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An investigation of the phenotypic plasticity of *Poa annua* L. with regard to altitudinal variations in Cumbria and County Durham.

Richard Field

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Durham University

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Summary

1. Four experiments were run simultaneously to investigate the importance of phenotypic plasticity relative to genetic differences in the morphological, physiological and growth responses of *Poa annua* to variations in altitude in County Durham and Cumbria. One experiment compared sites differing in altitude. Another tested for effects of temperature and nutrient status under controlled conditions in a 2x2 factorial design. The third investigated effects of light intensity. The fourth compared plants taken from populations growing at different altitudes, when grown under uniform conditions.

2. Reduced growth and development rates were found with altitude, along with increased fresh/dry mass ratios and relative dry matter allocation to roots. Total numbers of tillers, inflorescences and leaves showed no clear altitudinal trends, though tiller numbers per unit whole plant dry mass increased with altitude. Leaf and tiller lengths, total leaf area, specific leaf area and leaf area ratio decreased with altitude. Stomatal frequency on both leaf surfaces decreased with altitude. This was largely related to leaf size differences, but stomatal numbers per leaf decreased with elevation on the adaxial surface. An index of leaf folding was devised, and found to increase with altitude.

3. Nutrient status significantly affected almost all parameters measured, but was controlled for in the altitude experiment and therefore did not explain the variation found there.

4. Given adequate nutrient availability, lower temperature (15°C) decreased growth and dry matter partitioning to shoots, relative to higher temperature (21°C). Effects of temperature on other parameters were more minor.

5. Increased light intensity tended to increase growth and root weight ratio, but generally had little effect on other parameters. This factor probably explained little of the altitudinal variation.

6. The significant differences in measured variables with changed environmental conditions, in plants from the same stock, were taken to be plastic responses, though the genetic uniformity of the stock was not proved.

7. Plants from different populations grown under uniform conditions showed significant differences in growth, morphology and physiology. This was taken to indicate genetic variations, though the genotypes were not proved to be different. The apparent genetic variations seemed less related to altitude of origin than to other local site conditions affecting the source populations. Other possible explanations are discussed.

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List of Abbreviations

ANOVA	analysis of variance
a.s.l.	above mean sea level
DBG	Durham University Botanic Gardens
FAA	formalin acetic alcohol
GDF	Great Dun Fell
K	the allometric growth coefficient
LAR	leaf area ratio
L:S	leaf/shoot ratio (dry mass basis)
LWR	leaf weight ratio
N	nitrogen
N+P	nitrate and phosphate (or nitrogen and phosphorus)
PAR	photosynthetically active radiation
PCR	polymerase chain reaction
RGR	relative growth rate
RWR	root weight ratio (dry mass basis)
SLA	specific leaf area
S:R	shoot/root ratio (dry mass basis)
T_0	first harvest (at time zero)
T_x	*x*th harvest (time intervals between harvests vary)
UV	ultra violet
WBF	Widdybank Fell

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Acknowledgements

Many people helped during the course of this study. Special thanks must go to my supervisors, Drs. R. Baxter and J.A. Pearson, as well as to Alison Bradley at University College Stockton and Michael at Durham Botanic Gardens; and to Ian Findlay at Widdybank Fell, John Begg at Great Dun Fell, Neil Huntley at Hartside Nursery, Alston and Jo Cobb at the Botanic Gardens for their help and for permission to work at those sites. Many thanks are also due to Dr. Robert Whittaker; Michelle Metcalfe; Caroline Hartley; Dorothy Catling; Steve and the others at the Botanic Gardens; Jack, Gordon, Gillian, Paul, Chris, Richard and the other technical staff at Durham and University College, Stockton; and Audrey Warner at the Observatory. Thanks also to my parents and all my friends for their support and advice when I was working too hard.

Chapter 1. General Introduction

There is debate about the importance of phenotypic plasticity relative to genetic differences in determining plant responses to different environmental conditions (see Section 1.1). These responses may be similar to those of plants to environmental change over time. An understanding of the nature of plant responses to environmental variation is vital if prediction and management of the effects of landscape and climatic changes on the flora, and with it the fauna, of the world are to be achieved. In this, the physiological mechanisms involved, as well as the actual variations in plant response, are important.

Altitude provides readily-testable, complex, compound environmental variation. It involves several factors, such as temperature, exposure, and possibly nutrient status and light intensity and quality, depending on the exact locations chosen for study. To address the issues mentioned above, a study of the effects of altitude on a common C_3 grass species, *Poa annua* L., was undertaken.

1.1 Background

Phenotypic plasticity has been variously defined, both qualitatively (*e.g.* Bradshaw, 1965; Robinson and Rorison, 1988) and quantitatively (Scheiner and Goodnight, 1984). Basically it refers to the amount by which different environments change the phenotypic expression of a given genotype. Any trait in a plant genet which is affected by the magnitude of any environmental variable may be said to be phenotypically plastic to some degree. The capacity of individual plants to survive and grow under different altitudinal conditions can be achieved by phenotypic plasticity of morphological and physiological traits related to resource acquisition and utilisation (Schlichting, 1986; Kuiper and Kuiper, 1988).

The philosophy of the adaptationist school of evolutionary thought, which has tended to see the success of organisms in different environments as caused by genetic adaptation to local conditions, has recently been challenged (Lewontin, 1978; Gould and Lewontin, 1979) and modified by findings of constraints to evolution, and by information on the variation in responses of the phenotype of a given genotype to different environmental conditions. Phenotypic plasticity is often seen as an alternative way by which plants can adapt to environmental heterogeneity (Bradshaw, 1965; Marshall and Jain, 1968; Schlichting, 1986; Levin, 1988). Within the literature are studies investigating its role, and its importance relative to genotypic variation, in

populations of a range of grass species in relation to differences in several environmental factors (*e.g.* Scheiner and Goodnight, 1984; Platenkamp, 1990, 1991; Novak, Mack and Soltis, 1991; Poorter and Pothmann, 1992; Birch and Hutchings, 1992; Elberse and Berendse, 1993; Williams and Black, 1993; Miller and Fowler, 1994; Cheplick, 1995a, b; de Kroon and Hutchings, 1995; Williams, Mack and Black, 1995).

