To address this PET_p was substituted for soil water use to reflect the amount of water the plants were expected to have used in Spring when water was readily available (Section 5.3.2).

The potential soil moisture deficit, gives us an indication of how severe a drought was, is given by Equation 11:

Equation 11 $$\boldsymbol{PSMD(n) = \mathrm{SMD1} + \sum_1^n PETp - \sum_1^n R}$$

Where $PSMD_{(n)}$ is the potential soil moisture deficit (mm) on any given day, where SMD_1 is the soil moisture deficit (mm) when the cycle starts, PET_p is Penman-Monteith's potential evapotranspiration (mm) and R is the rainfall (mm). The results for the $PMSD_{(n)}$ are shown in Appendix J for Ladbrooks and Appendix K for Ashley Dene.

5.2.6 Water Use (WU)

WU (mm) is defined as the amount of water a crop uses. The WU for each regrowth cycle was interpolated from the difference between consecutive neutron probe readings as given by Equation 12:

Equation 12

$$\boldsymbol{WU = P(R) - (SMCd - SMCp) - D}$$

Where P(R) (mm) is the sum of precipitation between the two measurements, SMCd is the measured soil moisture content on the day of interest and SMCp is the measured soil moisture content from a previous measurement, D is drainage, expected to occur when water drained from the profile because of rain when the soil was saturated. Measurements of SMC occurred on herbage harvest days and mid-rotation as well.

The sum of water extracted from the profile between consecutive neutron probe measurements for the full regrowth cycle was the water use for each regrowth cycle. When the high water table confounded water use data (Section 5.2.5), PETp was used to determine water use (Section 5.3.3).

5.2.7 Water use efficiency (WUE)

Agronomically water use efficiency (WUE) is defined as the amount of dry matter produced per milimiter of water used per hectare, as shown in Equation 13

Equation 13

$$\boldsymbol{WUE = TDM/WU}$$

Where "WUE" is the water use efficiency, in kg DM mm^{-1} ha^{-1}, "TDM" is the total amount of dry matter produced in the considered interval of time (kg DM ha^{-1}) and "WU" is the water use (mm) defined in Equation 12. This was quantified for each plot as the slope of the least squares linear regression.

5.3 RESULTS

5.3.1 Plant Available Water Content (PAWC)

Experiment 1 –Ladbrooks

To calculate the PAWC, the maximum extraction depth was determined on both sites. This involved using measurements from the periods when the soil had dried out and was not influenced by a high water table. This meant measurements from 22/01/2019 were used.

A difference (P=0.040) between nitrogen treatments was observed. The fertilied pastures extracted water down to 1.67 m or 0.10 ± 0.010 m deeper than the snon-fertilised ones (Table 5-1). On average, all species extracted water down to 1.61 ± 0.010 m (P=0.160).

Table 5-1 Maximum water extraction depth (m), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2018 to July 2020 at Ladbrooks, Canterbury, New Zealand.

N+				N-			
BR	CF	PR	TF	BR	CF	PR	TF
1.65	1.70	1.65	1.65	1.60	1.60	1.55	1.55
Mean N	1.67					1.57	
BR		CF		PR		TF	Mean S
1.62		1.65		1.60		1.60	1.61
		P-value		SEM		LSD	
S		0.160		0.010		-	
N		0.040		0.010		0.030	
N*S		0.873		0.150		-	

Note: “BR” stands for brome, “CF” stands for cocksfoot, “PR” stands for perennial ryegrass, “TF” stands for tall fescue. “SEM” is the Standard Error of Means and “LSD” is the Least Significant Difference.

From the start of measurements in 2018 until their end in July 2020, the plant available water content was not different (P=0.203) amongst species and averaged 167 ± 4.8 mm. However, it was 196 ± 6.8 mm for +N pastures, which >40% higher (P=0.008) than the 131 ± 6.8 mm for the –N pastures (Table 5-2). This extra water came from extraction of greater extraction (lower LL values) at each individual layer rather than from differences in extraction depth. For example, Figure 5-1 shows how DUL – LL changed down the profile of two representative plots at Ladbrooks. At all depths DUL is relatively consistent but LL is lower in the N+ plot. The point where DUL equals LL indicates no water was being extracted and this was therefore considered as the maximum extraction depth (Section 5.2.4). At Ladbrooks, the maximum water extraction depth was 1.6 m.

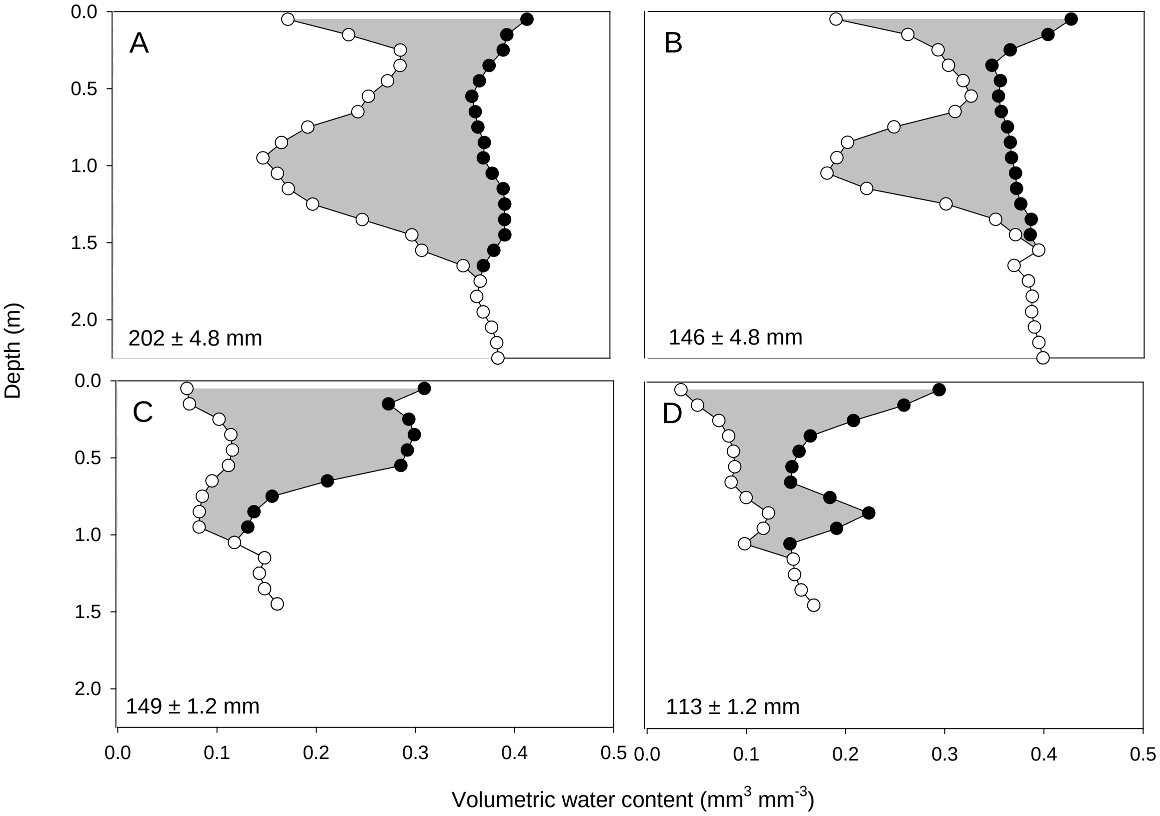


Figure 5-1 Drained Upper limit (●) and Lower Limit (○), in Ladbrooks (A,B) and Ashley Dene (C,D) for Plots CF 26 N+ (A,C) and CF 5 N-(B,D). The Measured Plant Available Water Content (mm³ mm⁻³) is represented by the shaded area. The text indicates the Plant Available Water content ± SEM Depth is Ladbrooks reached 1.65 m and in Ashley Dene, 1.05 m.

Table 5-2 Calculated maximum plant available water content (mm), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2018 to July 2020 at Ladbrooks, Canterbury, New Zealand.

N+				N-			
BR	CF	PR	TF	BR	CF	PR	TF
221	191	185	191	137	134	135	149
Mean N	197				138		
BR		CF		PR		TF	Mean S
179		162		160		169	167
		P-value		SEM		LSD	
S		0.495		9.2			
N		0.009		6.8		30.6	
N*S		0.532		13.5			

Note: "BR" stands for brome, "CF" stands for cocksfoot, "PR" stands for perennial ryegrass, "TF" stands for tall fescue. "SEM" is the Standard Error of Means and "LSD" is the Least Significant Difference at 5%.

Experiment 2 - Ashley Dene

In Ashley Dene, water extraction was not different among species (P=0.922), regardless of their nitrogen levels (P=0.215) with water extracted down to 0.99 ± 0.090 m (Table 5-3). However, there was less water availability in the layers from 0.4 to 1.0 m deep (Figure 5-1).

Table 5-3 Maximum water extraction depth (m), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2018 to July 2020 at Ashley Dene, Canterbury, New Zealand.

N+				N-			
BR	CF	PR	TF	BR	CF	PR	TF
1.00	1.02	1.00	1.01	1.00	1.00	0.98	0.98
Mean N	1.01				0.99		
BR		CF		PR		TF	Mean S
0.99		1.01		0.99		0.99	0.99
		P-value		SEM		LSD	
S		0.922		0.050		-	
N		0.215		0.038		-	
N*S		0.615		0.072		-	

Note: "BR" stands for brome, "CF" stands for cocksfoot, "PR" stands for perennial ryegrass, "TF" stands for tall fescue. "SEM" is the Standard Error of Means and "LSD" is the Least Significant Difference.

The PAWC averaged 118 ± 1.2 mm and was not different (P=0.684) amongst species. However, +N pastures averaged 13 mm more (P=0.011) PAWC than N- pastures (Table 5-4).

Table 5-4 Calculated maximum plant available water content (mm), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2018 to July 2020 at Ashley Dene, Canterbury, New Zealand.

N+				N-			
BR	CF	PR	TF	BR	CF	PR	TF
129	123	121	124	115	120	109	102
Mean N	124				111		

BR	CF	PR	TF	Mean S
122	121	115	113	118

	P-value	SEM	LSD
S	0.684	6.4	-
N	0.011	1.6	7.3
N*S	0.360	7.0	-

Note: “BR” stands for brome, “CF” stands for cocksfoot, “PR” stands for perennial ryegrass, “TF” stands for tall fescue. “SEM” is the Standard Error of Means and “LSD” is the Least Significant Difference at 5%.

5.3.2 Actual Soil Moisture Deficit (ASMD)

There were limitations in measuring soil water in early Spring (Section 5.2.5). This was because of a high water table that meant the interpolated values between measurements dates overestimated water extraction. For this reasons the ASMD was reset to the measured values which resulted in a vertical increase in ASMD just before the measurement date. The high water table was observed both at Ladbrooks and Ashley Dene, in Figure 5-2 and Figure 5-3.

The actual soil moisture deficit was based on the estimated actual evapotranspiration (PETa). This involved adjusting the potential evapotranspiration with an equation from NIWA (2019 estimates that account for the increased resistance to water extraction that occurs as a soil dries (Feddes *et al.*, 1976). Appendix L show the mean level of SMD when reductions in yield were observed.

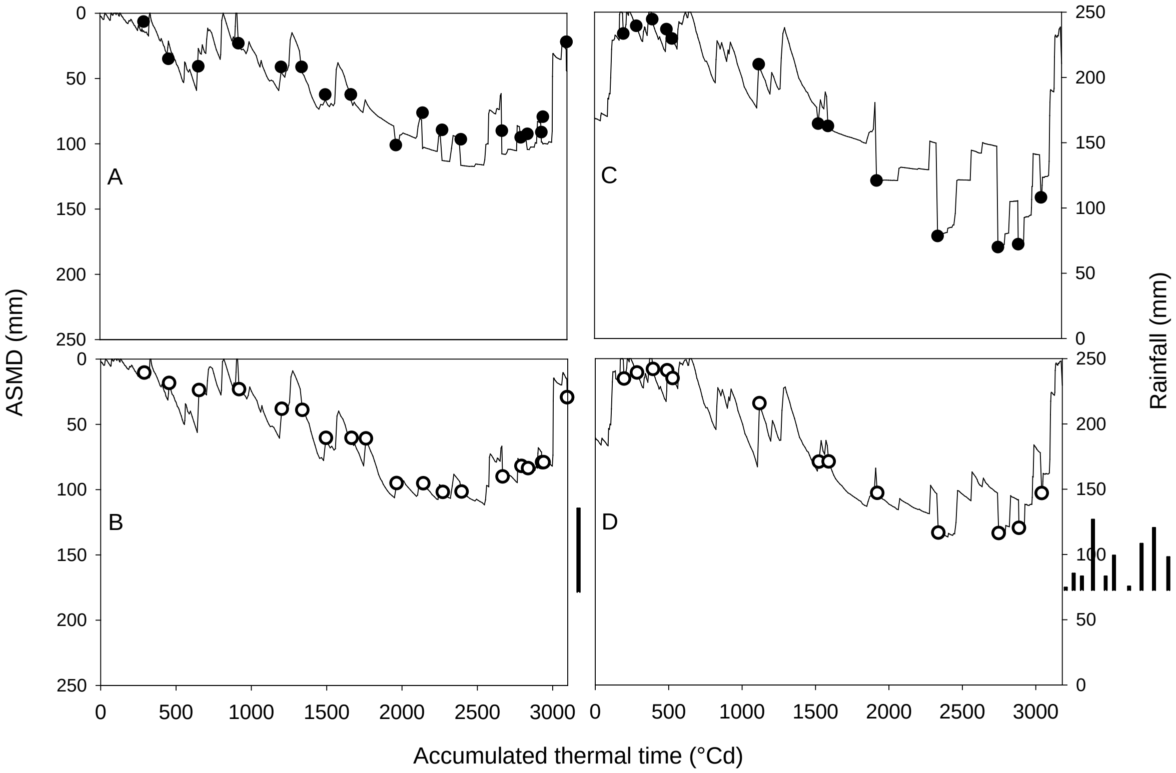


Figure 5-2 Mean actual soil moisture deficit (mm) for monocultures of brome fertilised with nitrogen (closed symbols; A,C) or not (open symbols; B,D), from July 2018 to July 2019 (A,B) and from July 2019 to July 2020 (C,D) grown at Ladbrooks, Canterbury New Zealand from July 2018 to July 2020. ● represents measurement days done with the neutron probe. Black bars indicate accumulated weekly rainfall (mm). The average plant available water content is 196 ± 6.8 mm for the N+ grasses and 138 ± 6.8 mm for the N- ones. Total rainfall from July 2018 to July 2019 was 623 mm, and from July 2019 to July 2020 was 786 mm.

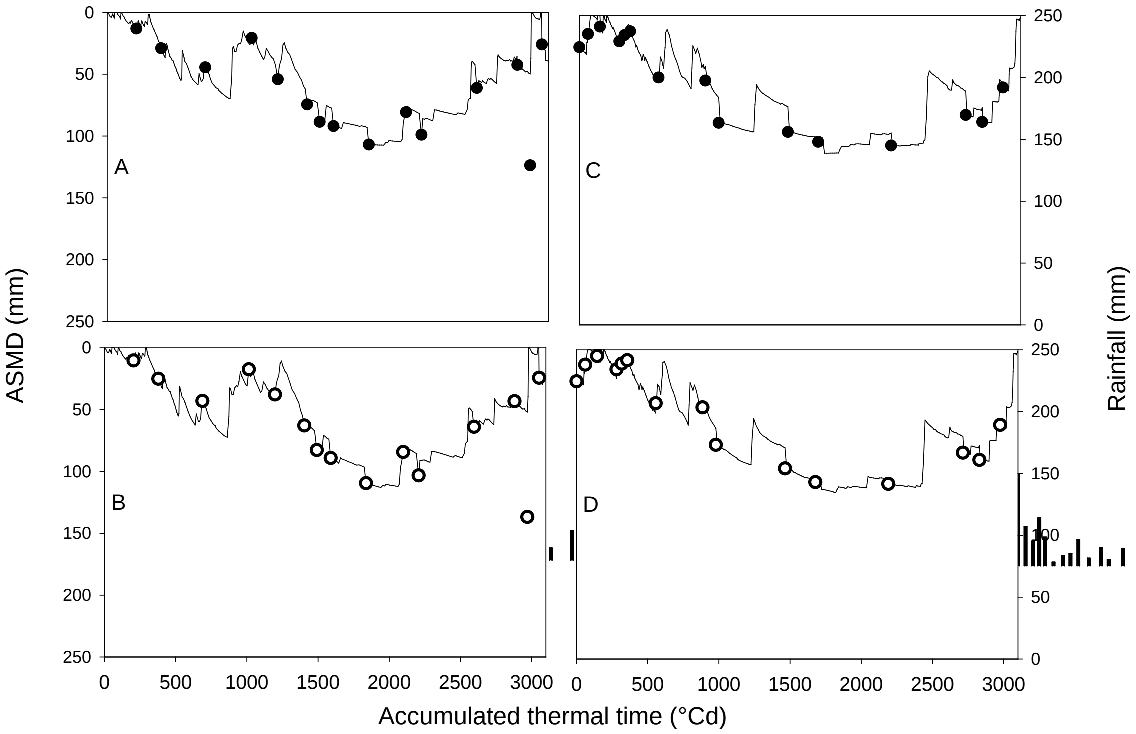


Figure 5-3 Mean actual soil moisture deficit (mm) for monocultures of brome fertilised with nitrogen (closed symbols; A,C) or not (open symbols; B,D), from July 2018 to July 2019 (A,B) and from July 2019 to July 2020 (C,D) grown at Ashley Dene, Canterbury New Zealand from July 2018 to July 2020. ● represents measurement days done with the neutron probe. Black bars indicate accumulated weekly rainfall (mm). The average plant available water content is 124 ± 2.7 mm for the N+ grasses and 111 ± 2.7 mm for the N- ones. Total rainfall from July 2018 to July 2019 was 566 mm, and from July 2019 to July 2020 was 529 mm.

Experiment 1 - Ladbrooks

Year 5 (2018/2019)

In Year 5, the ASDM was not different (P=0.787) amongst species but differed (P=0.008) between nitrogen treatments. On average the N- pastures had 11.1 mm higher ASMD than the N+ pastures, at 91.1 to 102.2 ± 2.74 mm, respectively (Figure 5-2).

The ASMD on 728 °Cd (02/11/2018), was 57.3 ± 3.21 mm for all grasses. By 1422 °Cd (04/01/2019) the ASDM was 65 mm for both nitrogen treatments and increased at a low rate until 2803 °Cd (04/05/19), when a week 40 mm of rain fell and it started to decrease it. By late June (3099 °Cd) it had reached 39.6 ± 1.75 mm for the N+ pastures and 45.6 ± 1.48 for the N- pastures.

Drainage was 32 mm higher (P=0.043) for N- pastures than N+ ones (Table 5-5).

Table 5-5 Estimated drainage (mm), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand.

N+				N-			
BR	CF	PR	TF	BR	CF	PR	TF
160	151	189	162	188	196	206	202
Mean N	166				198		
BR		CF		PR		TF	Mean S
174		174		198		182	182
		P-value		SEM		LSD	
S		0.368		10.3		-	
N		0.043		6.8		30.4	
N*S		0.341		1.9		-	

Note: “BR” stands for brome, “CF” stands for cocksfoot, “PR” stands for perennial ryegrass, “TF” stands for tall fescue. “SEM” is the Standard Error of Means and “LSD” is the Least Significant Difference at 5%.

Year 6 (2019/2020)

In Year 6, the ASMD was not different (P=0.974) amongst species but 5 mm higher for N- pastures (P<0.008), at 67.0 ± 2.0 mm, compared with N+ pastures at 72 ± 2.0 mm.

The ASMD after 101 °Cd (18/07/2019) was 62 ± 2.3 mm for the N+ pastures and 50 ± 2.3 mm for the N- pastures. This decreased to 11 mm for all pastures by 403 °Cd (19/09/2019) due to more than 125 mm of rainfall between those two dates. After 1093 °Cd (03/12/2019), when the deficit was 55 ± 1.7 mm for all pastures, there was > 80 mm of rain that fell over two weeks which resulted in drainage estimated to be >30 mm. The ASMD tended to increase from this date, until a maximum of 170 mm for the N+ pastures after 2747 °Cd (01/05/2020) and 136 mm for the N- pastures on the same date (Figure 5-2). From this time, there was a slow recovery to a final deficit of 5 ± 0.1 mm for all pastures after 3180 °Cd (21/07/2020).

Drainage was53 mm higher (P=0.029) for N- pastures than N+ ones (Table 5-6).

Table 5-6 Estimated drainage (mm), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand.

N+				N-			
BR	CF	PR	TF	BR	CF	PR	TF
147	140	162	142	185	190	222	207
Mean N	148				201		

BR	CF	PR	TF	Mean S
166	165	192	175	174

	P-value	SEM	LSD
S	0.339	11.0	-
N	0.029	9.6	43.0
N*S	0.745	16.0	-

Note: “BR” stands for brome, “CF” stands for cocksfoot, “PR” stands for perennial ryegrass, “TF” stands for tall fescue. “SEM” is the Standard Error of Means and “LSD” is the Least Significant Difference at 5%.

Experiment 2 - Ashley Dene

Year 5 (2018/2019)

In Year 5, ASDM was not different amongst species (P=0.876) but was 11.1 mm higher (P=0.03) for N- than the N+ pastures which averaged 91 ± 2.7 mm (Figure 5-3). From the start of this year until 372 °Cd (18/09/2018), the ASMD was always below 21 mm for the N+ pastures and 28 mm for the N- ones. Drainage of >20 mm was estimated during this period.

The ASMD, after 701 °Cd (02/11/2018), was at 42 ± 0.2 mm for the N+ pastures and 47 ± 0.2 mm for the N- pastures. It increased until a weekly rainfall of more than 25 mm reduced it to almost 0 mm for all pastures after 878 - 945 °Cd (20/11 – 27/11/2018). After 1403 °Cd (04/01/2019) the ASDM was 65 ± 1.3 mm for N+ grasses and 74 ± 1.3 mm mm for N-. It reached a midsummer maximum of 106 ± 1.5 mm for the N+ grasses and 115 ± 1.5 mm for the N- grasses after 1836 °Cd on 05/02/2019. ASMD then recovered with rainfall and was ~75 mm until 2532 °Cd (01/04/2019).

A single measurement taken on 30/05/2019 (2969 °Cd) shows an ASMD value of 125 mm which is inconsistent with the readings immediately before and after this so it was considered a “rogue reading” based on instrument failure and not included in calculations.

Drainage was estimated as 143 ± 6.2 mm for all species and N treatments (Table 5-7).

Table 5-7 Estimated drainage (mm), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2018 to July 2019 at Ashley Dene, Canterbury, New Zealand.

	N+				N-		
BR	CF	PR	TF	BR	CF	PR	TF
130	136	140	142	142	142	128	185
Mean N	137				149		

BR	CF	PR	TF	Mean S
136	139	134	164	143

	P-value	SEM	LSD
S	0.224	10.4	-
N	0.346	7.5	-
N*S	0.519	15.7	-

Note: “BR” stands for brome, “CF” stands for cocksfoot, “PR” stands for perennial ryegrass, “TF” stands for tall fescue. “SEM” is the Standard Error of Means and “LSD” is the Least Significant Difference at 5%.

Year 6 (2019/2020)

In Year 6, ASMD was not different among species (P=0.974) but the N- pastures had 8.5 mm more (P=0.008) ASMD than the N+ pastures (Figure 5-3). ASMD after 61 °Cd (18/07/2019) was 13 ± 0.8 mm for the N+ pastures and 18 ± 0.8 mm for the N- pastures. This decreased to 9 ± 0.5 mm to the N+ pastures and 13 ± 0.5 mm to the N- pastures by 403 °Cd (19/09/2019) due to more than 125 mm of rainfall. Most of the estimated drainage (>110 mm) in this year took place during this period. ASMD then increased to reach a maximum of 103 ± 1.4 mm, for the N+ pastures and 113 ± 1.4 mm, for the N- pastures, by 2200 °Cd (27/01/20). The ASMD then reduced after 2829 °Cd (13/05/2020), and after >80 mm of rain which returned plots to field capacity (DUL) by 3100 °Cd (11/07/2020).

Drainage was 151 ± 12.6 mm regardless of species and N treatment (Table 5-8).

Table 5-8 Estimated drainage (mm), for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen, from July 2019 to July 2020 at Ashley Dene, Canterbury, New Zealand.

	N+				N-		
BR	CF	PR	TF	BR	CF	PR	TF
134	155	128	166	163	147	117	202
Mean N	146				157		

BR	CF	PR	TF	Mean S
148	151	123	184	151

	P-value	SEM	LSD
S	0.082	14.3	-
N	0.579	13.3	-
N*S	0.623	22.5	-

Note: “BR” stands for brome, “CF” stands for cocksfoot, “PR” stands for perennial ryegrass, “TF” stands for tall fescue. “SEM” is the Standard Error of Means and “LSD” is the Least Significant Difference at 5%.

5.3.3 Water Use (WU) per year and regrowth cycle

Not all regrowth cycles could have their water use calculated solely from neutron probe readings due to high water table interference. For those readings PET_p was used to estimate their water use. Figure 5-4 shows the water use of each regrowth cycle calculated by neutron probe readings, total PET_p for each regrowth cycle and which regrowth cycles had PET_p considered as WU. During this period, when water is freely available PET_p is equivalent to PETa because the soil factor has not begun to reduce soil water extraction (NIWA 2019).

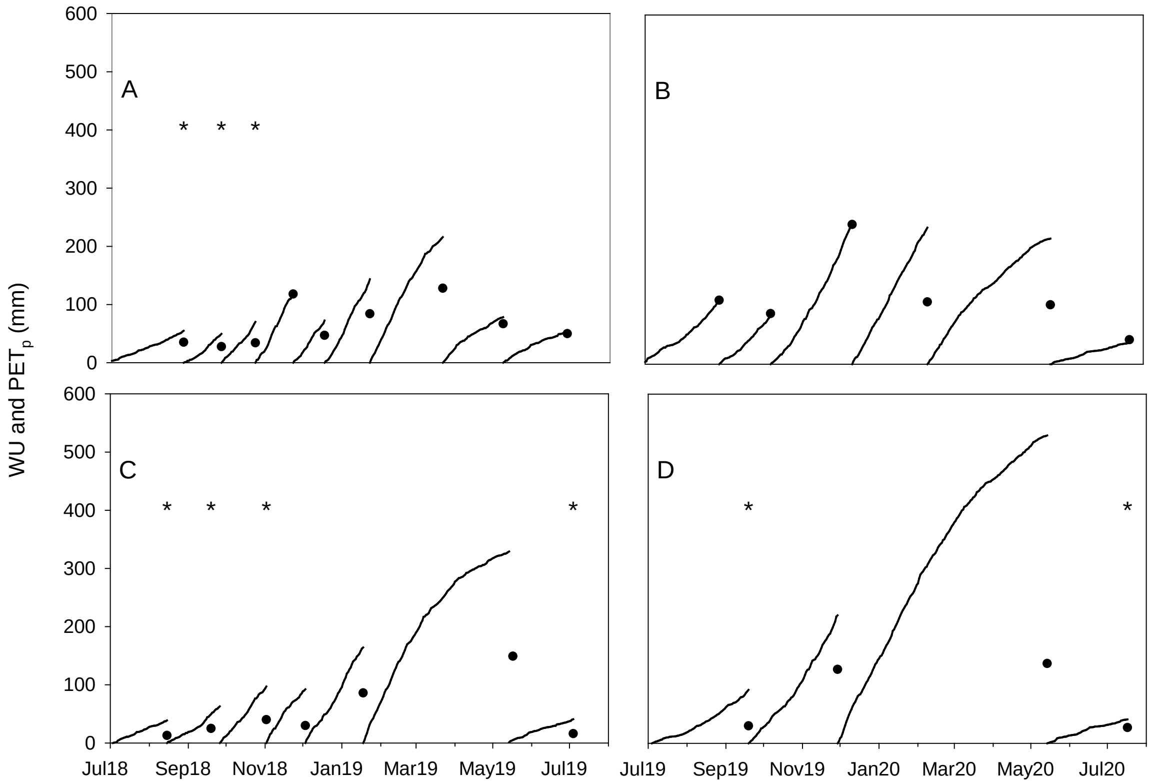


Figure 5-4 Mean measured water use (WU, mm) (●) and potential evapotranspiration (PET$_p$,mm) (solid line) from monocultures of brome, cocksfoot, p. ryegrass and tall fescue at Ladbrooks (A,B) and Ashley Dene (C,D), from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D). "*" represents regrowth cycles that when water use was estimated from PET$_p$.

Experiment 1 - Ladbrooks

In Year 5, at Ladbrooks, PET$_p$ values were used to estimate WU for the first three regrowth cycles. This was because the neutron probe readings gave a total WU during that period that was 40% lower than the PET$_p$ values in Spring (Section 5.3.2). The lack of reduction in actual soil moisture during this period reflects the high water table which means these values were considered inaccurate. In fact, a high water table reflected in the tube with an open end, from plot 15, to sometimes show water as low as 15 cm deep from the surface. From the fourth measurement on the water table dropped and the measured water use and PET$_a$ were the same, which suggests pastures were actively growing with no water limitation (Pronger *et al.*, 2019). After this water use was estimated from neutron probe measurements with daily water use values interpolated using Equation 10 for PET_a.

The total annual water use for all species was not different (P=0.248) at 667 ± 6.3 mm (Table 5-9). The accumulated water use was higher (P<0.001) for longer regrowth cycles. The regrowth period that started on the 22/01/2019 had the highest water use of 128 ± 2.6 mm, while those in Winter and early Spring (27/08-23/10/2018) had the lowest (Figure 5-5).

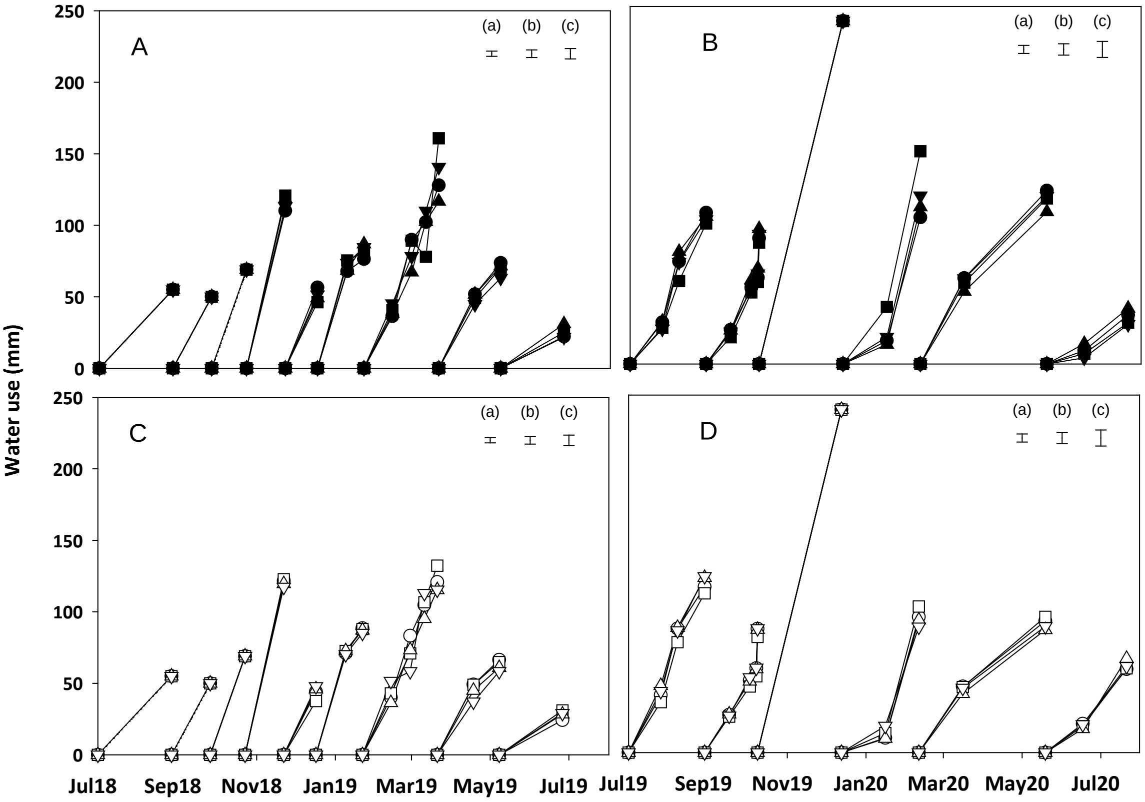


Figure 5-5 Water Use (mm) per regrowth cycle for cocksfoot (■,□), tall fescue(▼,▽) brome (●,○), perennial ryegrass(▲,△).sown in monoculture for Ladbrooks, from July-2018 to July-2019 (A,C) and from July-2019 to July-2020 (B,D) with (closed) or without nitrogen fertilisation (open). Standard Error of Means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

In Year 6, there were no restrictions to measurements due to the water table. In contrast to Year 5, the WU estimated from PET_p and the measured values were equal for the first three regrowth cycles so the measured values were considered valid. The WU was not different among species ($P=0.924$) or nitrogen ($P=0.739$) treatments and averaged 114 ± 3.9 mm per regrowth cycle. The mature third regrowth cycle (09/10-13/12/2019), had the highest ($P<0.05$) WU of 240 ± 3.9 mm, and sixth ending on 21/07/2020 had the lowest at 42 ± 3.9 mm. In this year the total water use was 691 mm (Table 5-10).

Year 5 (2018/2019)

Table 5-9 Water use (mm) for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, fertilised with nitrogen (N+) or not (N-), at Ladbrooks, Canterbury, New Zealand from July 2018 to July 2019.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
27/08/2018*	55	55	55	55	55	55	55	55	55 e
26/09/2018*	50	50	50	50	50	50	50	50	50 e
23/10/2018*	69	69	69	69	69	69	69	69	69 d
22/11/2018	110	121	119	113	121	123	120	117	118 b
17/12/2018	57	46	49	51	44	38	46	48	47 e
22/01/2019	76	81	87	84	88	88	87	85	84 c
21/03/2019	128	161	117	141	120	132	115	115	128 a
09/05/2019	74	70	72	63	66	65	61	63	67 d
28/06/2019	48	38	56	47	50	56	53	54	50 e
Mean Yearly N	676				660				
	BR		CF		PR		TF		Mean
Mean Yearly S	665		683		665		665		668

	P-value	SEM	LSD
S	0.248	6.8	-
N	0.288	7.7	-
S*N	0.174	10.8	-

Note: “BR” stands for brome, “CF” stands for cocksfoot, “PR” stands for perennial ryegrass, “TF” stands for tall fescue. “SEM” is the Standard Error of Means and “LSD” is the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the $\alpha = 0.05$ level). Regrowth cycles marked with “*” had PET_p considered as WU.

Year 6 (2019/2020)

Table 5-10 Water use (mm) by brome, cocksfoot, perennial ryegrass, tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen fertilisation at Ladbrooks, Canterbury, New Zealand, from July 2019 to July 2020.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
29/08/2019	106	98	103	101	116	111	122	123	110 b
09/10/2019	88	85	95	91	87	81	86	87	87 c
13/12/2019	240	240	240	239	239	238	239	238	240 a
11/02/2020	102	149	110	118	95	102	93	88	107 b
19/05/2020	121	115	99	117	92	95	86	88	102 b
21/07/2020	34	32	39	17	58	59	66	60	42 d
Mean Yearly N	695				688				
	BR		CF		PR		TF		Mean
Mean Yearly S	689		702		689		684		691
			P-value		SEM			LSD	
S			0.924		7.1			-	
N			0.769		5.2			-	
S*N			0.903		10.3			-	

Note: "BR" stands for brome, "CF" stands for cocksfoot, "PR" stands for perennial ryegrass, "TF" stands for tall fescue. "SEM" is the Standard Error of Means and "LSD" is the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Experiment 2 - Ashley Dene

The water use at Ashley Dene in Year 5 was unable to be calculated from neutron probe readings for the first four regrowth cycles and the last one (Table 5-11) due to the high water table. In these cases WU was estimated as equivalent to PET_p based on the assumptions of an actively growing green pasture with full canopy (Pronger *et al.*, 2019).

The WU was not different among species (P=0.720) or nitrogen (P=0.152) treatments (Table 5-11). The regrowth cycle harvested on 17/05/2019 used the greatest amount of water (149 mm) compared with <42 mm for the three Spring harvests in 2018 (Figure 5-6).

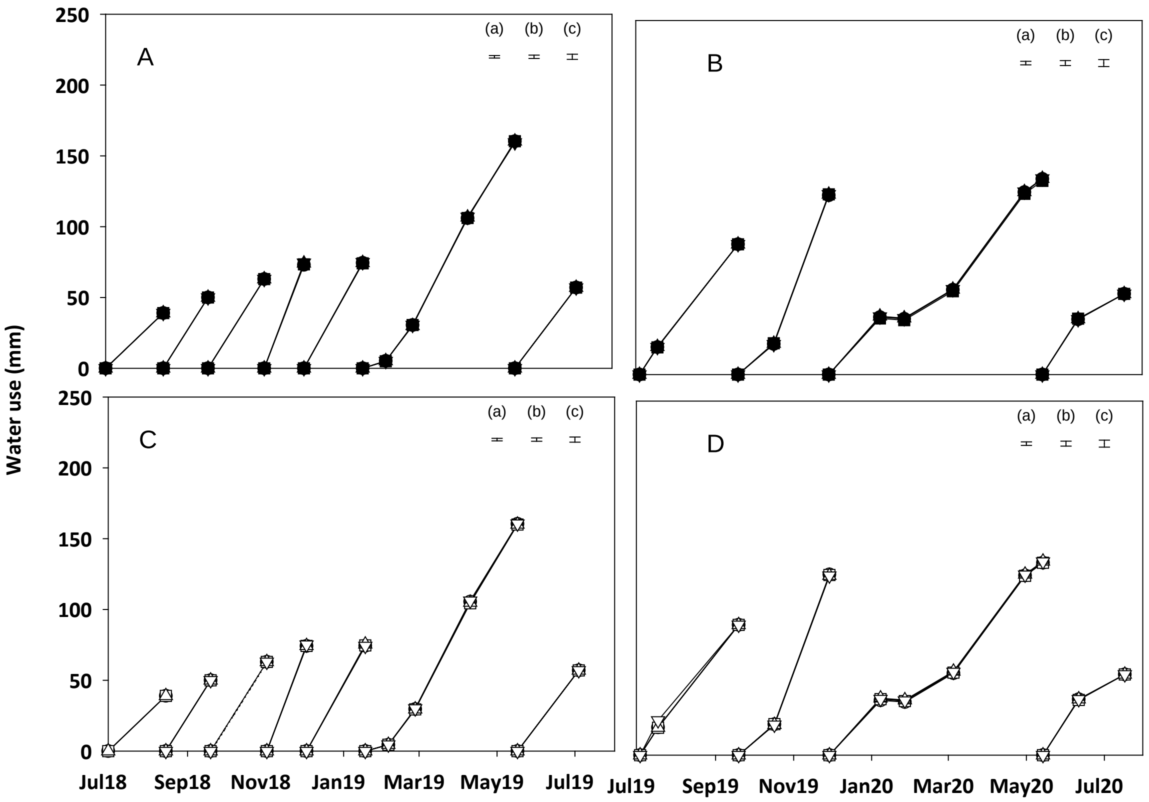


Figure 5-6 Water use (mm) per regrowth cycle for cocksfoot (■,□), tall fescue (▼,▽) brome (●,○), perennial ryegrass(▲,△) sown in monoculture at Ashley Dene from July-2018 to July-2019 (A,C) and from July-2019 to July-2020 (B,D) for all the species with (closed) or without nitrogen fertilisation (open).

In Year 6, the regrowth cycle harvested on 19/09/2019 had PET_p considered as WU because neutron probe readings were under the influence of the water table.

There was no difference in the water use per species (P=0.863) or nitrogen treatment (P=0.093) but there was a difference (P<0.001) in WU across regrowth cycles (Table 5-12). The last regrowth cycle had the lowest water use of 57 ± 4.3 mm for the period from 04/07 to 19/09/2019.

Year 5 (2018/2019)

Table 5-11 Water Use (mm) for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, fertilised with nitrogen (N+) or not (N-), at Ashley Dene, Canterbury, New Zealand from July 2018 to July 2019.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
15/08/18*	39	39	39	39	39	39	39	39	39 g
19/09/18*	50	50	50	50	50	50	50	50	49 f
02/11/18*	63	63	63	63	63	63	63	63	63 d
03/12/18*	73	73	73	73	73	73	73	73	73 c
18/01/19	86	86	86	85	86	86	85	86	86 b
17/05/19	149	149	149	150	149	149	149	148	149 a
04/07/19*	46	46	46	46	46	46	46	46	46 e
Mean Yearly N	506				506				
	BR		CF		PR		TF		Mean
Mean Yearly S	506		506		506		506		506
			P-value		SEM			LSD	
S			0.946		0.06			-	
N			0.926		0.02			-	
S*N			0.857		0.03			-	

Note: "BR" stands for brome, "CF" stands for cocksfoot, "PR" stands for perennial ryegrass, "TF" stands for tall fescue. "SEM" is the Standard Error of Means and "LSD" is the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level). Regrowth cycles marked with "*" had PET_p considered as WU.

Year 6 (2019/2020)

Table 5-12 Water Use (mm) for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, fertilised with nitrogen (N+) or not (N-), at Ashley Dene, Canterbury, New Zealand from July 2019 to July 2020.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
19/09/2019*	92	92	92	92	92	92	92	92	92 b
29/11/2019	127	127	127	127	127	127	127	127	127 c
14/05/2020	138	137	138	138	139	136	137	137	137 a
17/07/2020*	57	57	56	55	58	59	57	58	57 c
Mean Yearly N	414				414				
	BR		CF		PR		TF		Mean
Mean Yearly S	414		414		414		414		414
			P-value		SEM			LSD	
S			0.989		0.04			-	
N			0.907		0.01			-	
S*N			0.934		0.06			-	

Note: "BR" stands for brome, "CF" stands for cocksfoot, "PR" stands for perennial ryegrass, "TF" stands for tall fescue. "SEM" is the Standard Error of Means and "LSD" is the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level). Regrowth cycles marked with "*" had PET_p considered as WU.

5.3.4 Yearly Water Use Efficiency (WUE)

Experiment 1 - Ladbrooks

Year 5 (2018/2019)

Water use efficiency was not different among species (P=0.078) with mean a of 16.8 ± 1.96 kg DM ha^{-1} mm^{-1} (Table 5-13) but was 8.9 compared with 24.3 kg DM ha^{-1} mm^{-1} for N- and N+ treatments, respectively (Figure 5-7).

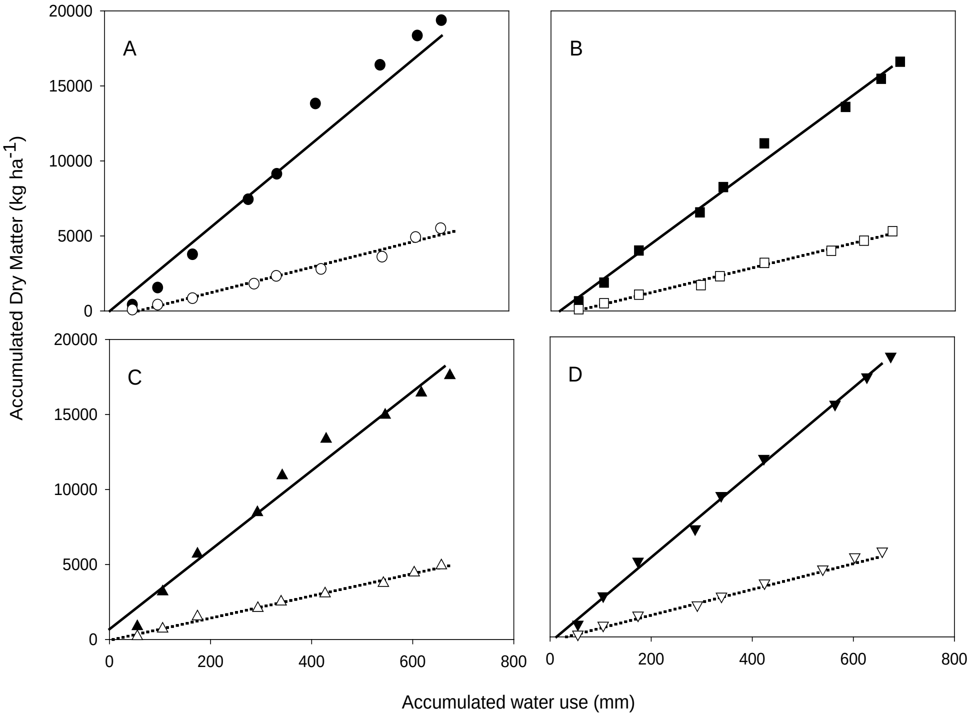


Figure 5-7 Accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by N+ (closed) and N- (open) monocultures of Brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) in 2018/2019 in Ladbrooks, Canterbury, New Zealand. Regression equation details for N+ and N- pastures are reported in Table 5-13.

Table 5-13 Regression equations for accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand.

Species	Equation (Y=)	SE Coef (x)	SE Coef	R^2	
Nitrogen level		N+			Mean S
Brome	27.04x-329	1.504	623	0.98	20.47
Cocksfoot	24.79x-442	0.993	430	0.98	16.52
P. ryegrass	26.42x+691	1.584	662	0.97	16.91
Tall fescue	28.29x-357	0.719	301	0.99	18.69
Mean	26.63				
Nitrogen level		N-			
Brome	8.52x-576	0.513	214	0.97	
Cocksfoot	8.25x-405	0.387	122	0.98	
P. ryegrass	7.40x-49	0.298	122	0.98	
Tall fescue	9.08x-289	0.551	226	0.98	
Mean	8.31			0.98	

	P-value	SEM	LSD
S	0.078	1.021	-
N	<0.001	0.563	2.253
N*S	0.059	1.299	-

Note: “SE Coef” stands for Standard Error of the Coefficient. “S” represents Species.

Year 6 (2019/2020)

In Year 6 at Ladbrooks, WUE was also not different among species (P=0.386) with a mean of 25.1 ± 1.56 kg DM ha^{-1} mm^{-1}. Nitrogen increased (P<0.001) it from 13.3 to 36.9 ± 0.92 kg DM ha^{-1} mm^{-1}, (Table 5-14 and Figure 5-8).

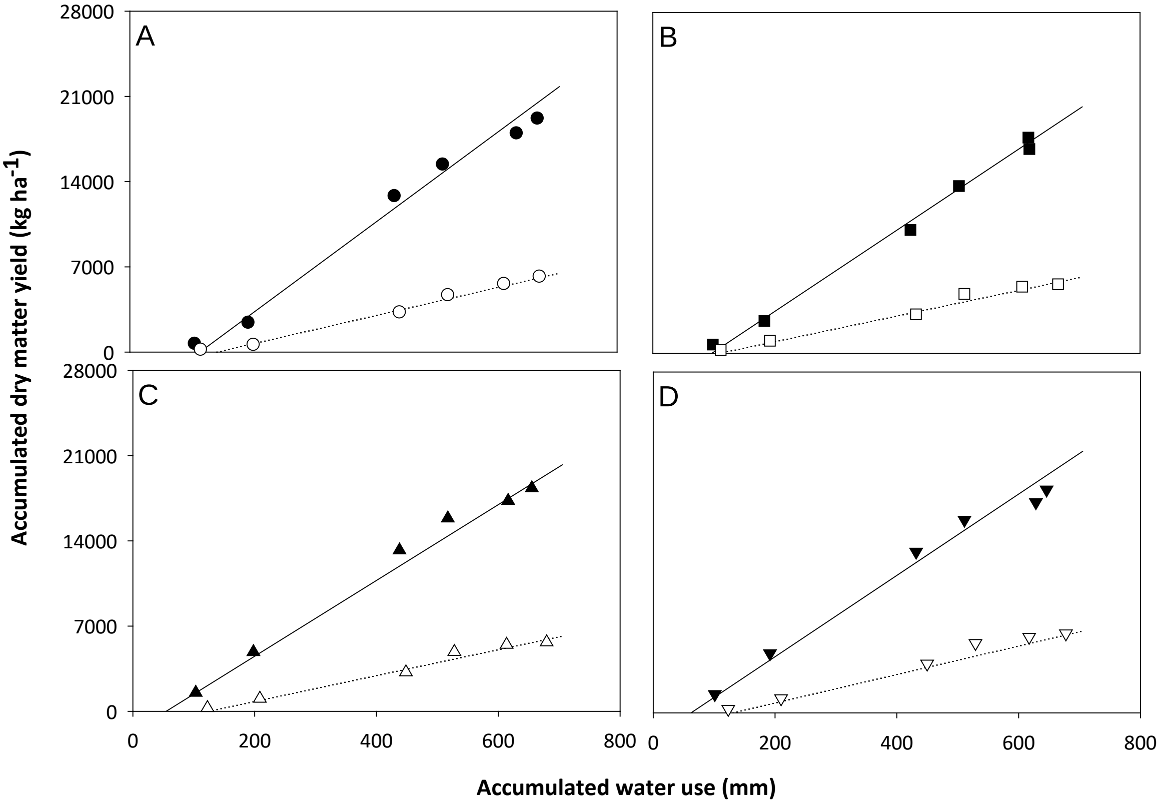


Figure 5-8 Accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by N+ (closed) and N- (open) monocultures of Brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) in 2019/2020 in Ladbrooks, Canterbury, New Zealand. Regression equation details for N+ and N- pastures are reported in Table 5-14.

Table 5-14 Regression equations for accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand.

Species	Equation (Y=)	SE Coef (x)	SE Coef	R^2	Mean S
Nitrogen level		N+			
Brome	41.3x-4238	1.65	695	0.90	27.5
Cocksfoot	35.7x-3213	1.29	572	0.93	24
P. ryegrass	37.2x-1695	1.40	620	0.90	24.8
Tall fescue	33.4x-2067	1.01	426	0.95	24
Mean	36.9				
Nitrogen level		N-			
Brome	13.7x-1670	0.72	290	0.89	
Cocksfoot	12.3x-1141	0.83	334	0.85	
P. ryegrass	12.5x-1327	0.75	289	0.85	
Tall fescue	14.6x-1531	0.68	316	0.90	
Mean	13.3				

	P-Value	SEM	LSD
S	0.386	1.6	-
N	<0.001	0.9	4.16
N*S	0.263	2.1	-

Note: ”SE Coef” stands for Standard Error of the Coefficient. “S” represents Species.

Experiment 2 - Ashley Dene

Year 5 (2018/2019)

In Year 5, WUE was not different (P=0.675) among species (Table 5-15) and averaged 15.1 ± 9.95 kg DM mm^{-1} ha^{-1}. Nitrogen increased (P=0.004), WUE from 11.8 to 18.4 ± 0.59 kg DM mm^{-1} ha^{-1} (Figure 5-9).

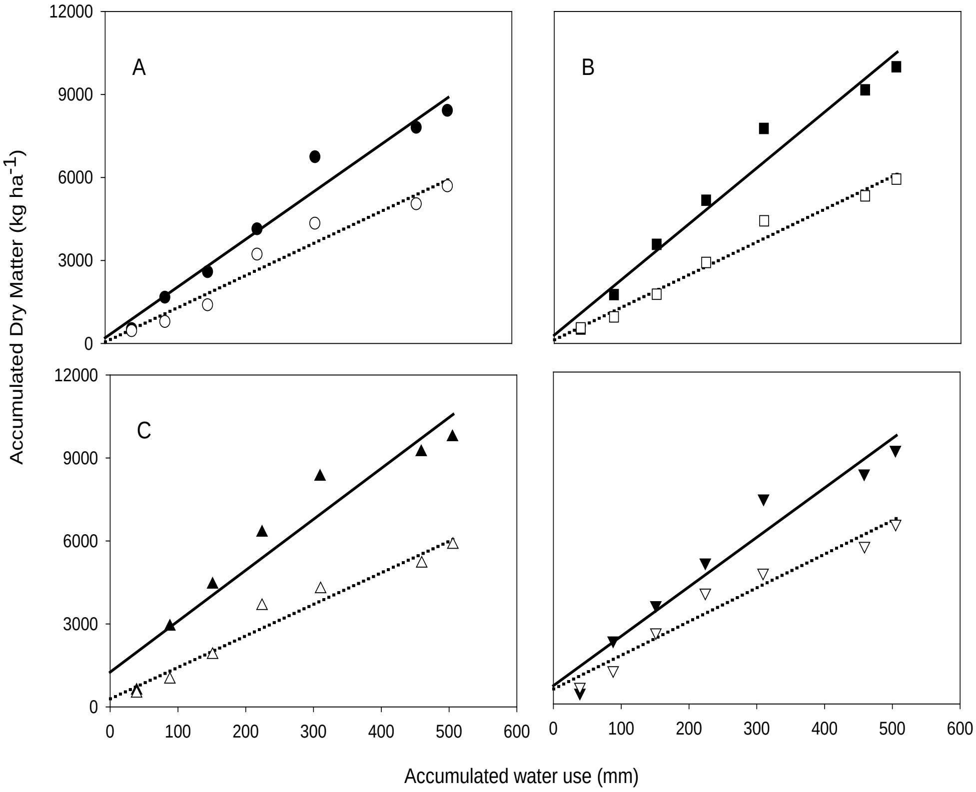


Figure 5-9 Accumulated yield (kg ha^{-1}) against accumulated water use (mm) by nitrogen fertilised N+ (closed) and non-fertilised N-(open) monocultures of Brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) in 2018/2019 in Ashley Dene, Canterbury, New Zealand. Regression equation details for N+ and N- pastures are reported in Table 5-15.

Table 5-15 Regression equations for accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2018 to July 2019 at Ashley Dene, Canterbury, New Zealand.

Species (S)	Equation	SE	SE Coef	R^2	
Nitrogen			N+		Mean S
Brome	17.17x+205	1.452	440	0.96	14.38
Cocksfoot	20.22x+301	1.655	501	0.97	16.03
P. ryegrass	18.42x+1255	2.490	755	0.91	14.91
Tall fescue	17.87x+661	1.884	571	0.95	15.03
Mean	18.42				
Nitrogen			N-		
Brome	11.60x+52	1.164	353	0.94	
Cocksfoot	11.84x+130	0.860	261	0.97	
P. ryegrass	11.40x+289	1.170	355	0.95	
Tall fescue	12.19x+541	1.153	385	0.95	
Mean	11.76				
	P-Value		SEM		LSD
S	0.675		0.948		-
N	0.004		0.591		2.659
S*N	0.634		1.270		-

Note: ”SE Coef” stands for Standard Error of the Coefficient. “S” represents Species.

Year 6 (2019/2020)

In Year 6 the WUE differed (P=0.045) among species (Table 5-10). Brome, cocksfoot and ryegrass had a higher WUE, 19.2 ± 1.39 kg DM mm^{-1} ha^{-1} than tall fescue 13.5 ± 1.39 kg DM mm^{-1} ha^{-1}. However, WUE was unaffected (P=0.685) by N application (Figure 5-10).

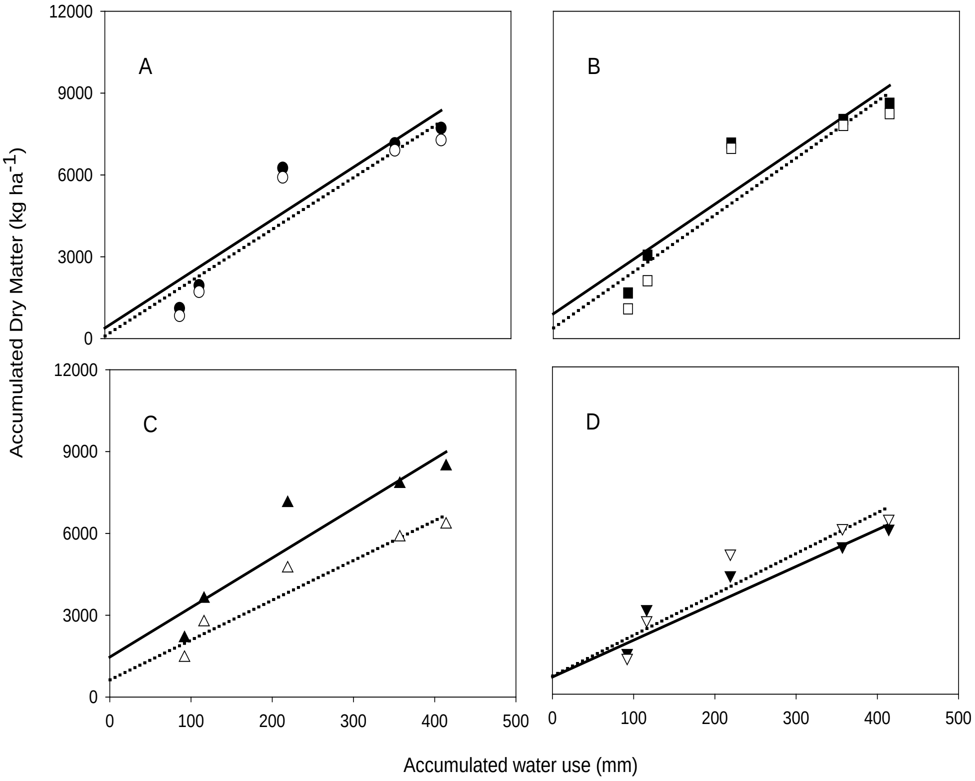


Figure 5-10 Accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by nitrogen fertilised N+ (closed) and non-fertilised N- (open) monocultures of Brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) from July 2019 to July 2020 in Ashley Dene, Canterbury, New Zealand. Regression equation details for N+ and N- pastures are reported in Table 5-16.

Table 5-16 Regression equations for accumulated yield (kg ha^{-1}) against accumulated water use (mm) by fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue in from July 2019 to July 2020 at Ashley Dene, Canterbury, New Zealand.

Species	Equation	SE Coef	SE Coef	R^2	
Nitrogen		N+			Mean S
Brome	19.26x+386	5.83	1737	0.85	19.11
Cocksfoot	20.21x+908	6.32	1881	0.84	20.54
P. ryegrass	18.17x+1466	5.70	1698	0.84	16.39
Tall fescue	13.51x+628	2.45	729	0.94	14.25
Mean	17.79				
Nitrogen		N-			
Brome	18.99x+95	5.80	1730	0.85	
Cocksfoot	20.86x+388	7.01	2090	0.82	
P. ryegrass	14.61x+628	3.11	927	0.91	
Tall fescue	14.98x+666	4.16	1240	0.85	
Mean	17.36				
	P-Value		SEM		LSD
S	0.045		1.39		4.47
N	0.685		0.68		-
S*N	0.597		1.82		-

Note: ”SE Coef” stands for Standard Error of the Coefficient. “S” represents Species.

5.3.5 Water use efficiency (WUE) per regrowth cycle

Experiment 1 - Ladbrooks

WUE differed (P=0.003) with regrowth cycle (Table 5-17). The regrowth cycles harvested on 17/12/18 and 22/01/18 had the highest WUE (Figure 5-11) with a mean value of 26.7 ± 2.6 kg DM mm^{-1} ha^{-1} (Table 5-17). The regrowth cycles harvested on 17/08/2018 and 21/03/2019 had the lowest WUE of 10.1 ± 2.6 kg DM mm^{-1} ha^{-1}. Nitrogen increased (P<0.001) WUE 2.3 times.

In Year 6 at Ladbrooks, regrowth cycles that took part in Spring or close to it, harvested from 09/10/2019 to 11/02/2020 differed from the first one this year, and had higher (P<0.001) values (Table 5-18).

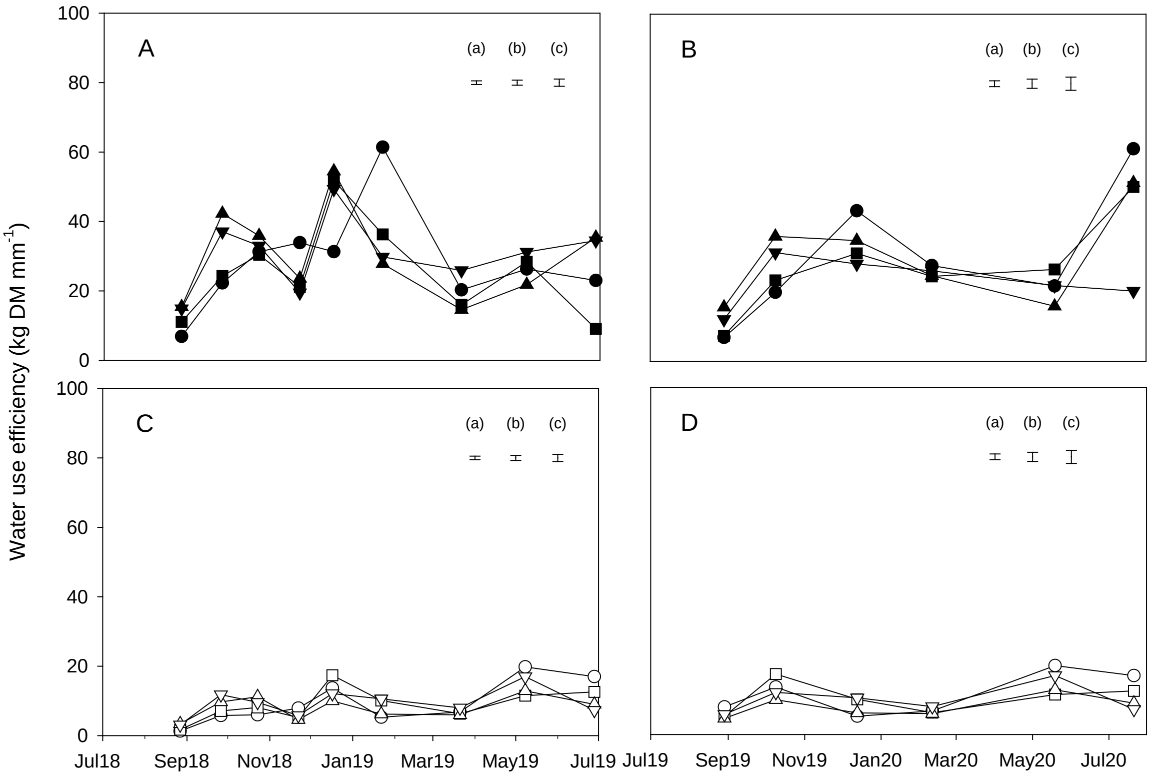


Figure 5-11 Water use efficiency (kg ha^{-1} mm^{-1}) per regrowth cycle for cocksfoot (■,□), tall fescue(▼,▽), brome(●,○), perennial ryegrass(▲,△) sown in monoculture at Ladbrooks from July-2018 to July-2019 (A,C) and from July-2019 to July-2020 (B,D) with (closed) or without nitrogen fertilisation (open). Standard Errors of Means are represented by (a) for Nitrogen, (b) for Species and (c) for Nitrogen*Species.

Year 5 (2018/2019)

Table 5-17 Water Use Efficiency (kg DM mm^{-1} ha^{-1}) for brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) or without (N-) nitrogen application, from July 2018 to July 2019 at Ladbrooks,Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
27/08/2018*	6.9	11.1	15.4	14.9	1.3	1.7	3.4	3.1	7.2 e
26/09/2018*	22.3	24.3	42.3	37.1	5.8	7.1	9.7	11.8	20.0 bd
23/10/2018*	31.3	30.4	35.9	33.0	6.0	8.1	11.2	9.6	20.7 b
22/11/2018	33.9	21.2	23.6	19.5	7.9	5.2	4.6	5.8	15.2 c
17/12/2018	31.3	51.8	54.5	49.3	13.7	17.4	10.0	12.1	30.0 a
22/01/2019	61.4	36.3	27.8	29.8	5.3	10.1	6.2	10.6	23.4 ab
21/03/2019	20.3	16.0	14.6	25.9	6.8	6.3	6.0	8.0	13.0 de
09/05/2019	26.3	28.4	21.8	31.2	19.8	11.5	12.9	17.0	21.1 bc
28/06/2019	23.0	9.1	35.4	34.5	17.0	12.6	9.0	7.3	18.5 bc

Mean Yearly N	28.8		8.9		
	BR	CF	PR	TF	Mean
Mean Yearly S	18.9	17.1	19.1	20.0	18.8

	P-value	SEM	LSD
S	0.397	1.11	-
N	<0.001	0.78	2.30
S*N	0.355	1.56	-

Note: “BR” stands for brome, “CF” stands for cocksfoot, “PR” stands for perennial ryegrass, “TF” stands for tall fescue. “SEM” is the Standard Error of Means and “LSD” is the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level). Regrowth cycles marked with “*”had PET_p considered as WU.

Year 6 (2019/2020)

Table 5-18 Water Use Efficiency (kg DM mm^{-1} ha^{-1}) for brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without nitrogen (N-), from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
29/08/2019	6.9	7.4	15.6	12.1	2.0	2.4	2.3	2.6	6.4 b
09/10/2019	19.9	23.4	36.0	31.4	5.0	9.8	10.6	10.3	18.3 a
13/12/2019	43.4	31.1	34.8	28.1	11.1	9.0	8.4	11.9	22.2 a
11/02/2020	27.6	24.5	24.7	26.1	15.0	16.4	15.5	20.4	21.3 a
19/05/2020	21.7	26.6	15.9	21.9	10.9	6.6	10.5	6.1	15.0 ab
21/07/2020	61.2	50.2	51.4	20.3	11.3	5.0	8.6	4.8	26.6 a

	N+	N-
Mean Yearly N	27.6	9.0

	BR	CF	PR	TF	Mean
Mean Yearly S	19.7	17.7	19.5	16.3	18.3

	P-value	SEM	LSD
S	0.786	2.68	-
N	<0.001	1.90	5.58
S*N	0.788	3.80	-

Note: “BR” stands for brome, “CF” stands for cocksfoot, “PR” stands for perennial ryegrass, “TF” stands for tall fescue. “SEM” is the Standard Error of Means and “LSD” is the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Experiment 2 - Ashley Dene

WUE for the regrowth cycles with the highest value (21 ± 0.95 kg DM mm^{-1} ha^{-1}, 15/08/2018, 03/12/2018, 18/01/2019) was more than three times (6.5 ± 0.95 kg DM mm^{-1} ha^{-1}) the value of WUE of the cycle (17/05/2019) with the lowest value (Figure 5-12).

In Year 6, a difference (P<0.001) was observed between regrowth cycles (Table 5-19). The regrowth cycle harvested on 29/11/19 had the highest WUE of 35.9 ± 1.26 kg DM mm^{-1} ha^{-1}. This was five times higher than the WUE of the regrowth cycle harvested on 14/05/20, 6.8 ± 1.26 kg DM mm^{-1} ha^{-1} (Figure 5-12).

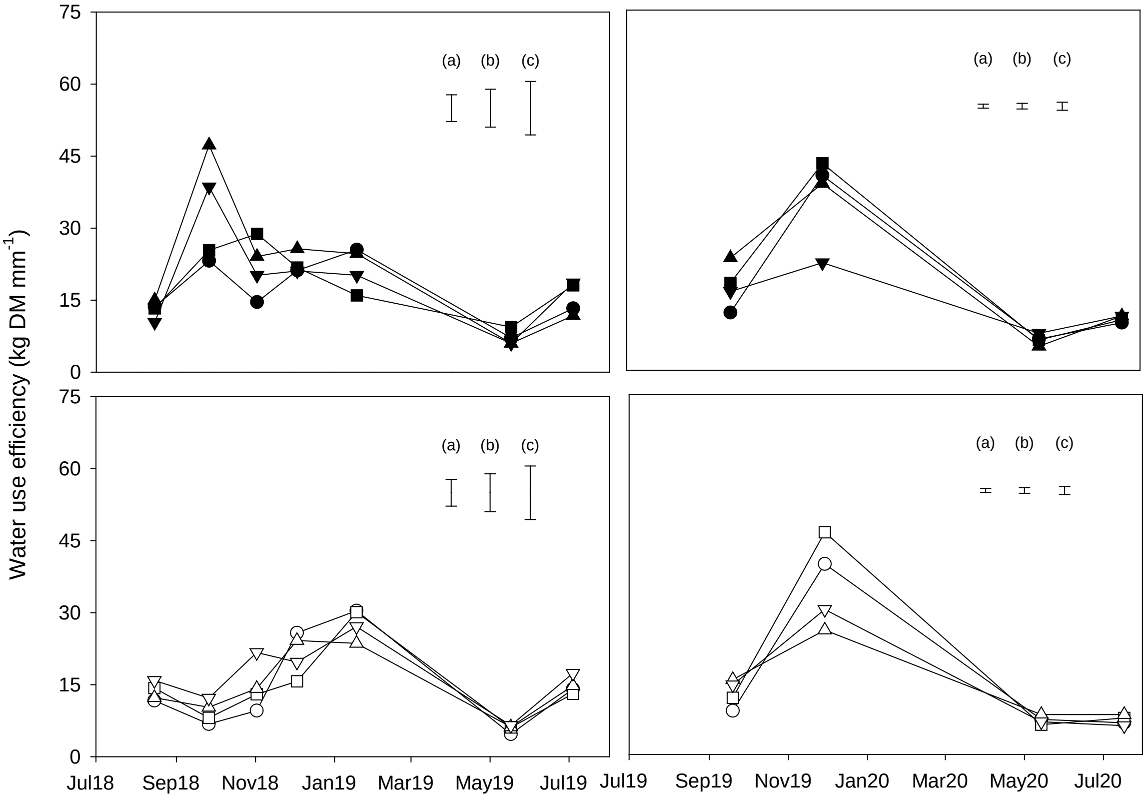


Figure 5-12 Water Use Efficiency (kg ha^{-1} mm^{-1}) per regrowth cycle for cocksfoot (■,□), tall fescue(▼,▽), brome (●,○), perennial ryegrass (▲,△) with (closed) or without nitrogen fertilisation (open), from July-2018 to July-2019 (A,C) and from July-2019 to July-2020 (B,D), sown in monoculture at Ashley Dene, Canterbury, New Zealand Standard Errors of Means are represented by (a) for Nitrogen, (b) for Species and (c) for Nitrogen*Species.

Year 5 (2018/2019)

Table 5-19 Water use efficiency (kg DM mm^{-1} ha^{-1}) of monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) or without (N-) nitrogen, from July-2018 to July 2019 at Ashley Dene, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
15/08/18*	13.6	13.3	15.0	10.4	11.7	14.3	12.3	15.9	13.3 c
26/09/18*	23.2	25.4	47.3	38.6	6.8	8.1	10.3	12.2	21.5 a
02/11/18*	14.6	28.8	24.1	20.2	9.6	13.0	14.2	21.7	18.3 b
03/12/2018	21.2	21.8	25.7	21.1	25.8	15.7	24.2	19.7	21.8 a
18/01/2019	25.5	16.0	24.7	20.2	30.4	30.1	23.6	27.1	19.7 ab
17/05/2019	7.1	9.4	6.0	6.0	4.7	6.1	6.2	6.5	6.5 d
04/07/2019*	13.3	18.1	11.8	18.6	14.1	13.1	14.8	17.4	15.1 c

	N+	N-
Mean Yearly N	20.2	13.0

	BR	CF	PR	TF	Mean
Mean Yearly S	14.9	16.8	17.3	17.4	16.6

	P-value	SEM	LSD
S	0.148	0.82	-
N	<0.001	0.58	1.72
S*N	0.303	1.17	-

Note: “BR” stands for brome, “CF” stands for cocksfoot, “PR” stands for perennial ryegrass, “TF” stands for tall fescue. “SEM” is the Standard Error of Means and “LSD” is the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the $\alpha = 0.05$ level). Regrowth cycles marked with “*” had PET_p considered as WU.

Year 6 (2018/2019)

Table 5-20 Water use efficiency (kg DM mm^{-1} ha^{-1}) of brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) or without (N-) nitrogen, from July-2019 to July 2020 at Ashley Dene, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
19/09/2019*	12.1	18.2	23.4	16.4	9.1	11.8	15.6	14.5	15.1 b
29/11/2019	40.6	43.1	38.9	22.4	39.7	46.3	25.9	30.2	35.9 a
14/05/2020	6.5	6.3	5.0	7.7	7.3	6.2	8.3	6.8	6.8 c
17/07/2020*	9.9	10.5	11.3	11.3	6.6	7.6	8.3	6.0	8.9 c

	N+	N-
Mean Yearly N	18.2	16.0

	BR	CF	PR	TF	Mean
Mean Yearly S	16.4	19.2	17.9	14.9	17.1

	P-value	SEM	LSD
S	0.084	1.13	-
N	0.075	0.80	-
S*N	0.443	1.60	-

Note: “BR” stands for brome, “CF” stands for cocksfoot, “PR” stands for perennial ryegrass, “TF” stands for tall fescue. “SEM” is the Standard Error of Means and “LSD” is the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the $\alpha = 0.05$ level). Regrowth cycles marked with “*” had PET_p considered as WU.

5.4 DISCUSSION

Chapter 4 demonstrated that nitrogen fertiliser increased pasture yields particularly in Spring. The differences in yield indicates differences in either resource (water and light) capture and their efficiency of use by the pastures. Specifically, yields at Ladbrooks, were double those at Ashley Dene and the fertilised pastures produced twice as much as the unfertilised. In Chapter 5 the aim was to investigate these differences in TDM production and relate them to water availability and its efficiency of use. To achieve this, the amount of plant available water content (PAWC) was quantified to compare sites, nitrogen and species effects (Objective 3). This included quantification of the actual soil moisture deficit from each treatment (Objective 4) from NIWA calculations of evapotranspiration interpolated between neutron probe measurements. Thus this chapter also quantifies differences in water extraction and water use efficiency to meet Objective 5.

5.4.1 Plant available water content and soil moisture deficit

The two sites used in this experiment were chosen because they have soils of different soil water holding capacity (Figure 5-2 and Figure 5-4). The Wakanui silt loam at Ladbrooks was expected to hold more water than the Lismore stony silt loam at Ashley Dene (Section 3.2.3). The results from this chapter confirm and quantify these differences. For example, the total plant available water content at Ladbrooks was estimated to be 167 mm (Table 5-2) compared with 118 mm at Ashley Dene (Table 5-4). Differences in plant available water are due to the texture and depth of the soil. Soil texture determines both the DUL and LL (Ratliff, 1983). The finer particle content in each layer at Ladbrooks resulted in higher DUL values than at Ashley Dene. For example the mean DUL was 37 mm at Ladbrooks compared with 24 mm at Ashley Dene (Figure 5-1). Importantly the fine textured soil at Ladbrooks was shown to hold more water in each layer than the stoney profile at Ashley. This is shown by the difference between DUL and LL in each layer. For example at 0.8 m depth the PAWC (difference between DUL and LL) was 16 mm at Ladrooks with no N fertiliser compared with 10 mm at Ashley Dene.

The PAWC was also affected by the addition of nitrogen. At Ladbrooks, the fertilised plantss had ~42% more PAWC than non-fertilised ones (from 138 to 197 mm, in fertilised plants plots, Table 5-2). This implies in greater water extraction at each soil layer which suggests a denser root system, that allowed the pastures to extract more water in every layer of soil (Skinner and Comas, 2010). The implication was that these well watered grasses had sufficient carbon available to them to fully explore the soil profile (Moot *et al.* 2021).

In contrast, at Ashley Dene, the PAWC difference between nitrogen treatments was only 10% (Table 5-4). This may have been caused by the compacted nature of the stones in the soil which physically restricted root growth (Harrison *et al.*, 1994). Also, roots at Ashley Dene only showed water extraction to a depth of 0.99 m (Table 5-3) compared with 1.61 m at Ladbrooks (Table 5-1). Thus, the neutron probe results have quantifed the expected differences in plant available soil water between these sites. The additional PAWC from N fertilised pastures highlights greater resource (water) capture from the same soil profile and which contributes to the higher pasture yield (Section 5.3.1).

The temporal patterns of calculated actual soil moisture deficit (ASMD) also different between sites (Figure 5-2 and Figure 5-3). The ASMD at Ladbrooks never reached <86% of the total PAWC. This means that, at any given moment, some water in the soil profile was available for the plants (especially from deep layers). In contrast, at Ashley Dene the ASMD reached almost 100% of the PAWC during February in both Years 5 and 6 (Figure 5-3). The greater stress at Ashley was caused by the lower PAWC, which means the actual evapotranspiration (PET_a) diverged from potential evapotranspiration (PET_p) earlier in the season as the soil water dries (Appendix M). In addition, rainfall differed but PET_p was consistent between the sites which are ~25 km apart. This highlights the need to measure rainfall on site. At Ladbrooks rainfall was 80 mm higher in Year 5 and 300 mm higher in Year 6 than at Ashley Dene (Figure 5-2 and Figure 5-3). Thus, the amount of water available from rainfall was higher at Ladbrooks which also delayed the onset of the stress factor when calculating soil water extraction (Appendix M).

At Ladbrooks, in Year 5 (Figure 5-2), until 30/01/2019 (or about 1787 °Cd), the maximum ASMD was 65 mm for all pastures. PET_p increased during summer but was equal to PET_a when the ASMD was ≤ 50% of PAWC. Frequent rainfall events kept the ASMD below the 85 mm expected to start to cause declines in DM accumulation (Sharifiamina, 2018). From early February 2019 (1800 °Cd), when DM accumulation began to reduce (Figure 4-1), especially for N- pastures, the ASMD reached values >100 mm (Figure 5-2). This reduction in yield caused by the water restriction was not alleveiated until June 2019 (3000 °Cd) when the ASMD went back to 40 mm, which would no longer be expected to reduce yields. In Year 6 at the same site, the ASMD reached a maximum of 108 mm for the N- pastures and 88 mm for the N+ ones by late January 2020 (1795 °Cd). From February 2020 on, the ASMD increased to a maximum of 136 mm for non-fertilised pastures and 170 mm to fertilised ones, and the timing of this aligned with observes reductions in DM accumulation (Figure 4-1). The ASMD then slowly reduced from early-May 2020 (2747 °Cd), but it was only reduced to levels that would not affect DM accumulation (<85 mm) in late June 2020 (3066 °Cd), when rainfall of >140 mm occurred over 3 weeks (Figure 3-2).

At Ashley Dene, in Year 5 (Figure 5-3), the ASMD reached a maximum value of 65 mm for the N+ grasses and 74 mm for N- grasses by early January 2019 (1405 °Cd) when a reduction in the temperature adjusted rate of dry matter accumulation was evident (Figure 4-2). From this time, the ASMD increased to a maximum of 106 mm for N+ pastures and 115 mm for N- pastures by early February 2019 (1836 °Cd). At times the ASMD reached 100% of the plot PAWC which meant no growth occurred unitl rainfall reduced the ASMD (Figure 5-3). Thus, sporadic rainfall events (Figure 5-3) led to short growth periods until the water was used, as shown by the weekly rainfall of 20 mm from 20-27/02/2019 (Figure 4-23 and Figure 5-3). When summer soil moisture deficits are very high, such as >100 mm at Ashley Dene, dry matter accumulation rate can be reduced to zero (Lelièvre *et al.*, 2011). Rainfall of 40 mm over 28 days from 24/04/2019 to 22/05/2019 reduced the ASMD to 46 mm for N+ plants and 51 mm to N- ones by early June 2019 (2974 °Cd).

In Year 6, at Ladbrooks, the measured observed deficit at the start of the year, of 63 mm for N+ plants and 67 mm for the N- ones, was observed on 14/07/2018 (88 °Cd) before it was lowered in the wet season, starting with a rainfall of 60 mm from 15-22/07/2018 (143 °Cd). From then, a period of unrestricted growth began (Table 4-47) which lasted until 17/11/2019 (885 °Cd) for the N- plants and 26/11/2019 (985° Cd), when the soil moisture deficit was, respectively, 40 mm and 47 mm, but began to increase rapidly due to the high PET_p. On 02/05/2020 (2760 °Cd), the ASMD reached a value of 141 mm for the N- grasses and 160 mm for the N+ ones, being reduced from now on by rainfall events. The total amount of rainfall that fell from 04/05/2020 (2784°Cd) until 21/07/2020 (3180°Cd) was 240 mm (Figure 5-2).

At Ashley Dene, in Year 6, water was non-limiting until 22/09/2019 (379 °Cd), when the soil moisture deficit quickly increased due to high PET_p (Figure 5-3 and Figure 5-4). It then reduced when rainfall events of >60 mm happened from 11/10/2019 (561 °Cd) for two weeks and >40 mm, in one week, from 08/11/2019 (815 ° Cd). From 29/11/19 (990 °Cd), the pastures remained at >75 mm of ASMD for most of the time (Figure 5-3). This was reflected in a low growth rate (Figure 4-24) until late-May 2020 (2891 °Cd). Recovery for N+ and N- grasses started only after a weekly rainfall that commenced on 15/05/2020 (2868 °C) took place. From this date on, the ASMD was gradually reduced due to successive rainfall events, which amounted to 116 mm until 17/07/2020 (3156 °Cd), combined with a lower PET_a at this time of the year.

5.4.2 Water use and water use efficiency

Restrictions to the use of neutron probe data to calculate the WU occurred in late Winter -early Spring due to high water tables, and waterlogging was seen on some plots especially at Ladbrooks. Water table level assesments were visually done using tube from plot 9 at Ladbrooks (tall fescue N-) and from plot 15 at both sites (cocksfoot N+). The tubes in these plots had an open end which allowed water to infiltrate. They were dried before each measurement but rapidly refilled, from the bottom upwards, with water when the water table was high. Water levels as close as 15 cm to the surface, measured with a ruler, were observed at both sites. Water table levels rise due to receiving more water than being able to drain. At certain parts the Canterbury Plains, the water table rises to 1.2 m deep during Winter, but it may rise up to the surface at other places (Lincoln) where soils shows poor drainage so the water level drops slowly (Duncan *et al.*, 2016).

The impact of the high water table was also shown by the fact that WU in early Spring was lower than PET_p. This is not expected to occur in Spring when there is no or a low soil moisture deficit (Figure 5.4). To correct those results, PET_p, which equals PET_a until soil moisture reaches 50% of the PAWC, was considered as the WU. This was then used to estimate water use efficiency.

The total annual water use was not different amongst species in Year 5, for both sites but water use at Ladbrooks (667 ± 6.3 mm, Table 5-9) was 11% higher than in Ashley Dene (516 ± 2.4 mm, Table 5-11). In Year 6, no difference was observed amongst species or nitrogen treatments at both sites, but water use at Ladbrooks (691 ± 13.4 mm, Table 5.10) was 70% higher than at Ashley Dene (414 ± 2.8 mm, Table 5.12). The difference between the two sites was due to PAWC. This was the result of different maximum extraction depths (60% deeper in Ladbrooks, as shown in Section 5.3.1), higher water extraction from each layer (Figure 5-1) and higher rainfall in both years (10% higher in Year 5, or 566 mm at Ashley Dene and 623 mm at Ladbrooks, and 49% higher in Year 6, or 529 mm at Ashley Dene and 786 mm at Ladbrooks). Previous results from this experiment have also shown differences in annual water use. Sharifiamina (2018) reported a WU of 823 mm for N+ pastures at Ladbrooks. Previous studies at Lincoln, New Zealand, have shown annual water use of over 700 mm on ryegrass/white clover pastures on a Wakanui silt loam soil (Black and Murdoch, 2013). For both sites, the water use was generally higher during warmer seasons of the year (Section 5.3.3), when PET_p is higher.

Nitrogen increased the WUE but only when there was water available. In Year 5, the WUE at Ladbrooks for non-fertilised plants was 8.3 kg DM mm^{-1} ha^{-1} (Table 5-13) and consistent throughout the year (Figure 5-11). However, the addition of N fertiliser almost tripled the annual WUE (Table 5-13 and Figure 5-7) and showed differences through the year. The highest WUE was in Spring when water was available (Table 5-17). In September and October 2018, at Ladbrooks, the WUE of all fertilised grasses was 30 kg DM mm^{-1} ha^{-1}. The high WUE values were observed due to the high dry matter accumulation in this part of the year (Table 4-1). The same was observed in Ashley Dene in this part of the same year, for example, in September 2019 the average WUE was 21.5 kg DM mm^{-1} ha^{-1} (Table 5-19), whilst the regrowth cycle harvested on 17/05/2019 the lowest WUE, at 6.5 kg DM mm^{-1} ha^{-1}. At Ladbrooks, the linear regression Year 5 (Table 5-13 and Figure 5-7) showed WUE was 26.4 kg DM mm^{-1} ha^{-1} for N+ grasses and 8.3 kg DM mm^{-1} ha^{-1} for the N- ones. At Ashley Dene, in Year 5, (Table 5-15 and Figure 5-9), the fertilised grasses had a water use efficiency (18.4 kg DM mm^{-1} ha^{-1}) about 1.5 times higher than the non-fertilised ones (11.8 kg DM mm^{-1} ha^{-1}).

Nitrogen fertilisation, with urea, has previously been shown to increase WUE from 19 to 63% on perenial ryegrass dominated pastures (McKenzie *et al.*, 2006) and about 20% for Ashley Dene in Year 2 of this experiment (Sharifiamina, 2018). When coupled with the results obtained from Ladbrooks this indicates the effect of nitrogen fertiliser on WUE depends on the water availability through the year (Moot *et al.* 2008). A lack of available water for plants, either from a deficit in rainfall and/or irrigation, will lead to lower WUE in pastures except for lucerne (Neal *et al.*, 2011).

The choice of management in Year 6 also had an impact on the WUE. The second regrowth cycle, at both Ladbrooks (Table 5-17) and Ashley Dene (Table 5-18), was harvested when plants were in an advanced reproductive stage. It is possible that carbon and nitrogen were remobilised from the roots, during this period which has previously been reported to increase the Spring WUE of lucerne (Moot *et al.* 2003). To confirm requires sequential digging of roots which was not possible in this experiment. However, the high WUE values at Ladbrooks of 36.9 ± 0.92 kg DM mm^{-1} ha^{-1} for the N+ pastures and 13.3 ± 0.92 kg DM mm^{-1} ha^{-1} for the N- ones suggest additional assimilates in above ground dry matter. This will be investigated further in Chapter 6 when radiation use efficiency (RUE) is calculated. A higher than average RUE during this period would support the idea of remobilization of root reserves. At Ashley Dene, in Year 6, this effect was not visible, and all species had the same WUE (17.6 ± 0.73 kg DM mm^{-1} ha^{-1}).

Water use efficiency is mostly increased by maximising dry matter accumulation during periods when vapour pressure deficit is low (Turner, 2004). This means increasing the dry matter accumulation on regrowth cycles that take place in the Winter. In this study these regrowth cycles had the lowest WUE at both Ladbrooks (27/08/19 and 29/08/19) (Tables 5.17 and 5.18) and Ashley Dene (15/08/18, 17/05/19 and 04/07/19) (Table 5-19 and Table 5-20) took place in the coldest season of the year, and nitrogen had little impact on these regrowth cycles. The implication is that temperature was the major factor limiting growth at this time which reduced water use efficiency. Peri *et al.* (2003) showed that when daily average temperatures were 17.0 °C the net photosynthesic (Pn) rates were 27.5 µmol CO_2 m^{-2} s^{-1} compared with 22 µmol CO_2 m^{-2} s^{-1} when the daily temperatures were 14.6 °C. The average daily temperature in August 2019 was 6.9 °C and 7.0 °C at Ashley Dene, in February 2020 (Pn = 6.5 µmol CO_2 m^{-2} s^{-1}), the average air temperature at Ladbrooks was 16.7 °C and 16.8 °C at Ashley Dene (Pn = 25.1 µmol CO_2 m^{-2} s^{-1}). The temperatures differences alone would imply, from August to February, in a net photosynthetic rate 386% higher at both sites, as suggested by Peri *et al.* (2003). The reduction in photosynthesis rates due to temperature would explain the reduction in water use effiency.

These data shows that being able to prospect water from greater depths or more intensely in each layer can increase plant yield and persistence. However, all four species used in these experiments did not differ in their resource (water) capture or the efficiency with which they used it except cocksfoot on th drier site. At summer dry sites like Ashley Dene, when irrigation is not available, the choice of species that can survive is important.

5.4.2.1 Yield differences within sites and between sites

At Ladbrooks in Year 5, an yield difference of 12715 kg DM ha^{-1} (Table 4-1) from plants that received nitrogen fertilisation (589 kg N ha^{-1} applied) and the ones who relied only in nitrogen from mineralization. In Year 6, at the same site, a difference of 13000 kg DM ha ^{1} (Table 4-2) was observed (405 kg N ha^{-1} applied). At Ashley Dene in Year 5, a difference of 3340 kg DM ha^{-1} (Table 4-3) was observed (467 kg N ha^{-1} applied) and in Year 6 (Table 4-4) the difference between nitrogen treatments was not significant. Those yield differences are not explained by the water use for it was the same for the fertilised and non-fertilised grasses both years at both sites (Table 5-9 to Table 5-12). Nonetheless, the efficiency on how the water resource was used by fertilised and non-fertilised pastures was different. The amount of differences explained by yield, due to WUE using the same resource is 12235 kg ha^{-1} at Ladbrooks in Year 5 and 13000 kg ha^{-1} at Year 6. At Ashley Dene, in Year 5, the WUE difference accounts for 3340 kg ha^{-1}, in Year 6, the differences in yield were non-significant, that means the difference in yield within sites due to nitrogen fertilisation comes mostly from how the resources are being used. As an appropriate level of nitrogen allows the plant to fully expand their canopy and intercept more radiation (Section 2.3.1), as long as increasing the use of this radiation to accumulate dry matter (Peri *et al.*, 2002b), which correlates to the WUE, the yield differences obtained from the nitrogen availability on radiation interceptance/use are explored in Chapter 6.

In Year 5, the fertilised plants had a difference, in yield, between Ladbrooks and Ashley Dene, of 8735 kg DM ha^{-1} (Table 4-1 and Table 4-3). The difference in yield that can be explained by water usage is 5026 kg DM ha^{-1}, from 168 mm of water used more at Ladbrooks and an yearly WUE of 26.63 kg DM mm^{-1} ha^{-1}, and a total of 3709 kg DM ha^{-1} (42%) is still not explained by water usage. In Year 6, the fertilised pastures had a yield difference, between the two sites, of 10385 kg DM ha^{-1}. The yield difference explained by the water usage is 9705 kg DM ha^{-1}, from the 263 mm of water used more at Ladbrooks with an yearly WUE of 36.90 kg DM mm^{-1} ha^{-1}. A total of 680 kg DM ha^{-1} was still not explained by the water usage, meaning that, in Year 6, the difference in the water use was able to explain most of the difference (93%) in yield. To be able to explain more of the differences, in yield, between sites, the impact of light interception and radiation use efficiency are investigated in Chapter 6.