Many of the above studies showed that both phenotypic plasticity and genotypic differences can be important in explaining variation in demography along environmental gradients and in responses to experimental changes in one or more environmental factor (*e.g.* Platenkamp, 1990; Miller and Fowler, 1994). Some earlier studies comparing congeneric species (Cumming, 1959; Marshall and Jain, 1968; Jain, 1979) suggested that one species in such a pairing tends to be more genetically variable and the other more phenotypically plastic. However, the general consensus of the literature cited above is that, in those species and circumstances at least, phenotypic plasticity tends to explain the variation better than do genotypic differences (Scheiner and Goodnight, 1984; Platenkamp, 1990, 1991; Novak *et al.*, 1991; Williams and Black, 1993; Williams *et al.*, 1995).

It has been recognised for a long time that phenotypic plasticity can itself be under genetic control (Bradshaw, 1965), and may be selected for when plants grow and reproduce in highly variable environments (Bradshaw, 1965; Schlichting, 1986; Kuiper and Kuiper, 1988; Hutchings, 1988; de Kroon and Knops, 1990; Birch and Hutchings, 1992), or where the distances involved in gene dispersal are large compared with the scale of environmental variation (Levin, 1988). Some (*e.g.* Bradshaw, 1965; Levins, 1963; Marshall and Jain, 1968; Jain, 1979) have argued that selection for genetic differences and for phenotypic plasticity are antagonistic, partly because phenotypic plasticity makes some genetic variation "invisible" to natural selection (Platenkamp, 1990). However, much current opinion seems to favour the idea that the two are not mutually exclusive, and that it is their interaction which determines the range and evolutionary potential of the species concerned (Wu and Jain, 1978; Silander and Antonovics, 1979; Scheiner and Goodnight, 1984; Scheiner, 1993; Williams and Black, 1993).

High levels of phenotypic plasticity may be more important in certain situations than in others. Founder populations of invading species, for instance, have little genetic base upon which to draw (Barrett and Richardson, 1985; Williams and Black, 1993). More relevant to the current study is the importance of phenotypic plasticity in the survival

and abundance of species which tend to reproduce asexually, and of species characteristic of environments which are heterogeneous in both space and time.

Both scenarios apply to *Poa annua*, which is characteristic of disturbed and bare ground. It is an important species in that it is frequently considered a weed, and can harbour crop pathogens such as the fungus *Mycosphaerella graminicola* (Chen, Boeger and McDonald, 1994) and the barley yellow dwarf virus (Masterman, Holmes and Foster, 1994), as well as cereal aphids (Masterman *et al.*, 1994). As such it has been the subject of work on biological control (*e.g.* Zhou and Neal, 1995) and control by nutrient regulation (*e.g.* Kuo, 1993a, 1993b). It has also been investigated for use as a possible biomonitor for heavy metal pollution (Djingova, Kuleff and Andreev, 1993). Therefore, understanding of the responses of *Poa annua* to potential environmental changes is important.

This study concentrates on the plasticity of response of *Poa annua* plants to variation in altitude, with respect to parameters of growth, morphology and physiology. Supporting this were similar investigations of the responses of plants from the same clump to different magnitudes of some environmental factors which may be constituents of altitudinal variation: temperature, nutrient availability and light intensity. A limited investigation into the variability of populations collected from different altitudes and grown under uniform conditions was undertaken for comparison.

With increasing evidence for, and concern about "global climate change", plant responses to environmental change constitute a large and burgeoning area of research which is of great importance. The transplanting involved in all the experiments in this study is equivalent to major and rapid environmental change (in temperature regime, for example). If one assumes some analogy between factor differences (such as altitude) and possible future climatic changes (an assumption which is difficult to test), then studies like this one have relevance to the research mentioned above. It is also important to gain knowledge of the mechanisms involved in plant responses to a broad range of environmental variation, and it is hoped that research like that undertaken here will help that aim.

1.2 Aim & objectives

Aim: To investigate the phenotypic plasticity of *Poa annua* with regard to altitudinal variations.

To achieve this aim, several objectives were defined:

1. To examine the effects of altitude on growth, morphology and physiology of grasses of the same genotype.
2. To examine the effects of environmental factors which change with altitude.
3. To analyse the responses of *Poa annua*, collected from populations growing at different altitudes, to the same environmental conditions.

The data collected are discussed in relation to the results of similar studies in the literature, and with respect to the debate about phenotypic plasticity and genetic difference discussed above. Growth, an end-product of morphology and physiological processes such as photosynthesis and nutrient usage, is used as the starting point for a reductionist approach which then assesses the contribution of components of the overall plant system to that growth.

Chapter 2. Materials and methods

2.1 Experimental design

To investigate the effects of altitude on the growth, morphology and physiology of grasses of the same genotype (Objective 1), trays of *Poa annua* plants from the same stock (see Section 2.3), were placed in three sites of widely differing altitudes (see next section), and destructively harvested at regular intervals (Chapter 3).

Altitude is not a single environmental variable. To investigate which aspects of altitude may be the most important in determining the responses found in Chapter 3 (Objective 2), possibly important components of altitude were considered. These included temperature, exposure, nutrient availability (which in many cases is reduced at higher altitudes - Friend and Woodward, 1990) and light regime. *Poa annua* plants were grown in controlled environment chambers in which temperature and light regime were pre-set. In these chambers a 2x2 factorial experiment was set up to investigate the effects of temperature and nutrients together and in isolation on the growth, morphology and physiology of *Poa annua* (Chapter 4). A shading frame used in a previous experiment (Ferris, 1991) was set up in the Durham University Botanic Gardens for analysis of the effect of light intensity (Chapter 5). Facilities were not available to study the effects of differences in exposure to either wind or ultra-violet light.