5.5 CONCLUSION

Chapter 5 dealt with Objectives 3, 4 and 5. Based on the obtained results, the conclusions are:

- Differences in the PAWC were observed according to different soil texture and structure and maximum water extraction depth at each site. At Ladbrooks, the PAWC was 167 mm, 41.5% higher than Ashley Dene's, at 118 mm. The PAWC was the same for species , nonetheless, nitrogen increased it at Ladbrooks (42%) and Ashley Dene (12%)
- The ASMD reached more restricted levels at Ashley Dene than at Ladbrooks. At Ashley Dene the deficit reached, in some cases, almost the totality of the PAWC.
- The WU was not affected by nitrogen fertilisation or different species.
- Nitrogen can increase WUE, nonetheless this increase is different through seasons. When water and temperature, are not restrictive, as in the Spring, the increase in WUE due to nitrogen fertilisation is higher, up to 5 times, than in other parts of the year. For non-fertilised pastures, there is little difference through the year.
- Cocksfoot had a higher WUE than the other species at Ashley Dene in Year 6.

The next chapter deals with explaining differences observed in yield by quantifying different levels in radiation interception and radiation use efficiency as caused by nitrogen and water resources availability.

6 LIGHT INTERCEPTION AND RADIATION USE EFFICIENCY OF GRASSES UNDER DRYLAND CONDITIONS

6.1 Introduction

In Chapter 4 differences in yield and quality under the contrasting nitrogen and water regimes were quantified to meet Objective 3. Pastures with N fertiliser had a higher yield then those unfertilised, particularly at Ladbrooks which was confirmed to have a higher PAWC in Chapter 5. The differences in temperature adjusted growth rates from Chapter 4 were also related to the estimated temporal pattern of actual soil moisture deficit to meet Objective 4. This also allowed water use efficiency to be calculated (Objective 5) which partly explained why the fertilised grasses had higher yields.

In this chapter the yield differences found in Chapter 4, not accounted for in Chapter 5, are examined in relation to how different nitrogen and water regimes affected radiation interception (radiation interceptance, or R/Ro) and radiation use efficiency (RUE) to meet Objective 6. Those are components used to describe yield accumulation (Equation 1) on Section 2.1.1.

6.2 Materials and Methods

A full description of management and experimental design was given in the materials and methods of Chapter 3 (Section 3.2). Methods to quantify canopy expansion and radiation interception are detailed below.

6.2.1 Leaf area index (LAI)

To measure LAI at the end of every regrowth cycle, a Sunscan plant canopy analyser (Delta-T Devices Ltd., Burwell, Cambridge, England) was used. To do this a metal channel of 1 x 0.04 x 0.03 m was inserted under the canopy and measured on 3 different occasions (20/11/2020, 30/12/2020 and 29/01/2021). This was when the compressed pasture height, measured with a plate meter (RPM; Filips Ltd, Feilding, New Zealand), was above 4 cm and enabled LAI to be determined from pasture yield and radiation interceptance, with one measurement done per plot. The Sunscan height (3 cm) required at least this level of pasture to cover for leaf area index be measured. Therefore it is less effective with a compressed pasture height lower than 4 cm. Below this the metal channel was not covered with leaves so no light interception and no leaf area index can be measured. Indeed, direct measurement of canopy, with the SunScan, when swards are short is inappropriate (Jonckheere *et al.*, 2004) for the readings are inaccurate, thus getting estimates of LAI pastures is difficult (Mills *et al.*, 2007).

Inserting channels at Ashley Dene was also difficult which did not allow frequent measurements. Therefore, a logarithmic regression between the Sunscan reading and the plate meter readings was created across the range of pasture yields measured (Figure 6-1).

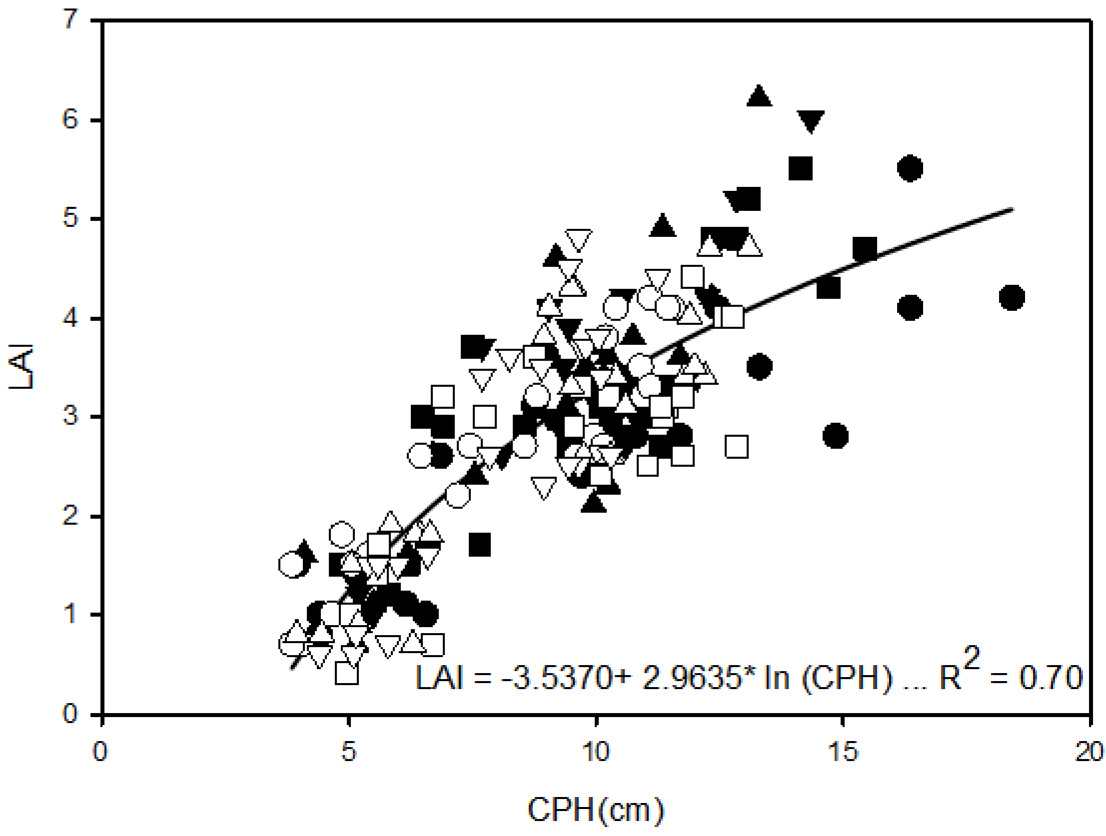


Figure 6-1 Leaf area index (LAI) against compressed pasture height (CPH, cm). Measurements were taken for nitrogen fertilised (closed) and non-fertilised (open) monocultures of brome (●,○), cocksfoot (■,□), perennial ryegrass(▲,△) and tall fescue (▼,▽) at both Ladbrooks and Ashley Dene, Canterbury, New Zealand, from September 2018 to July 2020.

6.2.2 Radiation Interceptance (R/Ro)

A Green Seeker Handheld Crop Sensor (Trimble Agriculture, USA) was also used to estimate the radiation interceptance (R/Ro), on every plot, from November 2018. Readings were taken at 7 to 21 day intervals with the exception of the Coronavirus lockdown period (April to July 2019) which prevented site access.

The Green Seeker records the amount of incident and received radiation in specific wavebands (brief bursts in the red and infrared light) and then measures the amount of each reflected back, by the canopy, to the sensor. A standard height for measurements of 1 m above soil level was used.

Readings were taken, once a day, between 10 a.m. and 4 p.m, and avoided times when dew or rain water was present on the leaves because it interferes with canopy light reflectance.

As plants develop and reach more advanced vegetative stages, their canopy increases in size. This results in more radiation in the visible part of the spectrum (VISI) being absorbed by photosynthesizing pigments and more radiation in the Near Infrared (NIRED) part of the spectrum is reflected due to multiple scattering by leaf tissue (Curran, 1981). Those values differ with species and nitrogen levels because they affect components inside leaves that will affect their colour and reflectance. Therefore, a difference in the reflectance of those two different wavelengths can be used to estimate radiation interceptance. The NDVI (Normalized Difference Vegetation Index) is then calculated to predict the interceptance (Tucker, 1986) using Equation 14:

Equation 14

$$\boldsymbol{NDVI = \frac{(NIRED-VISI)}{(NIRED+VISI)}}$$

In Equation 14, NIRED (774 nm) and VISI (656 nm) are used to determine the NDVI. Equation 14 requires calibration to avoid interference on the readings from the bare soil area. To do this bare soil readings were taken at each site. Their reading ranged from 0.10 to 0.23. A wet soil surface with higher contents of soil organic matter caused the bare soil reading to be higher, which could result in an underestimation of the interceptance, therefore, a small surface of 0.6 m x 0.6 m was kept, 1.5 m aways from Plot 2 at both sites, free of vegetable covering, where baresoil readings were taken with the Green Seeker.

Visual assessments on harvest days confirmed that the canopy never covered 100% of the surface, bare soil was always present so a corrective factor (C = 0.95) was used (Oliveira, 2011), as shown in Equation 15:

Equation 15

$$\boldsymbol{NDVI\ (R/Ro) = C * \frac{(NDVIr-NDVIs)}{(NDVImax-NDVIs)}}$$

Where NDVIr is the measured value for the plot, NDVIs is the bare soil reading, C is the corrective factor and NDVImax is the maximum reading for the plot. NDVImax for each regrowth rotation occurred on harvest day with maximum ground covering. The highest recorded value for each species was at Ladbrooks; 0.85 for brome, 0.88 for cocksfoot, 0.91 for perennial ryegrass and 0.90 for tall fescue. At Ashley Dene, the values were lower at 0.83; 0.85; 0.87 and 0.84, respectively.

Green Seeker readings were taken immediately before and after each plot harvest. The pre-harvest values provided the estimate for cover at the end of a regrowth cycle, while the post-harvest measurements provided the start value for the next regrowth cycle. Measurements were taken from 22/11/20 to 21/07/20 at Ladbrooks and from 03/12/2018 until 17/07/2020 at Ashley Dene.

6.2.3 Estimation of intercepted PAR in each regrowth cycle

To calculate PAR for every regrowth cycle, the amount of intercepted PAR daily had to be quantified. This was obtained from linear regressions between every reading taken, in sequential order, in regrowth cycles, to obtain the daily interceptance, using Equation 16 and Equation 17:

Equation 16

$$\boldsymbol{INTn = n * \frac{INTk - INTk-1}{(DDays)}}$$

Where INTk is the interceptance when the kth green seeker measurement and "INTk-1" is the previous measurement. "n" is the nth day after the day the kth measurement was taken and "DDays" is the difference in the number of days between the kth and the k-1th measurement.

Equation 17

$$\boldsymbol{PAR \ = \sum_{n=1}^{k} DIPARn * INTn}$$

Where "n" is the day when a regrowth cycle begins (the day after a harvest) and k is the harvest day that will end the respective regrowth cycle. DIPARn is the Daily Incident PAR on day “n” and INTn is the cover on day “n”. Daily intercepted PAR was summed up according to regrowth cycles.

6.2.4 Radiation Use Efficiency (RUE)

Radiation Use Efficiency (RUE, g DM m^{-2} MJ^{-1} PAR) is defined as the amount of dry matter (TDM, g m^{-2}) produced in a certain amount of time divided by the amount of PAR (MJ^{-1}) intercepted in that period, as shown in Equation 18. When plotting accumulated dry matter against accumulated PAR, the slope of the regression is the RUE.

Equation 18

$$\boldsymbol{RUE = TDM/PAR}$$

6.3 RESULTS

6.3.1 Leaf area index through seasons.

Experiment 1 - Ladbrooks

In Year 5, the LAI was affected by the interaction (P<0.001) between species and nitrogen. LAI doubled for brome and cocksfoot with applied N, but the increase for ryegrass and tall fescue was lower at 58% Table 6-1 and Figure 6-2).

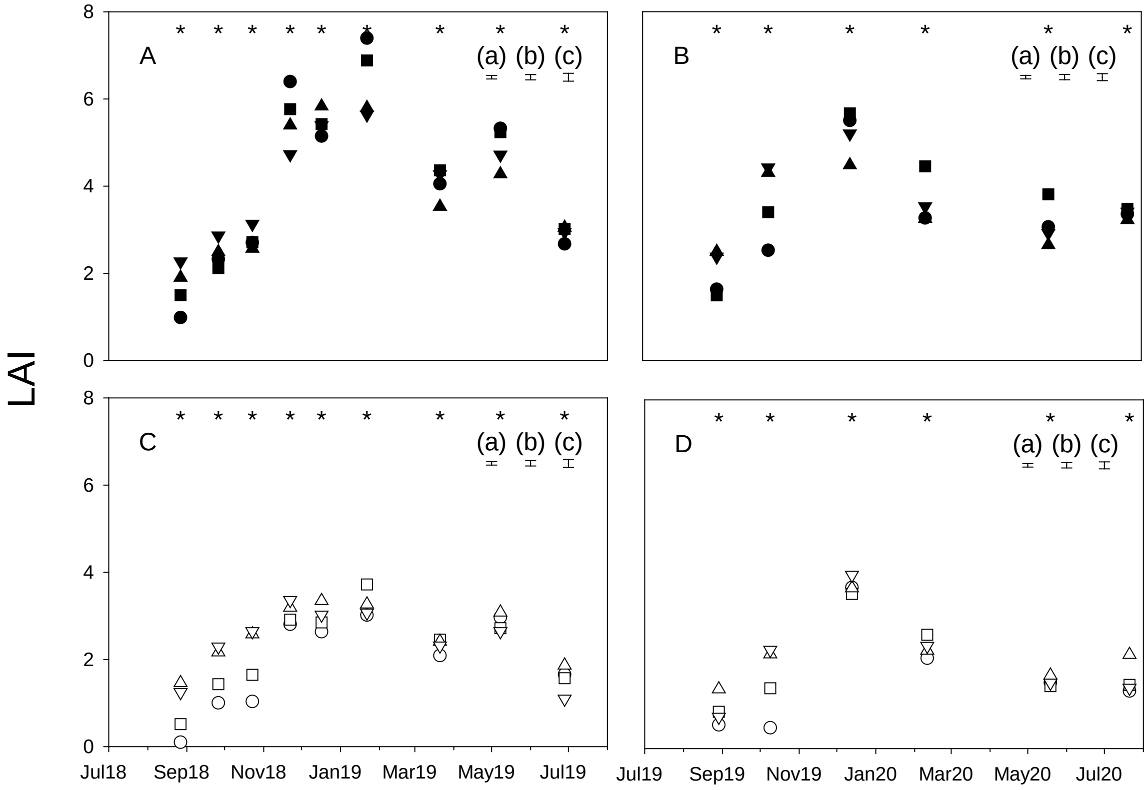


Figure 6-2 Leaf area index (LAI) from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) at Ladbrooks, Canterbury, New Zealand, for every regrowth cycle, for brome (•,○), cocksfoot (■,□), perennial ryegrass(▲,△) and tall fescue (▼,▽) with (closed) (A,B) or without nitrogen fertilisation (open) (B,D)."*" represents when differences were observed for the species. SEMs are represented for (a) nitrogen, (b) species and (c) nitrogen*species. LAI at 95% light interception is 5.07.

LAI was different (P<0.001) in Year 5 and Year 6, between different regrowth cycles. Leaf area expansion, for both years, was quickest at the end of Spring and into summer (Figure 6-2) consistent with the increase in ASMD (Figure 5-2).

The regrowth cycle harvested on 22/01/2019, at Ladbrooks in Year 5, had the highest LAI (P<0.001), 4.8 ± 0.06, which was four times the value of the regrowth cycle with the lowest LAI (27/08/2019), 1.2 ± 0.06, harvested on September 2018.

Table 6-1 Mean leaf area index (LAI), from monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without nitrogen (N-), harvested from July 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
27/08/19	1.0	1.5	1.9	2.3	0.1	0.6	1.4	1.2	1.2 g
26/09/18	2.3	2.1	2.5	2.9	1.0	1.4	2.1	2.3	2.1 f
23/10/18	2.7	2.7	2.6	3.1	1.0	1.6	2.6	2.6	2.4 e
22/11/18	6.4	5.8	5.4	4.7	2.8	2.9	3.2	3.4	4.3 b
17/12/18	5.0	5.4	5.8	5.4	2.6	2.8	3.3	3.0	4.2 b
22/01/19	7.4	6.9	5.8	5.6	3.0	3.7	3.3	3.1	4.8 a
21/03/19	4.0	4.4	3.5	4.3	2.1	2.5	2.4	2.3	3.2 d
09/05/19	5.3	5.2	4.3	4.7	2.9	2.7	3.1	2.6	3.8 c
28/06/19	2.7	3.0	3.0	2.9	1.6	1.5	1.9	1.1	2.2 ef
Mean Yearly N		4.0				2.3			

	BR	CF	PR	TF
Mean Yearly S	3.0	3.1	3.2	3.2
Yearly Mean		3.1		

	P-value	SEM	LSD
S	0.085	0.06	0.18
N	<0.001	0.04	0.13
S*N	<0.001	0.09	0.26

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "S" is for species, "N" for nitrogen and "N*S" for the interaction nitrogen*species."H" represents the harvest. "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Table 6-2 Mean leaf area index, for the interaction of brome, cocksfoot, perennial ryegrass and tall fescue, when grown in monoculture, with (N+) or without nitrogen (N-) measured from July 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand.

Nitrogen	BR	CF	PR	TF
N+	4.1	4.1	3.9	4.0
N-	1.9	2.2	2.6	2.4

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue. The P-value for the Nitrogen*Species interaction is <0.001, the standard error of means is 0.09 and the least significant difference at 5% of significance is 0.26.

In Year 6, LAI was also affected by the interaction (P=0.007) of nitrogen and species. Brome LAI doubled in response to nitrogen but the increase averaged 75% for the other species (Table 6-4). However, overall LAI of the brome was ~15% lower (P<0.001) than other species (Table 6-3) and nitrogen increased (P<0.001) the LAI by an average of ~84% through the year. The regrowth cycle harvested on 13/12/19, had a LAI of 4.5 ± 0.05. which was at least 50% higher (P<0.001) than the others.

Table 6-3 Mean leaf area index (LAI) from brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, from July 2019 to July 2020 grown with (N+) and without nitrogen (N-) at Ladbrooks, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
28/08/19	1.6	1.5	2.5	2.4	0.5	0.8	1.3	0.7	1.4 e
09/10/19	2.5	3.4	4.3	4.4	0.5	1.4	2.2	2.2	2.6 c
13/12/19	5.5	5.7	4.5	5.2	3.7	3.5	3.7	4.0	4.5 a
11/02/20	3.3	4.4	3.2	3.5	2.0	2.6	2.2	2.3	3.0 b
19/05/20	3.1	3.8	2.6	2.9	1.5	1.4	1.7	1.5	2.3 d
21/07/20	3.3	3.5	3.2	3.4	1.3	1.5	2.1	1.4	2.5 cd

	N+	N-
Mean Yearly N	3.5	1.9

	BR	CF	PR	TF	Mean
Mean Yearly S	2.4	2.8	2.8	2.8	2.7

	P-value	SEM	LSD
S	<0.001	0.06	0.18
N	<0.001	0.04	0.12
S*N	0.007	0.08	0.25

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "S" is for species, "N" for nitrogen and "N*S" for the interaction nitrogen*species, "H" represents the harvest."SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Table 6-4 Mean leaf area index, for the interaction of brome, cocksfoot, perennial ryegrass and tall fescue, when grown in monoculture, with (N+) or without nitrogen (N-) measured from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand.

Nitrogen	BR	CF	PR	TF
N+	3.2	3.7	3.4	3.6
N-	1.6	1.9	2.2	2.0

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue. The P-value for the Nitrogen*Species interaction is 0.007, the standard error of means is 0.08 and the least significant difference at 5% of significance is 0.25.

Experiment 2 - Ashley Dene

Measurements for Ashley Dene began on 03/12/2018. Unfertilised grasses had a mean LAI of 3.0 ± 0.05 which was lower ($P<0.001$) than the 3.4 ± 0.05 from N fertilised grasses. In Year 5, LAI differed amongst species ($P<0.001$). Cocksfoot had a mean LAI of 3.5 ± 0.06 which was higher than brome and tall fescue (3.1 ± 0.06) and p. ryegrass had the lowest mean LAI of 2.9 ± 0.06 (Table 6-5).

In both Year 5 and Year 6 the regrowth cycles harvested during Spring and early summer had a higher LAI ($P<0.001$) (Table 6-5and Table 6-6) than the ones harvested in Autumn or Winter (Figure 6-3).

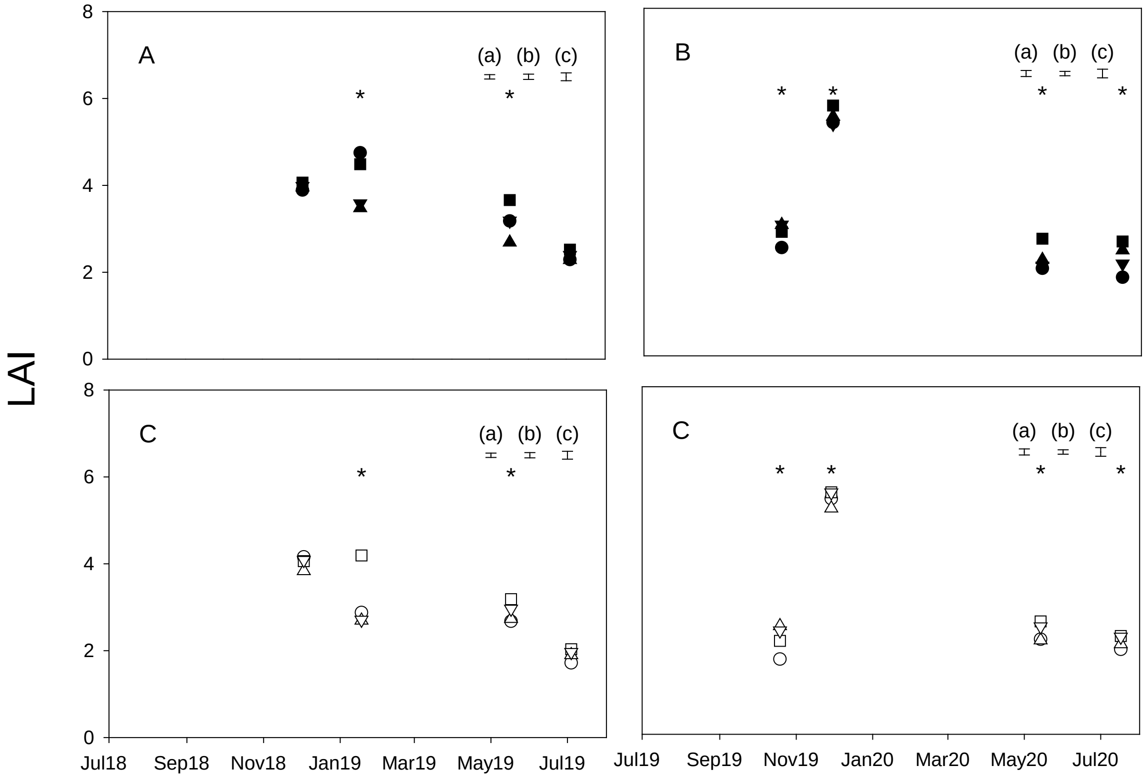


Figure 6-3 Leaf area index (LAI) from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) at Ashley Dene, Canterbury, New Zealand. For every regrowth cycle, for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△) and tall fescue(▼,▽) with (closed) (A,B) or without nitrogen fertilisation (open) (B,D). "*" represents when differences where observed for the species. SEMs are represented for (a) nitrogen, (b) species and (c) nitrogen*species. LAI at 95% light interception is 5.07. Data are absent for regrowth cycles harvested previous to 03/12/2018.

In Year 5, the regrowth cycle harvested on 03/12/2018 had the highest LAI, 4.0 ± 0.06, and the one with the lowest values was harvested in early July 2019, 2.1 ± 0.06, about 47% lower.

In Year 6, nitrogen fertiliser increased (P=0.002) mean LAI by 6%, from 3.0 ± 0.05 to 3.2 ± 0.05 (Table 6-6). The mean LAI was higher (P=0.018) at 3.3 ± 0.07 for cocksfoot than perennial ryegrass and tall fescue at 3.1 ± 0.07 and lowest for brome at 2.8 ± 0.07. The regrowth cycle harvested by late November 2019 had the highest LAI, of 5.4 ± 0.06, the one harvested in mid-July 2020 had the lowest LAI, 2.2 ± 0.06, a value 60% lower.

Year 5 (2018/2019)

Table 6-5 Mean leaf area index (LAI), of brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) and without (-N) nitrogen fertiliser measured from monocultures from July 2019 to July 2020, at Ashley Dene, Canterbury.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
03/12/18	3.9	4.1	4.0	4.0	4.2	4.1	3.8	4.1	4.0 a
18/01/19	4.7	4.5	3.5	3.6	2.9	4.2	2.7	2.7	3.6 b
17/05/19	3.2	3.7	2.7	3.2	2.7	3.2	2.7	3.0	3.0 c
04/07/19	2.3	2.5	2.3	2.4	1.7	2.0	1.9	2.0	2.1 d
Mean Yearly N		3.4					3.0		

	BR	CF	PR	TF	Mean
Mean Yearly S	3.2	3.5	2.9	3.1	3.2

	P-value	SEM	LSD
S	<0.001	0.06	0.19
N	<0.001	0.05	0.14
S*N	0.269	0.09	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "S" is for species, "N" for nitrogen and "N*S" for the interaction nitrogen*species, "Mean H" represents the mean for that regrowth cycle."SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 6-6 Mean leaf area index (LAI), of brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) and without (-N) nitrogen fertilisation harvested from July 2019 to July 2020, at Ashley Dene, Canterbury.

		N+					N-		
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
19/09/19	2.5	2.9	3.0	3.0	1.7	2.1	2.5	2.4	2.5 b
29/11/19	5.4	5.8	5.5	5.3	5.4	5.6	5.2	5.6	5.4 a
14/05/20	2.0	2.7	2.2	2.1	2.2	2.6	2.2	2.5	2.3 c
17/07/20	1.8	2.6	2.4	2.1	2.0	2.3	2.1	2.2	2.2 c
Mean Yearly N		3.2					3.0		
	BR		CF		PR		TF		Mean
Mean Yearly S	2.8		3.3		3.1		3.1		3.1
			P-value		SEM			LSD	
S			0.018		0.07			0.19	
N			0.002		0.05			0.15	
S*N			0.255		0.10			-	

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “S” is for species, “N” for nitrogen and “N*S” for the interaction nitrogen*species, “Mean H” represents the mean for that regrowth cycle.“SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

6.3.2 Intercepted PAR per regrowth cycle and year

Experiment 1 – Ladbrooks

Intercepted PAR, in Year 5, was increased ($P=0.002$) from 845 to 1195 ± 14.1 MJ m^{-2} by nitrogen fertiliser (Figure 6-4). This difference in PAR interception was generated ($P<0.001$) by nitrogen in regrowth cycles harvested until mid-January 2019 (Table 6-7).

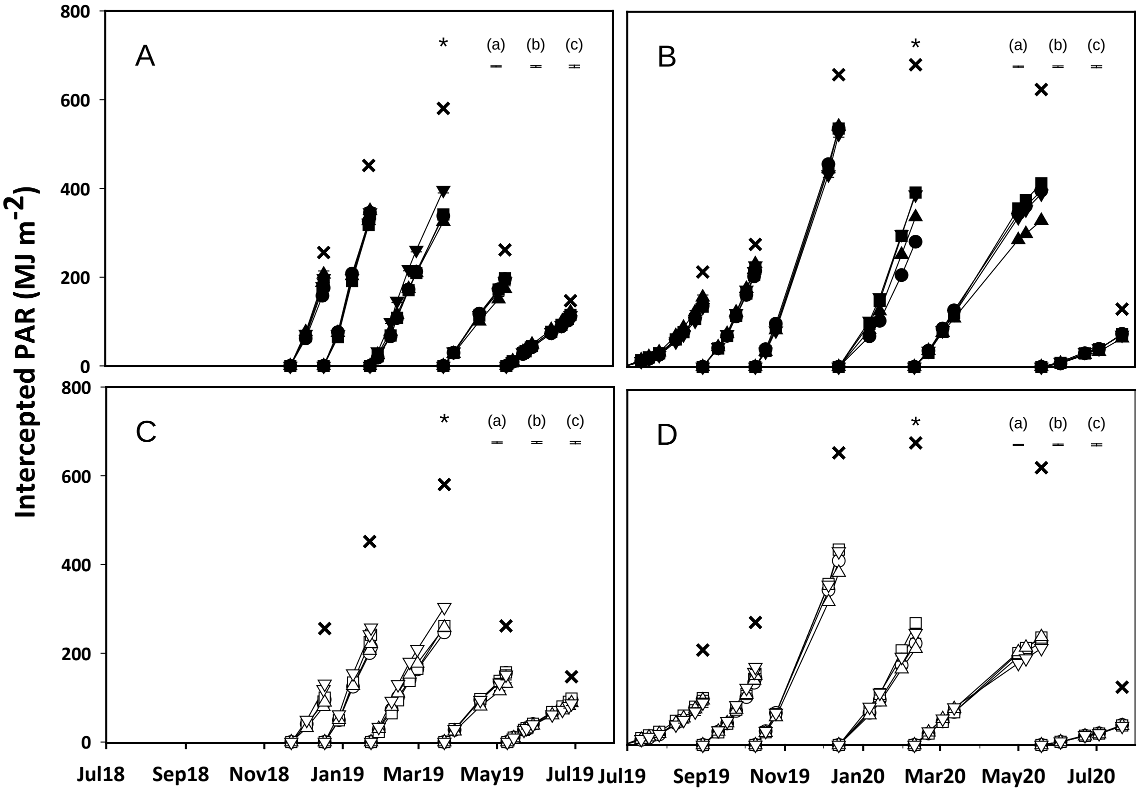


Figure 6-4 Intercepted PAR (MJ m^{-2}) from November 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D) at Ladbrooks, Canterbury, New Zealand, for every regrowth cycle, for brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△), tall fescue (▼,▽) with (closed) (A,B) or without nitrogen fertilisation (open) (B,D). "X" symbols represent incident PAR. "*" represents when differences were observed for the species. SEMs are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

PAR interception from tall fescue was 1085 ± 14.1 MJ m^{-2}, and ~10% more ($P<0.001$) than the other three species at 997 ± 14.1 MJ m^{-2} (Table 6-7). There was also a difference ($P<0.001$) among the regrowth cycles (Figure 6-4). Those that occurred in mid summer that had higher incident PAR (later Spring and summer) and/or lasted for a longer duration intercepted more PAR by up to 8 times compared with regrowth cycles harvested on 13/12/19 and 21/07/20.

In Year 6, the intercepted PAR was increased ($P=0.015$) by 41.5% (Table 6-8) by N fertiliser. Cocksfoot and tall fescue intercepted the most PAR ($P<0.001$) at 1500 ± 22.5 MJ m^{-2}.

Year 5 (2018/2019)

Table 6-7 Intercepted PAR per regrowth cycle (MJ m^{-2}) measured from December 2018 to July 2019, for brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) or without (N-) nitrogen, at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
17/12/18	175	190	210	200	95	100	90	130	150 b
22/01/19	345	340	350	345	215	240	220	260	290 a
21/03/19	340	340	325	400	250	260	260	305	310 a
09/05/19	195	200	175	190	155	155	130	150	170 b
28/06/19	110	115	120	115	95	100	90	90	105 c
Mean Yearly N	1195				845				

	BR	CF	PR	TF	Mean
Mean Yearly S	985	1020	985	1085	1020

	P-value	SEM	LSD
S	0.002	14.1	44.9
N	0.002	25.7	115.6
S*N	0.424	31.5	-

Note: "BR" stands for "Brome", "CF" for "Cocksfoot", "PR" for "Perennial Ryegrass" and "TF" for "Tall Fescue". "N" represents nitrogen; "S" represents species. "H" represents the harvests, "SEM" is the Standard Error of the Mean and "LSD" the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 6-8 Intercepted PAR per regrowth cycle (MJ m^{-2}) measured from July 2019 to July 2020, for brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) or without (N-) nitrogen, at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
29/08/19	140	135	155	135	105	105	100	95	120 e
09/10/19	215	220	230	230	150	160	155	175	190 d
13/12/19	540	535	540	525	415	440	390	435	480 a
11/02/20	280	395	340	390	230	275	215	250	300 b
19/05/20	400	415	330	390	235	240	245	220	310 c
21/07/20	75	75	65	75	45	45	45	45	60 f
Mean Yearly N		1705				1205			
	BR		CF		PR		TF		Mean
Mean Yearly S	1410		1520		1410		1480		1455

	P-value	SEM	LSD
S	<0.001	22.49	71.95
N	0.015	13.14	59.12
S*N	0.717	28.76	-

Note: “BR” stands for “Brome”, “CF” for “Cocksfoot”, “PR” for “Perennial Ryegrass” and “TF” for “Tall Fescue”. “N” represents nitrogen; “S” represents species, “H” represents the harvests. “SEM” is the Standard Error of the Mean and “LSD” the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Experiment 2 - Ashley Dene

Measurements for Year 5 at Ashley Dene account for the regrowth cycles that began in 04/12/18 and ended in 04/07/19. Cocksfoot and tall fescue intercepted ~10% more PAR ($P<0.001$) than the other species with a mean of 728 ± 15.7 MJ m^{-2} (Table 6-9).

The intercepted PAR was not different ($P=0.326$) between fertilised and unfertilised pastures at Ashley Dene for the measurement period of 04/12/18 to 04/07/19. However earlier rotations may have differed when water was available and nitrogen could be taken up by the plants (Figure 6-5).

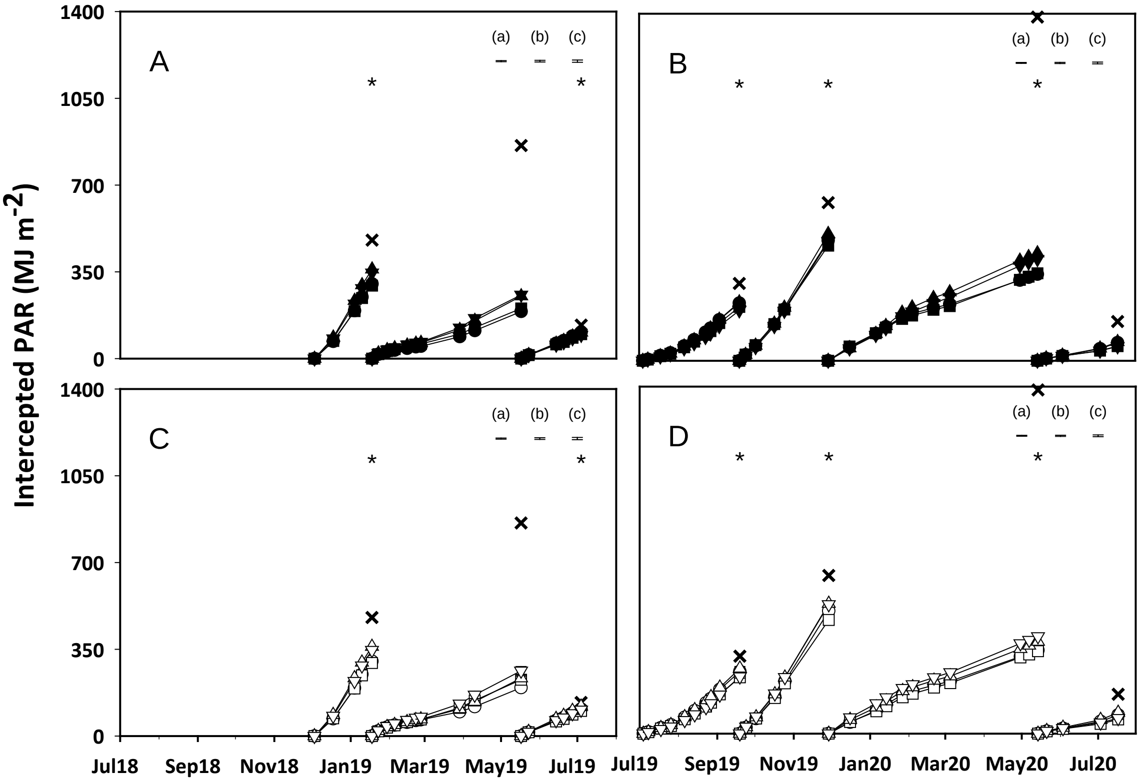


Figure 6-5 Intercepted PAR (MJ m^{-2}) at Ashley Dene, Canterbury, New Zealand for every regrowth cycle, for brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△), tall fescue (▼,▽) with (closed) or without nitrogen fertilisation (open) from December 2018 to July 2019 and from July 2019 to July 2020. "X" symbols represent incident PAR. Standard errors of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represents observed differences among species.

The intercepted PAR was different (P<0.001) amongst regrowth cycles. Plants in the first regrowth cycle intercepted, on average, 3.2 times more PAR than the last regrowth cycle (Table 6-9). Pastures in the second regrowth cycle intercepted, on average, 2.1 times that value. Longer regrowth cycles tended to intercept more PAR than shorter regrowth cycles, but the one on 17/05/19 also intercepted less than others.

Measurements for intercepted PAR in Year 6 started on 04/07/19 and ended on 17/07/20 at Ashley Dene. There was a difference (P=0.025) amongst the species, cocksfoot and tall fescue intercepted about 10% more PAR than the other species (Table 6-10).

Measurements for Year 6 in Ashley Dene started on 04/07/19 and ended on 17/07/20. In Year 6, nitrogen increased (P=0.003) intercepted PAR from July 2019 to December 2019. Regrowth cycles in late Spring and summer or were longer in duration had higher (P<0.001) intercepted PAR than the others. In addition, cocksfoot and tall fescue intercept ~10% more (P<0.025) PAR than the other species (Table 6-10). The last regrowth cycle this year intercepted less PAR (P<0.001) than the half of the first one (Table 6-6).

Year 5 (2018/2019)

Table 6-9 Intercepted PAR per regrowth cycle (MJ m^{-2}) for brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) or without (N-) nitrogen fertilisation, measured from December 2018 to July 2019 at Ashley Dene, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
18/01/19	305	370	355	380	300	355	310	365	340 a
17/05/19	195	255	200	235	205	260	230	265	230 b
04/07/19	110	110	120	120	100	100	105	105	105 c
Mean Yearly N		685				675			
	BR		CF		PR		TF		Mean
Mean Yearly S	605		720		655		735		680

	P-value	SEM	LSD
S	<0.001	15.7	50.3
N	0.326	7.4	-
S*N	0.855	19.4	-

Note: "BR" stands for "Brome", "CF" for "Cocksfoot", "PR" for "Perennial Ryegrass" and "TF" for "Tall Fescue". "N" represents nitrogen; "S" represents species, "H" represents the harvests. "SEM" is the Standard Error of the Mean and "LSD" the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 6-10 Intercepted PAR per regrowth cycle (MJ m^{-2}) for brome, cocksfoot, perennial ryegrass and tall fescue, grown with (N+) and without (N-) nitrogen measured from July-2019 to July-2020 at Ashley Dene, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
19/09/19	240	245	260	270	225	210	235	240	240 c
29/11/19	495	520	500	530	470	490	465	530	500 a
14/05/20	350	435	340	375	355	410	335	395	375 b
17/07/20	75	75	75	85	60	60	60	60	70 e
Mean Yearly N	1220				1150				
	BR		CF		PR		TF		
Mean Yearly S	1130		1220		1130		1240		1185

	P-value	SEM	LSD
S	0.025	25.8	82.4
N	<0.001	3.5	15.6
S*N	0.472	28.8	-

Note: "BR" stands for "Brome", "CF" for "Cocksfoot", "PR" for "Perennial Ryegrass" and "TF" for "Tall Fescue". "N" represents nitrogen; "S" represents species, "H" represents the harvests. "SEM" is the Standard Error of the Mean and "LSD" the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

6.3.3 Radiation Use Efficiency (RUE) per year

Experiment 1 - Ladbrooks

Measurements in Year 5 were only from 22/11/18 to 28/06/19 (Section 6.2.2) which will have affected the linear regressions which is discussed in Sections 6.4.1 and 6.4.2.

Photosynthetically active radiation (PAR) that is absorbed by the plants has a positive relation with their growth rate (Gallagher, 1978; Section 2.1.4). There was strong ($R^2 \geq 0.85$ for most cases) linear regression between the accumulated DM and the total intercepted PAR (Figure 6-6).

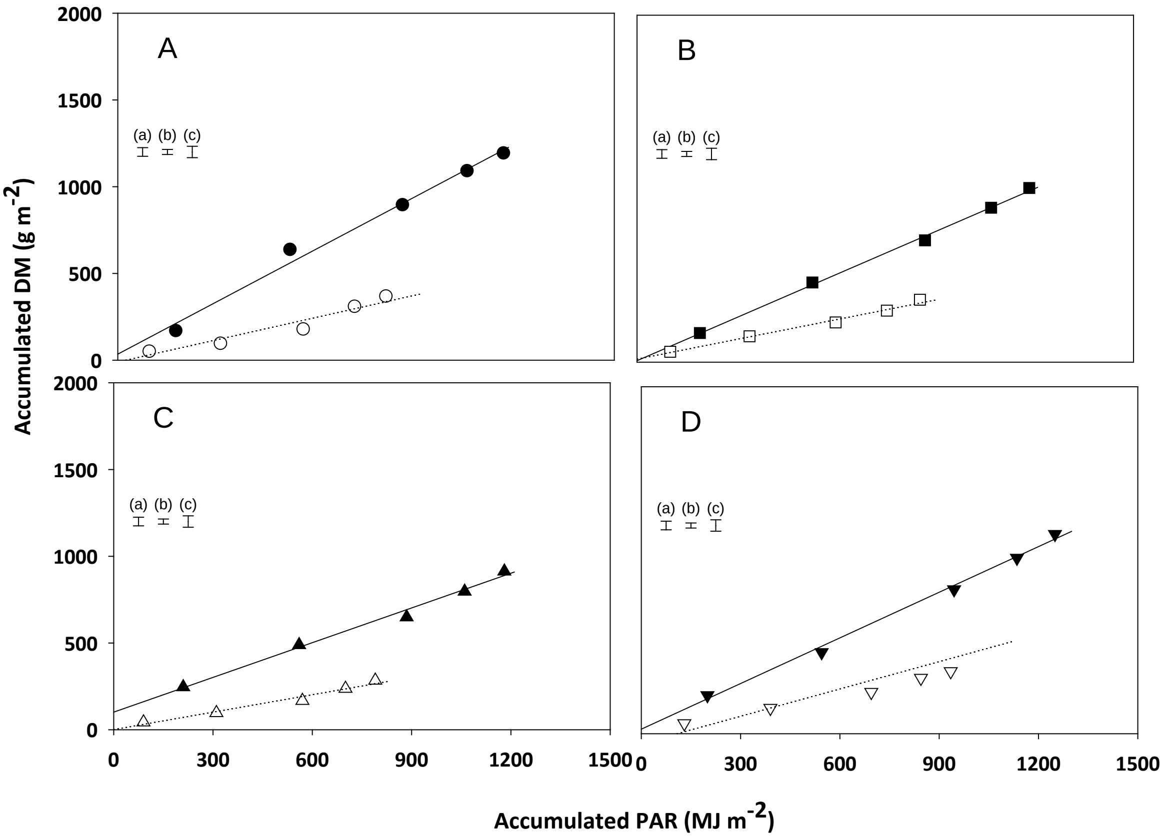


Figure 6-6 Accumulated yield (g m^{-2}) against accumulated photosynthetically active radiation (PAR; MJ m^{-2}) by N+ (closed) and N- (open) monocultures of brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) and tall fescue (D) (▼,▽) from November 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand. Regression equation details for N+ and N- pastures are reported in Table 6-11. Error bars are shown for (a) species, (b) nitrogen and (c) nitrogen*species.

RUE, in Year 5 at Ladbrooks, was not different amongst species (P=0.056) with a mean of 0.66 ± 0.03 g DM MJ ^{1} m^{-2}, but RUE was affected (P=0.001) by N level (Table 6-11). N fertiliser increased RUE by ~55%, or from 0.38 to 0.84 ± 0.03 g DM MJ^{1}.

In Year 6, RUE was not different (P=0.053) among species with a mean of 0.81 ± 0.10 g DM MJ^{-1} (Table 6-12). N increased (P<0.001) RUE from 0.53 to 1.09 g DM MJ^{-1} m^{-2} (Figure 6-7).

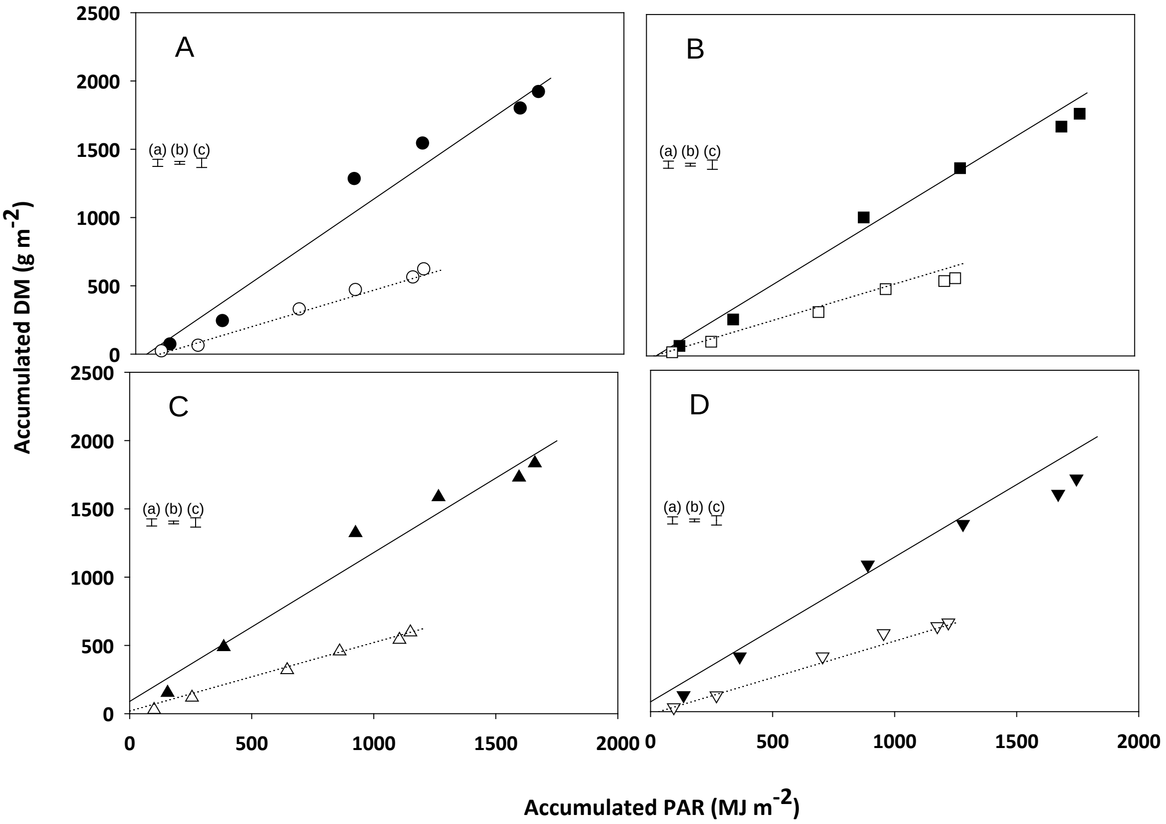


Figure 6-7 Accumulated yield (g DM m^{-2}) against accumulated photosynthetically active radiation (PAR) (MJ m^{-2}) for nitrogen fertilised N+ (closed) and unfertilised N- (open) monocultures of brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) and tall fescue (D) (▼,▽) from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand. Symbols are represented for harvests. Regression equation details for N+ and N- pastures are reported in Table 6-12. Error bars are shown for (a) species, (b) nitrogen and (c) nitrogen*species.

Year 5 (2018/2019)

Table 6-11 Regression equations for accumulated yield (g DM m^{-2}) against accumulated intercepted photosynthetically active radiation (MJ m^{-2}) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from November 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand.

Species (S)	Equation(Y=)	SE Coef(X)	SE Coef(Y)	R^2	
N level (N)		+N			Mean S
Brome	1.003x+39	0.05	36	0.90	0.728
Cocksfoot	0.822x+10	0.05	39	0.89	0.600
P. ryegrass	0.661x+106	0.03	25	0.89	0.499
Tall fescue	0.886x+19	0.04	33	0.87	0.618
Mean	0.843				
N level (N)		-N			
Brome	0.453x-20	0.03	19	0.70	
Cocksfoot	0.378x+17	0.03	17	0.78	
P. ryegrass	0.337x-4	0.02	10	0.82	
Tall fescue	0.350x-50	0.04	27	0.61	
Mean	0.380				

	P-value	SEM	LSD
S	0.056	0.049	-
N	0.001	0.029	0.129
S*N	0.301	0.064	-

Note: “SE Coef” stands for Standard Error of the Coefficient. “S” represents Species.

Year 6 (2019/2020)

Table 6-12 Regression equations for accumulated yield (g DM m^{-2}) against accumulated intercepted photosynthetically active radiation (MJ m^{-2}) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue from July 2019 to July 2020 at Ladbrooks, Canterbury, New Zealand.

Species (S)	Equation(Y=)	SE Coef(X)	SE Coef(Y)	R^2	
N level (N)		+N			Mean S
Brome	1.254x-55	0.03	25	0.94	0.910
Cocksfoot	1.048x-42	0.02	25	0.96	0.760
P. ryegrass	1.094x+90	0.02	23	0.96	0.811
Tall fescue	0.963+72	0.02	23	0.96	0.765
Mean	1.089				
N level (N)		-N			
Brome	0.567x-53	0.02	12	0.93	
Cocksfoot	0.473x-16	0.02	17	0.90	
P. ryegrass	0.528x-20	0.02	11	0.91	
Tall fescue	0.567x-18	0.01	10	0.94	
Mean	0.533				

	P-value	SEM	LSD
S	0.053	0.036	-
N	<0.001	0.014	0.065
S*N	0.120	0.047	-

Note: “SE Coef” stands for Standard Error of the Coefficient. “S” representes Species.

Experiment 2 - Ashley Dene

Measurements were taken from 03/12/18 to 04/07/19 for Year 5 at Ashley Dene (Section 6.2.2). The lack of Spring measurements probably affected the linear regressions and is addressed in Sections 6.4.1 and 6.4.2. In Year 5 at Ashley Dene, RUE was increased by nitrogen fertilisation (P=0.045) only for cocksfoot, from 0.41 to 0.60 ± 0.05 g DM MJ^{-1} (Figure 6-8).

In Year 6 at Ashley Dene, a difference was observed for species (P=0.041). Brome, cocksfoot and p. ryegrass had a higher mean RUE of 0.66 ± 0.05 g DM MJ^{1} than tall fescue at 0.47 ± 0.050 g DM MJ^{1} (Table 6-14). Nitrogen fertiliser did not affect (P=0.778) RUE at Ashley Dene (Figure 6-9).

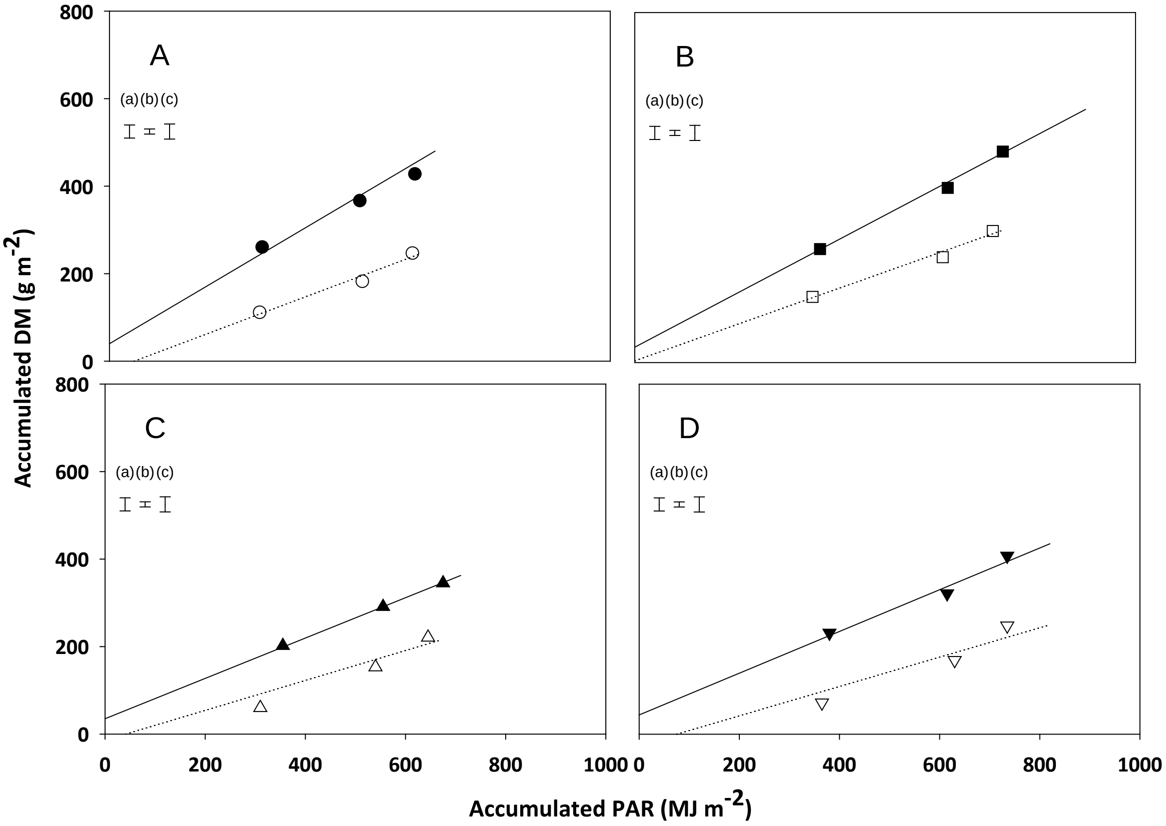


Figure 6-8 Accumulated yield (g DM m^{-2}) against accumulated photosynthetically active radiation (PAR) (MJ m^{-2}) by N+ (closed) and N- (open) monocultures of brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) and tall fescue (D) (▼,▽) from December-2018 to July-2019 at Ashley Dene, Canterbury, New Zealand. Symbols are represented for harvests. Regression equation details for N+ and N- pastures are reported in Table 6-13. Error bars are shown for (a) species, (b) nitrogen and (c) nitrogen*species.

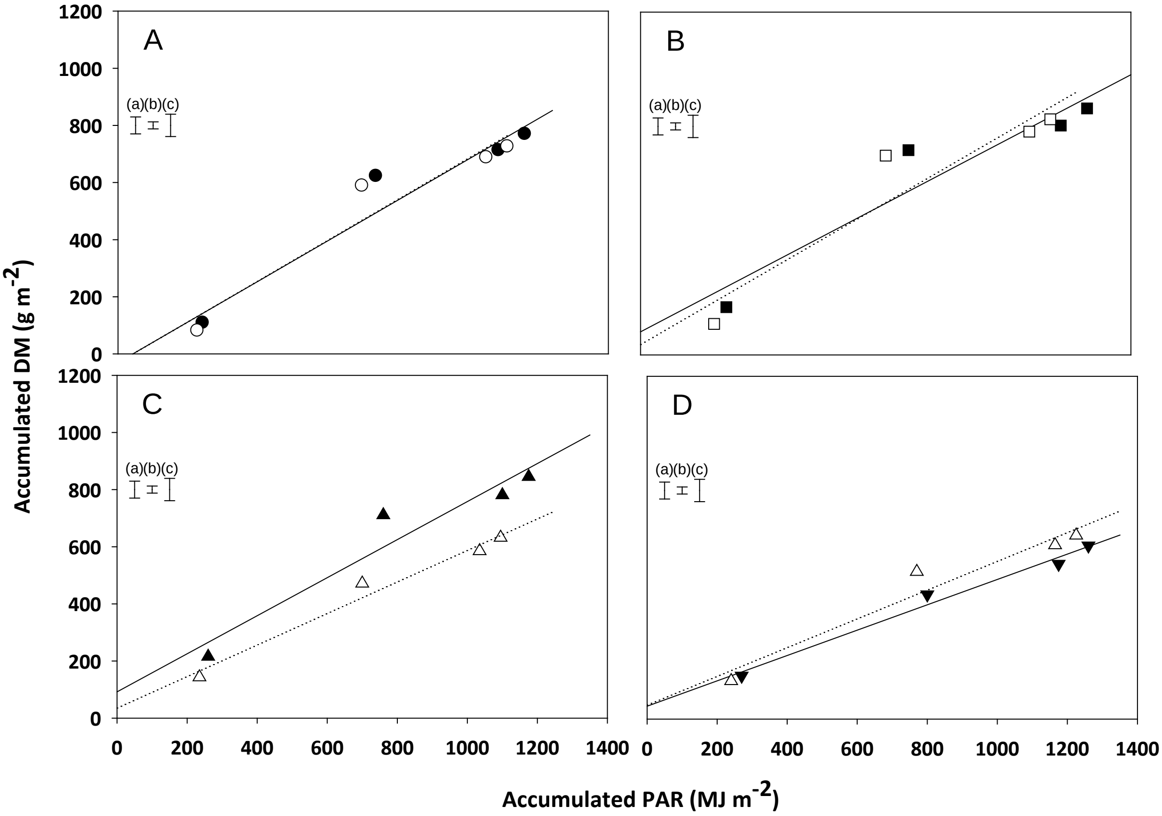


Figure 6-9 Accumulated yield (kg ha^{-1}) against accumulated photosynthetically active radiation (PAR) (MJ m^{-2}) by N+ (closed) and N- (open) monocultures of brome (A) (●,○), cocksfoot (B) (■,□), perennial ryegrass (C) (▲,△) and tall fescue (D) (▼,▽) from July-2019 to July 2020, at Ashley Dene, Canterbury, New Zealand. Symbols are represented for harvests. Regression equation details for N+ and N- pastures are reported in Table 6-14. Error bars are shown for (a) species, (b) nitrogen and (c) nitrogen*species.

Year 5 (2018/2019)

Table 6-13 Regression equations for accumulated yield (g DM m^{-2}) against accumulated intercepted photosynthetically active radiation (MJ m^{-2}) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, from December 2018 to July 2019, at Ashley Dene, Canterbury, New Zealand.

Species (S)	Equation(Y=)	SE Coef(X)	SE Coef(Y)	R^2	
N level (N)		+N			Mean S
Brome	0.494x+40	0.05	18	0.72	0.462
Cocksfoot	0.604x+35	0.05	19	0.61	0.505
P. ryegrass	0.461x+35	0.06	24	0.62	0.465
Tall fescue	0.477x+44	0.05	22	0.90	0.465
Mean	0.509				
N level (N)		-N			
Brome	0.430x-21	0.05	22	0.72	
Cocksfoot	0.407x+4	0.04	17	0.54	
P. ryegrass	0.470x-14	0.09	39	0.95	
Tall fescue	0.453x-25	0.05	20	0.94	
Mean	0.440				

	P-Value	SEM	LSD
S	0.883	0.044	-
N	0.069	0.017	-
S*N	0.045	0.051	0.154

Note: “SE Coef” stands for Standard Error of the Coefficient.

Year 6 (2019/2020)

Table 6-14 Regression equations for accumulated yield (g DM m^{-2}) against accumulated intercepted photosynthetically active radiation (MJ m^{-2}) by nitrogen fertilised (N+) and non-fertilised (N-) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue, from July 2019 to July 2020, at Ashley Dene, Canterbury, New Zealand.

Species (S)	Equation(Y=)	SE Coef(X)	SE Coef(Y)	R^2	
N level (N)		+N			Mean S
Brome	0.692x-30	0.02	21	0.93	0.704
Cocksfoot	0.643x+81	0.02	18	0.91	0.676
P. ryegrass	0.666x+92	0.02	16	0.92	0.614
Tall fescue	0.444x+45	0.01	7	0.97	0.474
Mean	0.611				
N level (N)		-N			
Brome	0.715x-30	0.05	42	0.65	
Cocksfoot	0.710x+37	0.02	17	0.93	
P. ryegrass	0.552x+35	0.01	9	0.95	
Tall fescue	0.503x+49	0.02	14	0.89	
Mean	0.620				
	P-value		SEM		LSD
S	0.041		0.050		0.162
N	0.778		0.021		-
S*N	0.627		0.066		-

Note: “SE Coef” stands for Standard Error of the Coefficient.

6.3.4 Radiation Use Efficiency (RUE) per regrowth cycle

Experiment 1 - Ladbrooks

RUE in Year 5, differed (P=0.010) among regrowth cycles (Table 6-15). The periods of early February 2019 until late May 2019, averaged 0.58 ± 0.022 g DM MJ^{-1} m^{-2}, compared with 0.77 ± 0.022 g DM MJ^{-1} m^{-2} from other periods. In Year 6 RUE also differed (P<0.001) among regrowth cycles (Table 6-16) being higher in Spring and summer except for the last harvest (Figure 6-10).

RUE was affected by the interacton of N and regrowth cycle (P<0.001). Specifically, RUE values were higher for N+ pastures in Spring until the end of January 2020 and then they were not different. Species responses were also affected by the interaction with regrowth cycle (P=0.004). Brome had higher values during Winter (Table 6-16) and in the harvest in late January 2020 when it produced more dry matter than the other species.

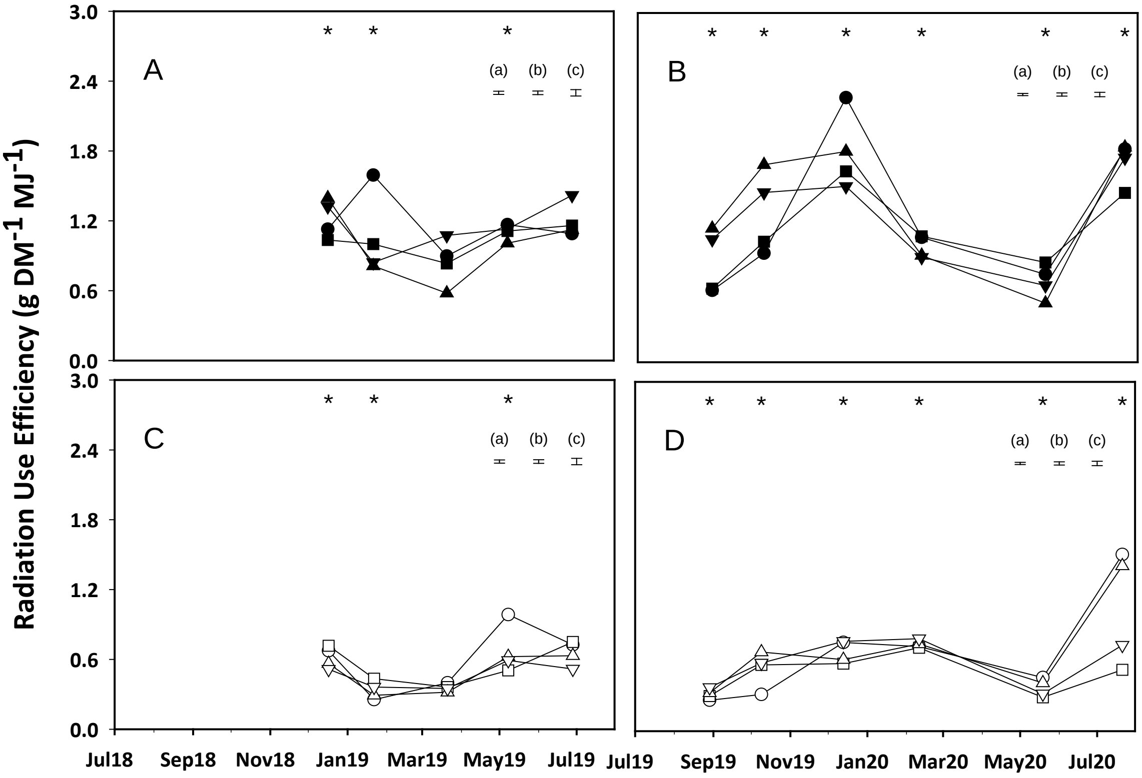


Figure 6-10 Radiation use efficiency (g DM MJ^{-1} PAR m^{-2}), for brome(●,○), cocksfoot (■,□), perennial ryegrass(▲,△) and tall fescue(▼,▽) with (closed) or without nitrogen fertilisation (open) at Ladbrooks, from July 2018 to July 2019 and from July 2019 to July 2020. Standard errors of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species. "*" represents when differences were observed among species.

P. ryegrass was the species that had the lowest mean RUE (P<0.001) from January 2020 until late May 2020 (Figure 6-10).

Year 5 (2018/2019)

Table 6-15 Radiation Use Efficiency (g DM MJ^{-1} PAR) for brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) or without (-N) nitrogen fertilisation, from November 2018 to July 2019 at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
17/12/18	0.96	0.88	1.19	1.12	0.57	0.61	0.48	0.44	0.78 a
22/01/19	1.35	0.85	0.69	0.71	0.21	0.37	0.25	0.31	0.59 b
21/03/19	0.76	0.71	0.49	0.91	0.34	0.31	0.27	0.30	0.51 b
09/05/19	0.99	0.94	0.86	0.96	0.83	0.42	0.53	0.50	0.76 a
28/06/19	0.93	0.99	0.95	1.21	0.61	0.64	0.54	0.44	0.79 a
Mean Yearly N	0.93				0.45				

	BR	CF	PR	TF	Mean
Mean Yearly S	0.76	0.67	0.62	0.69	0.69

	P-value	SEM	LSD
S	0.144	0.039	-
N	<0.001	0.027	0.081
S*N	0.362	0.055	-

Note: "BR" stands for "Brome", "CF" for "Cocksfoot", "PR" for "Perennial Ryegrass" and "TF" for "Tall Fescue". "N" represents nitrogen; "S" represents species, "H" represents the harvests. "SEM" is the Standard Error of the Mean and "LSD" the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2029/2020)

Table 6-16 Radiation Use Efficiency (g DM MJ^{-1} PAR) for brome, cocksfoot, perennial ryegrass and tall fescue, with (+N) and without (-N) nitrogen fertilisation, sown in monoculture, from July 2019 to July 2020 in Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
29/08/19	0.53	0.54	0.98	0.90	0.22	0.26	0.28	0.31	0.50 c
09/10/19	0.80	0.88	1.44	1.24	0.26	0.48	0.59	0.49	0.77 b
13/12/19	1.93	1.39	1.54	1.28	0.65	0.49	0.52	0.65	1.05 a
11/02/20	0.91	0.92	0.78	0.76	0.62	0.61	0.64	0.67	0.74 b
19/05/20	0.64	0.73	0.43	0.56	0.39	0.25	0.35	0.27	0.45 c
21/07/20	1.56	1.23	1.57	1.49	1.29	0.45	1.21	0.63	1.18 a
Mean Yearly N	1.04				0.53				

	BR	CF	PR	TF	Mean
Mean Yearly S	0.82	0.69	0.86	0.77	0.78

	P-value	SEM	LSD
S	0.001	0.027	0.079
N	<0.001	0.019	0.056
S*N	0.925	0.038	-

Note: "BR" stands for "Brome", "CF" for "Cocksfoot", "PR" for "Perennial Ryegrass" and "TF" for "Tall Fescue". "N" represents nitrogen; "S" represents species, "H" represents the harvests. "SEM" is the Standard Error of the Mean and "LSD" the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Experiment 2 - Ashley Dene

For RUE, in Year 5, there was an interaction between species and regrowth cycle (P=0.017) and nitrogen and regrowth cycles (P<0.001). For example, tall fescue and cocksfoot had higher RUE values from May 2019 to July 2019. The increase in RUE from nitrogen fertiliser was higher in the first regrowth cycle than later ones (Table 6-17).

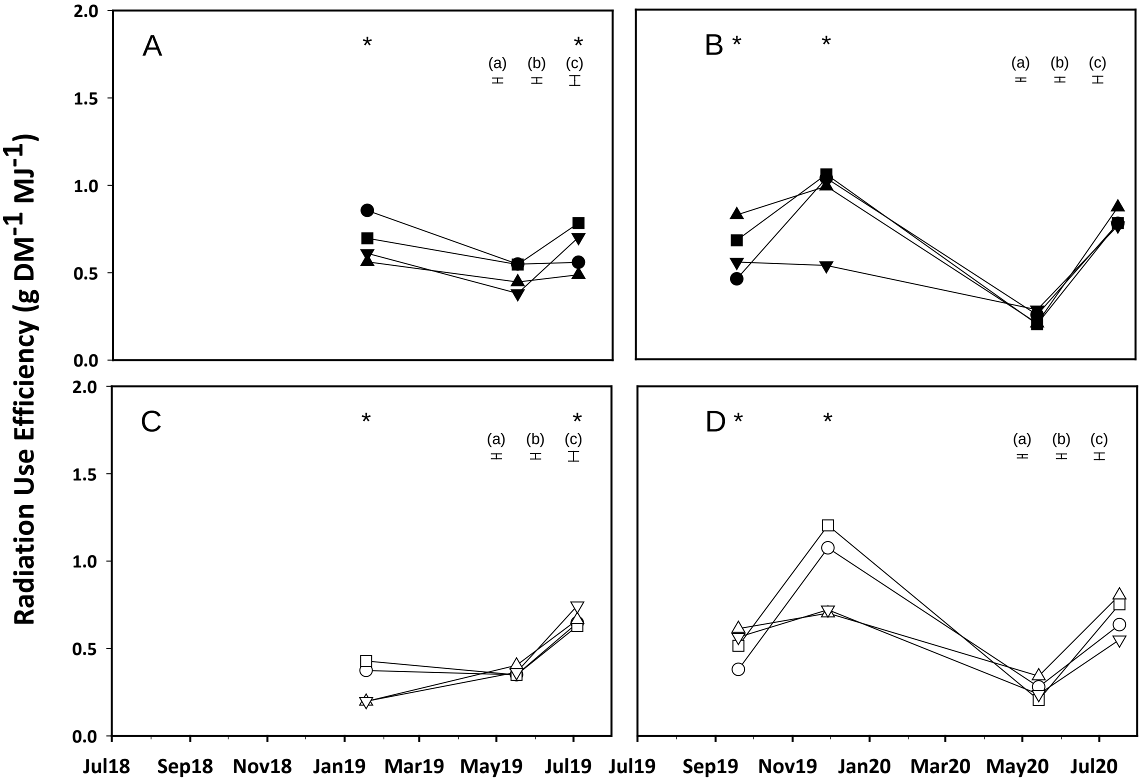


Figure 6-11 Radiation Use Efficiency (g DM MJ^{-1} PAR m^{-2}) for brome (●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) with (closed) or without nitrogen fertilisation (open). From July 2018 to July 2019 (A;C) and from July 2019 to July 2020 (B,D) at Ashley Dene, Standard errors of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

RUE, in Year 6, was different (P<0.001) for regrowth cycles (Table 6-17). The last regrowth cycle (harvested on 04/07/2019) in this year had a RUE more than 50% higher than the previous cycle (17/05/2019) (Figure 6-11).

Year 5 (2018/2019)

Table 6-17 Radiation Use Efficiency (g DM MJ^{-1} PAR m^{-2}) for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen fertilisation, from December 2018 to July 2019, at Ashley Dene, Canterbury, New Zealand.

		N+				N-			
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
18/01/19	0.86	0.70	0.56	0.61	0.37	0.42	0.20	0.20	0.49 b
17/05/19	0.55	0.54	0.45	0.38	0.35	0.35	0.40	0.37	0.42 c
04/07/19	0.56	0.78	0.48	0.71	0.65	0.63	0.66	0.75	0.65 a
Mean Yearly N		0.60				0.45			
	BR		CF		PR		TF		Mean
Mean Yearly S	0.56		0.57		0.46		0.50		0.52
			P-value		SEM			LSD	
S			0.017		0.025			0.073	
N			<0.001		0.018			0.052	
S*N			0.238		0.035			-	

Note: "BR" stands for "Brome", "CF" for "Cocksfoot", "PR" for "Perennial Ryegrass" and "TF" for "Tall Fescue". "N" represents nitrogen; "S" represents species, "H" represents the harvests. "SEM" is the Standard Error of the Mean and "LSD" the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Year 6 (2019/2020)

Table 6-18 Radiation Use Efficiency (g DM MJ^{-1} PAR m^{-2}) for brome, cocksfoot, perennial ryegrass and tall fescue, sown in monoculture, with (N+) or without (N-) nitrogen fertilisation, from July 2019 to July 2020, at Ashley Dene, Canterbury, New Zealand.

		N+				N-			
Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
19/09/19	0.46	0.68	0.82	0.55	0.38	0.52	0.61	0.56	0.58 c
29/11/19	1.04	1.05	0.54	0.54	1.08	1.21	0.72	0.72	0.92 a
14/05/20	0.25	0.20	0.28	0.28	0.28	0.20	0.24	0.24	0.25 d
17/07/20	0.77	0.78	0.76	0.76	0.64	0.76	0.81	0.55	0.74 b
Mean Yearly N		0.65				0.60			
	BR		CF		PR		TF		Mean
Mean Yearly S	0.61		0.67		0.67		0.53		0.62
			P-value		SEM			LSD	
S			0.074		0.030			-	
N			0.314		0.042			-	
S*N			0.839		0.059			-	

Note: "BR" stands for "Brome", "CF" for "Cocksfoot", "PR" for "Perennial Ryegrass" and "TF" for "Tall Fescue". "N" represents nitrogen; "S" represents species, "H" represents the harvests. "SEM" is the Standard Error of the Mean and "LSD" the Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

6.4 DISCUSSION

Chapter 5 quantified the differences in TDM production reported in Chapter 4 in relation to moisture and N levels. To achieve this, the amount of plant available water content (PAWC) at each site had to be quantified, along with understand how nitrogen fertilisation affected it (Objective 3), then a study of how the soil moisture deficit was seasonally affected according to each species and nitrogen levels (Objective 4) could be proceeded with.

Not all of the differences in yield were explained by the water use, so this chapter explored differences in resource capture and utilization of light. This was done through the intercepted PAR and RUE. The amount of intercepted PAR and its use to produce dry matter, represented by RUE, are important components of the TDM yield, as shown in Equation 1. Objective 6 aimed to quantify each of these components separatedly to further explain the yields differences described in Chapter 4.

6.4.1 The effect of nitrogen fertilisation and water availability on the LAI, intercepted PAR and RUE

LAI was significantly increased by the nitrogen fertilisation at both Ladbrooks and Ashley Dene in both Year 5 and Year 6. The average increase in LAI was much higher in Ladbrooks (74% and 84%, for Year 5 and Year 6; Table 6-1 and Table 6-3) than in Ashley Dene (13% and 7%; Table 6-5 and Table 6-6). This increase was more apparent in Spring 2019 and 2020 and also early summer 2019 at Ladbrooks due to warmer temperatures (Figure 3-2) and availability of water in those periods (Figure 5-2 and Figure 5-3). During the colder seasons, lower temperatures imply a lower leaf area expansion rate, thus, the effect of nitrogen fertilisation on LAI is less. This would restrict LAI development, for example, for harvests in mid June (28/06/19) after 3100 °Cd had accumulated in Year 5 and late Winter (28/08/19 after 261 °Cd in Year 6) at Ladbrooks. A restriction in LAI would also occur under a water deficit such as on 04/07/19 (3110 °Cd in Year 5) at Ashley Dene. The N+ pastures from the regrowth cycle harvested on 22/01/2019 (1665 °Cd) was the last one in Year 5 at Ladbrooks to have non limiting conditions in Year 5 when LAI could develop without restrictions of water, nitrogen or temperature. When the plants are affected by the water stress, the leaf expansion rates are reduced before the net photosynthetic rate (Wardlaw, 1969; Bunce, 1978).

The higher LAI results in more intercepted PAR when grasses are under no stress. For example, N fertilised pastures resulted in increased intercepted PAR for both years at Ladbrooks (Table 6-7 and Table 6-8) by an average of 41% in Year 5 and Year 6. This occurred even in the absence of early Spring measurements in Year 5, when high water availability, prior to mid-November are expected to enhance the effects of nitrogen fertiliser. In contrast the lower PAWC at Ashley Dene meant differences were not detected in Year 5 (Table 6-9) because water restrictions (Figure 5-3) had started by mid-November 2018 which would impede the effect of nitrogen. In Year 6 at Ashley Dene, nonetheless, an increase of 6% in intercepted PAR was observed (Table 6-10) when fertilising with nitrogen. The reductions in LAI and intercepted PAR are not always similar for their relationship is not linear.

RUE was also affected by the nitrogen fertiliser when water was available. This is demonstrated by the increases at Ladbrooks in RUE values (Figure 6-10 and Figure 6-11). These increments were higher in regrowth cycles that took place when water was available (Table 6-16 and Table 6-18). The lack of measurements, at both sites, from June 2018 to November 2018 produced a 30% lower annual RUE for Year 5 (Figure 6-6 and Figure 6-8, Table 6-11 and Table 6-13) than Year 6 (Table 6-12 and Table 6-13). However, this is an artefact of the lack of Spring measurements in Year 5. The increases in RUE, due to nitrogen fertiliser rangedfrom 58 to 63% on the harvests from 29/08/2019 to 13/12/2019 (Table 6-16) at Ladbrooks. At Ashley Dene they increased from 20 to 46% for harvests from 11/02/2020 to 21/07/2020 (Table 6-18), which are regrowth cycles which grown under water stress (Figure 5-2). Overall, through the Year 5 at Ladbrooks, N fertilisation increased RUE from 0.38 to 0.84 ± 0.28 g DM m^{-2} MJ^{-1} (Table 6-11) and, in Year 6, from 0.53 to 1.09 ± 0.28 g DM m^{-2} MJ^{-1} (Table 6-12). At Ashley Dene, the effect of nitrogen fertilisation through the year was not visible on the whole of either Year 5 (mean of 0.48 ± 0.09 g DM m^{-2} MJ^{-1}, Table 6-13) or Year 6 (mean of 0.61 ± 0.08 g DM m^{-2} MJ^{-1}, Table 6-14). The results at both sites in Year 6 are likely to have been influenced by carbon remobilisation from roots to aerial parts on the reproductive harvests (13/12/19 at Ladbrooks and 29/11/19 at Ashley Dene), as demonstrated in Table 6-16 and Table 6-18 as those harvests that had the highest RUE. This lack of response is consistent with the impacts on WUE discussed in Section 5.4.2.

6.4.2 Impact of PAR interceptance and RUE changes in relation to species.

Tall fescue and cocksfoot were the species that intercepted PAR the most at both environments, except in Year 5 at Ladbrooks (Table 6-7 to Table 6-10). In Year 5, only tall fescue intercepted more PAR, probably due to the lack of data rather than a true species difference. The higher interceptance is shown in Table 6-7 to Table 6-10, and averaged 10% more at Ladbrooks in Year 5 (tall fescue only), 7% more in Year 6, 14% more at Ashley Dene, in Year 5 and 8% more in Year 6. These results suggest there were consistent morphological differences in the species that led to differences in light capture. These results are consistent with the description of tall fescue as having an erect growth habit, which allows it to capture more light (Maamouri *et al.*, 2015) due to higher leaf angle. In contrast, cocksfoot is a species that covers the ground by producing longer and wider leaves. The results for all species at Ashley Dene were also under the influence of the weed content, which was especially high for tall fescue being 18% in Year 5 (Table 4-21) and 46% in Year 6 (Table 4-22). Weeds covering the ground are not differentiated by the Green Seeker which means their quantification through botanical compostion measurements is important to interpret results (Figure 4-9 and Figure 4-10). It is possible that the higher weed content and erect habit of tall fescue resulted in higher PAR interceptance, at Ashley Dene in Year 6, whereas for cocksfoot it is explained by the higher VDM (Table 4-12). The results in this thesis are consistent with previous reports where cocksfoot and tall fescue have been shown to have similar levels of PAR interceptance but higher than other C_3 grasses (Kørup *et al.*, 2018) under the same growth conditions. It should be noted that this higher light interception did not translate to differences in total annual dry matter production which suggests other mechanisms restricted growth. Differences in species responses at the plant level are examined in Chapter 7.

The difference in lifecycle of Brome spp may also have influenced the intercepted PAR results. In each regrowth cycle its early reproductive development was used as an indicator of time to harvest. The earlier development also meant its leaves began to senesce earlier than the other species which can be expected to reduce green leaf area and intercepted PAR.

The RUE of each species were highest and not different in the non-limiting conditions, of nitrogen, water and temperature, in Spring as shown by the results for Ladbrooks in both years (Table 6-11 and Table 6-12), and for Year 5 at Ashley Dene (Table 6-13). However there was a difference among species at Ashley Dene in Year 6. Tall fescue had the lowest RUE and brome the highest (48% higher than tall fescue), followed by cocksfoot (42% higher than tall fescue), and ryegrass (29% higher than tall fescue). Given the total amount of dry matter produced was the same but light interception was different then RUE was calculated to be different, particularly for tall fescue all species at Ashley Dene in Year 6. The implication is that the four grasses employed different strategies to achieve the same TDM. For example, tall fescue and cocksfoot intercepted more light than brome and p. ryegrass (Table 6-10) but their yields were the same (Table 4-4). By definition this means RUE had to differ which implies differences in the photosynthetic strategies employed by each species, particularly when water was limiting.

The results in this chapter are consistent with those from Druille *et al.* (2019) who showed that RUE was more responsive to nitrogen then water and light availability. This is because RUE is dependent on the net photosynthetic rate, and it is affected by nitrogen, water, light intensity, temperature and leaf age (Peri *et al.*, 2003). The physiological basis of differences in RUE response at the plant (photosynthetic) level are investigated further in Chapter 7.

6.4.2.1 Yield differences within sites and between sites

Due to the lack of measurements in the start of Year 5, actions were taken to examine how the yield differences could be explained by differences in PAR interception and RUE. The incident radiation was conservative throughout the five years of the experiment and with historical values (Table 3-2). Furthermore the fraction of intercepted radiation/incident radiation at Ladbrooks and Ashley Dene was not different within sites between Years 5 and 6. Therefore yield differences between nitrogen treatments can be partially explained by differences in the intercepted PAR and RUE. In Year 5 at Ladbrooks, an extra 500 MJ m^{-2} was intercepted by the N+ pastures which accounts for an extra 4245 kg DM ha^{-1} (with a RUE of N+ plants of 0.849 g MJ^{-1} m^{-2} year^{-1}; Table 6-11). Similarly, for Year 6, the extra 5445 kg ha^{-1} comes from the additional 500 MJ m^{-2} at an RUE for N+ pastures of 1.089 g MJ^{-1} m^{-2} year^{-1} (Table 6-12). At Ashley Dene, in Year 5, the extra 70 MJ m^{-2} intercepted by N+ pastures only explains 335 kg ha^{-1} (RUE of all plants 0.475 g MJ^{-1} m^{-2} year^{-1}; Table 6-13). In Year 6, no differences in yield were observed.

Thus, overall the higher RUE of intercepted light accounted for 5580 kg ha^{-1} in Year 5 at Ladbrooks and 6700 kg ha^{-1} in Year 6. At Ashley Dene, the RUE were the same for the different nitrogen treatments. These differences added to those explained by the differences in PAWC and WUE (Section 5.4.2.1) account for all of the observed yield differences.

For the differences observed between sites, in Year 5, for the N+ grasses, the higher water use at Ladbrooks explained 5026 kg DM ha^{-1} of yield difference, but 3709 kg DM ha^{-1} was unexplained. The difference of extra 485 MJ m^{-2} intercepted at Ladbrooks explains an extra yield of 4118 kg DM ha^{-1} in Year 5 (RUE of N+ grasses at 0.849 g DM MJ^{-1} m^{-2}, Table 6-11) and 5281 kg DM ha^{-1} in Year 6 (RUE of N+ grasses at 1.089 g DM MJ^{-1} m^{-2}, Table 6-13). This means that in both Year 5 and 6, light interception explained 100% of the yield that was not explained by differences in water use.

Overall, the results show that restrictions in the soil moisture restrict radiation interceptance which decreases TDM accumulation. Nitrogen fertiliser allows plants to fully expand their canopy and maximize the capture of incident radiation. However, when the soil moisture is low, plants have a reduction in their interceptance but also the inability to utilize applied N. These results highlight that the measurement of PAWC and light interception can be used to explain yields differences in pastures. Optimizing yield therefore becomes an exercise in capturing and utilizing light and water as efficiently as possible. The results in this chapter and in Chapter 5 show the importance of nitrogen to do that but also how species may use different strategies to achieve the same result.

Mechanisms to study what happens physiologically to the plants that modify the canopy structure according to moisture and nitrogen levels, according to species, are detailed in Chapter 7.

6.5 CONCLUSION

- Nitrogen fertilisation is essential for the grasses to expand their canopy and intercept light. It increased LAI which resulted in an overall increase of 29% of intercepted light at Ladbrooks (both years) and from no increase up to 5 % at Ashley Dene.
- The mean light interceptance and RUE were 18% higher at Ladbrooks, compared with Ashley Dene, due to less restrictive soil moisture conditions and additional impact this had on nitrogen availability.
- Cocksfoot and tall fescue intercepted more light but this did not results in greater TDM yields.
- Tall fescue has an erect habit that allows it to intercept more light but it is unable, under restrictive conditions, to accumulate more TDM than the other species, which resulted in a lower RUE observed at Ashley Dene on Year 6.

In Chapter 7, plant physiological mechanisms that explain how grasses had their leaf area, related to the intercepted PAR, and photosynthetic rate, that is related to RUE, affected by the water stress, under different nitrogen level, is explored for each species.

7 PHYSIOLOGICAL TRAITS

7.1 Introduction

In Chapter 4 the agronomic yield and botanical composition of four grass species were quantified under two nitrogen and water regimes. Yield differences were related to water extraction and light interception in Chapters 5 and 6. Specifically, the yield of pastures was not different amongst species on most harvest dates. Yield was most influenced by the plant available water at each site and how the water availability according to season on each site allowed nitrogen to be utilized. Chapter 4 showed that cocksfoot maintained more vegetative dry matter than other species, particularly when the pastures were left to grow to a mature state. Further, in Chapter 6 differences in light interception were detected amongst species with higher radiation interception found for cocksfoot. Thus, yield results were not different amongst species but the quality of herbage was. These results indicate that the species utilised different strategies to achieve the same yields. Furthermore, cocksfoot, tall fescue and brome have all been previously recommended as more drought tolerant than perennial ryegrass (Section 2.6). In this chapter the emphasis is on the plant physiological traits that explain the differences in species responses reported in Chapters 4 to 6. Measurements focused on how they responded to water and nitrogen stress and whether there were common or different strategies employed by the different species.

7.2 Materials and Methods

Measurements for this chapter were taken from December 2018 to May 2020 at both Ladbrooks and Ashley Dene, for a total of 21 different occasions (16 at Ladbrooks and 5 at Ashley Dene).

7.2.1 Osmotic Potential

The leaf water potential was measured on the first fully expanded leaf. To do this three leaves were plucked from each plot, and stored in a microfuge tube.

Tubes were inserted into liquid nitrogen to freeze the tissues, left to defrost to rupture the cells and then centrifuged (Centrifuge 5415D, Eppendorf, Hamburg, Germany) at 12000

RPM for 3 minutes. 10 µL of the liquid part inside the tube was removed and loaded into a Vapor Pressure Osmometer VAPRO 5520 (Wescor Medicine and Science, Logan – UT, USA) for analysis.

The osmotic potential (Ψs) was calculated using Van't Hoff equation (Equation 19):

Equation 19 $$\Psi s = RT.Cj$$

In this equation RT is the gas constant (N m^2 mol^{-1}) at a specified temperature and Cj is total osmolality (mol kg^{-1}).

7.2.2 Proline Accumulation

To measure proline, six of the youngest, fully expanded, leaf laminas were randomly picked from for brome, cocksfoot and tall fescue and 12 leaves for p. ryegrass. Samples were immediately frozen in liquid nitrogen and stored in a -40 °C freezer. The frozen leaves were hand-ground, with a mortar and pestle, using liquid nitrogen until powder particle size.

Ground samples were then vacuum dried at Lincoln University Riddols Laboratory, for 48 h. After this, samples were weighed 10±0.6 mg and placed inside 2 mL centrifuge tubes. 1.2 mL of SSA reagent (Sulphosalicylic acid 3% weight/volume) was added to each tube, before they were vortexed then centrifuged for 7 min at 12000 RPM.

After the centrifuge, 1 mL of the supernatant was removed to another centrifuge tube and then centrifuged again using the same centrifuge conditions. 0.5 mL from the centrifuged tube was transferred to a glass vial and mixed with 2 mL of deionized water.

Another 2 mL of Ninhydrin reagent (1.5 g of Ninhydrin mixed with 60 mL glacial acetic acid and 40 mL of water) was added to the supernatant and this mixture was then put in a water bath for 1 hr at 100 °C. The reaction mixture was extracted with 2 mL toluene and the chromophore containing toluene was aspirated then cooled to room temperature. This mix had absorbance measured at 520 nm (Bokhari; 1985) with a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

For every day when proline was chemically analysed in the laboratory, standards were used to make the reference absorbance X concentration linear regression. Standards had the concentration of L-proline of 0; 1; 2.5; 5; 10; 20 and 30 mg L^{-1}. Proline accumulation was measured in µg g^{-1}.

7.2.3 Photosynthetic Rate and Conductance

Photosynthesis rate and conductance were measured using the LICOR 6400 Portable Photosynthesis System. This emits H_20 and CO_2 gases in a known controlled flow rate, then measures the gases output, to account for leaf respiration.

This device measures leaf temperature using a thermocouple that touches the leaf being used for photosynthesis measurements, and the light intensity is controlled to be kept at a constant level.

To measure photosynthesis, the youngest fully expanded leaf was selected, one per plot, and inserted into the machine´s measuring chamber (Evans, 2014). The photosynthesis rate and conductance measurements were taken when the reading is stable.