The experiments of Chapters 3-5 address the issue of phenotypic plasticity. However, it was not possible to confirm that the plants used were all of the same genotype, without recourse to techniques well beyond the scope of this study. To maximise the likelihood of genetic uniformity, all the plants used were taken from one small clump (Section 2.3). But the possibility remains that the plants were not from the same genet, given the abundance of *Poa annua* nearby. Other studies with different species have used similar assumptions without formal checking of the genotypes (*e.g.* Scheiner & Goodnight, 1984; Williams and Black, 1993).

An experiment was set up to analyse the responses of *Poa annua*, collected from populations growing at different altitudes, to the same environmental conditions (Objective 3): Chapter 6. Here the analogy is between the separate populations and different genotypes, but again genetic differences could not feasibly be proved.

2.2 Study sites

Site 1: Durham University Botanic Gardens (DBG)

Altitude 100 m a.s.l. Grid reference: NZ 274409.

This site was used not only to study the effects of altitude (Chapter 3), but also those of light intensity (Chapter 5) and of different source populations (Chapter 6). Daily weather data are not routinely collected for the Botanic Gardens, but were obtained from the nearby Observatory (approximately the same altitude, Grid ref. NZ 267415). An exclosure in which plants are nursed (Plate 2.1) was used for the experiments, but was found not to be effective in excluding rabbits. Therefore tables were constructed within the exclosure, and all the plants placed on them, which proved highly effective against herbivory.

Site 2: Widdybank Fell (WBF)

Altitude 513 m a.s.l. Grid reference: NY 818298.

WBF is a relatively exposed moorland site in the Pennines situated next to a major reservoir (Cow Green). Because of the rarity and conservation value of its flora it is part of an important National Nature Reserve. The site, which was at a meteorological station on the Fell, was chosen because of its altitude, accessibility, and ready availability of daily weather data (Figure 1.1). An exclosure, housing the meteorological station, was used to prevent interference with the experiment by grazing rabbits and sheep.

Site 3: Great Dun Fell (GDF)

Altitude: 848 m a.s.l. Grid reference: NY 711322.

GDF, with the nearby Little Dun and Cross Fells, forms a high ridge in the Pennines, which includes the highest point in England outside of the Lake District. It consists of moorland which is exposed to regularly high winds. The site, which was at the summit of the fell, was chosen because it was the highest place with reasonable accessibility from Durham - there is a road leading to the top (Figure 1.1). Weather data are also collected on a daily basis (though not at weekends or bank holidays) at the study site. An exclosure was constructed using wooden stakes and wire netting (Plate 2.2) to prevent rabbit and sheep grazing.

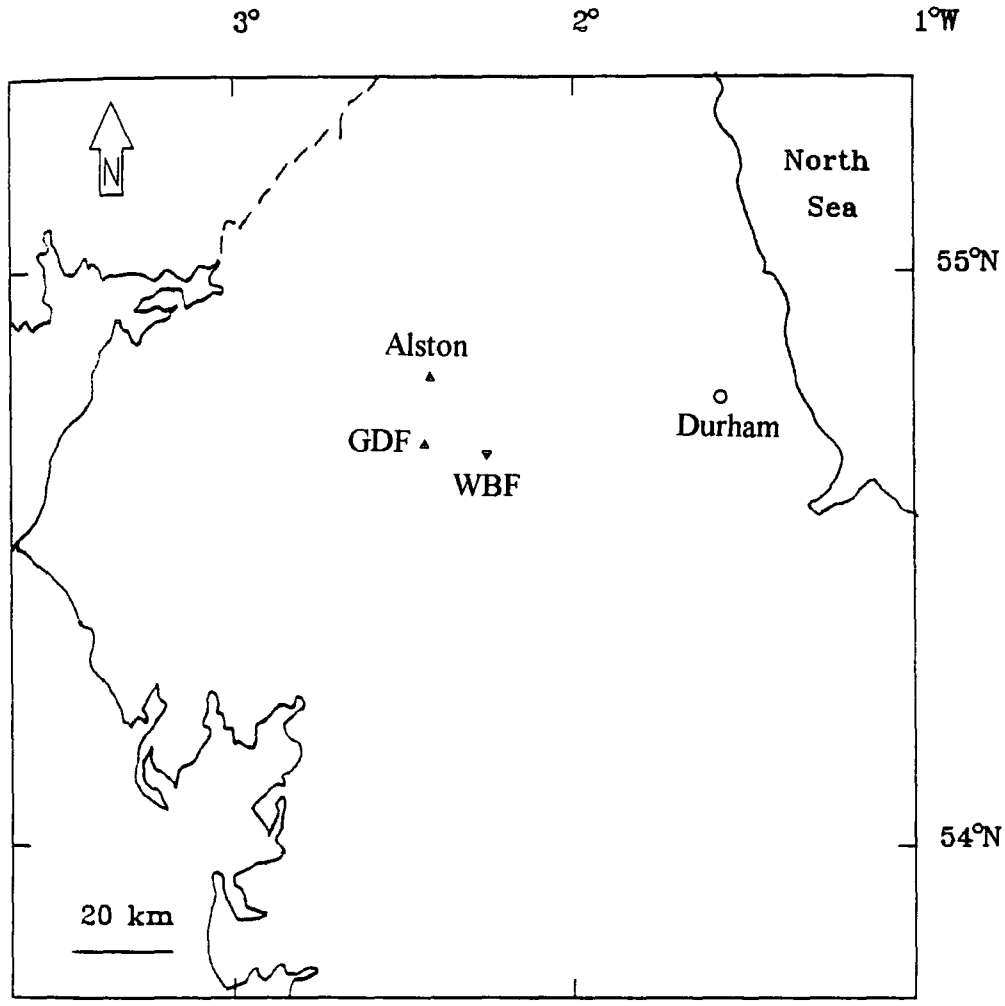


Figure 1.1: Map of the study sites.

Key: WBF = Widdybank Fell; GDF = Great Dun Fell;
Alston = Hartside Nursery, near Alston.

Plate 2.1: The exclosure at Durham University Botanic Gardens. The locations of (a) the altitude experiment DBG site and population difference experiment (table), and (b) the light intensity experiment, are indicated.