The photosynthetic rate was adjusted for leaf area inside the chamber. To do this the leaf width was measured with a Vernier and leaf length is always 30 mm, which is the chamber's length. The final output is measured in µmol CO_2 m^{-2} s^{-1}. Conductance , measured in mol H_20 m^{-2} s^{-1} , was also corrected for leaf area as well.

Operational restrictions for using the LICOR, due to low temperatures (air temperature lower than 8 °C), happened in the colder seasons of the year. Other procedures required changes to the light intensity in the portable measuring device (Table 7-1), from 1000 to 1500 µmol m^{-2} s^{-1} photosynthetic photon flux density, or vice-versa.

Table 7-1 Date, Site, Light Intensity and Average Leaf Temperature (Ave. Leaf Temp.) from taken LICOR measurements for brome, cocksfoot, perennial ryegrass and tall fescue, grown with or without nitrogen, from December 2018 to March 2020 in Ladbrooks (LB) and Ashley Dene (ASH), Canterbury, New Zealand. PPFD represents the photosynthetic photon flux density.

Date	Site	Light Intensity (μmol m^{-2} s^{-1} PPFD)	Ave Leaf Temp (°C)
07/12/2018	LB	1500	23.5
18/12/2018	ASH	1500	22.5
10/01/2019	ASH	1500	29.6
29/01/2019	LB	1500	26.8
13/02/2019	LB	1500	28.9
11/03/2019	LB	1000	22.0
10/04/2019	ASH	1500	24.4
3/05/2019	LB	1000	17.2
24/05/2019	LB	1000	14.9
28/08/2019	LB	1000	16.6
3/10/2019	LB	1000	13.5
21/11/2019	ASH	1000	26.1
16/01/2020	LB	1000	20.7
31/01/2020	LB	1000	24.1
16/03/2020	LB	1000	22.4

7.2.4 Chlorophyll Concentration

To estimate the amount of chlorophyll in the leaves, a SPAD (Soil Plant Analysis Development) device was used which makes non-destructive measurements. Three measured points on each leaf (base, medium and end) were averaged for each plot on the youngest fully expanded leaf. The SPAD measures the difference between the transmittance of red light (650 nm) and infrared light (940 nm) through the leaf. This measurement relates to the total amount of chlorophyll and does not differentiate between chlorophyll "A" or "B".

A calibration was made for each species, to obtain the specific concentration of chlorophyll (g mL^{-1}) with results from a DMF (N,N-Dimethylformamide, 5 mL used) extraction. The DMF extraction was based on a modified method proposed by Hofmann *et al.* (2003). This uses five leaf parts of each species for a known surface area with dimensions measured using a caliper and nitrogen from the SPAD value. Samples were submerged in a falcon tube with

5 mL of DMF. The tube was immediately closed and wrapped in aluminium foil, to prevent any contact between vegetable tissue and light, then kept for 24 h in a fridge.

The day after, the solution was measured in an UV-1800 spectrophotometer in two wavelengths, 664.5 and 647 nm, and the total amount of chlorophyll in the DMF solution estimated from Equation 20:

Equation 20

$$[\boldsymbol{Chloro}] = \mathbf{17.9} * \boldsymbol{A}\mathbf{647} + \mathbf{8.08} * \boldsymbol{A}\mathbf{664.5}$$

Where [Chloro] is the chlorophyll concentration in the solution (µg mL^{-1}), “A647” is the absorbance value at 647 nm and “A664.5” the absorbance value at 664.5 nm. The total amount of chlorophyll (mg) in the measured leaf area part is given by [Chloro]*5 (5 mL of DMF). The values used to make the regression chlorophyll content (mg m^{-2}) against SPAD values are shown in Table 7-2.

Table 7-2 Regressions used to obtain the Chlorophyll content (mg m-2) from the SPAD reading (X), for brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen.

Species	Nitrogen	Equation	R^2
Brome	N+	15.663*X-201.2	0.90
Brome	N-	$270.89*e^{0.0049*X}$	0.68
Cocksfoot	N+	13.346*X-98.402	0.94
Cocksfoot	N-	$108.51*e^{0.0319*X}$	0.81
Perennial Ryegrass	N+	11.283*X+33.271	0.89
Perennial Ryegrass	N-	55.748*X-1414.4	0.72
Tall Fescue	N+	3.4175*X+416.29	0.62
Tall Fescue	N-	$199.04*e^{0.023*x}$	0.52

7.2.5 Relative Water Content (RWC)

Leaf relative water content (RWC) reflects the balance between water supply to the leaf lamina tissue and transpiration rate (Lugojan and Ciulca 2011). A high RWC is an indicator of plant tolerance (Clarke and McCaig, 1982) to dry environments.

The method to determine RWC was described by Cyriac *et al.* (2018). A fully expanded leaf was taken from each plant and inserted into a Falcon tube of known weight and reweighed to obtain the leaf freshweight (FW).

The tube + leaf was then filled with water and left inside a fridge for 24 h to return the leaf to maximum turgor. The leaf was then removed and surface dried with a tissue before measuring its turgid weight (TW). The final step was to dry the leaf inside a forced air oven for at least 24h to determine its dry weight (DW).

RWC was then determined using Equation 21:

Equation 21 $$RWC = (FW - DW) / (TW - DW)$$

RWC is represented as a percentage.

7.2.6 Statistical Analyses

Repeated Measures ANOVAs were used to analyze the effect of nitrogen fertilisation, species and weather season for each variable separately.

Differences between sites are shown as differences between dates in the repeated measures ANOVAs. GenStat was used for those analyses. RWC needed ArcSin transformation to fit ANOVA assumptions.

A PCA (principal component analysis) was run, after the data as standardized, to analyse how the variables correlate to each other as well to visualize the effect of the nitrogen fertilisation and species on them. PCAs were run for each site separatedly as well as for the whole dataset (General Data, Section 7.3.6.1) to provide explanation of a general answer of the treatments under the different stress scenarions. The R Software with RStudio version 4.0.3 was used for this analysis and build some of the graphics related to it, others were done using Sigmaplot, as in other chapters. Wilk's Lambda values and variable correlations to each Principal Component are shown on Appendix Q.

Packages used in the RStudio were "readxl", "FactoMineR", "FactoInvestigate", "Factoshiny", "ggplot2", "factoextra", "devtools", "remotes" and "ggbiplot". PCAs were done for the whole dataset and for each site separately as well.

7.3 RESULTS

7.3.1 Osmotic Potential

Ladbrooks

The osmotic potential was increased (P<0.001) by nitrogen fertilisation. Plants that received fertiliser had a higher mean of -1.81 ± 0.008 MPa than non-fertilised plants, -1.86 ± 0.008 MPa.

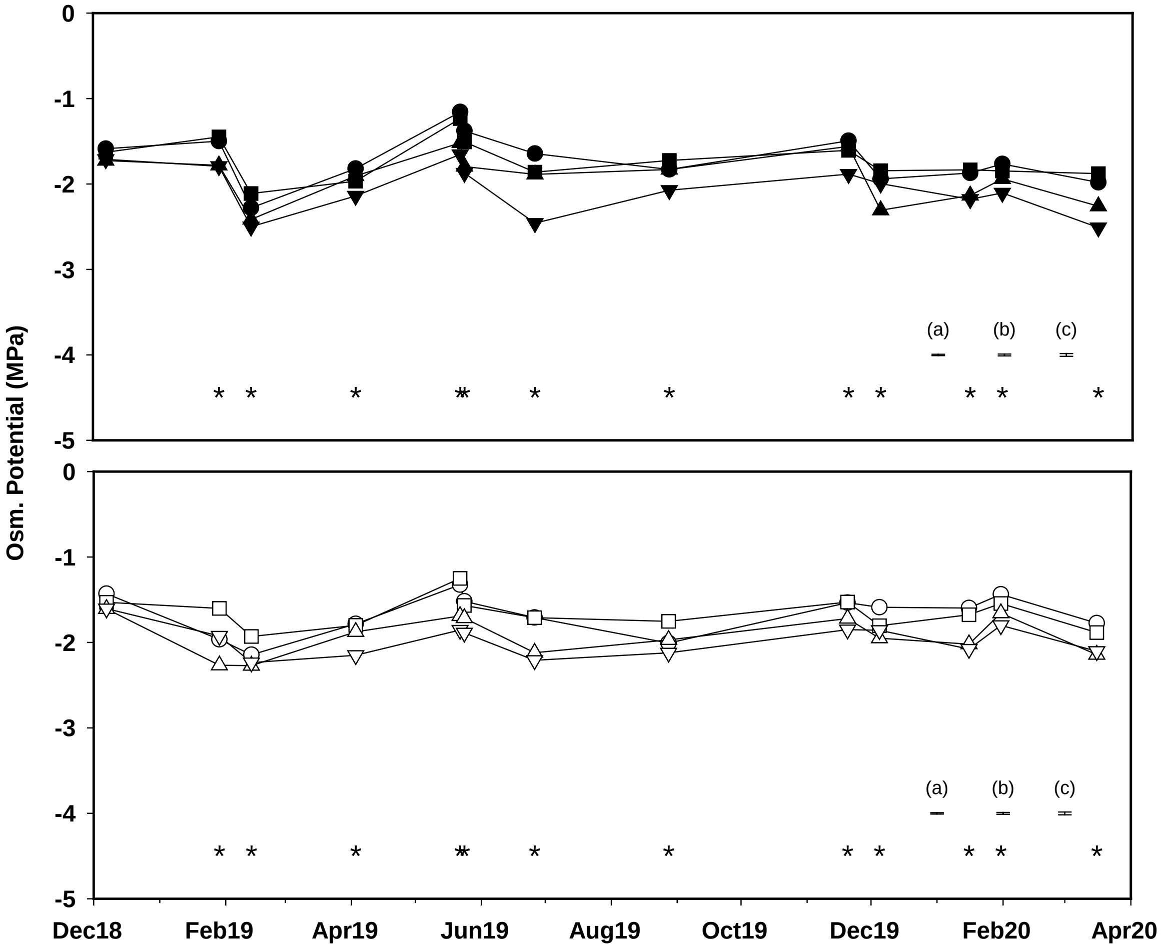


Figure 7-1 Osmotic Potential of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△)and tall fescue (▼,▽) from December 2018 to February 2020 at Ladbrooks, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. "*" represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

The osmotic potential was different amongst species (P<0.001). Cocksfoot and brome averaged -1.69 ± 0.011 MPa, but these values were higher than for perennial ryegrass, -1.93 ± 0.011 MPa and tall fescue, -2.02 ± 0.011 MPa (Table 7-3) across the measurement period (Figure 7-1).

The osmotic potential differed across measurement dates (P<0.001). The highest values were found in Ladbrooks when soil water content was high, for example,the value was -1.46 ± 0.18 MPa on 22/05/19 in Ladbrooks.

There was an interaction between dates and nitrogen fertilisation (P<0.001), with a reduction in osmotic potential more noticeable from June 2019 to November 2019, when water was available in the system. An interaction (P<0.001) between date and species was also observed with lower osmotic potential during dry periods (from December 2019 to May 2019 and from December 2020 to May 2020) for perennial ryegrass and tall fescue than brome and cocksfoot.

Table 7-3 Mean osmotic potential (MPa) from December 2018 to March 2020, from Ladbrooks, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D).

	N+				N-				
Date	BR	CF	PR	TF	BR	CF	PR	TF	Mean D
07/12/18	-1.586	-1.628	-1.725	-1.711	-1.430	-1.530	-1.607	-1.603	-1.603 e
29/01/19	-1.498	-1.448	-1.781	-1.792	-1.964	-1.601	-2.266	-1.923	-1.784 d
13/02/19	-2.280	-2.111	-2.414	-2.499	-2.144	-1.929	-2.271	-2.235	-2.235 a
03/04/19	-1.819	-1.966	-1.905	-2.140	-1.782	-1.800	-1.875	-2.149	-1.930 c
22/05/19	-1.156	-1.234	-1.518	-1.653	-1.323	-1.250	-1.690	-1.854	-1.460 f
24/05/19	-1.378	-1.507	-1.795	-1.871	-1.518	-1.566	-1.714	-1.887	-1.655 e
26/06/19	-1.644	-1.861	-1.887	-2.458	-1.708	-1.710	-2.120	-2.208	-1.950 c
28/08/19	-1.829	-1.724	-1.829	-2.072	-2.007	-1.752	-1.970	-2.121	-1.913 c
20/11/19	-1.493	-1.605	-1.560	-1.883	-1.533	-1.527	-1.719	-1.849	-1.646 e
05/12/19	-1.940	-1.844	-2.307	-1.995	-1.588	-1.803	-1.950	-1.856	-1.910 c
16/01/20	-1.871	-1.834	-2.134	-2.178	-1.597	-1.675	-2.020	-2.077	-1.923 c
31/01/20	-1.765	-1.847	-1.940	-2.104	-1.436	-1.543	-1.657	-1.799	-1.761 d
16/03/20	-1.981	-1.878	-2.260	-2.510	-1.744	-1.883	-2.142	-2.102	-2.063 b
Mean N	-1.808				-1.858				

	BR	CF	PR	TF	Mean
Mean S	-1.694	-1.694	-1.925	-2.020	-1.833

	P-Value	SEM	LSD
S	<0.001	0.011	0.033
N	<0.001	0.008	0.024
S*N	0.054	0.016	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Ashley Dene

At Ashley Dene, the osmotic potential differed amongst species (P<0.001). Perennial ryegrass and tall fescue had a 20% lower osmotic potential, -2.18 ± 0.075 MPa than brome and cocksfoot, -1.77 ± 0.075 MPa (Table 7-4). Nitrogen fertilisation did not affect (P=0.471) osmotic potential at Ashley Dene (Figure 7-2).

The osmotic potential was also different among dates (P<0.001) with the lowest value of -3.04 ± 0.009 MPa on 05/02/19 during a period of water scarcity.

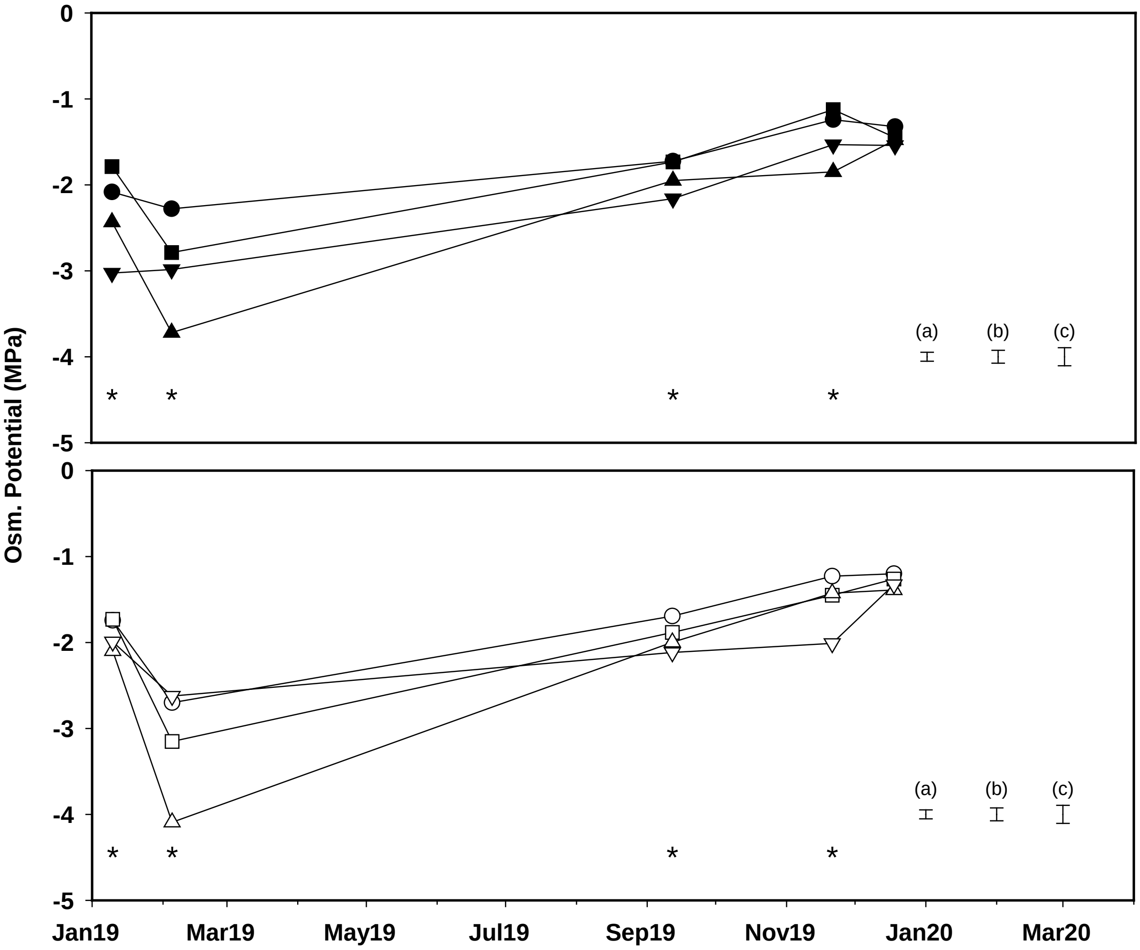


Figure 7-2 Osmotic Potential of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ashley Dene, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. ”*” represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

The lowest value for osmotic potential was -4.09 MPa found for non-fertilised ryegrass on 05/02/19 when ASMD was 108 mm, at Ashley Dene. The highest value was -1.12 MPa for fertilised cocksfoot on 21/11/19 when ASMD of 42 mm.

Table 7-4 Mean osmotic potential (MPa) from December 2018 to March 2020, from Ashley Dene, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D).

	N+				N-				
Date	BR	CF	PR	TF	BR	CF	PR	TF	Mean D
18/12/18	-1.323	-1.450	-1.478	-1.542	-1.201	-1.262	-1.389	-1.326	-1.371 c
10/01/19	-2.084	-1.788	-2.433	-3.027	-1.744	-1.731	-2.098	-1.992	-2.112 b
05/02/19	-2.279	-2.788	-3.719	-2.985	-2.701	-3.152	-4.092	-2.623	-3.042 a
12/09/19	-1.725	-1.733	-1.950	-2.160	-1.694	-1.884	-1.998	-2.117	-1.908 b
21/11/19	-1.242	-1.125	-1.849	-1.532	-1.229	-1.450	-1.426	-2.010	-1.483 c
Mean N	-1.956				-2.088				

	BR	CF	PR	TF	Mean
Mean S	-1.722	-1.836	-2.243	-2.131	-1.983

	P-Value	SEM	LSD
S	<0.001	0.075	0.219
N	0.471	0.053	-
S*N	0.422	0.105	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P. Ryegrass, “TF” represents Tall Fescue, “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

7.3.2 Proline accumulation

Ladbrooks

Proline accumulation was affected by the interaction of species and nitrogen fertiliser (P<0.001). Proline accumulation increased more for perennial ryegrass and tall fescue, superior to 4.50 ± 1.175 mg g^{-1} DM, on cocksfoot, the increase was 2.00 ± 1.175 mg g^{-1} DM and brome 0.3 ± 1.175 mg g^{-1} DM (Table 7-6).

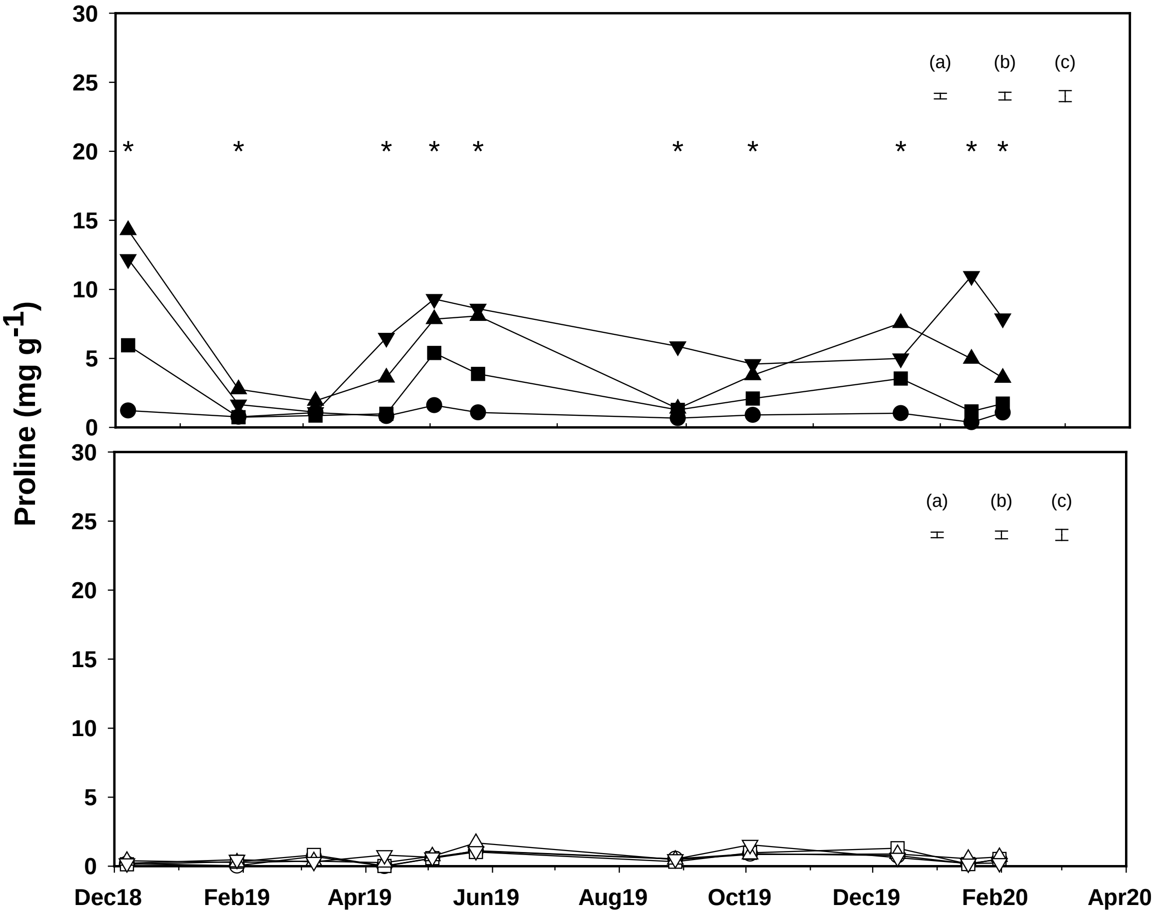


Figure 7-3 **Proline accumulation (mg g^{-1} DM) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ladbrooks and Ladbrooks, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation."*" represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.**

Table 7-5 Mean proline accumulation (mg g^{-1} DM) for brome, cocksfoot, p. ryegrass, cocksfoot and tall fescue, grown with (N+) and without (N-) nitrogen, from December 2018 to January 2021, at Ladbrooks, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D).

	N+				N-				
Date	BR	CF	TF	PR	BR	CF	TF	PR	Mean D
07/12/18	1.22	5.95	14.29	12.19	0.19	0.14	0.40	0.22	4.32 a
29/01/19	0.77	0.74	2.76	1.66	0.04	0.33	0.28	0.48	0.88 e
07/03/19	1.08	0.86	1.93	1.11	0.71	0.82	3.61	0.32	1.30 de
10/04/19	0.82	0.99	0.26	6.49	0.00	0.02	7.85	0.81	2.15 bc
03/05/19	1.61	5.40	0.73	9.30	0.58	0.59	0.73	0.65	2.45 b
24/05/19	1.09	3.88	8.08	8.60	1.13	1.03	1.70	1.10	3.33 ab
28/08/19	0.67	1.27	1.37	5.88	0.54	0.33	0.48	0.52	1.38 cde
03/10/19	0.91	2.09	3.78	4.59	0.91	0.98	0.85	1.56	1.96 cde
13/12/19	1.03	3.54	7.57	5.00	0.77	1.31	0.89	0.62	2.59 bc
16/01/20	0.38	1.16	4.98	10.97	0.19	0.16	0.55	0.20	2.32 bcd
31/01/20	1.07	1.72	3.60	7.90	0.50	0.53	0.68	0.23	2.03 cde
Mean N	3.77				0.58				
	BR		CF		PR		TF		Mean
Mean S	0.80		1.54		2.94		3.43		2.18

	P-Value	SEM	LSD
S	<0.001	0.282	0.831
N	<0.001	0.200	0.587
S*N	<0.001	0.399	1.175

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass,"TF" represents Tall Fescue, "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Table 7-6 Mean proline accumulation (mg g^{-1} DM), for brome, cocksfoot, perennial ryegrass, cocksfoot and tall fescue, grown with (N+) and without (N-) nitrogen, in Ladbrooks, Canterbury, New Zealand, from, for the interaction Species x Nitrogen.

	Species			
Nitrogen	BR	CF	PR	TF
+N	0.99	2.54	5.27	6.29
-N	0.62	0.53	0.61	0.57

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, the standard error of means is 0.282 for species, 0.200 for nitrogen and 0.399 for the interaction. and the Least Significant Difference at 5% are 0.831 for species, 0.587 for nitrogen and 1.175 for the interaction.

Ashley Dene

Proline accumulation was not the same for the species according to different nitrogen levels (P<0.001). Tall fescue and p. ryegrass responded in a different way having a much higher increase (at least 10.6 ± 1.415 mg g^{-1} DM) than brome and cocksfoot (maximum 2.80 ± 1.415 mg g^{-1} DM) (Table 7.8). There was a difference (P=0.018) in the proline accumulation amongst the different days of measurement. Dates in January and February 2019 had the highest proline values (Figure 7-4).

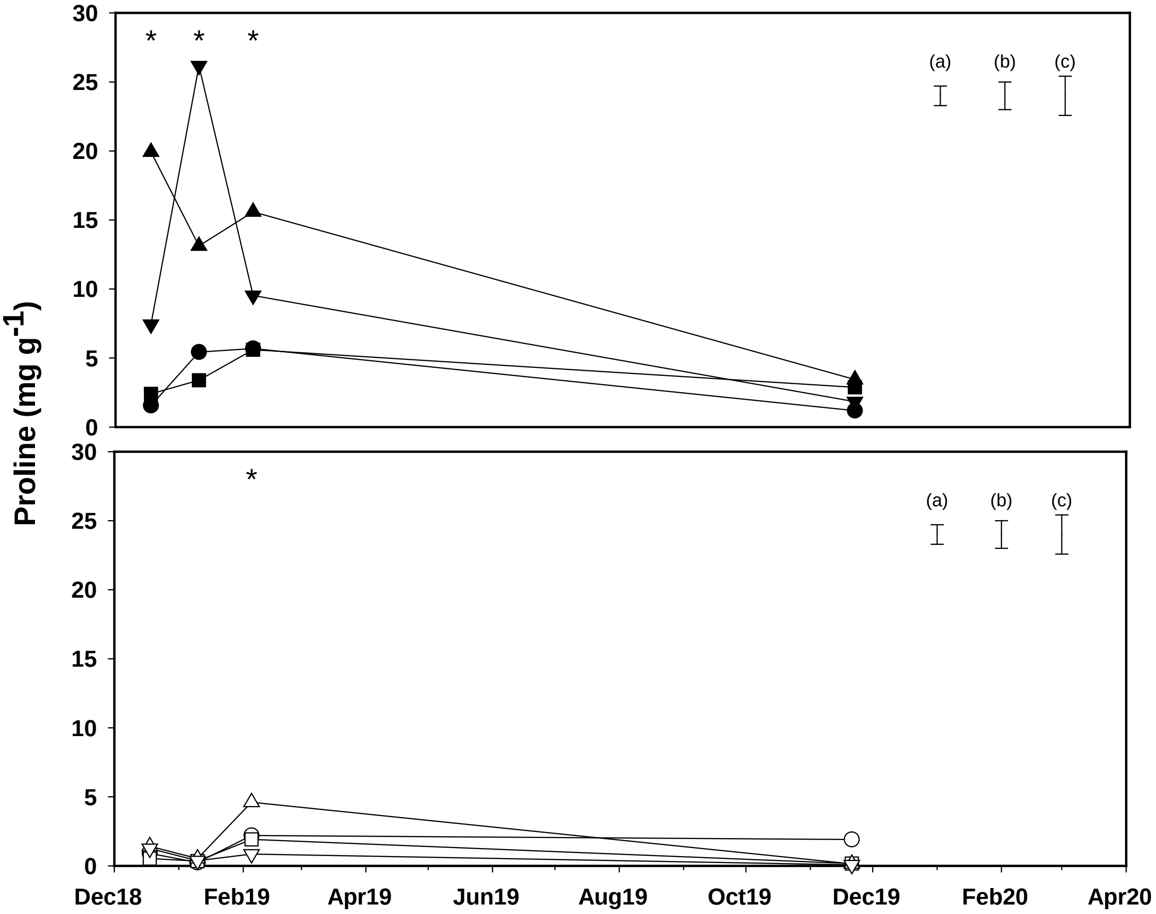


Figure 7-4 Proline accumulation (mg g^{-1} DM) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ladbrooks and Ashley Dene, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. "*" represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

Table 7-7 Mean proline accumulation (mg g^{-1} DM) for brome, cocksfoot, perennial ryegrass, cocksfoot and tall fescue, grown with (N+) and without (N-) nitrogen, from December 2018 to January 2021, in Ashley Dene, Canterbury, New Zealand according to species (S), nitrogen fertilisation (N) and date (D).

	N+				N-				
Date	BR	CF	TF	PR	BR	CF	TF	PR	Mean D
18/12/18	1.55	2.41	19.93	7.43	0.91	0.53	1.42	1.26	4.43 ab
10/01/19	5.43	3.39	13.12	26.18	0.24	0.35	0.53	0.37	6.20 a
05/02/19	5.69	5.60	15.59	9.53	2.20	1.91	4.60	0.85	5.74 a
21/11/19	1.19	2.88	3.44	1.84	1.91	0.17	0.14	0.08	1.45 b
Mean N	7.89				1.09				
		BR		CF		PR		TF	Mean
Mean S		2.39		2.15		7.35		5.94	4.49

	P-Value	SEM	LSD
S	0.002	1.000	2.942
N	<0.001	0.707	2.080
S*N	0.004	1.415	4.160

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass,"TF" represents Tall Fescue, "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Table 7-8 Mean proline Concentration (mg g^{-1} DM), for brome, cocksfoot, perennial ryegrass, cocksfoot and tall fescue, grown with (N+) and without (N-) nitrogen, in Ashley Dene, Canterbury, New Zealand, from, for the interaction Species x Nitrogen.

	Species			
Nitrogen	BR	CF	PR	TF
+N	3.46	3.57	13.02	11.24
-N	1.31	0.74	1.67	0.64

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue. The P-value for the interaction is 0.004, the standard error of means is 1.415 for the interaction Nitrogen*Species and the Least Significant Difference at 5% is 4.160 mg g^{-1} DM for the interaction Nitrogen*Species.

7.3.3 Photosynthetic Rate and Conductance

Photosynthetic Rate

Ladbrooks

The net photosynthetic rate (Pn) was higher (P=0.034) for brome, tall fescue and p. ryegrass, 13.4 ± 0.54 µmol CO_2 m^{-2} s^{-1}. Cocksfoot had a Pn of 12.0 ± 0.54 µmol CO_2 m^{-2} s^{-1} (Table 7-9).

Pn was increased (P=0.041) by nitrogen fertilisation, particularly for regrowth cycles that took place when water was available (P=0.015) such as on 29/01/19, 24/05/2019, 28/08/19 and 03/10/2019 (Figure 7-5).

There was a difference in the measured dates (P<0.001). The dates where water restrictions were higher showed a lower net photosynthetic rate, such as in 16/01/20; 31/01/20 and 16/03/20.

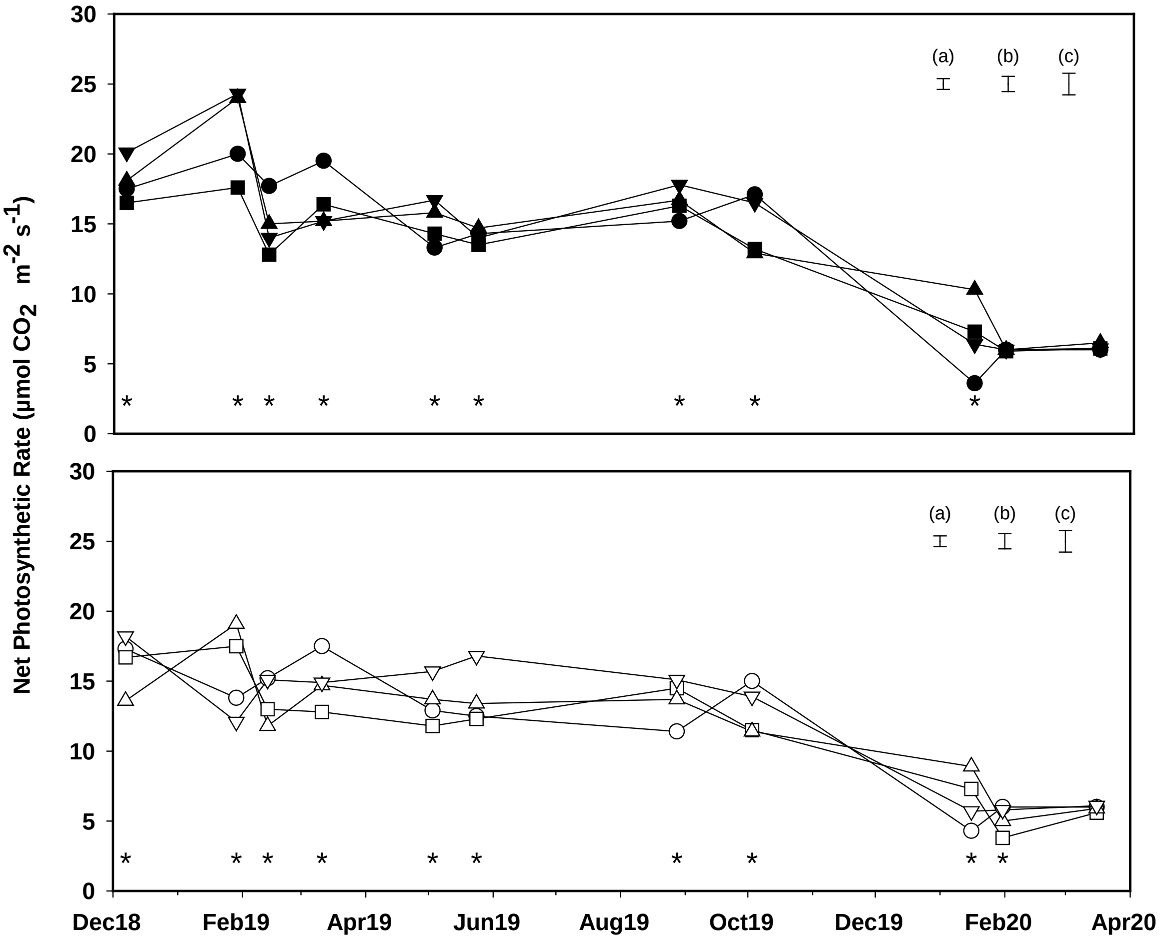


Figure 7-5 Net photosynthetic rate (μmol of CO_2 m^{-2} s^{-1}) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ladbrooks, Canterbury, New Zealand with (closed) or without (open) nitrogen fertilisation."*" represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

Table 7-9 Phostosynthetic rate (µmol CO_2 m^{-2} s^{-1}) of brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen from December 2018 to March 2020 at Ladbrooks, Canterbury, New Zealand.

	N+				N-				
Date	BR	CF	PR	TF	BR	CF	PR	TF	Mean D
07/12/18	17.5	16.5	18.1	20.1	17.3	16.7	13.6	18.2	16.9 ab
29/01/19	20	17.6	24	24.3	13.8	17.5	19.1	12.1	18.5 a
13/02/19	17.7	12.8	15	14	15.2	13	11.8	15.1	14.3 bc
11/03/19	19.5	16.4	15.2	15.2	17.5	12.8	14.7	14.9	15.8 abc
03/05/19	13.3	14.3	15.8	16.7	12.9	11.8	13.7	15.7	14.3 bc
24/05/19	14.3	13.5	14.7	17.1	12.5	12.3	13.4	16.8	16.6 abc
28/08/19	15.2	16.3	16.7	17.8	11.4	14.5	13.7	15.1	15.1 bc
03/10/19	17.1	13.2	12.9	16.5	15	11.5	11.4	13.9	13.9 c
16/01/20	3.6	7.3	10.3	6.4	4.3	7.3	8.9	5.7	6.7 d
31/01/20	6	5.9	6	6	6	3.8	5	5.8	5.5 d
16/03/20	6	6.1	6.5	6.1	6	5.6	5.9	6.1	6 d
Mean N	14.1				12.0				

	BR	CF	PR	TF	Mean
Mean S	12.8	12.1	13.0	14.4	13.0

	P-Value	SEM	LSD
S	0.034	0.54	1.60
N	0.041	0.41	1.27
S*N	0.160	0.77	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Ashley Dene

Brome and p. ryegrass had a higher(P<0.001) Pn, 10.73 ± 0.483 µmol CO_2 m^{-2} s^{-1}, than cocksfoot and tall fescue, 7.54 ± 0.483 µmol CO_2 m^{-2} s^{-1} (Figure 7-6). This means brome and p. ryegrass had a Pn rate more than 40% higher than cocksfoot and tall fescue, in Ashley Dene, for the duration of the experiment (Table 7-10).

Different measurement dates were significantly different (P<0.001), in part because of environmental variables, in part because of differences in the LICOR operational characteristics (Figure 7-6).

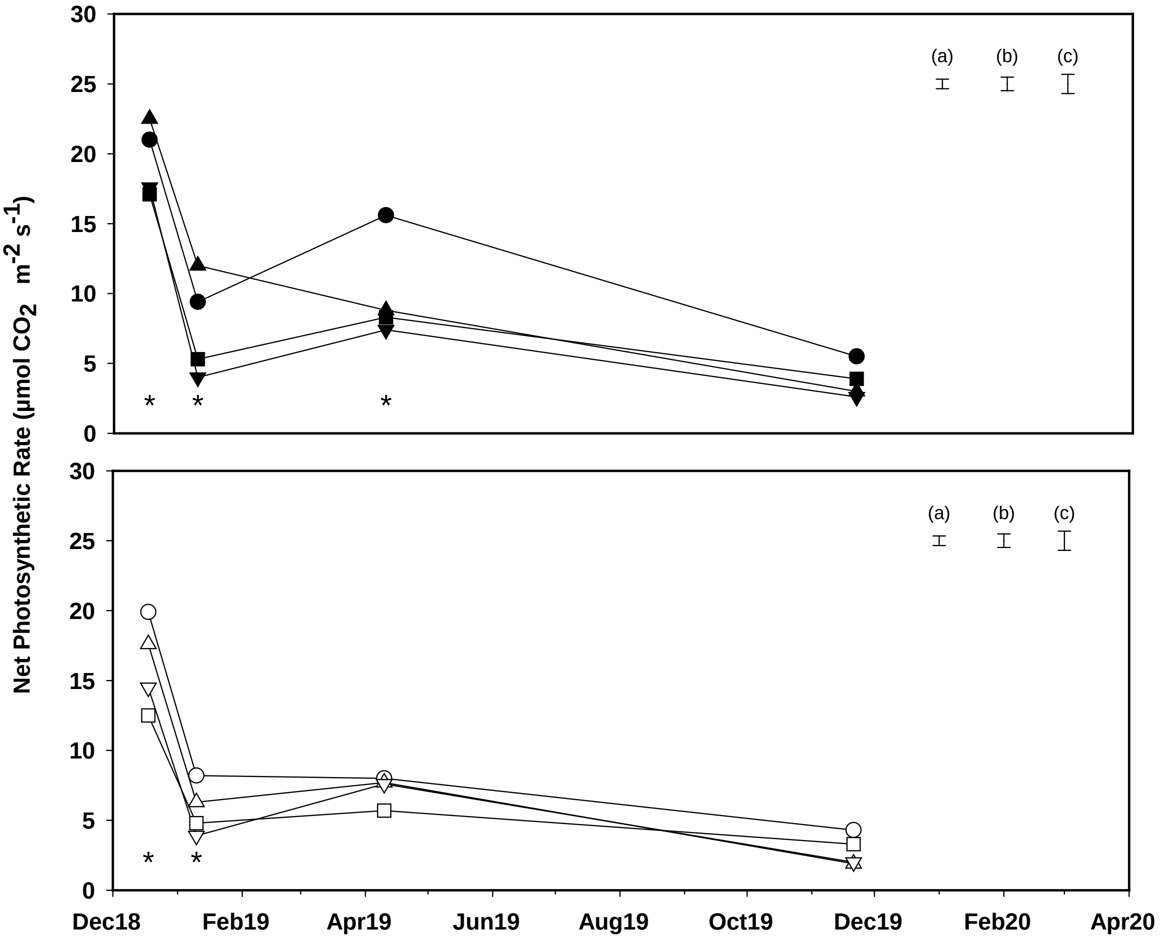


Figure 7-6 Photosynthetic rate (µmol of CO_2 m^{-2} s^{-1}) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ashley Dene, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. "*" represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

The Pn rate was increased (P=0.042) by nitrogen fertilisation by 25%, or from 8.0 to 10.2 ± 1.00 µmol of CO2 m^{-2} s^{-1} (Table 7-10), and there was an interaction amongst date and nitrogen (P=0.002). Pastures fertilised with nitrogen had a higher Pn rate than those unfertilised except on 21/11/2019.

Table 7-10 Net phostosynthetic rate (μmol CO_2 m^{-2} s^{-1}) of brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen from December 2018 to March 2020 at and Ashley Dene, Canterbury, New Zealand.

	N+				N-				
Date	BR	CF	PR	TF	BR	CF	PR	TF	Mean D
18/12/18	21.0	17.1	22.5	17.6	19.9	12.5	17.6	14.5	17.8 a
10/01/19	9.4	5.3	12	4	8.2	4.8	6.3	3.9	6.7 c
10/04/19	15.6	8.3	8.8	7.4	8	5.7	7.7	7.6	8.6 b
21/11/19	5.5	3.9	3	2.6	4.3	3.3	1.9	2	3.3 d
Mean N			10.2				8.0		
	BR		CF		PR			TF	Mean
Mean S	11.5		7.6		10.0			7.5	9.1

	P-Value	SEM	LSD
S	<0.001	0.48	1.42
N	0.042	0.34	1.00
S*N	0.085	0.68	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Stomatal conductance

Ladbrooks

Pastures fertilised with nitrogen had a higher (P=0.024) stomatal conductance, 0.02 ± 0.006 mol H_2O m^{-2} s^{-1} than non-fertilised ones.

Stomatal conductance was the highest for tall fescue (P=0.004), at 0.42 ± 0.008 mol H_2O m^{-2} s^{-1}, followed by brome, then cocksfoot and p. ryegrass which were not different amongst themselves and averaged 0.305 ± 0.008 mol H_2O m^{-2} s^{-1} (Table 7-11).

Average values per measurement date differed (P<0.001) and tended to be lower (Figure 7-7) when the Pn was low as well, for example, the measurements done on 16/01/20, 31/01/20 and 16/03/20 had conductance values of 0.057; 0.035 and 0.044 ± 0.004 mol H_2O m^{-2} s^{-1}.

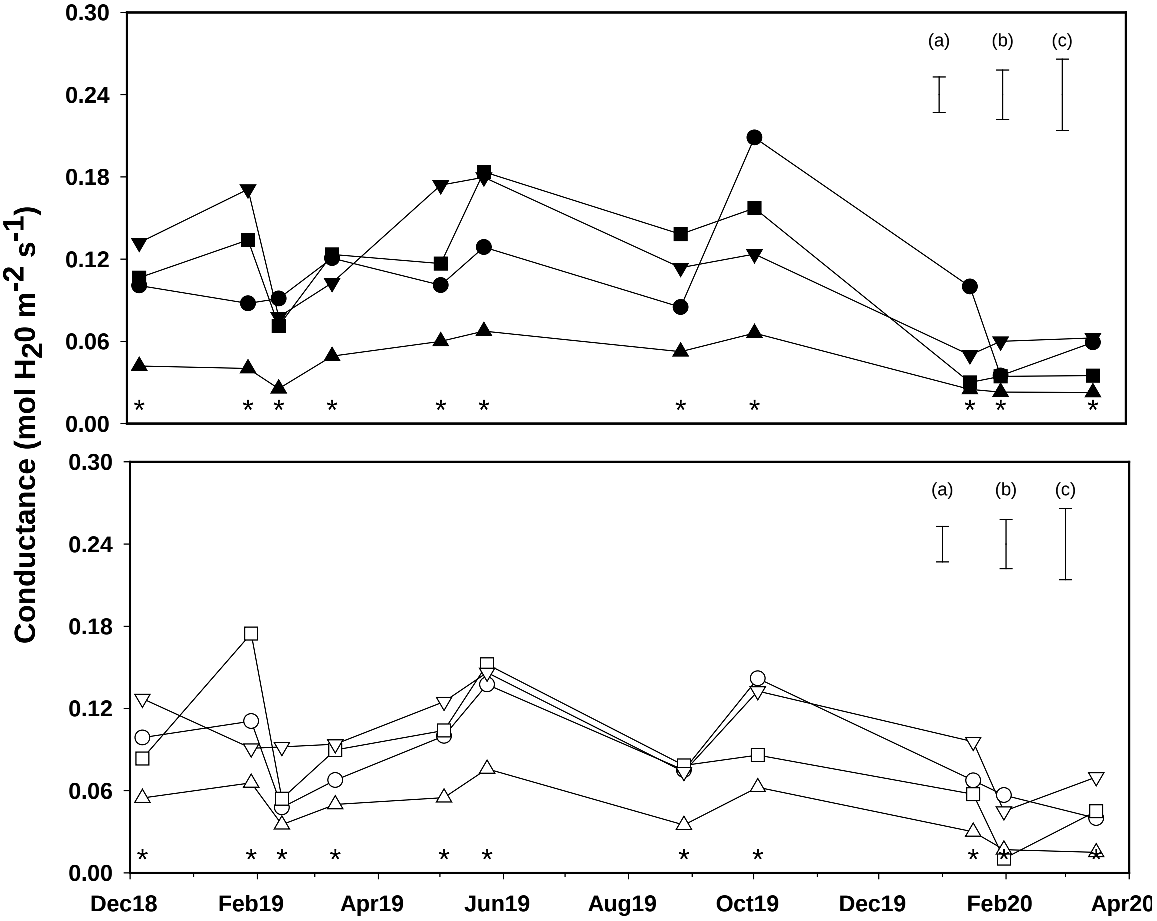


Figure 7-7 Stomatal conductance (mol H_2O m^{-2} s^{-1}) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ladbrooks, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. "*" represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

Table 7-11 Mean stomatal conductance (mol H_20 m^{-2} s^{-1}) of brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen from December 2018 to March 2020, at Ladbrooks, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D).