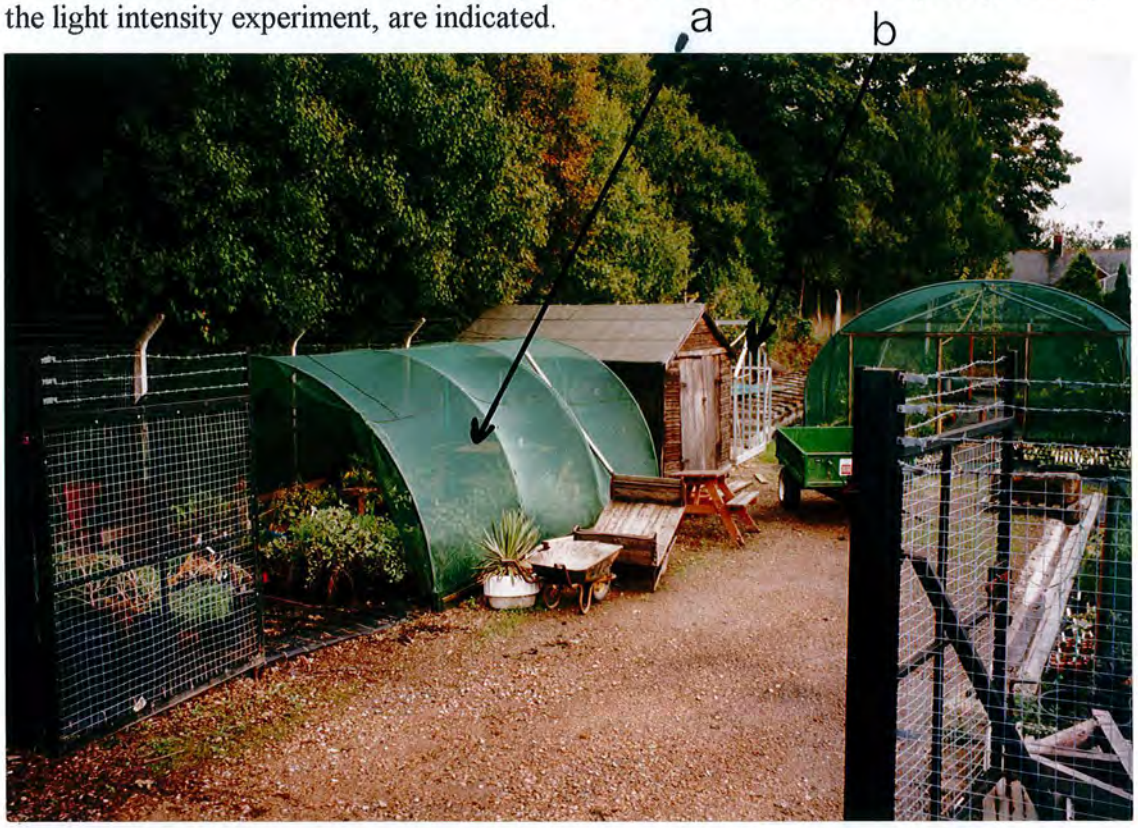


Plate 2.2: The exclosure at Great Dun Fell.

2.3 Plant stock: Poa annua

Poa annua was used to investigate responses to altitude because it fulfilled the following criteria:

1. Capacity to survive and grow naturally at widely-varying altitudes, *i.e.* high altitudinal amplitude.
2. Ease of use.
3. High abundance and wide range of occurrence.

Poa annua is the commonest and most widespread grass of bare and disturbed ground in Britain, being found throughout the country (Fitter, Fitter and Farrer, 1984). It tends to thrive in the field on coarser soils, with relatively low pH and low extractable calcium concentration (Kuo, 1993a; Ervio *et al.*, 1994). It is an annual or short-lived perennial C_3 grass, with a capacity for rapid growth. Stems may attain 30cm or more, but are usually much shorter in the field; occasionally they may root.

Phenotypic plasticity experiments (Chapters 3-5)

To ensure maximum capacity to survive and grow at very different altitudes, the stock was taken from a mid-altitude site (Hartside Nursery, Alston - 330 m a.s.l.; grid ref. NY 707447). To try to minimise genotypic variability, all the *Poa annua* used in these experiments was taken from a small, isolated clump in a gravel car park.

Population difference experiment (Chapter 6)

In order to obtain genotypes from as widely-varying altitudes as possible, it was intended that the plant stock used in this experiment be collected from the different sites used in the altitude experiment. However, though it is known to occur there, attempts to find *Poa annua* at the top of Great Dun Fell were unsuccessful - *Poa trivialis* being found where *Poa annua* might have been expected. Because of this, material was again collected from the same clump in Alston, which became the intermediate source population for the experiment, being approximately half way in altitude between DBGs (100 m) and WBF (513 m). From each site material was again collected from one small clump.

All experiments

Single uniform tillers of *Poa annua* were carefully separated from the stock. In the altitude experiment each transplant consisted of three small, joined tillers with roots. The amount of root stock which could be separated with each tiller was often not uniform. Therefore first harvest values of root dry mass and derived variables (such as root weight ratio, and shoot/root ratio) have little biological meaning on their own.

They are presented on the graphs because they may influence the subsequent balance of growth in the plant.

2.4 Methods

Transplanting the *Poa annua*

In the altitude and population difference experiments (Chapters 3 and 6), tillers from the Alston stock were planted into trays containing an all purpose seed and potting compost (J. Arthur Bowers, Ltd.). Eight plants were spaced uniformly in each tray in the altitude experiment, and six in the population difference investigation. In the other two experiments, tillers were transplanted into pots of horticultural sand, one per pot. This transplanting regime ensure no differences in soil depth within experiments, a factor which was found to be a major factor controlling growth of *Sesleria caerulea* in a previous study of changes with altitude in County Durham (West, 1975).

Treatments

Effects of altitude

Two trays were placed at GDF (Plate 2.3), two in DBG, and one in the intermediate site, WBF. In all cases, the trays were put in the exclosures. A set of plants was also analysed in the laboratory to provide a baseline for the study. No nutrients were applied, as the compost used contained sufficient. At GDF and WBF water was not applied, except during establishment of the tillers. At DBG the plants often received water from the sprinklers in the exclosure. Harvests were every four weeks, with one week allowed at the beginning for establishment:

$$T_0 = 0 \text{ weeks}, \quad T_1 = 5 \text{ weeks}, \quad T_2 = 9 \text{ weeks}, \quad \text{and } T_3 = 13 \text{ weeks}$$

Because of time constraints, only two plants from each of the sites were collected at each harvest. Although the standard error bars are generally small this low replicate number must be borne in mind when drawing conclusions. Only certain variables were measured at the final harvest. Here, a greater number of replicates was sampled: 12 for each of DBG and GDF and three for WBF. In the latter case one plant had been shaded out (Plate 2.5) and was excluded from the analysis. The results for DBG at the final harvest were affected by the fact that the plants were suffering from senescence. This appeared to be caused by old age, a supposition supported by observation of plants in a spare tray left after the final harvest, which continued to die back, no leaves remaining green after 3 further weeks.

Plate 2.3: The *Poa annua* plants initially transplanted to Great Dun Fell. Each consisted of three small, joined tillers with roots.

Plate 2.4: Comparison of the plants harvested from Widdybank Fell and Great Dun Fell after 5 weeks, showing plant and leaf size differences. The plants on the right are *Glyceria plicata*, which were grown for comparison, but are not reported in this study.

Plate 2.5: Shading by surrounding vegetation at Widdybank Fell after 13 weeks (top), and (bottom) the effects of that shading on one of the *Poa annua* plants, (bottom right of the top tray), which was therefore excluded from the analyses. The plants in the bottom tray are again *Glyceria plicata*, which is not reported here.

Significance values were obtained from two-way analysis of variance (ANOVA). Plate 2.4 illustrates growth and leaf size differences between GDF and WBF at the second harvest.

Effects of temperature and nutrients

120 pots were placed in growth rooms (photoperiod 16 hours light, 8 hours dark) at 15°C. 24 of these were randomly allocated to each of five harvests, to be made 3 weeks apart. Within each set, six pots were randomly allocated to each of the following four combinations:

low temperature, high nutrient	high temperature, high nutrient
low temperature, low nutrient	high temperature, low nutrient

Because the light regime in the growth rooms was found not to be entirely uniform, the pots assigned to the different harvests were distributed in a stratified random manner within each treatment to remove any bias caused by the differences in light regime.

After two days' acclimation to the growth room environment the first harvest (T_0) was made. The temperatures of the two growth rooms were then set at two different levels: 21°C ("high") and 15°C ("low"). Within each growth room, half the pots were assigned high nutrient status and the other half low (Plate 2.6). All the pots were administered a nutrient solution twice weekly (Mondays and Thursdays), and watered with deionised water daily on other week days, and once per weekend. The nutrients used for the different treatments approximated to the Long Ashton Nutrient Solution (Hewitt, 1966; Smith, Johnston and Cornforth, 1983), which has been successfully used for a long time for the sand or water culture of a wide range of crop plants. Following Baxter *et al.* (1994b), the only nutrients which were varied between the treatments were nitrate and phosphate (see Table 2.1).

The time interval between harvests was set at 3 weeks. However, from week 4 a rust badly affected many of the plants (Plate 2.7). Therefore all the remaining plants were harvested at T_2 and those intended for T_3 and T_4 used for variability and normality assessment. The plants most affected were those in the 21°C growth room, primarily in the high nutrient treatment; the low temperature high nutrient plants at week 6 appeared very vigorous and healthy (Plate 2.7). The number of replicates for each treatment was five per harvest; the sixth was preserved in formalin acetic alcohol (FAA). Significance values were obtained from three-way ANOVA.

Table 2.1: The nutrient regimes used in the temperature and nutrient factorial experiment. 100 ml of these solutions were applied twice weekly to each pot (Mondays and Thursdays). Pots were watered daily to field capacity on other week days, and once per weekend, using deionised water.

Nutrient	Stock soln. (g/l)	Vol. of stock used in 10 l of nutrient soln. (ml) High nutrient treatment	Low nutrient treatment
$MgSO_4.7H_2O$	184.0	4.0	4.0
Fe EDTA (mono-sodium complex)	37.3	1.0	1.0
$MnSO_4.4H_2O$	22.3	0.2	0.2
K_2SO_4	87.0	8.0	8.0
$CaCl_2.6H_2O$	219.0	8.0	8.0
Micronutrients:			
$CuSO_4.5H_2O$,	2.5		
$ZnSO_4.7H_2O$,	2.9		
H_3BO_3,	31.0	1.0	1.0
NaCl,	58.5		
$Na_2MoO_4.2H_2O$	1.2		
Nitrate variable:			
NH_4NO_3	80.0	25.0	4.0
Phosphate variable:			
$NaH_2PO_4.H_2O$	13.8	50.0	8.0

Figure 2.1: Diagrammatic representation of the shading frame.
The 18 compartments are shown. In each, the light intensity afforded is given: high (95% light transmission), medium (60% light transmission) and low (40% light transmission). These values are based on mean values of readings taken at plant level in each compartment, under relatively low and uniform light conditions (overcast day). The bottom number in each compartment represents the mean red/far red ratio (660/730 nm) found for each treatment.

Medium 60 % 1.08	Low 40 % 1.06	Medium 60 % 1.08	Low 40 % 1.06	Medium 60 % 1.08	Low 40 % 1.06
High 95 % 1.10	Medium 60 % 1.08	High 95 % 1.10	Medium 60 % 1.08	High 95 % 1.10	Medium 60 % 1.08
Low 40 % 1.06	High 95 % 1.10	Low 40 % 1.06	High 95 % 1.10	Low 40 % 1.06	High 95 % 1.10

Plate 2.6: The 21°C growth room after 3 weeks. The high nutrient status plants are those nearest the camera, and the low N+P plants are those furthest away.