	N+				N-				
Date	BR	CF	PR	TF	BR	CF	PR	TF	Mean D
07/12/18	0.101	0.107	0.042	0.132	0.099	0.084	0.055	0.127	0.093 bc
29/01/19	0.088	0.134	0.040	0.171	0.111	0.175	0.066	0.091	0.109 abc
13/02/19	0.091	0.071	0.026	0.078	0.048	0.054	0.035	0.092	0.062 cd
11/03/19	0.121	0.124	0.049	0.103	0.068	0.090	0.050	0.094	0.087 bd
03/05/19	0.501	0.117	0.060	0.174	0.100	0.104	0.055	0.125	0.154 a
24/05/19	0.129	0.184	0.068	0.180	0.138	0.152	0.076	0.146	0.134 ab
28/08/19	0.085	0.138	0.053	0.114	0.075	0.079	0.035	0.074	0.081 bc
03/10/19	0.209	0.157	0.066	0.124	0.142	0.086	0.063	0.133	0.122 ab
16/01/20	0.100	0.030	0.025	0.050	0.068	0.058	0.030	0.096	0.057 cd
31/01/20	0.035	0.035	0.023	0.060	0.057	0.011	0.017	0.045	0.035 d
16/03/20	0.059	0.035	0.023	0.063	0.040	0.045	0.015	0.070	0.044 d
Mean N			0.099				0.079		

	BR	CF	PR	TF	Mean
Mean S	0.112	0.094	0.044	0.106	0.089

	P-Value	SEM	LSD
S	<0.001	0.008	0.025
N	0.024	0.006	0.018
S*N	0.172	0.012	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Ashley Dene

Brome had the highest ($P<0.001$) stomatal conductance at Ashley Dene of 0.08 ± 0.004 mol H_2O m^{-2} s^{-1}, followed by cocksfoot and tall fescue at 0.06 ± 0.004 mol H_2O m^{-2} s^{-1}. Stomatal conductance for perennial ryegrass was lowest at 0.04 ± 0.004 mol H_2O m^{-2} s^{-1} (Table 7-12).

The date with the lowest ($P<0.01$) stomatal conductance of 0.02 ± 0.006 mol H_2O m^{-2} s^{-1} was on 10/01/19, which was less than a fifth of the that on 18/12/19 (Figure 7-8).

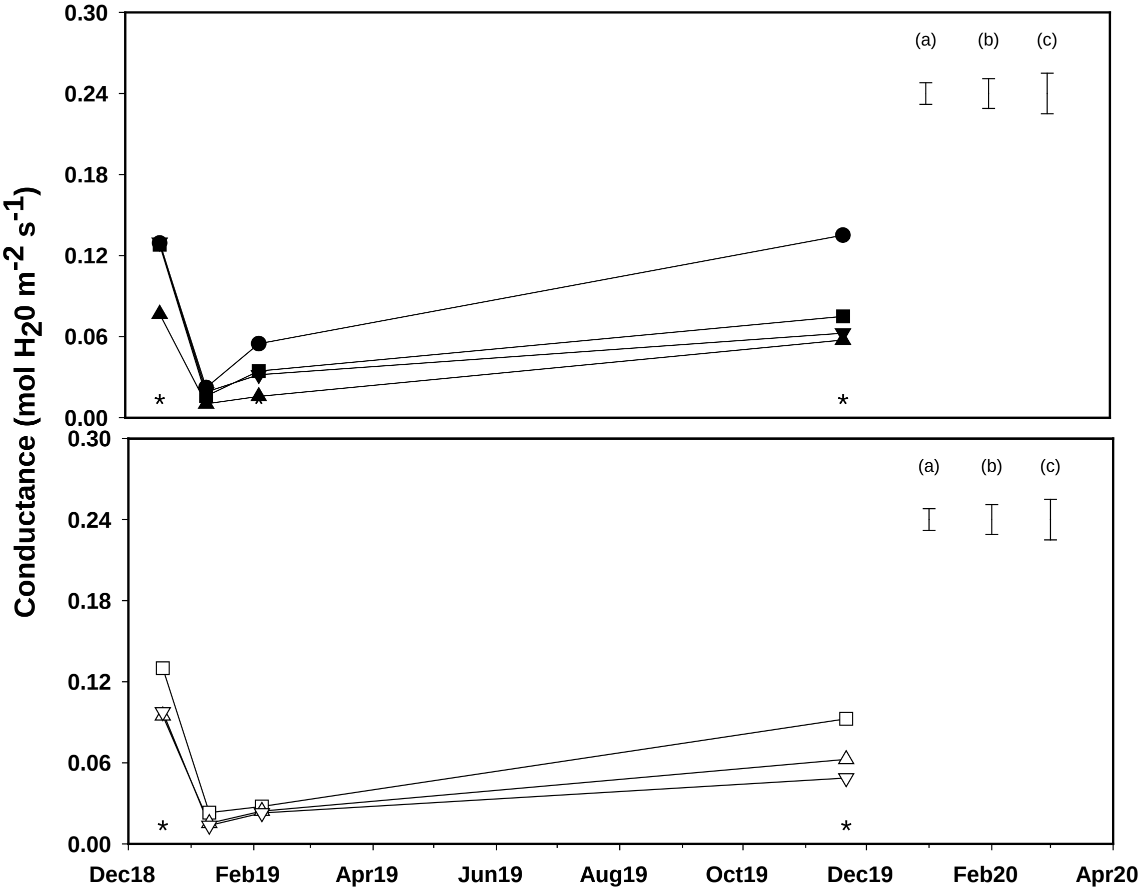


Figure 7-8 Stomatal conductance (mol H_2O m^{-2} s^{-1}) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to February 2020 at Ashley Dene, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. "*" represents when differences amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

Table 7-12 Mean stomatal conductance (mol H_20 m^{-2} s^{-1}) of brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen from December 2018 to March 2020, at Ashley Dene, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D).

	N+				N-				
Date	BR	CF	PR	TF	BR	CF	PR	TF	Mean D
18/12/18	0.129	0.128	0.077	0.130	0.151	0.109	0.081	0.115	0.115 a
10/01/19	0.022	0.016	0.010	0.019	0.028	0.018	0.013	0.015	0.018 c
10/04/19	0.055	0.035	0.016	0.032	0.024	0.032	0.017	0.029	0.030 c
21/11/19	0.135	0.075	0.058	0.063	0.100	0.085	0.040	0.058	0.077 b
Mean N			0.062				0.057		

	BR	CF	PR	TF	Mean
Mean S	0.080	0.062	0.039	0.057	0.060

	P-Value	SEM	LSD
S	<0.001	0.004	0.013
N	0.238	0.003	-
S*N	0.924	0.006	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P. Ryegrass, “TF” represents Tall Fescue, “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

7.3.4 Chlorophyll concentration

Ladbrooks

The chlorophyll concentration (CC) was different (P=0.033) on different measurement dates. The measurement on 03/10/19 had the highest average, of 562.8 ± 34.78 mg m^{-2}, 40% higher than the other days, which had an average of 400 ± 34.78 mg m^{-2} (Figure 7-9).

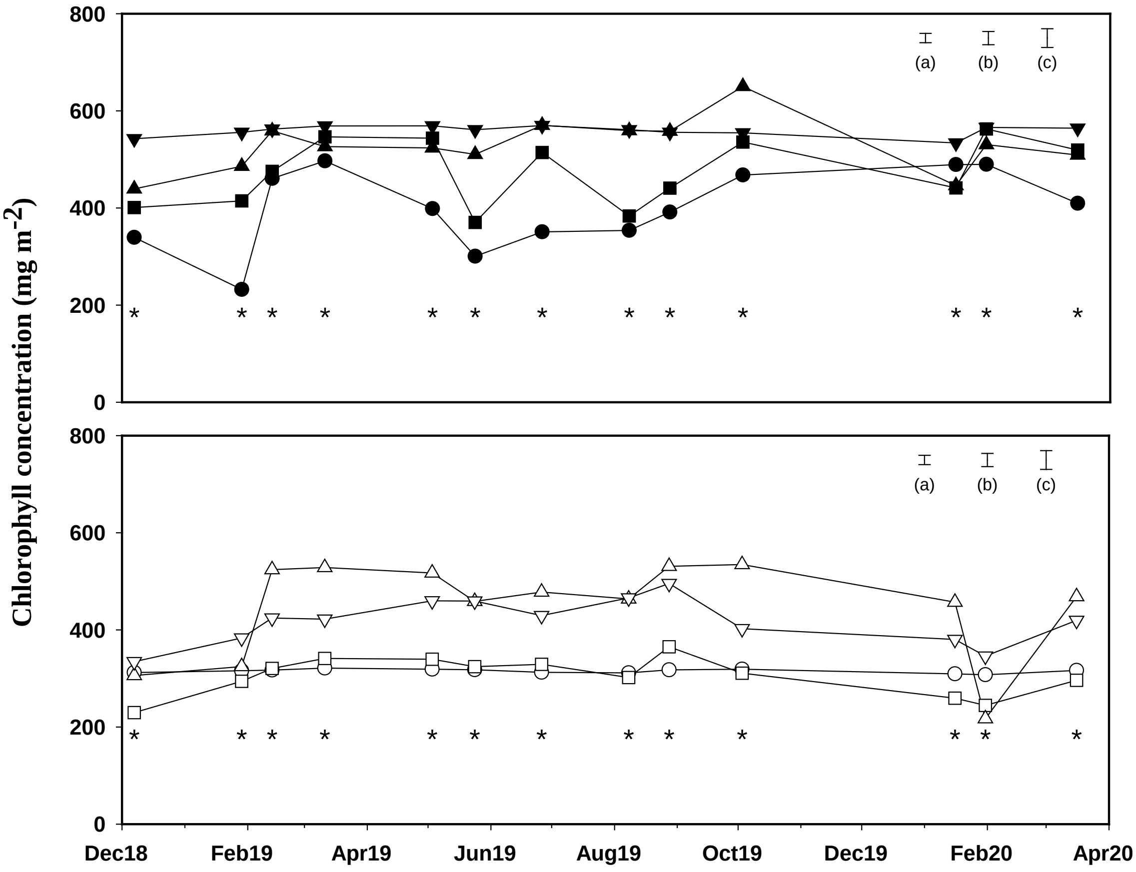


Figure 7-9 Chlorophyll concentration (mg m^{-2} of leaf area) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to April 2020 at Ladbrooks, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. "*" represents when differces amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

For the CC, there was an interaction between nitrogen and species (P<0.001). It increased CC more for cocksfoot, 169 ± 19.3 mg m^{-2}, than other species, which had a maximum increase of 142 ± 19.3 mg m^{-2} (Table 7.13).

Table 7-13 Mean chlorophyll concentration (mg m^{-2}) for brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen, from December 2018 to February 2020 at Ladbrooks, Canterbury, New Zealand with (N+) or without (N-) nitrogen fertilisation. (S), nitrogen fertilisation (N) and date (D).

	N+				N-				
Date	BR	CF	PR	TF	BR	CF	PR	TF	Mean D
07/12/18	340	400	440	540	310	230	310	330	360 c
29/01/19	230	420	490	560	320	290	320	380	380 c
13/02/19	460	480	560	560	320	320	520	420	460 bc
03/05/19	520	510	560	570	330	360	990	510	540 b
24/05/19	400	540	520	570	320	340	520	460	460 bc
26/06/19	300	370	510	560	320	320	460	460	410 c
08/08/19	330	350	420	530	320	310	770	420	430 bc
03/10/19	350	380	560	560	310	300	660	470	660 a
05/12/19	470	540	650	550	320	310	535	403	470 bc
16/01/20	490	440	450	530	310	260	460	380	410 c
31/01/20	490	560	530	570	310	240	220	346	410 c
16/03/20	410	520	510	560	320	300	470	420	440 bc
Mean N			490				415		
	BR		CF		PR		TF		Mean
Mean S	360		390		570		490		450

	P-Value	SEM	LSD
S	<0.001	13.6	40.1
N	<0.001	9.6	28.4
S*N	<0.001	19.3	56.7

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass,"TF" represents Tall Fescue, "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Table 7-14 Mean chlorophyll concentration (mg m^{-2}), for brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen, at Ladbrooks, Canterbury, New Zealand for the interaction of Nitrogen and Species.

	Species			
Nitrogen	BR	CF	PR	TF
N+	399	473	528	559
N-	315	304	616	417

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass,"TF" represents Tall Fescue, the P-Value for the interaction Nitrogen*Species is <0.001, the Standard Error of the Mean is 19.3 mg m^{-2}, and the Least Significant Difference at 5% is 56.7 mg m^{-2}.

Ashley Dene

The CC was different ($P<0.001$) for different dates. It was 20% lower, 420 ± 8.1 mg m^{-2} (Table 7-15), when compared to periods of higher soil water available to plants (Figure 7-10).

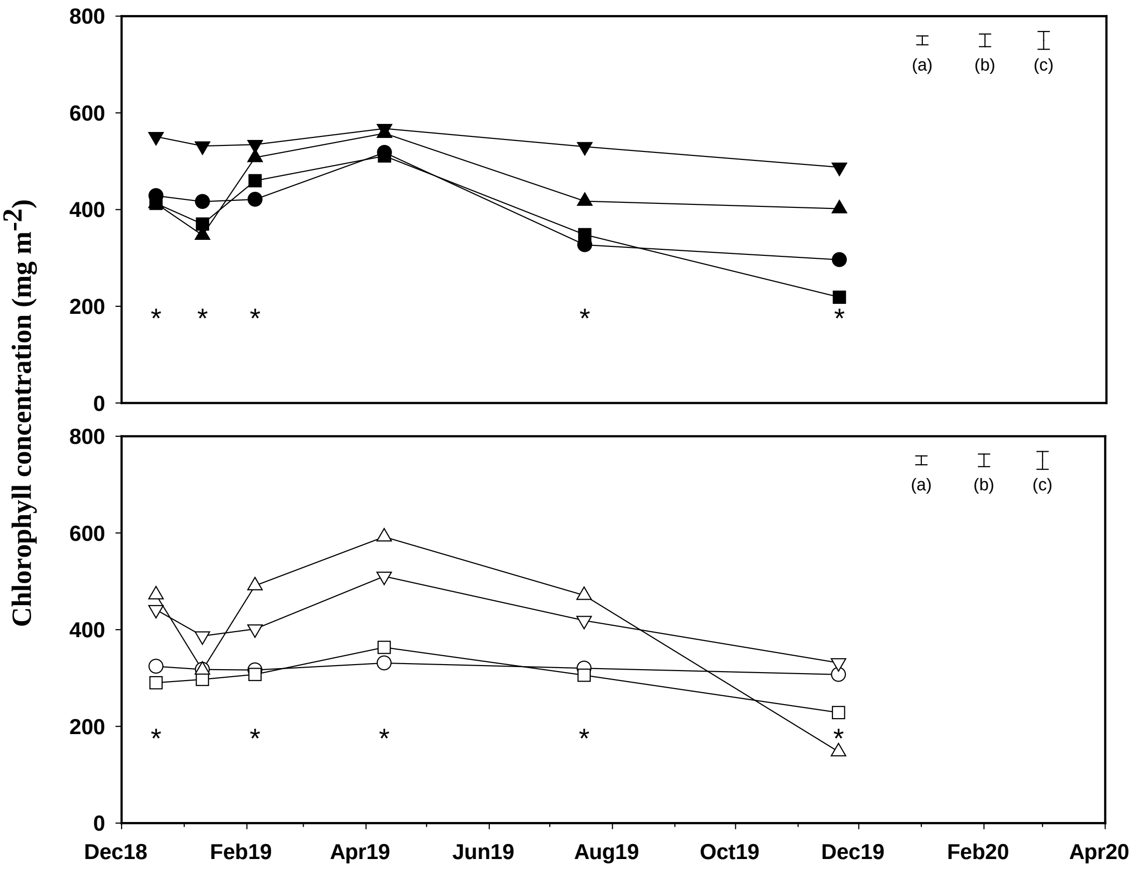


Figure 7-10 Chlorophyll concentration (mg m^{-2} of leaf area) of brome (●,○), cocksfoot (■,□), p. ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to April 2020 at Ashley Dene, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. "*" represents when differces amongst the species were observed. Standard error of means are represented for (a) nitrogen, (b) species and (c) nitrogen*species.

There was an interaction ($P<0.001$) between species and nitrogen for chlorophyll content (Table 7-15). The effect of nitrogen fertilisation was higher for cocksfoot (Table 7-16), an increase of 188 mg m^{-2}, whilst, this increase in other species was of, maximum 118 mg m^{-2}.

Table 7-15 Mean chlorophyll concentration (mg m^{-2}) for brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen, from December 2018 to February 2020 at Ashley Dene, Canterbury, New Zealand with (N+) or without (N-) nitrogen fertilisation. (S), nitrogen fertilisation (N) and date (D).

	N+				N-				
Date	BR	CF	PR	TF	BR	CF	PR	TF	Mean D
18/12/18	430	410	410	550	320	290	470	440	420 ab
10/01/19	420	370	350	530	320	300	320	390	370 b
05/02/19	420	460	510	530	320	310	490	400	430 ab
10/04/19	500	550	530	570	320	340	530	420	470 a
18/07/19	350	510	570	570	310	330	550	430	420 ab
21/11/19	300	220	400	490	310	230	150	331	300 c
Mean N			440				380		

	BR	CF	PR	TF	Mean
Mean S	360	345	460	475	410

	P-Value	SEM	LSD
S	<0.001	13.0	38.2
N	<0.001	9.2	27.0
S*N	0.002	18.4	54.0

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass,“TF” represents Tall Fescue, “SEM” represents Standard Error of the Mean and “LSD” represents Least Significant Difference at 5%. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Table 7-16 Mean chlorophyll concentration (mg m^{-2}), for brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen, at Ashley Dene, Canterbury, New Zealand for the interaction of Nitrogen and Species.

	Species			
Nitrogen	BR	CF	PR	TF
N+	401	387	440	533
N-	319	299	482	415

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P. Ryegrass,“TF” represents Tall Fescue. The P-Value for the interaction Nitrogen*Species is 0.002, the Standard Error of the Mean is 18.4 mg m^{-2} and the Least Significant Difference at 5% is 54.0 mg m^{-2}.

7.3.5 Relative water content (RWC)

Ladbrooks

The RWC was different (P=0.003) for species at Ladbrooks (Table 7-17). Brome, cocksfoot and p. ryegrass had the highest average value of 93 ± 0.2 %. Tall Fescue had the lowest value, 89 ± 0.2% (Table 7-17). Dates with a higher soil moisture deficit had higher values

(P<0.001), such as in 13/02/19, with an ASMD of 109 mm, and 05/12/19, with an ASMD of 86 mm (Figure 7-11).

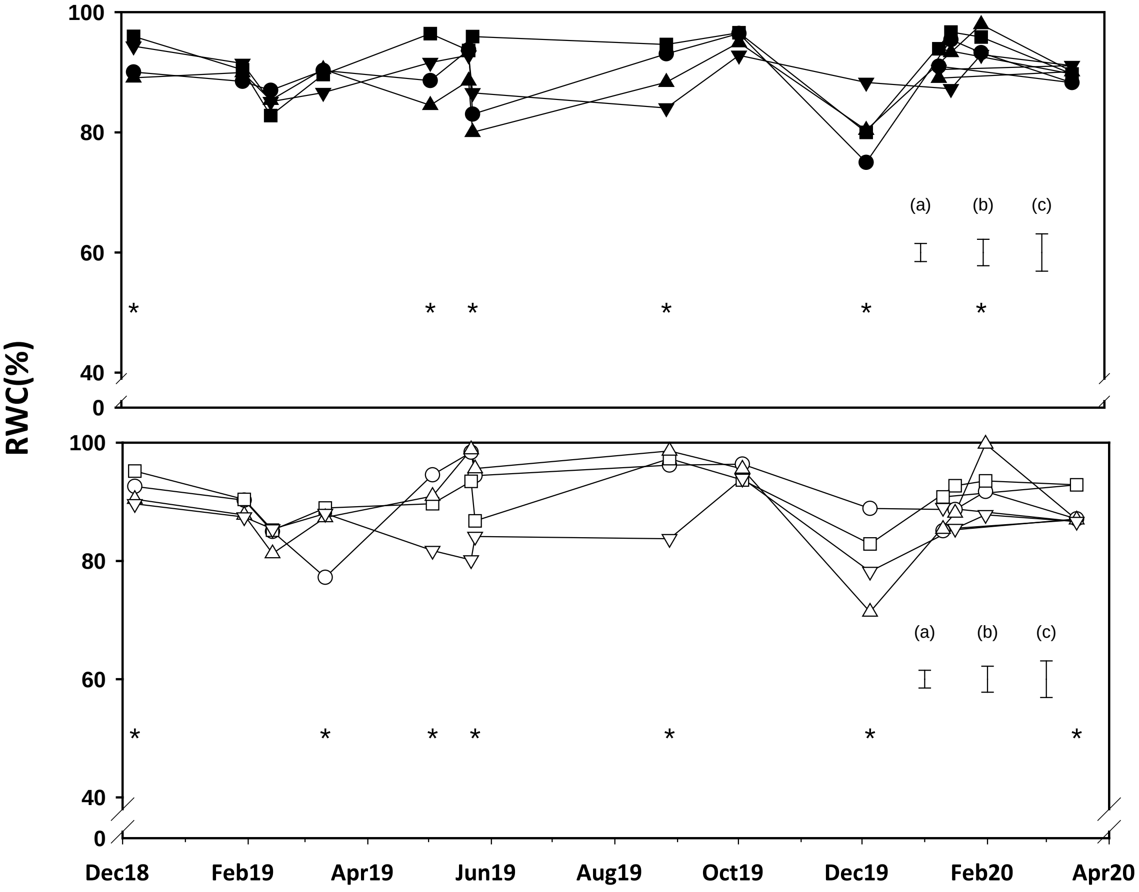


Figure 7-11 Relative water content (RWC) (%) of brome (•,○),cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to April 2020 in Ladbrooks, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation. “*” symbols represents when differences amongst the species were observed. Standard error of means is represented for (a) nitrogen, (b) species and (c) nitrogen*species.

Table 7-17 Mean relative water content (%) for brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen from December 2018 to March 2020, at Ladbrooks, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D).

	N+				N-				
Date	BR	CF	PR	TF	BR	CF	PR	TF	Mean D
07/12/18	91	96	92	94	93	95	95	92	94 ab
29/01/19	89	91	90	92	90	90	88	88	90 bc
13/02/19	87	84	86	86	86	85	82	86	85 cd
11/03/19	91	92	90	87	78	90	89	89	88 c
03/05/19	89	97	86	92	95	93	91	82	91 bc
22/05/19	97	94	91	97	100	97	99	81	96 a
24/05/19	87	99	81	91	97	90	97	88	92 b
28/08/19	93	98	90	88	98	98	93	88	95 a
03/10/19	97	97	95	94	97	94	96	94	96 a
05/12/19	75	80	82	89	89	83	72	79	81 d
10/01/20	91	94	92	91	86	91	86	89	90 bc
16/01/20	97	98	94	88	89	93	89	86	92 b
31/01/20	91	98	99	93	92	94	100	88	96 a
16/03/20	88	91	90	91	88	93	88	87	90 bc
Mean N			92				91		
	BR		CF		PR		TF		Mean
Mean S	92		93		92		89		92

	P-Value	SEM	LSD
S	0.029	0.022	0.064
N	0.435	0.015	-
S*N	0.103	0.031	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P. Ryegrass, "TF" represents Tall Fescue, "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. P-Values, SEM and LSD are shown for analysed transformed ArcSin data. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

Ashley Dene

Different dates had significant differences (P<0.001) (Table 7-18). On 05/02/19 (1836 °Cd) (Figure 7-12), when the ASMD was 107 mm, the RWC was at its lowest value of 67 ± 0.01%.

The RWC differed (P<0.001) among species. Brome and cocksfoot had an average of RWC of 87 ± 0.082 or ~15% higher than the average of p. ryegrass and tall fescue, 80 ± 0.082 (Table 7-18).

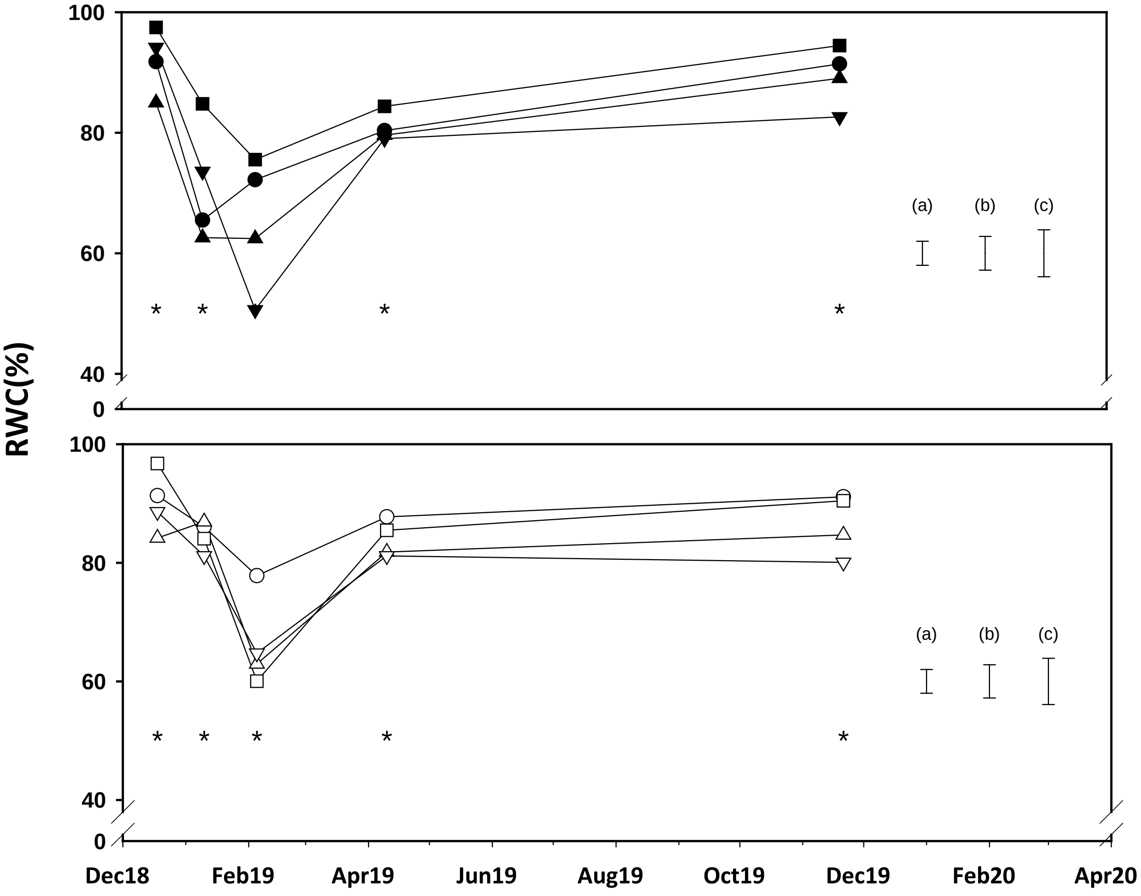


Figure 7-12 Relative water content (RWC) (%) of brome (●,○),cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽) from December 2018 to April 2020 at Ashley Dene, Canterbury, New Zealand with (filled with black) or without (filled with white) nitrogen fertilisation."*" symbols represents when differences amongst the species were observed. Standard error of means is represented for (a) nitrogen, (b) species and (c) nitrogen*species.

Table 7-18 Mean relative water content (%) for brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen from December 2018 to March 2020, at Ashley Dene, Canterbury, New Zealand, according to species (S), nitrogen fertilisation (N) and date (D).

	N+				N-				
Date	BR	CF	PR	TF	BR	CF	PR	TF	Mean D
18/12/18	93	98	86	97	92	97	84	89	93 a
10/01/19	66	86	63	74	86	85	90	82	80 b
05/02/19	74	76	64	51	80	64	64	66	67 c
10/04/19	81	86	80	80	91	86	82	82	84 b
21/11/19	92	96	92	84	91	93	85	80	90 a
Mean N			83				85		

	BR	CF	PR	TF	Mean
Mean S	86	89	80	80	84

	P-Value	SEM	LSD
S	<0.001	0.028	0.082
N	0.234	0.020	-
S*N	0.162	0.039	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "SEM" represents Standard Error of the Mean and "LSD" represents Least Significant Difference at 5%. P-Values, SEM and LSD are shown for analysed transformed ArcSin data. Means within a column with different letters are significantly different (LSD at the α = 0.05 level).

7.3.6 Principal Component Analysis (PCA)

7.3.6.1 General Data

For this PCA, principal component 1 (PC1), PC2 and PC3 accounted, respectively, for 44.96%; 36.38% and 9.41% of the total variation.

PCA results indicate that the Relative Water Content (RWC) was negatively related to chlorophyll concentration and proline accumulation (Figure 7-13 and Table 7-19). This means that the lower the amount of water in a cell the higher the concentration of solutes and proline accumulation inside the cell.

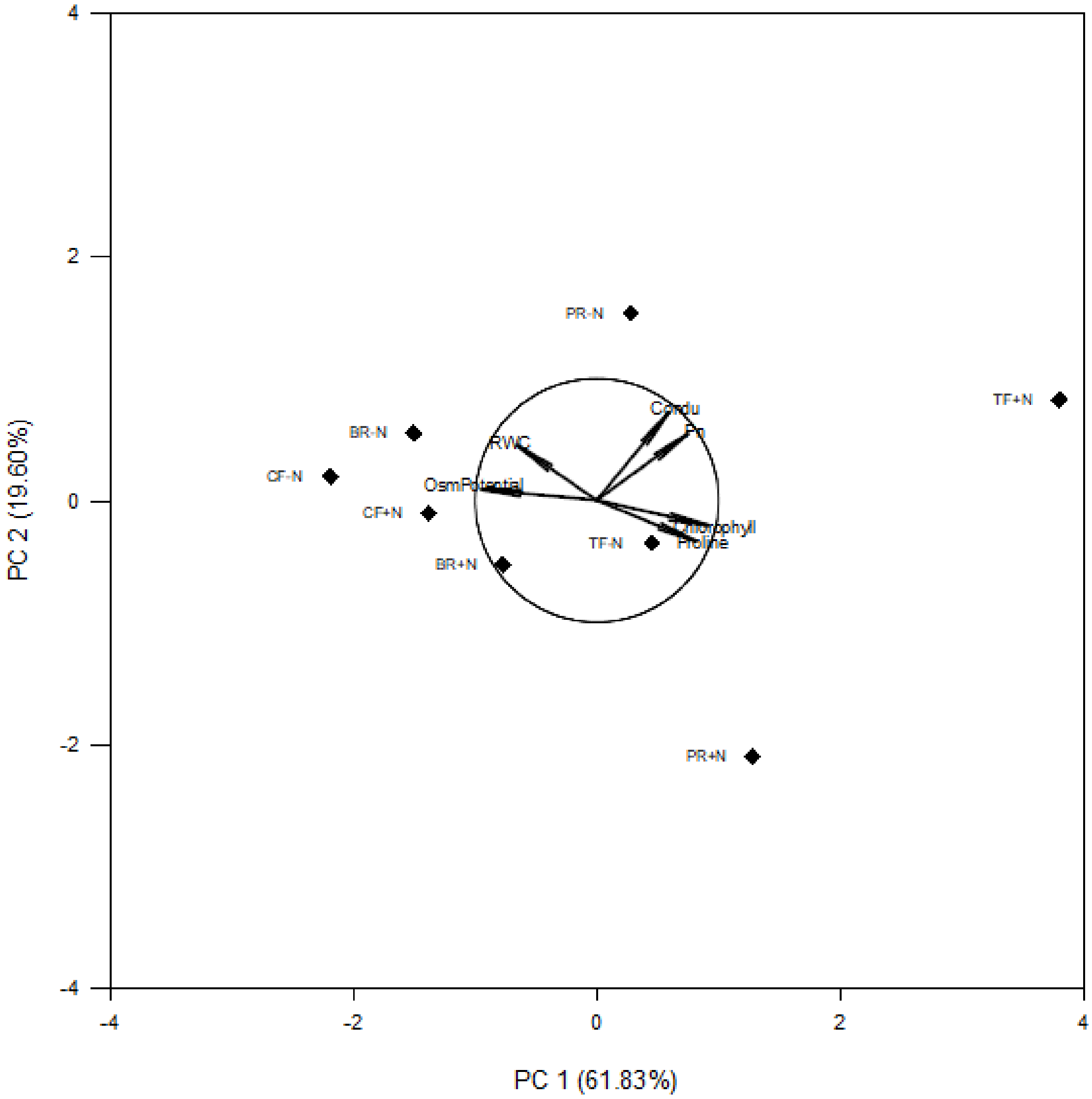


Figure 7-13 Component Loadings with individual scores for monocultures of brome (BR), cocksfoot (CF), perennial ryegrass (PR) and tall fescue (TF) grown, with (+N) and without (-N) nitrogen, at Ladbrooks and Ashley Dene, Canterbury, New Zealand, from December 2018 to March 2020. "RWC" is the relative water content, "Condu" is the stomatal conductivity, "Pn" is the net photosynthetic rate, "Chlorophyll" is the chlorophyll concentration, "Proline" is the proline accumulation, "OsmPotential" is the osmotic potential.

The photosynthetic rate was strongly related (>87%) to stomatal conductivity, which is because there is more gas exchange happening at higher photosynthetic rates. This means that increased CO_2 assimilation with increased photosynthetic rates leads to greater water vapour loss which can lead to reduced turgor in the cells.

The vectors for all variables, except RWC and Osmotic Potential, were positively and strongly linked to PC1 (Figure 7-13). That means, that an increase in one of these variables is associated with an increae in the others but the opposite occurs for RWC.

The PCA analysis showed the effect of nitrogen fertilisation for perennial ryegrass, according to the PCA, is positively related to an increase in the proline accumulation and the chlorophyll concentration, but negatively for the RWC, Pn and stomatal conductance. For tall fescue, N+ was positively related to an increase in the chlorophyll content and proline accumulation, regardless of the site. For cocksfoot and brome, N+ linked to a decrease in the osmotic potential and minor increases in the proline accumulation, chlorophyll concentration.

Cocksfoot and brome, regardless of nitrogen fertilisation, showed a higher value of RWC and lower values for net photosynthetic rate (Pn), chlorophyll content and proline accumulation than the other two species. Of note, the N+ and N- samples of p. ryegrass and t. fescue located on the positive side of the x-axis(PC1) (Figure 7-13), or the opposite size of that to brome and cocksfoot. This means that they have a positive relation to chlorophyll concentration, proline accumulation, Pn and stomatal conductance, whilst brome and cocksfoot were more related to a higher osmotic potential and RWC.

Table 7-19 Physiological variables Principal Components Analysis (PCA) Correlation Matrix for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown, with and without nitrogen fertilisation, at Ladbrooks and Ashley Dene, Canterbury, New Zealand, from December 2018 to March 2020.

	RWC	Chlorophyll	Proline	Pn	Conductivity	Osm.Potential
Chlorophyll	-0.184	-				
Proline	-0.521	0.672	-			
Pn	0.377	0.331	-0.119	-		
Conductivity	0.269	0.300	-0.102	0.872	-	
Osm.Potential	0.357	-0.763	-0.626	-0.234	-0.360	-

Note: RWC stands for Relative Water Content, Chlorophyll is the chlorophyll concentration, Proline is the proline accumulation, Pn stands for Net Photosynthetic Rate, Conductivity is the stomatal conductivity and Osm.Potential is the Osmotic Potential.

A hierarchical clustering (Figure 7-14), done with the standardised data and the “Single linkage method”, separated N+ Tall Fescue from Ladbrooks from all the other treatments. Treatments from Ashley Dene and Ladbrooks were never in the same group except for the group containing non-fertilised brome at Ashley Dene, non-fertilised cocksfoot and brome and fertilised brome at Ladbrooks.

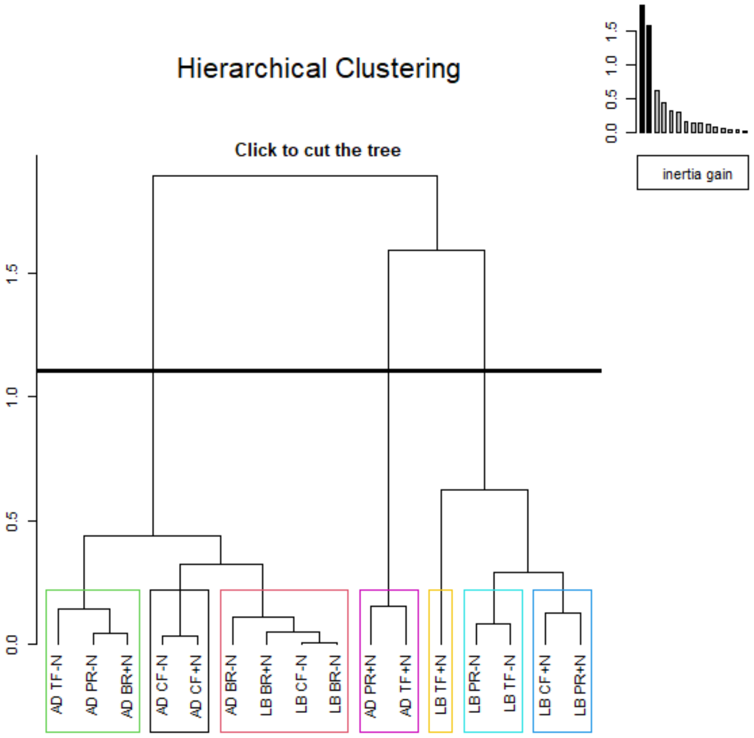


Figure 7-14 Hierarchical Clustering from Individuals according to the PCA analysis for monocultures of brome (BR), cocksfoot (CF), perennial ryegrass (PR) and tall fescue (TF) grown, with (N+) and without (N-) nitrogen, at Ladbrooks (LB) and Ashley Dene (AD), Canterbury, New Zealand, from December 2018 to March 2020.

7.3.6.2 Ladbrooks

For this PCA, PC1, PC2 and PC3 accounted for, respectively, 62.76%; 17.07% and 14.87% of the total variation.

Pn was closely related to CC (Figure 7-15), and those variables were not correlated with the RWC. At Ladbrooks, the effect of nitrogen fertilisation for cocksfoot was linked to an increase in the RWC.

N fertiliser had a lower effect on Pn and the CC for brome and p. ryegrass, and a greater effect for tall fescue (Figure 7-15), especially for an increase on conductance, CC, Pn and the proline accumulation.

As at Ladbrooks the water stress was lower than in Ashley Dene most of the time (Section 5.3.2) and proline accumulation levels were lower, the net photosynthetic rate was dependent mostly in the chlorophyll content present in the leaves.

Table 7-20 Physiological variables Principal Components Analysis (PCA) Correlation Matrix for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown, with and without nitrogen, in Ladbrooks, Canterbury, New Zealand, from December 2018 to March 2020.

	RWC	Chlorophyll	Proline	Pn	Conductivity	Osm.Potential
Chlorophyll	-0.325	-				
Proline	-0.235	0.908	-			
Pn	-0.141	0.753	0.766	-		
Conductivity	-0.279	0.328	0.278	0.760	-	
Osm.Potential	0.594	-0.725	-0.578	-0.715	-0.564	-

Note: RWC stands for Relative Water Content, Chlorophyll is the chlorophyll concentration, Proline is the proline accumulation, Pn stands for Net Photosynthetic Rate, Conductivity is the stomatal conductivity and Osm.Potential is the Osmotic Potential.

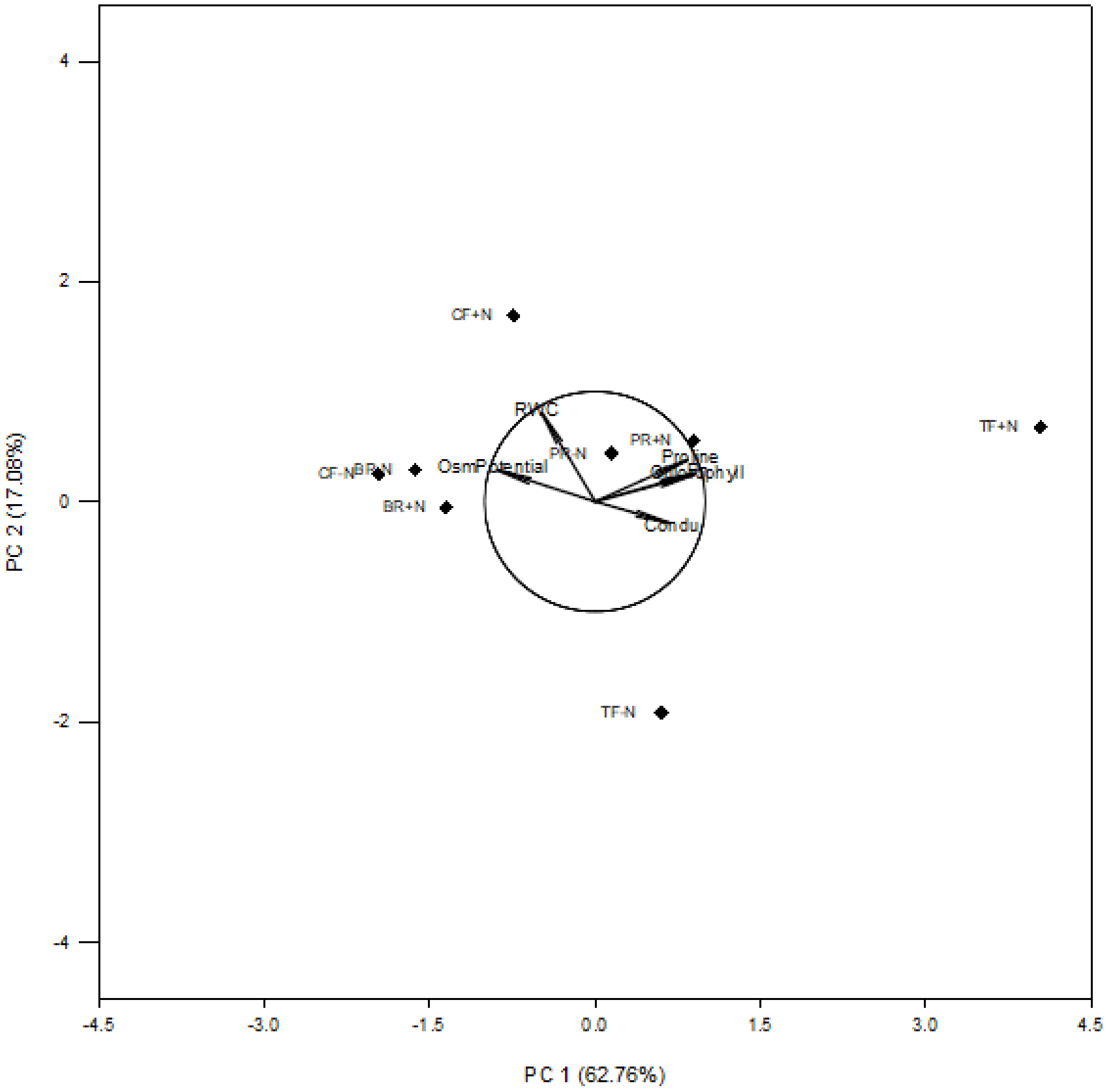


Figure 7-15 Component Loadings with individual scores for monocultures of brome (BR), cocksfoot (CF), perennial ryegrass (PR) and tall fescue (TF) grown, with (+N) and without (-N) nitrogen, in Ladbrooks, Canterbury, New Zealand, from December 2018 to March 2020. "RWC" is the relative water content, "Condu" is the stomatal conductivity, "Pn" is the net photosynthetic rate, "Chlorophyll" is the chlorophyll concentration, "Proline" is the proline accumulation, "OsmPotential" is the osmotic potential.

7.3.6.3 Ashley Dene

For this PCA, PC1, PC2 and PC3 accounted for, respectively, 56.96%, 32.89% and 5.51% of the total variation.