Plate 2.7: Plants from the four treatments after 6 weeks. Anticlockwise from top left: low temperature high N+P; low temperature low N+P; high temperature low N+P; high temperature high N+P, which were suffering from infection by a rust. Note the large number of inflorescences in the high temperature high N+P treatment.

Effects of light intensity

The shading frame was set up in the exclosure in the Botanic Gardens (Plate 2.8). It consisted of 18 compartments, six of each of three shading levels, designated "low", "medium" and "high" according to the light intensity afforded (Figure 2.1). The differences in light intensity in terms of both photosynthetically-active radiation (PAR) and near red/far red ratio (660 nm/730 nm wavelength) were measured using lightmeters (Skye models SKP200 (PAR) and SKP100 (660 nm/730 nm)). Little variation in red/far red ratio was found between the compartments (Figure 2.1).

Four pots were placed in every compartment, on a table to protect from rabbit grazing, the frame being suspended above. Dark dustbin liners were attached to the sides so that light only entered through the frame (Plate 2.8). The nutrient and watering regimes were the same as those used for the high nutrient treatments in the temperature and nutrient experiment.

Each of the four pots per compartment was designated to a different harvest: T_0-T_3. The relevant pot from each compartment was removed at each of the first three harvests. Again at T_2 (8 weeks) all the remaining plants were taken, those originally destined for T_3 being used for variability and normality assessment. Replicate number was five, the sixth being preserved. Only certain variables were measured on the plants from the medium light intensity treatment. Significance values were obtained from two-way ANOVA.

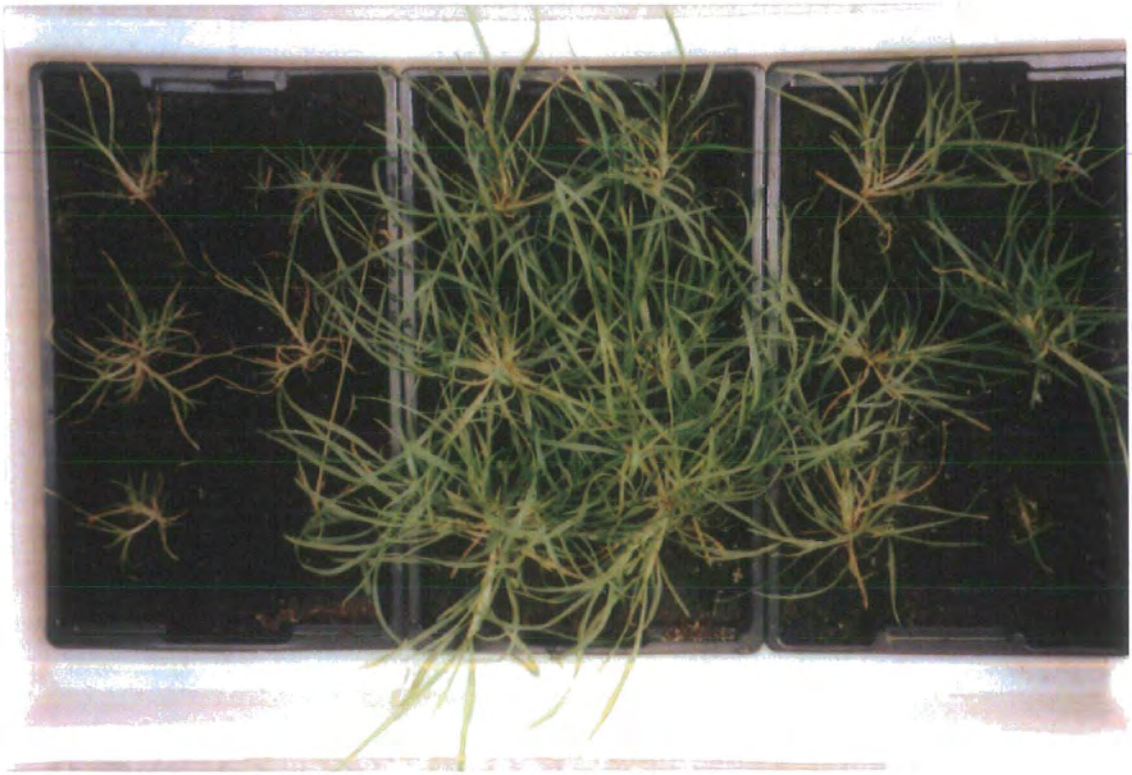
Population difference experiment

To ensure uniform mesic conditions, the trays were placed next to each other, on the same table as the DBG plants of the altitude experiment, and watered regularly.

Unfortunately, due to lack of time (while other experiments were already running), it was not possible to run this experiment along exactly the same lines as the others. Because of this, similar analysis of the growth and development of the plants was not possible, and only the first and last harvests are reported here. Replicate number was again five per treatment, the sixth being preserved in FAA. Significance values were obtained from one-way ANOVA with Tukey's multiple comparison of means, and from two-sample t-tests. Plate 2.9 illustrates growth and leaf size differences between the plants at the final harvest.

Plate 2.8: The shading frame used, with three pots in each compartment. Photograph taken between the first and the second harvest.

Plate 2.9: The plants in the population difference experiment, at the final harvest. The tray on the left contains the Durham plants; the Alston plants are in the middle; and the WBF plants on the right. Size and leaf length differences can clearly be seen.



All experiments

The first harvest (T_0) does not relate to the site or treatment concerned, but to the conditions in the gravel car park at Alston from which the stock was collected (Chapters 3-5), or to the conditions experienced by the source populations (Chapter 6). Therefore any consideration of morphological changes with development should disregard the first point. In most cases this only leaves two time points, so discussion of trends with plant development is limited.

Variables measured

The importance of studying combinations of plant variables, in investigations of plant responses to different environmental conditions, has recently been stressed (*e.g.* Lambers *et al.*, 1989; Konings, 1989; van Hinsberg, 1994). In many cases, there appear to be genetic correlations between traits (van Hinsberg, 1994), and it seems that plants cannot always vary one feature while keeping others constant (Robinson and Rorison, 1988). A large number of variables was therefore measured at each destructive harvest:

* Dry masses: whole plant, roots, shoots, "stems" and leaves.
* Dry mass partitioning: root weight ratio (RWR), shoot/root ratio (S:R), leaf weight ratio (LWR) and leaf/shoot ratio (L:S).
* Numbers of tillers and inflorescences (mature and emerging).
* Numbers of mature and senescent leaves per plant and per tiller.
* Leaf length (every leaf), total leaf area and length of longest tiller.
* Specific leaf area (SLA) and leaf area ratio (LAR).
* Degree of leaf folding and the whole plant wet/dry mass ratio.
* Number of stomata per unit area and per leaf on each surface of the lamina.

Definitions of the terms used

The terms used are explained in the glossary (Appendix 1). Most are standard, but some have been specifically defined for this study:

Emerging inflorescence - an inflorescence which is emerging from the leaf sheath.

Leaf folding - The degree to which the leaf is folded in cross-section. The leaf folding index was defined as:

1 - (width of unflattened leaf / width of flattened leaf)

Thus a leaf with no folding has an index of zero, and a completely folded leaf an index of one.

Mature inflorescence - Any inflorescence which has emerged from the leaf sheath, including senescent inflorescences.

Mature leaf - Any leaf which has developed a full ligule, which was taken to indicate full expansion.

Mature tiller - Any tiller with at least one mature or senescent leaf and/or at least one mature inflorescence.

Shoot - any above-ground part of the plant, *i.e.* leaves and structural material. Dead material was also included.

"Stem" - Any above-ground part of the plant which is not live leaf tissue. Thus dead leaves were included as "stem".

Analyses carried out

The laboratory analyses were designed to obtain the variables listed above as accurately and efficiently as possible given the constraints of time and facilities. A standard procedure was adopted for the analysis of all the plants from the different experiments:

1. As soon as possible after removing the plants from the respective sites, a random selection of mature leaves from each treatment was measured for degree of cross-sectional leaf folding. Callipers were used to measure the width of each selected leaf (with any folding) two thirds of the way from the ligule to the tip. The leaf was then flattened and the width re-measured at the same location. The folding index was calculated as defined above.
2. In the experiments involving trays, the plants were carefully separated from each other if necessary. Where pots were used, separation of the roots from the muslin lining the bottom was required, which presented problems as the roots were prone to breaking and the muslin to disintegrating.
3. All soil or sand was then washed from the roots, and the leaves and stems rinsed. Again great care was required because the finer roots tended to break easily in the process.
4. Each plant was laid out and the length of the longest tiller (not including inflorescences) measured to the nearest centimetre using a ruler. Each tiller present was recorded, and the length of each leaf on it measured to the nearest millimetre. These lengths were recorded in order of insertion for each tiller. Where inflorescences

were present, the location of each on the tiller was recorded. The status (mature, immature or senescent) of each leaf and inflorescence was also noted.

5. The plants were patted dry with tissue, and the fresh mass of each measured.

6. The roots were cut from the plant and their mass determined separately. The rest of the plant was then re-weighed (because loss of mass occurred continuously once the roots were removed from water). The live leaves were carefully separated from the stems and senescent leaves: each was cut off at the ligule. Total fresh leaf mass and total fresh mass of "stems" were determined. The material was then dried at 70°C for a minimum of 48 hours, transferred to a desiccator containing silica gel, and re-weighed at room temperature after a further 24 hours.

7. A number of leaves was selected for epidermal peels. These were determined in the leaf measuring stage, according to the plastochron index (Erickson and Michelini, 1957) of the leaves on the primary tiller, so as to minimise the variation due to the developmental stage of the leaf. To keep track of the index, at each harvest the most recent fully-expanded leaf on the primary tiller was marked. However, leaf turnover was rapid and the marked leaf was often either senescent or no longer locatable. The selected leaves were laid flat on a glass sheet and clear nail varnish carefully applied in a uniform layer to the abaxial surface. The varnish was allowed to dry and then peeled from each leaf using tweezers, and placed flat on a microscope slide. The same procedure was then used to obtain an epidermal peel of the adaxial surfaces of the same leaves. Later, the peels were placed under a microscope and magnified 400 times (Appendix 2). Because stomatal frequency can vary with position on the leaf (Meidner and Mansfield, 1968), counts of stomata present in each of 15 fields of view were performed at one quarter, one half and three quarters of the way from the ligule to the tip on each surface of each leaf. The mean of the resulting 45 counts was calculated, and was divided by the area of a field of view to estimate the stomatal frequency for each surface. The length of each leaf was recorded. From the leaves used to determine specific leaf area (see point 9, this section), approximate relationships between the length and area of mature leaves were noted for the different treatments in each experiment. These were used to derive estimates of the areas of each of the peeled leaves. Stomatal frequencies were multiplied by estimated areas to give total stomatal numbers per leaf surface. The mean and standard error of both stomatal frequencies and stomatal numbers per leaf surface were determined for eight replicates of each treatment in the altitude experiment, and for three replicates in the other experiments.

8. All the live leaves were laid flat on a glass plate, and another glass plate was placed on top. They were then photocopied, and later the images of the leaves were cut out by hand and the mass of these pieces of paper determined using an accurate balance.

This mass was divided by a constant to give total leaf area, which therefore represents half the total leaf surface. The constant was determined by weighing several known areas (each 100mm^2) of the photocopied paper and finding the mean.

9. Some of the youngest mature leaves were selected to determine specific leaf area, again according to the plastochron index. These were marked on the photocopies, and the leaves themselves put in separate envelopes. The envelopes were then dried and the masses of their contents later determined as above. In the altitude experiment, eight leaves were used for each plant, and in the other investigations three per plant.