At Ashley Dene, the osmotic potential and the RWC are negatively related to the proline accumulation and the CC (Figure 7-16). Proline accumulation and the CC linked were very related one another.

Fertilising the plants with nitrogen was, for all species, to a higher proline accumulation (Figure 7-4 and Figure 7-5) and chlorophyll concentration (Figure 7-9 and Figure 7-10), though the increase was different for the species, higher for p. ryegrass and tall fescue, and lower for brome and cocksfoot.

Cocksfoot was related to a higher RWC (Figure 7-16), regardless of fertilisation levels and also an increased osmotic potential. Brome and p. ryegrass were related to a higher conductance and photosynthetic rate whilst tall fescue was related to a higher proline accumulation and chlorophyll content.

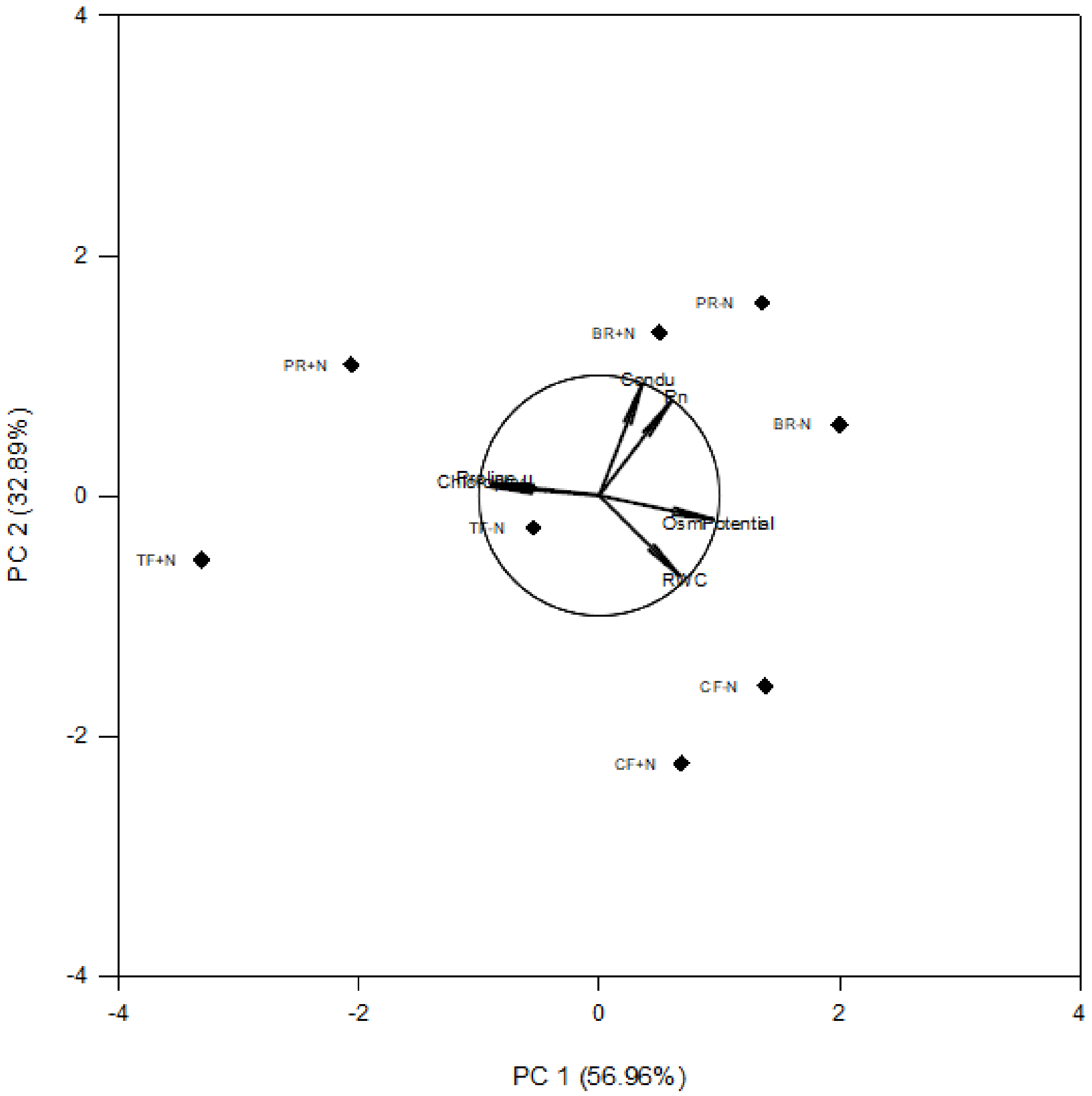


Figure 7-16 Component Loadings with individual scores for monocultures of brome (BR), cocksfoot (CF), perennial ryegrass (PR) and tall fescue (TF) grown, with (+N) and without (-N) nitrogen, in Ashley Dene, Canterbury, New Zealand, from December 2018 to March 2020. "RWC" is the relative water content, "Condu" is the stomatal conductivity, "Pn" is the net photosynthetic rate, "Chlorophyll" is the chlorophyll concentration, "Proline" is the proline accumulation, "OsmPotential" is the osmotic potential.

Table 7-21 Physiological variables Principal Components Analysis (PCA) Correlation Matrix for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown, with and without nitrogen, in Ashley Dene, Canterbury, New Zealand, from December 2018 to March 2020.

	RWC	Chlorophyll	Proline	Pn	Conductivity	Osm.Potential
Chlorophyll	-0.576	-				
Proline	-0.600	0.752	-			
Pn	-0.078	-0.448	-0.401	-		
Conductivity	-0.356	-0.216	-0.219	0.937	-	
Osm.Potential	0.780	-0.825	-0.802	0.417	0.161	-

Note: RWC stands for Relative Water Content, Chlorophyll is the chlorophyll concentration, Proline is the proline accumulation, Pn stands for Net Photosynthetic Rate, Conductivity is the stomatal conductivity and Osm.Potential is the Osmotic Potential.

7.4 DISCUSSION

Objectives 3, 5 and 6 quantified mechanisms that justified the yield differences under different water and nitrogen availabilities. Differences in the water use, water use efficiency, intercepted PAR and RUE are able to explain 100% of the yield differences under the different water and nitrogen regimes.

In Objective 6, cocksfoot was demonstrated to be the species that had the highest PAR interceptance and the highest VDM in water restricted conditions.

Objective 7 aims justifying how physiological traits alters the yield generating components, intercepted PAR and RUE, in brome, cocksfoot, perennial ryegrass and tall fescue.

7.4.1 How do grasses react to changes in the environment?

A reduction in the net photosynthetic rate (Pn) is noticed as one or more resources (water or temperature) are reduced. This is demonstrated by the measurements at Ladbrooks in 2020, for measurements done in water stress times of 16/01/20 (1560 °Cd), 31/01/20 (1795 °Cd) and 16/03/20 (2337 °Cd), had the lowest average of 6.1 µmol CO_2 m^{-2} s^{-1} (Table 7-9) and at Ashley Dene, on 10/01/19 (1492 °Cd), 10/04/19 (2595 °Cd) and 21/11/19 (990 °Cd in Year 6), had, respectively, 6.7; 8.6 and 3.3 µmol CO_2 m^{-2} s^{-1} (Table 7-10).

The lower Pn aligns with the RUE values on Table 6-16 and Table 6-17, for lowest RUE values are found for regrowth cycles that had had water and temperature restrictions. The decrease in photosynthetic activity as the soil moisture increases is in accordance with the observed for *L. perenne* and *F. arundinacea* by Fariaszewska and Staniak (2015), and cocksfoot (Ramos *et al.* 1996), which had a reduction of photosynthetic rate of 2.13 µmol CO_2 m^{-2} s^{-1} per each bar of water potential decreased (Peri *et al.*, 2002).

The Pn, at the experiment, was not at it's peak even with full open stomata. Though stomatal conductance and the Pn are closely related, as shown by the PCAs on Figure 7-13; Figure 7-15 and Figure 7-16, open stomata would not necessarily imply in higher Pn when resources were restricted, such as temperature and soil moisture, as observed for 03/05/19 (2189 °Cd) at Ladbrooks (Table 7-11), when the Pn rate was 14.3 µmol CO_2 m^{-2} s^{-1} and the conductance was 0.154 mmol H_2O m^{-2} s^{-1}.

The Pn rate, for all studied species, is also very dependent on the CC and preventing its degration allows the photosynthetic apparatus to maintain the gas-exchange rates. A reduction in the CC occured in association with reductions in Pn. For example, at Ashley Dene on 18/12/18 (1181 °Cd) the Pn was 17.8 µmol CO_2 m^{-2} s^{-1} and the CC was 420 mg m^{-2}. This reduced to 6.7 µmol CO_2 m^{-2} s^{-1} on 10/01/19 (1491 °Cd) and 370 mg m^{-2}, and to 3.3 µmol CO_2 m^{-2} s^{-1} and 300 mg m^{-2} on 21/11/19 (990 °Cd in Year 6). The CC is linked to nitrogen levels, as shown in Table 7-13, for N fertilisation increased the CC by 20% at Ladbrooks and 17% at Ashley Dene, and Figure 7-13 shows that, for all species, N fertiliser makes the pastures more related to higher Pn rates.

Proline was accumulated in higher amounts at the water restricted environment and differently amongst species when nitrogen was supplied. This is show by the proline accumulation at Ashley Dene, 4.49 mg g^{-1} DM (Table 7-7), and at Ladbrooks, 2.18 mg g^{-1} DM (Table 7-5). For the species, when under nitrogen fertilisation, ryegrass had an increase of 4.41 mg g^{-1} DM, Table 7-6, and tall fescue, of 2.51 mg g^{-1} DM, whilst for brome and cocksfoot the increase was lower than 1.6 mg g^{-1} DM. This happened to protect the chlorophyll molecules from biochemical stress generated species like reactive oxygen species (ROS). This phoenomenum is shown by the PCAs (Figure 7-13; Figure 7-15 and Figure 7-16).

Specifically, proline is more related to the chlorophyll content in Ashley Dene than in Ladbrooks (Figure 7-15 and Figure 7-16). Its accumulation (Table 7-13) nonetheless did not prevent equal amounts of CC in the plants as in Ladbrooks (Table 7-15), either because the degradation due to stress was so high the proline accumulation was not enough to avoid it or because plants had a limitation in resources not allowing more chlorophyll to be synthesized.

Losses in cell turgor were observed as the soil dried. This is shown by the fact that, when water was plently available, the pastures had a higher RWC (at Ladbrooks, 94%, 95% and 96% on 07/12/18, 28/08/19 and 03/10/19, respectively, on Table 7-17, and at Ashley Dene, 90% on 21/11/19, on Table 7-18) and osmotic potential (at Ladbrooks, -1.603; -1.913 and -1.646 MPa on 07/12/18, 28/08/19 and 03/10/19, respectively, on Table 7-3, and at Ashley Dene, -1.483 on 21/11/19, on Table 7-4) than in other times of the year. A loss in cell water and turgor has previously been observed in tall fescue as the soil dried (Huang *et al.*, 1998), and the same was observed in this experiment not only for tall fescue, but for all species.

An accumulation of solutes inside the cell decreased photosynthesis for all species at Ladbrooks. This is because photosynthetic rate was negatively correlated with osmotic potential (Table 7-15). For example, the lowest Pn (6 µmol CO_2 m^{-2} s^{-1}, Table 7-9) was observed on one of the days (16/03/20) when the osmotic potential was at its lowest (-2.063 MPa, Table 7-3). In this experiment the osmotic potential never reached this low a value, but a decrease in the net photosynthetic rate as the osmotic potential decreased was measured (Table 7-3 and Table 7-9). At both sites, the osmotic potential was correlated to the RWC, shown by the PCAs depicted in Figures 7.15 and 7.16. As the higher content increased it dilutes cell solutes increasing the osmotic potential. Fu *et al.*(2010) observed, for tall fescue, values of RWC around 73% when osmotic potential values were around -1.85 MPa, and RWC around 58% when the osmotic potential was around -4.1 MPa. Values of similar order of magnitude were found for all grasses at Ashley Dene such as on 05/02/19 (2758 °Cd) when the osmotic potential was -3.0 MPa (Table 7-4) and the RWC 67% (Table 7-18). On 12/09/19 (318 °Cd on Year 6) the osmotic potential was -2.0 MPa (Table 7-4) and the RWC 84% (Table 7-18).

When the water resource was more abundant and plants could change their strategy whether to save or waste water. This is shown by the osmotic potential being directly opposed to the stomatal conductivity (Figure 7-15), regulating the photosynthetic process.

Under high stress, the situation required pastures to focus on surviving, particularly ryegrass and tall fescue, hence their higher proline accumulation of 7.35 and 5.94 mg g^{-1} DM respectively (Table 7-7)., in this case, the link between osmotic potential and stomatal conductivity was not as pronounced, as shown on Figure 7-16. This point was probably past the minimum osmotic potential, -4.4 MPa (Wilson *et al.* 1980), where it influenced the stomatal ouverture and conductance.

Though the water scarcity more severe in Ashley Dene than in Ladbrooks (Section 5.3.2), it never reached values that would cause continuous irreparable damage to the plants. The RWC never reached values lower than 55% (observed for p. ryegrass, on 05/02/19 in Ashley Dene, Table 7-18). In the experiment conducted by Huang *et al.* (1998), permanent damage to chloroplasts was observed when the RWC reached values under than 30%, never recovering ever after the cells were hydrated.

A high RWC is an indicator of high dry matter yield. For example, during the Spring 2019 at Ladbrooks, temperature adjusted growth rates were high, especially for N+ pastures, at rates of 13.2 kg DM °Cd^{-1} ha^{-1}, and the N- at 7.4 kg DM °Cd^{-1} ha^{-1} (Table 4-47), the RWC values were 95% on 28/08/19 (243°Cd on Year 6) and 96% at 03/10/19 (448 °Cd) (Table 7-17), and when the ASMD increases, and dry matter accumulation is reduced, like on 10/01/19 (1488 °Cd), when the ASMD was 95 mm for both N levels (Figure 5-2), and the temperature adjusted growth rate was 3.4 kg DM °Cd^{-1} ha^{-1} for N+ pastures and 1.6 kg DM °Cd^{-1} ha^{-1} for N- pastures, the average RWC was 90%. At Ashley Dene, the measurement on 05/02/19 (1836 °Cd in Year 5) had an ASMD of 107 mm (Figure 5-3), the temperature adjusted growth rate was 0.4 kg DM °Cd^{-1} ha^{-1} and the average RWC was 67% (Table 7-18), which contrats to the measurements on 21/11/19 (885 °Cd, ASMD of 41 mm), when the average temperature growth rate was 6.7 kg DM °Cd^{-1} ha^{-1} for all species (Table 4-49) and the average RWC was 90%. RWC has been shown to correlate with dry matter yield (Ebrahimiyan *et al.*, 2013), due to the maintenance of a higher cell turgor meaning a maintenance of a higher leaf area and an appropriate amount of water for a high photosynthetic rate.

7.4.2 The role of nitrogen

Nitrogen fertiliser allowed the fertilised plants' cells to maintain turgor. The significant ($P<0.001$) action of nitrogen in the osmotic potential at Ladbrooks (Table 7-3) justifies the grass canopy expansion, as discussed by Munns (1988), allowing the N+ plants to intercept more PAR (Table 6-7 and Table 6-8). Data showing that fertilising with urea proved to have an effect on all traits on the studied grasses except the RWC (Table 7-17) at Ladbrooks and the osmotic potential (Table 7-4), RWC (TablTable 7-18), and stomatal conductance (Table 7-12) at Ashley Dene. At Ashley Dene, the osmotic potentic and stomatal conductance were probably shown to be not affected by nitrogen fertilisation for the measurements done for those variables took place mostly when the ASMD was increasing or high (Figure 5-3) and not on periods when water was plently available such as the Spring 2018.

Nitrogen affected the Pn when water and temperature were not in restrictive levels. This is shown by the lack of difference between N+ and N- measurements on 16/01/20, 31/01/20 and 16/03/20 at Ladbrooks (Table 7-9) and 29/11/19 at Ashley Dene (Table 7-10). Peri *et al.* (2003) reported, for cocksfoot, an increase of 9.05 g CO_2 m^{-2} d^{-1} per 1% of increase in the ratio kg N DM/ kg DM. Similar values were found for cocksfoot and brome in this thesis under no water restricted situations (03/10/19), but not for other species, also, the interference of water availability and temperature on other days prevents this comparison, on other dates, amongst species. The fact that nitrogen did affect the photosynthetic rate in this experiment is in accordance with previous findings (Peri *et al.*, 2002) as well as the increase in RUE (Table 6-15 to Table 6-18).

Nitrogen was associated with an increase in proline accumulation, particularly for water stressed plants. It is demonstrated by the substantial increase in proline accumulation in fertilised plants, 6.5 times the value of non-fertilised plants at Ladbrooks (Figure 7-3) and 7.1 times at Ashley Dene (Figure 7-4). This increase was more evident in traditional dry periods of the year, from January to May (both Year 5 and Year 6), except for the measurement of 07/03/19 (2206 °Cd) at Ladbrooks, 1.30 mg g^{-1} DM, probably due to the fact the measurements were done close to rainfall days. An increase in the amount of sacrifice-compounds (proline) aims protecting plants from ROS species (Section 2.5.4). As discussed in Section 7.4.1, plants with higher proline accumulation had a higher chlorophyll concentration, but the chlorophyll content was also higher when more nitrogen was available.

7.4.3 Any differences amongst the species ?

P. ryegrass and tall fescue tend to protect their photosynthetic apparatus under stress. This is shown by their higher proline accumulation (for both, 3.19 ± 0.282 mg g^{-1} DM at Ladbrooks, Table 7-5, and, 6.64 ± 1.000 mg g^{-1} DM at Ashley Dene, Table 7-7) and higher chlorophyll concentration (respectively, 570 and 490 ± 13.6 mg m^{-2}, at Ladbrooks, Table 7.13, and, for both, 467 ± 13.0 mg m^{-2} at Ashley Dene, Table 7-15), when compared to cocksfoot and brome (for proline accumulation, both had the value of 1.17 ± 0.282 mg g^{-1} DM at LB and 2.27 ± 1.000 mg g^{-1} DM at AD, and for the CC, 375 ± 13.6 mg m^{-2} for both at LB and 352 ± 13.0 mg m^{-2} for both at AD). Proline has been reported to be more accumulated in p. ryegrass than in cocksfoot under the same growth conditions (Mela and Rand, 1979; Volaire *et al.* 1998), and the same was observed in this experiment. Tall fescue did also have a high proline accumulation, at Ladbrooks, while brome was not different from cocksfoot, 1.12 mg g^{-1} DM. While the chlorophyll concentration was closely related to proline accumulation and opposed to osmotic potential and RWC on both sites (PCA Figure 7-13), in Ashley Dene it was less related to Pn (PCA Figure 7-16) than in Ladbrooks (PCA Figure 7-15). This is likely due to biochemical efforts from the plants to protect chlorophyll in environments with a higher soil moisture deficit at the cost of sacrificing a portion of Pn.

Cocksfoot, when under stress, tend to preserve cell turgor while not protecting the photosynthetic apparatus as the other species do. This is due to this species being more related to maintaining a higher cell osmotic potential (at least 15% higher than the others, as shown on Table 7-3 and Table 7-4) and RWC (93% at Ladbrooks, Table 7-17, and 89% at Ashley Dene, Table 7-18), while having lower proline accumulation (less than 50% of the values ryegrass and tall fescue had, as shown on Table 7-5 and Table 7-7) and chlorophyll concentration (at least 25% lower tah ryegrass and tall fescue, shown on Table 7-13 and Table 7-15). This was observed in both sites and happened because either their leaf cells have more water, then proline and chlorophyll have lower concentrations are diluted or because those species naturally produce less proline and chlorophyll molecules, or a combination of both.

Cocksfoot's strategy of preserving turgor is associated with a higher leaf area maintenance and radiation interceptance (10% higher LAI at Ashley Dene on Year 5, Table 6-5, and 5% higher on Year 6, Table 6-6). The maintenance of cell turgor has been associated with the maintenance of the canopy structure (Huang *et al.* 1998) and leaf area (Volaire *et al.* 1998), which implies in higher radiation interceptance, as demonstrated for cocksfoot in Section 6.3.2.

7.5 CONCLUSIONS

This chapter dealt with the objective of determining physiological reasons for agronomical differences observed on the field:

- The intensity on how the four grass species all react to water stress is what justify the observed agronomic differences, mostly linked to preserving leaf area and vegetative dry matter.
- Cocksfoot and brome have a strategy of preserving leaf area and sacrificing the net photosynthetic rate when under water stress. Perennial ryegrass and tall fescue tend do preserve chlorophyll and protect the photosynthetic apparatus but sacrifice the leaf area.
- Nitrogen fertilisation interfered with some but not all measured physiological traits. Even though, it had a beneficial effect by allowing fertilised plants to keep cell turgor and leaf area, increasing the net photosynthetic rate and reducing the damage from stress by increasing proline accumulation.
- As the water stress was more intense at Ashley Dene, the studied physiological traits were more affected in this site than in Ladbrooks. Cell water losses were higher and had a higher effect in the plants than at Ladbrooks.

8 GENERAL DISCUSSION AND CONCLUSIONS

The main aim of this thesis was to evaluate the effects of nitrogen and moisture on the growth of dryland monocultures of brome, cocksfoot, perennial ryegrass and tall fescue. Further investigation at the plant level examined the physiology behind the yield reduction mechanisms for each species. The assumption was that understanding how each species reacts to these stresses would provide information to improve the management of dryland grazing systems. To do so, two field experiments 25 km apart were established in 2014. The two sites were chosen because they differ in soil characteristics, specifically their water holding capacity. This focuses on explaining production and persistence results from measurements taken in Years 4 and 5 being the agronomic years of 2018/2019 and 2019/2020.

In this chapter, the main results from each result chapter are integrated to address the overall aim of the study leading to suggestions for dryland pasture management and future research areas.

8.1 Pasture production, quality and persistence

Five and six years after being established, the pastures showed an expected yield reduction most notably at Ashley Dene where weed invasion was higher and vegetative production lower than at Ladbrooks. Of note the TDM yield was not different amongst species at Ladbrooks (LB) and Ashley Dene (AD), but brome always had lower annual vegetative dry matter, due to its propensity to seed throughout the year. When left for an extended period at Ashley Dene, cocksfoot maintained the highest propotion of vegetative herbage and thus had the highest quality feed for livestock.

8.1.1 Temperature

When soil moisture was non limiting, nitrogen enabled a three fold increase in temperature adjusted growth rates. Maximum growth rates occurred in Spring and were largely linear until water limited growth in late Spring or summer. These results highlight previous knowledge that a minimum soil water content and rainfall are required to enable uptake and response to nitrogen (Mills *et al.*, 2009; Sharifiamina, 2018),. Also as expected (Li et al, 2011; Minneé *et al.*, 2013), the majority of dry matter produced at both LB and AD (at least 70%) was produced in the first six months of the agronomic year (July – December). This is when Winter rainfall has recharged soil moisture levels and low night temperatures restrict respiration to maximise radiation and water use efficiency. In practice, this means dryland farmers aim to maximise pasture production and quality during Spring, when feed demand is the highest for lactating animals and their off-spring. The temperature adjusted growth rates for all species show that the use of nitrogen fertiliser can aid pasture production at this time.

As the season progresses and water becomes restricted, the impact of nitrogen fertiliser was less efficient, particularly at AD. This was shown during the dry season by higher temperature adjusted growth rates of fertilised plots at LB (2.4 kg DM ha^{-1} °Cd^{-1} higher in Year 5 and 1.9 kg DM ha^{-1} °Cd^{-1} higher in Year 6) but no response at AD. At LB, the amount of water in the soil profile during dry periods was sufficent to allow some of the nitrogen fertiliser to be absorbed by the plants. In this water-restricted phase, at LB, the temperature adjusted growth rate of the fertilised plant were reduced to about a third (3.6 kg DM ha^{-1} °Cd^{-1} in Year 5 and 3.4 kg DM ha^{-1} °Cd^{-1} in Year 6, Table 4-46 and Table 4-47) of those in Spring (8.3 kg DM ha^{-1} °Cd^{-1} in Year 5 and 13.1 kg DM ha^{-1} °Cd^{-1} in Year 6) when the water was available. The non-fertilised grasses had growth rates reduced to about half (from 2.8 to 1.2 kg DM ha^{-1} °Cd^{-1} in Year 5 and from 3.4 to 1.6 kg DM ha^{-1} °Cd^{-1} in Year 6). The reduction at AD was to one eight/fourteenth for fertilised pastures (from 6.4 to 0.8 kg DM ha^{-1} °Cd^{-1} in Year 5 and from 7.1 to 0.5 kg DM ha^{-1} °Cd^{-1} in Year 6, Table 4-47 and Table 4-48) and one sixth/twenty for non-fertilised ones (from 3.7 to 0.6 kg DM ha^{-1} °Cd^{-1} in Year 5 and from 6.2 to 0.3 kg DM ha^{-1} °Cd^{-1} in Year 6). The growth in this part of the year was accounted for by broken-stick linear regressions, but in practice this probably represents periods of high growth rates when rain fall and then no growth once the moisture has been used which creates a “saw tooth” pattern.

Due to soil characteristics and also rainfall in Year 6, the water scarcity at AD started earlier and finished later than at LB. In June, the end of Autumn, and July, start of the Winter, some days had air temperatures lower than the base temperature of 3 °C (Figure 3-2) which reduces the rate of dry matter accumulation. June is the month when soils generally have their profiles recharged and the pastures enter a recovery phase. It is possible that the deeper roots at LB allowed the pastures at this site to be able to obtain water for longer than at AD. The temperature adjusted growth rate values of 8.3 kg DM ha^{-1} $°Cd^{-1}$ found in this study slightly higher, at LB, to those reported by Sharifiamina (2018) and for irrigated fertilised cocksfoot by Mills (2006), probably due to a difference when the dry period started, but not similar at AD, where a five-stage broken stick model was reported. A five stage model was justified this year due to rainfall events during the traditional dry period, which, for a certain amount of time, increased the temperature adjusted growth rate until the soil became dry again.

8.1.2 Quality

Nitrogen is frequently the most limiting mineral element for pasture production (Mills *et al.*, 2009), particularly for drylands pasture soils, which are low in nitrogen levels (Gultekin *et al.*, 2020). Nitrogen also plays an important role in pasture quality. It increases the annual amount of protein, (kg ha^{-1} $year^{-1}$;) and energy (GJ ha^{-1} $year^{-1}$,) produced by influeing the amount and quality of feed on offer to animals. Fibre is also expected to be increased, in kg ha^{-1}, with nitrogen fertilisation because taller plants require greater amounts of structure. Increases in the crude amount of protein (up to 7 times at LB and 2 times at AD; Table 4-27 to Table 4-31), metabolisable energy (up to 4 times at LB and 1.2 times at AD; Table 4-32 to Table 4-35) and fibre (up to 3.8 times for ADF and 4 times for NDF at LB and up to 1.6 times for ADF and 1.5 times for NDF at AD; Table 4-36 to Table 4-45) due to nitrogen fertilisation were measured at both sites but more at LB than at AD because of the higher yields.

In environments that face severe water restriction in some parts of the year, such as AD, nitrogen fertiliser is important especially when moisture is available. This is justified by an increase in the higher quality of vegetative dry matter (Hides, 1983), that allows a higher offer of protein and metabolisable energy. This was also observed, in a lower scale, when pastures were harvested in an advanced reproductive stage, for reproductive dry matter has lower quality standards (Section 2.3.3) than vegetative ones.

Nitrogen recovery (NR) was higher at LB (0.82 ± 0.021 kg N kg N^{-1} and 1.9 ± 0.322 kg N kg N^{-1}, in Year 5 and 6, respectively, Table 4-23 and Table 4-24) than at AD (0.47 ± 0.021 kg N kg N^{-1} and 1.0 ± 0.230 kg N kg N^{-1}, in Year 5 and 6, respectively, Table 4-25and Table 4-26). This was probably due to the higher available soil moisture, that allowed a greater nitrogen absorption by the roots. The shorter period of summer dry may also have produced lower soil surface temperatures at LD which would reduce volatilization losses (Jones *et al.*, 2007). It is unlikely that any nitrogen was lost by leaching because the nitrogen balance showed carry over effects from one rotation to the next and even from one season to the next. For example, the first regrowth cycle, in Year 6, harvested, at both LB and AD, did not receive any nitrogen fertiliser and the N+ plots still had a higher TDM yield (Table 4-2 and Table 4-4) and their respective NR values (7.43 ± 0.041 kg N kg N^{-1} at LB, Table 4-24, and 3.22 ± 0.036 kg N kg N^{-1} at AD, Table 4-26) demonstrate that the N+ plots had a higher N% content than the N- ones.

Perennial ryegrass, was expected to produce higher quality, per kg DM, than the other species (Reed, 1996) but it was only able to express its full potential when its nitrogen requirements were met. In contrast, at AD cocksfoot consistently provided more high quality feed because it retained vegetative growth and held it for longer than the other species (Figure 4-6).

The management of allowing the plants to reach advanced phenological stages represented the common practice that occurs on many dryland farms. Specifically, grasses go reproductive rapidly in late Spring when feed supply exceeds demand. This provides a management challenge, particularly on hill country properties where mechanical topping is impossible. The results showed that this deferred grazing produced a high pasture yield but the loss in quality did not compensate. Thus, it is important to control grazing to maximize pre-reproductive stages which also allowed a new regrowth cycle to start sooner, especially when water was still available by the end of November and December. This was most apparent in 2019 at both sites and would have produced a greater amount of high quality VDM for the production year.

8.1.3 Persistence

The higher weed proportion found on AD five and six years after sowing is explained by the water restrictions which limits leaf extension and therefore reduces ground cover, which allows weeds to establish. The weed content has consistently been higher for tall fescue since its slow establishment (Easton *et al.*, 1994). At LB, as the plants had more water and nitrogen could be properly absorbed, the pasture covered the ground more fully with extensive tillering observed. This minimized weed ingress and contributed to greater persistence at this site. At LB, all the species had similar levels of TDM reduction and persistence six years after sowing.

This implication is that the pastures at AD reach economic non-viable levels earlier than at LB, allowing the progressive yield reduction and weed ingress (Waller, 2011; Tozer, 2011b).

There was a reduction in dry matter production all species from those reported by Sharifiamina (2018) in the early years of this experiment. However, the data suggest that cocksfoot had greater persistence and could offer the animals the highest amount of quality feed six years after sowing. This corroborates with findings of Moot (2012), in which cocksfoot was stated to have a greater persistence than p. ryegrass, especially when in a mix with subterranean clover.

8.2 Soil water availability

The soil water availability was the major difference between the two selected sites. The higher PAWC at LB (30% higher) resulted from soil differences and root extraction depth. Also, in Year 6, a significant difference in rainfall was observed between sites, with 260 mm more rainfall at LB through the agronomic year (Figure 3-2).

The data from Years 5 and 6 suport the previous observation by Sharifiamina (2018) that the most important factor affecting how plants used water was nitrogen. At LB, the water use efficiency was, roughly, three times higher in both years, with no differences among species.

At AD, the amount of water used was not different among species, but 30% lower than LB in Year 5 and 66% lower in Year 6 (this year under the influence of a higher 260 mm rainfall at LB). This reflects the soil characteristics at AD, which is not as deep, and full of stones which restricted root development (Sharifiamina, 2018). At both sites nitrogen fertiliser increased the WUE during Spring due to a higher soil moisture availability, but not at other times of the year. The increase in WUE at AD in Year 5 was 50% but no increase was observed in Year 6, due to no increase in TDM with the same water use amongst nitrogen treatments.

The efficiency with which the pastures use water is linked to higher radiation interceptance and RUE caused by nitrogen fertilisation. There is a high positive correlation between WUE and RUE in grasses (Kørup *et al.*, 2017). This happens because the leaf area and photosynthetic capacity of leaves is increased (Peri, 2002b) under high water availability. At both LB and AD, the regrowth cycles had the highest WUE increases due to nitrogen (Figure 5-11 and Figure 5-12) also had RUE increases (harvest cycles that took place until early summer both years, Figure 6-10 and Figure 6-11) probably due to their greater chlorophyll content (Table 7-13).

In this study, pastures were likely to have had their yield accumulation affected by ASMD values between 65 – 80 mm. Those values seem low when compared to other studies, but they are justified due to the fact PET_a had reductions and the ASMD never reached values such as the ones seen on Appendices J and K at 2500 °Cd (around the start of April) at both sites in both years. Though some rain events through the summer dry period were able to give the pasture some large growth responses, they were insufficient to recharge the soil and allow a continuous growth. This was the reason for the lower temperature adjusted growth rates which are only maximal and linear from the start of June each year. At this time temperature is the most restrictive factor. The exception was for LB in Year 5, when even though the ASMD was high, it never reached values higher than 85% of the PAWC, so water was available for the plants even with a difficult extraction as shown by Equation 10.

The expectation is that in summer dry conditions WUE will be low. However, at this time irrigated pastures can have high WUE from high available water and nitrogen (McKenzie *et al.*, 2006). WUE has been reported to be, in some cases, higher in summer than that of Spring, under irrigation system, using tall fescue or perennial ryegrass (Minée *et al.*, 2010).

8.3 Yield reduction mechanisms and their physiology

The observed yield differences between the two sites are explained mostly by differences in the intercepted radiation due to reductions in leaf area and water use.The species used in this research adopted different strategies to deal with nitrogen and water stress. Tall fescue and perennial ryegrass tended to preserve their photosynthetic apparatus to detriment of their leaf area and therefore canopy cover. In contrast, brome and cocksfoot preserved their leaf area and radiation interceptance (Figure 7-13).

When the abiotic stresses of water and nitrogen occur, reactive oxygen species (ROS, generally free radicals) appear and damage different cell apparatuses, including those involved in photosynthesis. Perennial ryegrass and tall fescue appeared to protect their chlorophyll content by accumulating the amino acid proline which was used as a sacrifice compound for ROS. This is observed especially when plants, in a water-restricted environment, are provided with nitrogen (Monreal *et al.*, 2006; Spayd *et al.*; 1994), a building block for proline (increased 6.5 times at LB at 7 at AD), and also reflected in higher chlorophyll concentration (between 15 and 20% higher, at AD and LB respectively). Both produced more chlorophyll and protected those molecules with more accumulated proline. However, chlorophyll concentration is affected by the water stress (Schlemmer, 2005) through degradation and its concentration inside the cells differs with RWC.

Under water stress, the reduction in leaf area and the leaves relative position to the sun limit the ability of plants to obtain light resources (Collino *et al.*, 2001). Therefore, a reduction in the amount of intercepted photosynthetically active radiation takes place and the pastures can no longer grow at their maximum potential for the given production environment. Increased soil moisture deficit reduces the leaf area expansion measured by the leaf area index (Liu *et al.*; 2021) even on developed and expanded leaves, and they change their architecture to intercept sunlight. A reduction in LAI was observed during dry periods, from the end of January until mid-May at LB and from early January to mid-June at AD. These occurred with reductions in LAI of up to 70% those measured in regrowth cycles that took place when water was available. This implies a reduction of up to 88% in intercepted PAR, which explained why ~70% of the TDM is produced during Spring, when neither temperature nor water are restricting canopy expansion.

Brome and cocksfoot preserved their leaf area and canopy expansion by maintaining cell turgor, with a higher osmotic potential (Table 7-17 and Table 7-18) and relative water content (Table 7-3 and Table 7-4). Nonetheless, cocksfoot and tall fescue were the species that intercepted the most radiation at both LB and AD. Though tall fescue did not utilise maintainence of cell turgor and leaf area as a strategy, it still maintained a high interceptance due to its erect habit (Maamouri *et al.*, 2015). However, weed ingress may also have confounded interception results for tall fescue, especially at AD. Brome, maintained its leaf area rather than its photosynthetic apparatus, but had lower radiation interceptance probably due to earlier leaf senescence earlier, which was used as the basis for most harvests.

In environments with severe water restrictions, such as AD, the strategy of maintaining the cell turgor and higher osmotic potential, and keeping an expanded LAI and high interceptance, was reflected in higher VDM for cocksfoot. This result confirms the findings of Sharifiamina (2018) in the early years of the same experiment.

8.4 Implications for farming systems

These results indicate that these monocultures of the four studied grasses were severely restricted in their yields when there was a lack of resources namely water and nitrogen. At times they ceased growth such as in the summer dry periods at AD. In a longer term, this will affect persistence by allowing weed ingress, particularly flat weeds. The results from this study, particularly at AD represent flat dryland, lacking access to irrigation systems, and hill country areas on east regions of New Zealand that are typically affected by summer dry conditions, particularly through February.

The results from LB, where water was available more frequently to plants, can be used to interpret results for areas where irrigation is available. They suggest that any of the species used would be appropriate and coud be managed to produce high quality feed. The addition on nitrogen was the main requirement to maintain yield, particularly in Spring. However, with current N restrictions of 190 kg N/ha in place it is clear that yield potential will be lost in summer safe or irrigated pastures. The implication is that carrying capacity of farms will be reduced unless other farm systems can be developed that ensure nitrogen is available to plants when they are actively growing. The reduction in nitrogen use is likely to lead to greater clover content in pastures, which can partially offset the N restrictions. For example , Myint *et al*. (2021) recently showed that pasture mixes of grasses and clover, especially p. ryegrass and white clover, under the application of 25 kg N/ha, can produce as much high quality feed as grasses being fully provided nitrogen.

For more summer dry conditions, cocksfoot was the most appropriate grass. It was most productive on the shallower soil at AD which his susceptible to more intense summer dry conditions. In particularly cocksfoot maintained green leaf material and produced greater quantities of forage for animals under water stressed conditions. These results contradict the comments by Kemp and Culvernor (1994) that species adapted to surviving dry environments generally have agronomic traits that are not ideal, like slow growth, lower palatability combined with poor grazing tolerance. In this research cocksfoot was able to provide protein and metabolisable energy in levels either similar or superior to the other species at both sites.

Applying nitrogen on soils of low water holding capacity, similar to hill country areas, produced a response in Spring, season when soil water was available (Talamini Junior *et al.* 2021). However, the use of legume can also be recommended to overcome the nitrogen deficiency in these environments. The experiments for this research used monocultures of grasses but the use of pasture mixes can be considered with more than one species known to increase pasture performance (Moloney, 1991) for yield and/or quality.

8.5 Recommendations for future research

The results chapters highlighted areas that require further investigation as a complement to the present thesis. These include:

The mechanisms of maintenance on how cocksfoot tends to maintain its interceptance are still not fully explained. Though the physiology data suggests its maintenance of leaf area (Section 7.4.3), an study on tillering number is suggested for further quantifying and complementing the understanding of it.

The nitrogen applied from fertilisers seems to have stayed in the soil profile as suggested by the nitrogen recovery data (Section 4.4.2). More research on understanding the dynamics of nitrogen edaphic residuals in pasture systems, on different soil types, is required to improve management tools such as Overseer. Additionally, further studies with different levels of nitrogen fertilisation can help quantifying better the effect of water stress with nitrogen deficiency on the pastures.

The management type proposed by regenerative agriculture was taken into account on only one harvest (Sections 4.4.1 and 4.4.3), when the pastures were allowed to reach advanced reproductive stages. A long-term study quantifying the influence of this kind of management on dry matter yield, WU, WUE, intercepted PAR, RUE and persistence is required to compare it with other grazing managements.

The experiments focused mainly on plants aerial parts. More information on root biomass according to nitrogen and water levels according to each season and physiological traits related to root development should be investigate to evaluate the effects of stress on the whole of pasture plants for the studied species.

This study focused on monocultures of brome, cocksfoot, perennial ryegrass and tall fescue. As pasture mixes of grasses and legumes are common components of grazing systems in New Zealand. The effect of the interaction of those species since their establishment on how each species reacts to stress should, thus, be investigated.

8.6 Conclusions

The present research from this thesis provided explanation on how physiological traits change the yield generating components, from Equation 1, according to the different temperatures and water and nitrogen availability. The agronomic performance (Chapter 4) through the stations had to be demonstrated and explained by the water use and its efficiency (Chapter 5) along the PAR interception and its use efficiency (Chapter 6) so that the physiology traits (Chapter 7) could justify the differences. Specific conclusions include:

- Cocksfoot maintained its leaves more expanded that other species during dry season stress times. This was done by maintaining a higher RWC and osmotic potential. This results in a greater VDM maintenance and PAR interception.
- The strategy of preserving the photosynthetic apparatus, as used by p. ryegrass and tall fescue, ultimately resulted in a lower amount of quality feed that can be offered to animals in a resource restricted environment such as Ashley Dene. Brome used a strategy closer to cocksfoot´s.
- Around 70-75% of the year total dry matter is accumulated during the Spring, when water is available and the temperatures were high. When the dry season starts, the yield reduction is explained by a reduction in the canopy cover combined with a reduction in the net photosynthetic rate.
- Nitrogen fertilisation is important to increase yield by allowing a full canopy expansion. On dryland environments, it showed to still be important on preserving the pasture quality. During stress times, nitrogen fertilisation results in plants protecting their cell components by allowing them to accumulate more proline.
- Six years after sowing, tall fescue was the species had that the worst persistence at a resource restricted environment due to its low VDM yield and high weed ingress.

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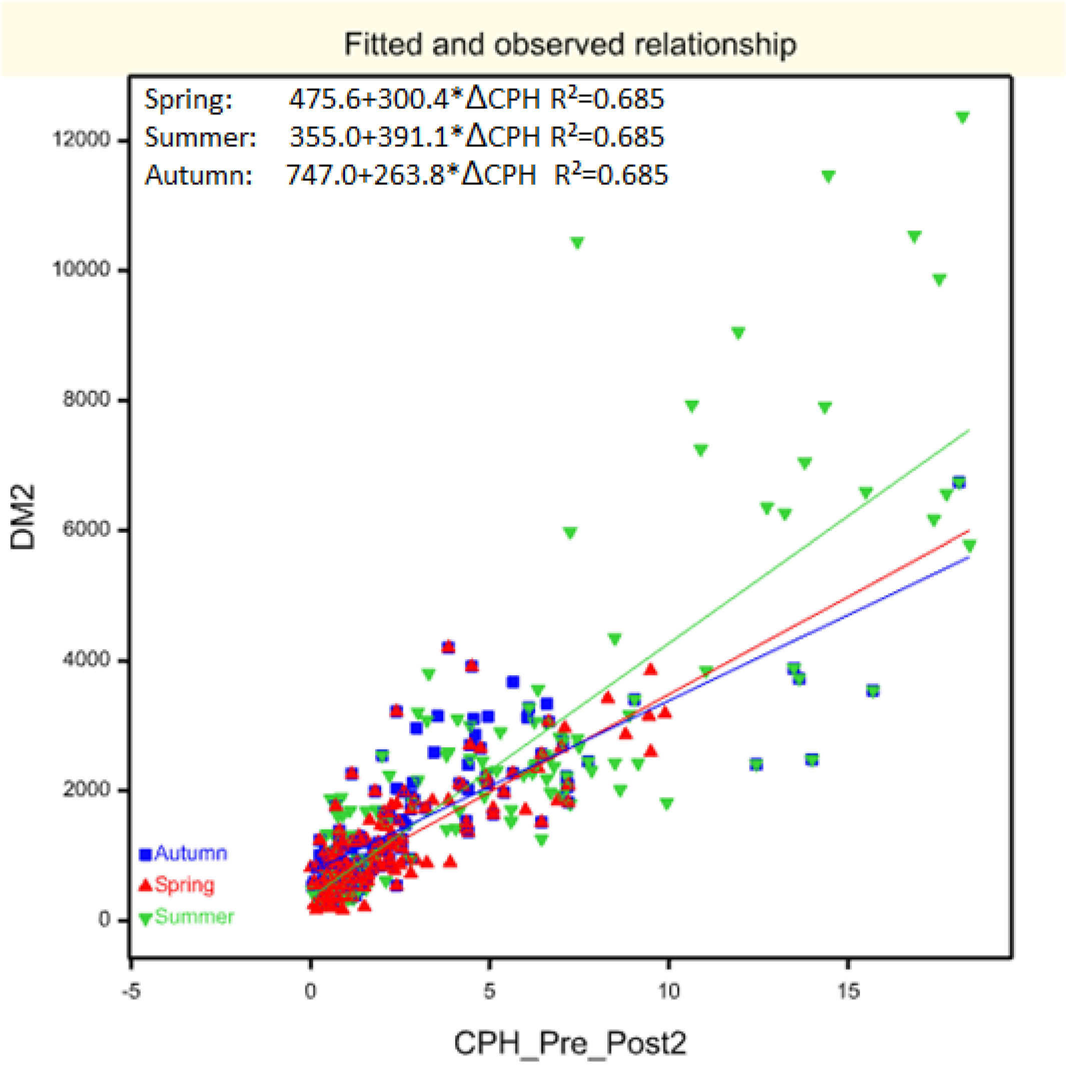
APPENDICES

Appendix A Linear regressions Compressed Plate Height x Dry Matter Production used for different seasons.



Appendix B Protein content (% of dry matter) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ladbrooks.

		N+				N-				
Origin	Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
Vegetative	23/10/18	30.1	28.2	26.5	27.1	16.9	15.9	12.6	14.8	21.5
	22/11/18	26.0	26.6	25.9	25.0	13.9	14.0	12.7	13.3	19.7
	17/12/18	30.5	26.8	25.0	24.1	16.9	17.1	16.1	14.2	21.3
	22/01/19	24.7	21.8	22.8	21.5	15.7	13.9	14.8	13.4	18.6
	21/03/19	25.6	23.9	24.2	19.3	18.0	14.9	16.6	15.5	19.7
	09/05/19	29.4	31.2	29.1	28.0	18.7	19.0	16.8	17.7	23.7
	28/06/19	26.9	26.8	25.9	25.3	19.7	19.3	16.2	18.3	22.3
	29/08/19	20.7	28.1	23.8	23.1	15.5	18.0	13.3	17.1	20.0
	09/10/19	32.1	31.2	28.9	27.5	18.2	16.1	13.4	16.6	23.0
	13/12/19	14.3	17.0	19.7	15.8	11.3	10.0	10.6	9.5	13.5
	11/02/20	22.0	18.8	19.2	19.9	10.8	9.9	10.2	9.7	15.1
	19/05/20	22.7	21.7	20.6	21.7	16.5	13.2	14.6	13.4	18.0
	21/07/20	22.9	23.3	21.0	21.7	20.9	24.1	22.6	22.9	22.4
Reproductive	23/10/18	14.3	16.5	19.6	13.5	7.1	8.3	7.4	5.5	11.5
	22/11/18	12.3	15.5	19.2	12.4	5.9	7.4	7.6	4.9	10.7
	17/12/18	14.5	15.6	18.4	12.1	7.1	9.0	9.5	5.4	11.4
	22/01/19	11.8	12.7	16.8	10.8	6.7	7.3	8.8	5.0	10.0
	21/03/19	12.1	13.9	17.8	9.6	7.7	7.8	9.8	5.8	10.6
	09/05/19	14.0	18.2	21.4	13.9	8.0	9.9	9.9	6.6	12.7
	28/06/19	12.8	15.7	19.1	12.6	8.3	10.2	9.6	6.9	11.9
	29/08/19	-	-	-	-	-	-	-	-	-
	09/10/19	-	-	-	-	-	-	-	-	-
	13/12/19	6.7	10.0	14.5	8.1	4.8	5.2	6.2	3.5	7.4
	11/02/20	-	-	-	-	-	-	-	-	-
	19/05/20	-	-	-	-	-	-	-	-	-
	21/07/20	-	-	-	-	-	-	-	-	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “N” represents nitrogen, “S” represents species, “N*S” the interaction among nitrogen and species, “H” represents the harvest.

Appendix C Protein content (% of dry matter) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ashley Dene

		N+				N-				
Origin	Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
Vegetative	2/11/18	25.4	26.0	23.9	22.3	15.4	14.0	13.5	15.8	19.5
	3/12/18	28.0	26.7	26.1	27.6	18.3	15.6	15.5	17.0	21.9
	18/01/19	21.9	20.7	20.6	18.0	14.2	11.3	12.1	12.4	16.4
	17/05/19	24.0	25.1	26.7	26.6	21.1	19.8	20.1	19.6	22.9
	04/07/19	29.0	31.3	30.4	28.1	23.3	23.3	19.6	20.5	25.7
	19/09/19	26.8	27.6	21.0	23.7	21.5	22.7	16.4	19.2	22.4
	29/11/19	20.1	19.3	15.0	16.1	9.2	8.7	7.4	8.0	13.0
	14/05/20	25.4	25.4	25.5	25.8	19.2	15.6	16.9	16.6	21.3
	17/07/20	25.4	27.2	24.7	26.3	20.2	22.4	19.8	21.2	23.4
Reproductive	2/11/18	-	-	-	-	-	-	-	-	-
	19/09/19	26.8	27.6	21.0	23.7	21.5	22.7	16.4	19.2	22.4
	29/11/19	20.1	19.3	15.0	16.1	9.2	8.7	7.4	8.0	13.0
	14/05/20	25.4	25.4	25.5	25.8	19.2	15.6	16.9	16.6	21.3
	17/07/20	25.4	27.2	24.7	26.3	20.2	22.4	19.8	21.2	23.4
	19/09/19	-	-	-	-	-	-	-	-	-
	29/11/19	13.1	15.7	13.2	13.6	7.9	6.6	6.4	5.6	10.3
	14/05/20	-	-	-	-	-	-	-	-	-
	17/07/20	-	-	-	-	-	-	-	-	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “N” represents nitrogen, “S” represents species, “N*S” the interaction among nitrogen and species, “H” represents the harvest.

Appendix D Metabolisable energy (MJ kg^{-1}) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ladbrooks.

		N+				N-				
Origin	Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
Vegetative	23/10/18	11.1	11.1	11.1	10.9	10.6	11.5	11.6	10.4	11.0
	22/11/18	10.6	10.9	11.2	10.7	10.1	11.0	11.4	10.0	10.7
	17/12/18	11.0	11.3	11.7	11.1	10.4	11.0	11.5	10.1	11.0
	22/01/19	10.2	10.5	12.0	10.9	10.1	10.9	11.7	10.2	10.8
	21/03/19	10.9	11.0	11.7	10.3	10.5	11.1	11.7	10.4	11.0
	09/05/19	11.2	11.4	12.1	11.3	10.7	11.3	12.2	11.0	11.4
	28/06/19	11.6	11.7	12.1	11.5	11.3	11.7	12.7	12.0	11.8
	29/08/19	12.0	12.0	12.3	11.9	11.4	12.2	12.9	11.8	12.1
	09/10/19	11.4	11.1	11.6	11.0	10.7	11.9	12.1	11.0	11.3
	13/12/19	9.5	9.9	10.6	10.0	9.9	11.1	11.5	10.0	10.3
	11/02/20	10.3	11.0	11.7	10.7	9.8	11.6	12.6	11.1	11.1
	19/05/20	10.9	11.2	11.7	10.9	10.2	11.5	12.4	11.2	11.3
	21/07/20	10.7	10.6	10.8	10.6	10.6	11.1	11.5	11.1	10.9
Reproductive	23/10/18	7.1	8.1	9.3	8.1	8.2	7.9	8.8	7.7	8.1
	22/11/18	6.8	7.9	9.3	7.9	7.8	7.6	8.7	7.4	7.9
	17/12/18	7.0	8.2	9.8	8.2	8.0	7.6	8.8	7.5	8.1
	22/01/19	6.5	7.6	9.9	8.0	7.8	7.5	8.9	7.6	8.0
	21/03/19	7.0	8.0	9.7	7.6	8.1	7.7	9.0	7.7	8.1
	09/05/19	7.2	8.3	10.0	8.3	8.2	7.8	9.3	8.1	8.4
	28/06/19	7.4	8.4	10.1	8.5	8.7	8.1	9.7	8.8	8.7
	29/08/19	-	-	-	-	-	-	-	-	-
	09/10/19	-	-	-	-	-	-	-	-	-
	13/12/19	6.1	7.1	8.8	7.4	7.6	7.6	8.8	7.4	7.6
	11/02/20	-	-	-	-	-	-	-	-	-
	19/05/20	-	-	-	-	-	-	-	-	-
	21/07/20	-	-	-	-	-	-	-	-	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "N" represents nitrogen, "S" represents species, "N*S" the interaction among nitrogen and species, "H" represents the harvest.

Appendix E Metabolisable energy (MJ kg^{-1}) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ashley Dene

		N+				N-				
Origin	Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
Vegetative	2/11/18	11.0	11.2	11.6	11.2	10.9	11.3	11.9	11.0	11.3
	3/12/18	10.5	10.9	10.6	10.7	10.1	10.3	10.4	10.2	10.5
	18/01/19	10.7	10.6	11.7	10.6	10.5	11.1	10.8	10.9	10.9
	17/05/19	11.1	11.2	11.7	11.2	10.9	11.2	11.6	10.9	11.2
	04/07/19	11.4	11.7	12.2	11.5	11.4	11.5	12.7	11.7	11.8
	19/09/19	11.6	11.9	12.2	11.9	11.3	11.5	12.4	11.7	11.8
	29/11/19	10.2	10.7	10.2	9.8	9.4	11.2	10.6	10.2	10.3
	14/05/20	10.8	11.8	11.2	11.3	10.4	11.8	11.6	11.2	11.3
	17/07/20	11.0	11.5	11.8	10.6	10.6	11.4	11.5	11.2	11.2
Reproductive	2/11/18	-	-	-	-	-	-	-	-	-
	19/09/19	8.4	9.6	10.0	9.7	9.0	7.9	9.2	8.6	9.0
	29/11/19	8.6	9.4	10.9	9.6	9.4	8.5	9.5	9.2	9.4
	14/05/20	-	-	-	-	-	-	-	-	-
	17/07/20	-	-	-	-	-	-	-	-	-
	19/09/19	-	-	-	-	-	-	-	-	-
	29/11/19	8.1	9.5	9.6	8.8	8.4	8.6	9.2	8.6	8.9
	14/05/20	-	-	-	-	-	-	-	-	-
	17/07/20	-	-	-	-	-	-	-	-	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "N" represents nitrogen, "S" represents species, "N*S" the interaction among nitrogen and species, "H" represents the harvest.

Appendix F Acid Detergent Fiber (% of Dry Matter) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ladbrooks.

		N+				N-				
Origin	Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
Vegetative	23/10/18	26.8	27.1	27.7	26.4	29.8	26.7	26.0	29.6	27.5
	22/11/18	31.3	29.5	27.8	26.9	32.7	29.6	27.0	31.4	29.5
	17/12/18	26.0	26.7	25.4	25.3	31.6	30.3	26.3	30.6	27.8
	22/01/19	30.6	30.8	24.4	26.6	31.5	30.3	25.0	30.2	28.7
	21/03/19	30.2	30.3	26.9	30.7	33.5	30.1	27.0	31.4	30.0
	09/05/19	27.7	25.7	23.8	25.1	29.7	27.5	23.8	27.3	26.3
	28/06/19	23.9	22.9	21.5	23.4	26.1	24.0	20.8	21.7	23.0
	29/08/19	21.7	22.2	21.6	22.7	24.5	22.4	20.2	22.9	22.3
	09/10/19	24.8	26.4	26.3	27.5	28.1	25.3	24.2	27.8	26.3
	13/12/19	37.8	37.0	32.3	31.8	36.3	31.7	26.9	32.0	33.2
	11/02/20	31.0	30.7	24.3	27.8	33.4	27.1	21.7	26.5	27.8
	19/05/20	29.3	28.2	25.0	28.3	31.7	26.4	22.9	26.3	27.3
	21/07/20	30.8	31.9	31.4	31.2	31.9	29.6	28.1	29.6	30.6
Reproductive	23/10/18	34.4	33.8	32.1	36.4	34.9	36.9	36.2	41.5	35.8
	22/11/18	40.2	36.7	32.2	37.1	38.3	40.9	37.6	44.1	38.4
	17/12/18	33.4	33.3	29.4	34.8	37.0	42.0	36.5	42.9	36.2
	22/01/19	39.4	38.3	28.2	36.7	36.9	41.9	34.7	42.3	37.3
	21/03/19	38.8	37.7	31.2	42.3	39.2	41.7	37.5	44.0	39.1
	09/05/19	35.6	31.9	27.5	34.7	34.8	38.0	33.0	38.2	34.2
	28/06/19	30.8	28.5	24.9	32.3	30.6	33.2	28.9	30.5	30.0
	29/08/19	-	-	-	-	-	-	-	-	-
	09/10/19	-	-	-	-	-	-	-	-	-
	13/12/19	48.5	46.0	37.4	43.8	42.4	43.8	37.3	44.8	43.0
	11/02/20	-	-	-	-	-	-	-	-	-
	19/05/20	-	-	-	-	-	-	-	-	-
	21/07/20	-	-	-	-	-	-	-	-	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “N” represents nitrogen, “S” represents species, “N*S” the interaction among nitrogen and species, “H” represents the harvest.

Appendix G Neutral Detergent Fiber (% of Dry Matter) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ladbrooks.

		N+				N-				
Origin	Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
Vegetative	23/10/18	53.7	52.6	51.1	51.7	53.6	49.7	47.9	55.4	52.0
	22/11/18	57.7	55.9	51.5	51.9	57.6	54.6	48.3	58.6	54.5
	17/12/18	50.0	51.4	46.5	48.1	56.2	56.2	48.1	57.2	51.7
	22/01/19	60.9	60.1	47.4	52.0	56.2	55.9	45.6	55.4	54.2
	21/03/19	58.9	60.1	51.2	58.8	62.4	56.4	49.7	57.4	56.9
	09/05/19	54.9	52.2	46.4	50.2	54.5	51.5	44.3	51.1	50.6
	28/06/19	48.9	47.2	42.2	46.9	49.0	46.8	39.8	41.3	45.3
	29/08/19	41.9	45.1	41.7	43.8	44.0	44.3	38.2	41.9	42.6
	09/10/19	51.1	53.8	49.6	53.3	51.2	50.2	45.1	51.7	50.8
	13/12/19	62.4	65.5	55.7	56.5	60.6	57.1	47.4	56.8	57.8
	11/02/20	58.9	58.1	44.8	51.6	58.1	51.3	41.4	49.0	51.7
	19/05/20	57.0	55.8	47.6	55.0	56.9	49.6	42.9	49.0	51.7
	21/07/20	57.3	58.1	57.4	60.0	54.9	53.4	48.1	50.0	54.9
Reproductive	23/10/18	69.0	61.5	57.4	65.8	65.3	65.0	65.4	73.4	65.4
	22/11/18	74.1	65.4	58.0	66.1	70.2	71.4	66.2	77.6	68.6
	17/12/18	64.2	60.1	52.3	61.3	68.5	73.6	65.8	75.7	65.2
	22/01/19	78.3	70.3	53.3	66.2	68.5	73.1	62.3	73.3	68.2
	21/03/19	75.8	70.2	57.6	74.8	76.1	73.9	67.9	76.0	71.6
	09/05/19	70.6	61.0	52.1	63.8	66.4	67.4	60.4	67.7	63.7
	28/06/19	62.9	55.2	47.5	59.8	59.7	61.4	54.4	54.8	56.9
	29/08/19	-	-	-	-	-	-	-	-	-
	09/10/19	-	-	-	-	-	-	-	-	-
	13/12/19	80.3	76.5	62.6	71.9	73.9	74.7	64.8	75.3	72.5
	11/02/20	-	-	-	-	-	-	-	-	-
	19/05/20	-	-	-	-	-	-	-	-	-
	21/07/20	-	-	-	-	-	-	-	-	-

Note: "BR" represents Brome, "CF" represents Cocksfoot, "PR" represents P.Ryegrass, "TF" represents Tall Fescue, "N" represents nitrogen, "S" represents species, "N*S" the interaction among nitrogen and species, "H" represents the harvest.

Appendix H Acid Detergent Fiber (% of dry matter) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ashley Dene

		N+				N-				
Origin	Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
Vegetative	2/11/18	29.0	28.7	26.5	27.7	29.9	27.8	25.1	28.9	28.0
	3/12/18	29.9	29.0	29.2	28.3	33.7	32.7	31.6	31.3	30.7
	18/01/19	31.1	31.7	26.9	30.1	32.1	30.5	29.8	29.4	30.2
	17/05/19	29.8	31.5	28.0	27.3	30.4	29.7	28.1	28.9	29.2
	04/07/19	24.7	24.4	22.1	23.9	25.5	25.7	20.5	23.8	23.8
	19/09/19	25.3	25.6	24.9	24.3	27.2	27.2	24.4	26.1	25.6
	29/11/19	34.1	33.8	34.0	34.7	37.7	31.2	32.1	32.4	33.8
	14/05/20	28.0	24.4	25.5	25.2	30.6	25.7	25.4	26.5	26.4
	17/07/20	27.4	27.2	24.6	23.8	29.1	26.2	24.3	25.5	26.0
Reproductive	2/11/18	-	-	-	-	-	-	-	-	-
	19/09/19	35.4	33.0	30.9	32.1	35.1	42.9	36.7	38.7	35.6
	29/11/19	37.1	36.0	28.4	34.0	33.4	40.2	34.5	36.4	35.0
	14/05/20	-	-	-	-	-	-	-	-	-
	17/07/20	-	-	-	-	-	-	-	-	-
	19/09/19	-	-	-	-	-	-	-	-	-
	29/11/19	40.4	38.3	35.8	39.3	39.3	40.9	36.8	40.1	38.9
	14/05/20	-	-	-	-	-	-	-	-	-
	17/07/20	-	-	-	-	-	-	-	-	-

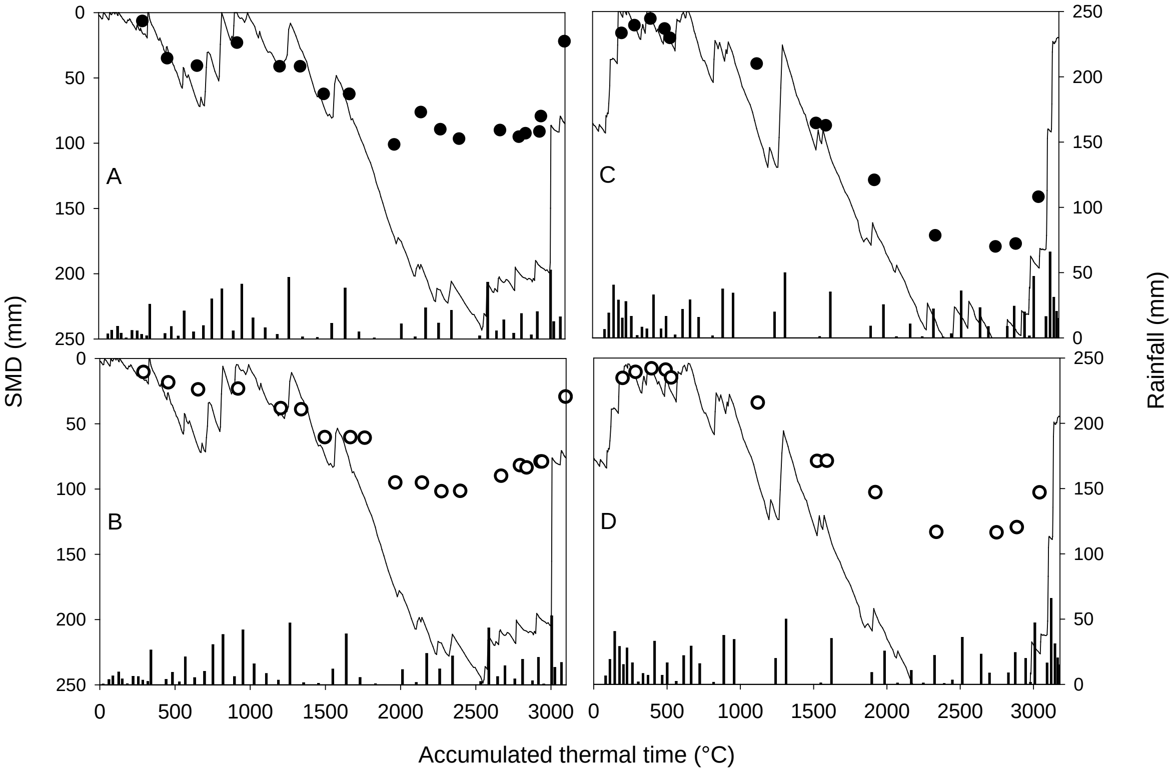
Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “N” represents nitrogen, “S” represents species, “N*S” the interaction among nitrogen and species, “H” represents the harvest.

Appendix I Neutral Detergent Fiber (% of dry matter) from brome, cocksfoot, perennial ryegrass and tall fescue, with (N+) and without (N-) nitrogen fertilisation, from vegetative and reproductive botanical components, harvested from July 2018 to July 2020 at Ashley Dene

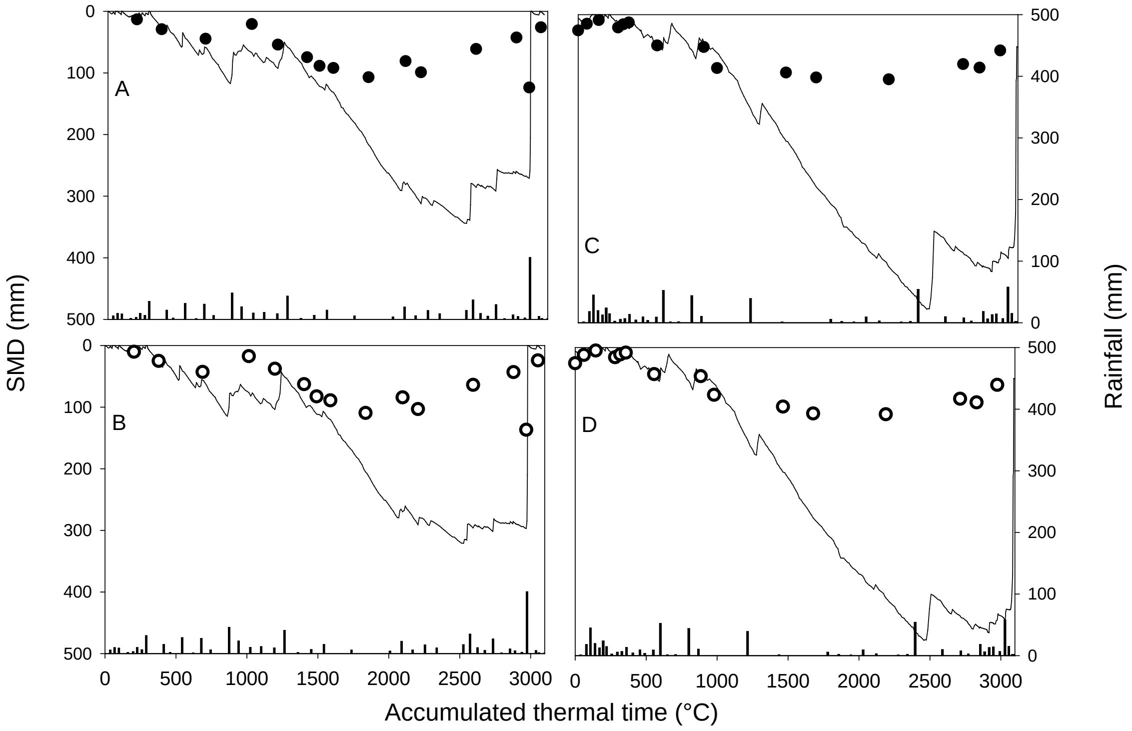
		N+				N-				
Origin	Harvest	BR	CF	PR	TF	BR	CF	PR	TF	Mean H
Vegetative	2/11/18	53.1	51.7	44.1	47.8	56.0	53.6	48.3	55.3	51.2
	3/12/18	55.8	52.5	53.1	52.9	63.3	61.9	59.8	60.4	57.5
	18/01/19	59.5	61.1	47.4	56.8	59.5	56.7	56.5	55.6	56.6
	17/05/19	55.1	51.0	44.7	51.4	55.5	56.1	50.6	54.9	52.4
	04/07/19	50.7	48.0	40.3	48.2	49.5	51.3	39.7	48.0	47.0
	19/09/19	50.9	49.4	45.3	45.8	50.9	53.7	43.8	48.8	48.6
	29/11/19	63.0	59.8	59.3	61.0	68.1	59.2	59.5	59.7	61.2
	14/05/20	56.1	51.0	49.5	51.8	58.3	50.7	48.0	52.5	52.2
	17/07/20	51.9	47.1	41.3	47.0	53.6	50.1	44.2	48.2	47.9
Reproductive	2/11/18	-	-	-	-	-	-	-	-	-
	19/09/19	64.6	56.0	57.0	59.1	65.4	76.0	66.8	70.9	64.5
	29/11/19	69.4	64.9	50.6	62.9	61.5	69.7	63.0	65.2	63.4
	14/05/20	-	-	-	-	-	-	-	-	-
	17/07/20	-	-	-	-	-	-	-	-	-
	19/09/19	-	-	-	-	-	-	-	-	-
	29/11/19	73.1	62.9	63.1	67.5	70.4	72.6	65.7	70.0	68.2
	14/05/20	-	-	-	-	-	-	-	-	-
	17/07/20	-	-	-	-	-	-	-	-	-

Note: “BR” represents Brome, “CF” represents Cocksfoot, “PR” represents P.Ryegrass, “TF” represents Tall Fescue, “N” represents nitrogen, “S” represents species, “N*S” the interaction among nitrogen and species, “H” represents the harvest.

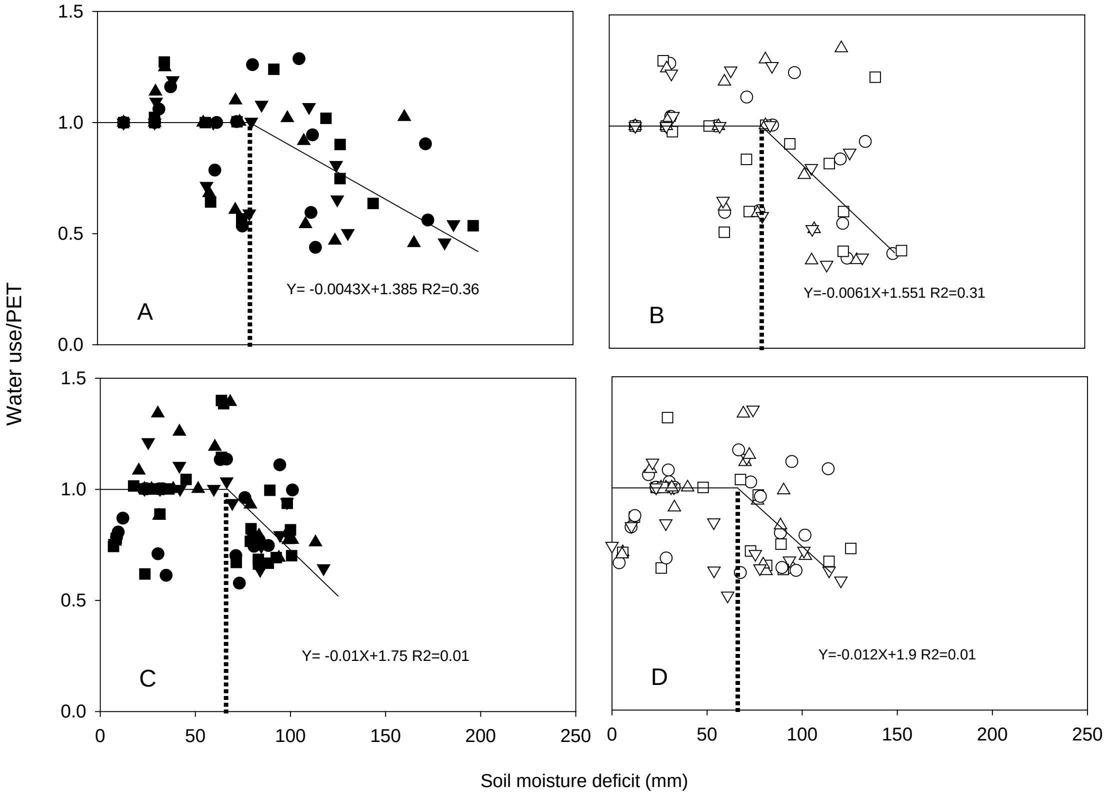
Appendix J Potential soil moisture deficit (mm) when NIWA's proposed PET$_a$ reduction is not considered at Ladbrooks, from July 2018 to July 2019 and from July 2019 to July 2020 for fertilised (closed) and non-fertilised (open) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue. "●" symbols represent days when neutron probe measurements were done.



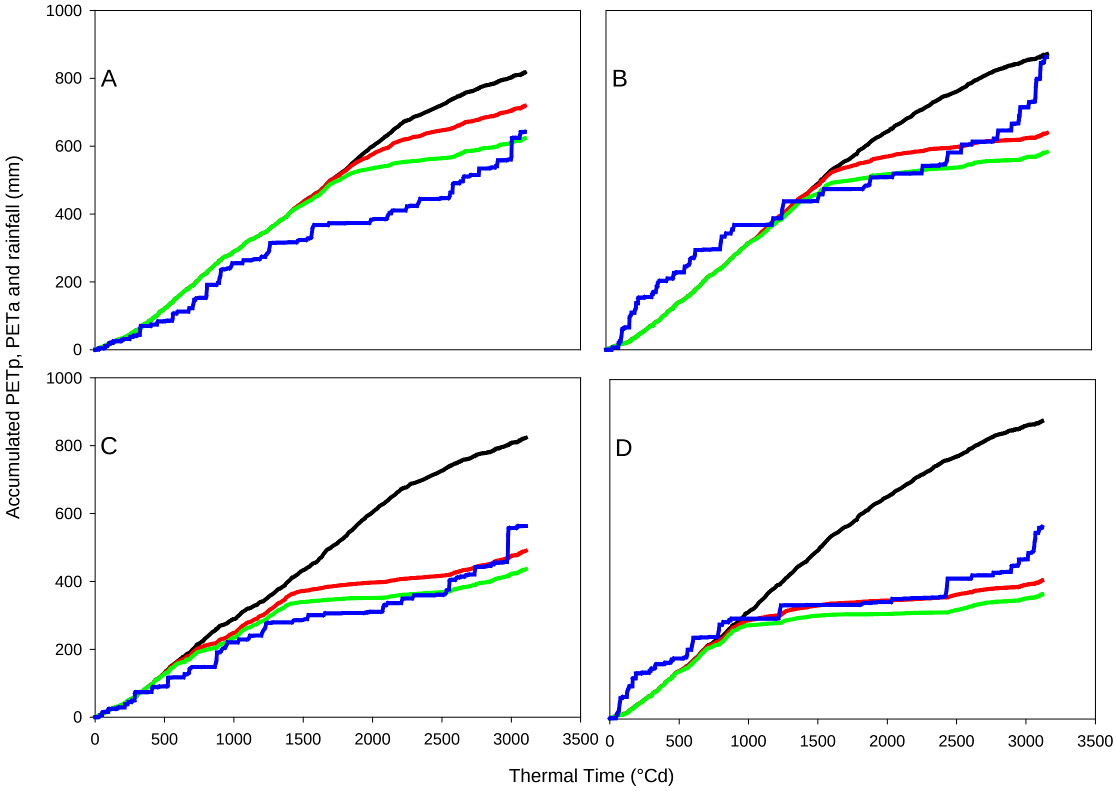
Appendix K Potential soil moisture deficit (mm), when NIWA's proposed PET$_a$ reduction is not considered at Ashley Dene, from July 2018 to July 2019 and from July 2019 to July 2020 for fertilised (closed) and non-fertilised (open) monocultures of brome, cocksfoot, perennial ryegrass and tall fescue. "●" symbols represent days when neutron probe measurements were done.



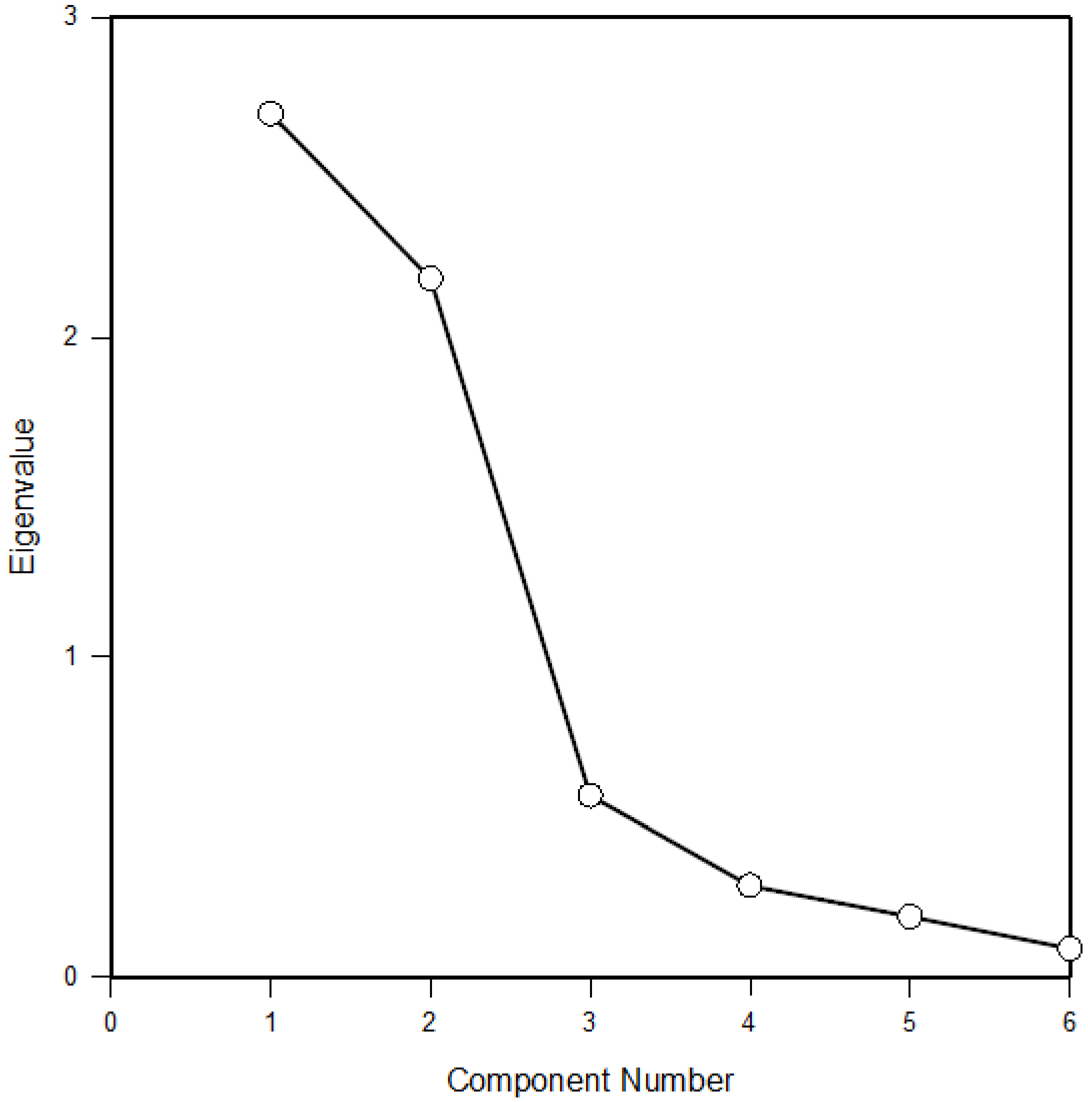
Appendix L Mean soil moisture deficit (mm) in relation to WU/PET$_p$ for mono cultures of for brome(●,○), cocksfoot (■,□), perennial ryegrass (▲,△) and tall fescue (▼,▽), with (closed) or without nitrogen fertilisation (open) at Ladbrooks (A,B) and Ashley Dene (C,D) from Jully 2018 to July 2020.



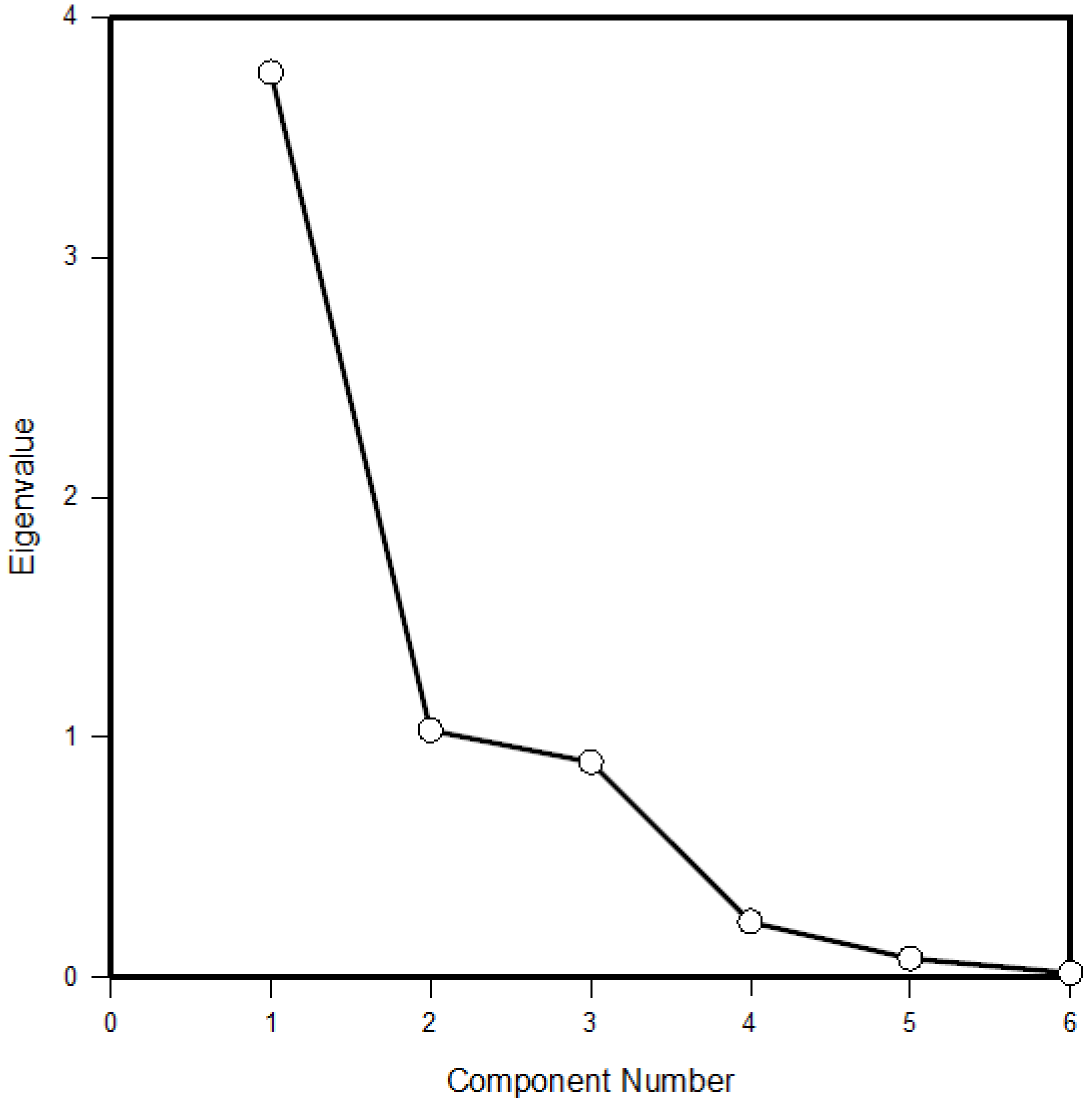
Appendix M Accumulated PET_p (black, mm), PET_a for N+ grasses (red, mm), PET_a for N- grasses (green, mm) and rainfall (blue, mm) for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue with (N+) or without (N-) nitrogen fertilisation at Ladbrooks (A,B) and Ashley Dene (C,D) from July 2018 to July 2019 (A,C) and from July 2019 to July 2020 (B,D).



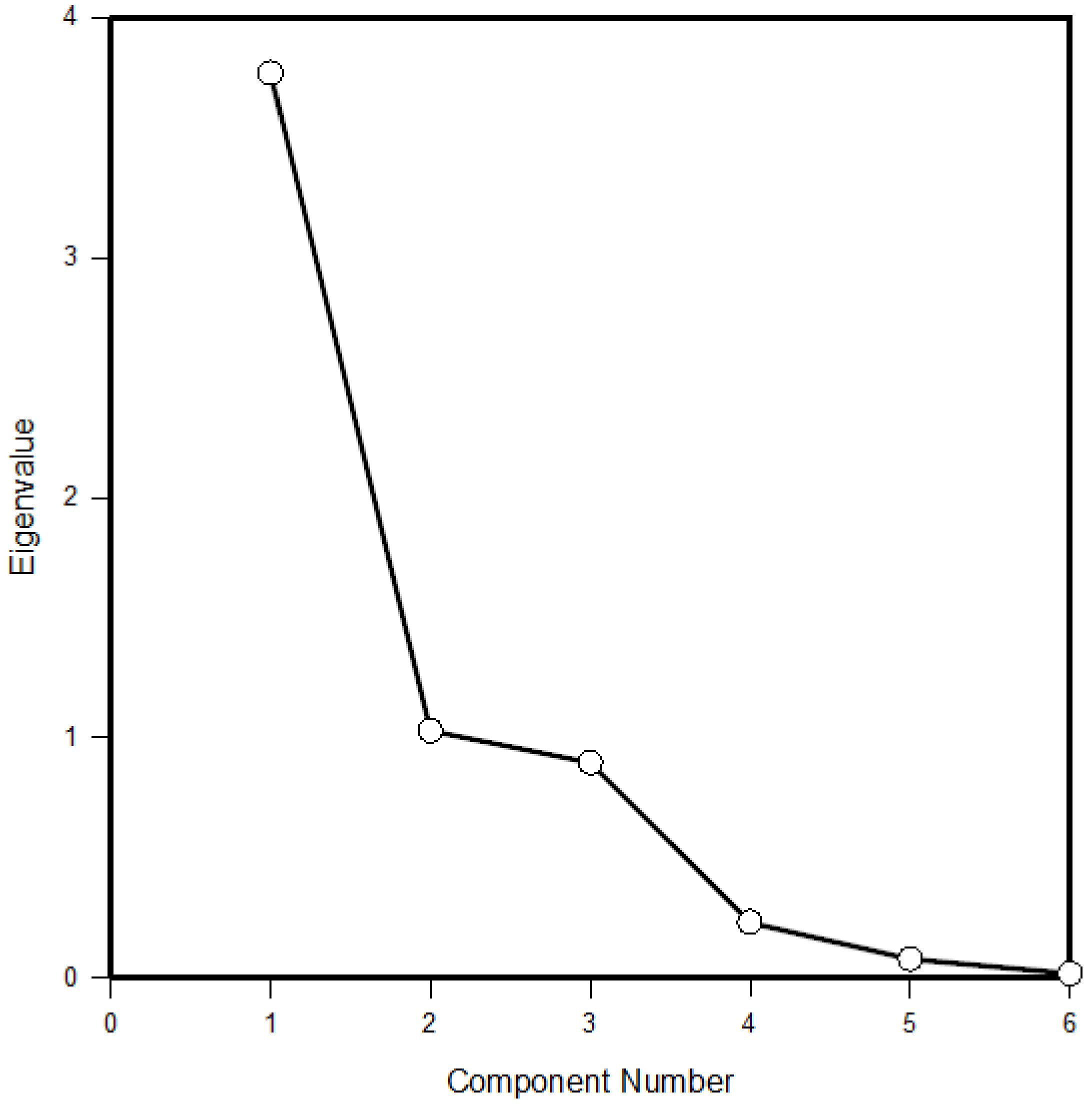
Appendix N Scree Plot from Components in the PCA for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown, with and without nitrogen, in Ladbrooks and Ashley Dene, Canterbury, New Zealand, from December 2018 to March 2020.



Appendix O Scree Plot from Components in the PCA for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown, with and without nitrogen, in Ladbrooks, Canterbury, New Zealand, from December 2018 to March 2020.



Appendix P Scree Plot from Components in the PCA for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown, with and without nitrogen, in Ashley Dene, Canterbury, New Zealand, from December 2018 to March 2020.



Appendix Q Variables' correlations with each Principal Components and Wilk's Lambda Value from PCAs from monocultures of brome (BR), cocksfoot (CF), perennial ryegrass (PR) and tall fescue (TF) grown, with (+N) and without (-N) nitrogen, at Ashley Dene and Ladbrooks and Combined (General Data), Canterbury, New Zealand, from December 2018 to March 2020. "RWC" is the relative water content, "Sto.Condu." is the stomatal conductivity, "Pn" is the net photosynthetic rate, "Chlorophyll" is the chlorophyll concentration, "Proline" is the proline accumulation, "Osm.Pot." is the osmotic potential.

PCA - General						
Wilk's Lambda	0.07071					
Variable	PC1	PC2	PC3	PC4	PC5	PC6
RWC	-0.337	-0.413	0.673	-0.285	0.423	0.049
Chlorophyll	0.473	0.188	0.269	-0.457	-0.248	0.631
Proline	0.421	0.306	0.515	-0.004	-0.108	-0.672
Pn	0.388	-0.502	0.239	0.694	-0.119	0.212
Sto. Condu.	0.307	-0.664	-0.315	-0.478	-0.188	-0.318
Osm.Pot.	-0.490	-0.084	0.231	0.001	-0.835	-0.034
PCA -						
Wilk's Lambda	0.05279					
RWC	-0.246	0.791	-0.326	-0.389	-0.054	-0.231
Chlorophyll	0.458	0.237	0.347	-0.065	-0.771	0.125
Proline	0.431	0.372	0.343	0.413	0.414	-0.465
Pn	0.468	0.255	-0.320	0.022	0.326	0.712
Sto. Condu.	0.344	-0.189	-0.743	0.280	-0.273	-0.376
Osm.Pot.	-0.454	0.282	-0.041	0.771	-0.225	0.259
PCA - Ashley						
Wilk's Lambda	0.18887					
RWC	0.361	-0.476	-0.530	-0.197	0.529	0.207
Chlorophyll	-0.487	0.055	-0.311	-0.787	-0.184	-0.099
Proline	-0.481	0.073	-0.654	0.570	-0.074	0.068
Pn	0.321	0.557	-0.288	-0.020	0.247	-0.665
Sto. Condu.	0.192	0.659	-0.108	-0.127	0.030	0.707
Osm.Pot.	0.511	-0.140	-0.317	-0.013	-0.786	-0.025