Given the time-consuming nature of the measurements, inaccuracies could potentially have arisen if plants had continued to grow after harvesting. The problem was minimised by storing the plants in a refrigerator (for up to 72 hours) until the measurements could be performed

In all cases, some leaves were preserved in formalin acetic alcohol (FAA). This was to allow investigation of internal leaf morphology and physiology, using microtome techniques. However, there was insufficient time to pursue this line.

At the last harvest in each experiment, all remaining plants were removed to the laboratory. The normal number of replicates was analysed in full. The less time-consuming analyses were carried out on the remaining plants in order to assess variability and to help determine (by increasing replicate numbers) which data transformations (if any) were required.

2.5 Statistical Analysis

Data transformation

Finney (1989) states:

> "A sound general principle is that data are usually most clearly interpreted on the original scale of measurement. Therefore transformations should be avoided unless clearly necessary."

Sparks (unpublished) states:

> "Presentation of results where some data are transformed and others not is confusing. It is perhaps preferable to sacrifice a little apparent optimality in order to achieve consistency."

All the data were checked using histograms for clear departures from normality. Bearing in mind the two statements above, and the fact that ANOVA and t-tests are

relatively robust to departures from normality in data (Evans, 1972; Mead and Curnow, 1983; Bailey, 1995; Sokal and Rohlf, 1995), the following data transformations were used consistently throughout all the experiments in testing for significance (but not in data presentation):

* The angular transformation (*i.e. arcsin (√(variable))*) for all percentage data, *i.e.* bounded absolutely by 0 and 1 (Mead and Curnow, 1983). This refers to RWR, LWR, and the leaf folding index.

* The square root transformation (*i.e. √(variable + 0.375)*) for counts, as recommended by Anscombe (1948) and Kihlberg, Herson and Schutz (1972). This refers to numbers of tillers, inflorescences and leaves.

* The logarithmic transformation (*i.e. ln (variable)*) when variances and means were clearly related and tests assuming equal variances were to be used (Zar, 1984). The data concerned were all dry mass measures.

Critical level of significance

The 5% level ($p \leq 0.05$) was used throughout this study, and is the critical value referred to unless otherwise stated.

2.6 Weather data

The experiments were conducted over a very warm and dry summer in 1995. Weather conditions may significantly affect the results of the outdoor experiments (chapters 3, 5 and 6). The weather variables recorded were:

<u>All sites</u>

Maximum air temperature (10 a.m.-10 a.m.).
Minimum air temperature (10 a.m.-10 a.m.).
Daily rainfall (10 a.m.-10 a.m.).
Wind speed at 10 a.m..
Relative humidity at 10 a.m. (via wet bulb and dry bulb air temperatures).

<u>Durham and Widdybank Fell</u>

Number of sunshine hours (10 a.m.-10 a.m.).
Minimum grass temperature (10 a.m.-10 a.m.).

These data are presented as graphs in Appendix 3. They were tested for significant differences between sites using one-way ANOVA and Tukey's multiple comparison of means, the results of which are shown in Table 2.2.

Table 2.2: Summary of statistical significance of the effects of altitude on weather variables in the three sites during the study period.

A one-way ANOVA was performed, with Tukey's multiple comparison of means to establish significant differences between the sites. Significance notation for the ANOVA: n.s., not significant (P>0.05); * P<0.05; ** P<0.01; *** P<0.001. Sig. is significance; DF is degrees of freedom. DO is Durham Observatory (assumed equiavlent to DBG weather data). Significance notation for the multiple comparison of means: the letters indicate significance at the 5% level - if two sites have no common letters they are significantly different. No sunshine hours or minimum grass temperature data were available for GDF. The data are presented in graphical form in Appendix 3.

			Tukey's comparison of means		
Weather variable	**ANOVA**		**Site**	**Mean**	**Sig.**
Daily rainfall	**Sig.**	***	DO	0.748	a
(error DF: 243)	F	11.24	WBF	2.000	a
	DF	2	GDF	3.847	b
Sunshine hours	**Sig.**	**n.s.**	DO	5.804	a
(error DF: 179)	F	0.19	WBF	6.053	a
	DF	1			
Wind speed at 10 a.m.	**Sig.**	***	DO	2.933	a
(error DF: 243)	F	161.85	WBF	12.678	b
	DF	2	GDF	18.092	c
Maximum air temperature	**Sig.**	***	DO	17.052	a
(error DF: 243)	F	31.35	WBF	14.280	b
	DF	2	GDF	11.077	c
Minimum air temperature	**Sig.**	***	DO	7.963	a
(error DF: 243)	F	14.33	WBF	5.999	b
	DF	2	GDF	4.451	b
Minimum grass temperature	**Sig.**	*	DO	4.964	a
(error DF: 179)	F	6.17	WBF	3.364	b
	DF	1			
Relative humidity	**Sig.**	***	DO	74.33	a
(error DF: 243)	F	13.59	WBF	82.13	b
	DF	2	GDF	82.23	b

Chapter 3. The effects of altitude on the growth, morphology and physiology of *Poa annua* from the same population.

3.1 Null Hypothesis

Altitude had no significant effect on the growth form, physiology and dry matter accumulation of plants from the same population grown at different elevations.

3.2 Results

Growth

In terms of absolute changes in dry matter, most of the variables measured showed exponential increases with time (Figure 3.1), and in each case (apart from DBG week 13) smaller increases were found with altitude ($P < 0.001$, Table 3.1). The exception was root dry mass, which showed no significant difference with altitude, and suggested sigmoidal growth, possibly indicating a degree of pot-binding.

Dry matter partitioning

Measures of dry matter partitioning between shoots and roots showed greater relative allocation to roots with altitude ($P < 0.001$; Figure 3.2). Differences between the sites in partitioning between leaves and stems were not significant, while relative stem allocation increased with time.

Tillers, inflorescences and leaves

No significant differences in numbers of mature inflorescences (per plant or per tiller) were found between sites. Numbers of emerging inflorescences per plant tended to decrease with altitude, but this was strongly dependent on time ($P < 0.001$ for the interaction; Figure 3.3e; Table 3.2.).

Leaf senescence showed little change with altitude up to week 9 (Figure 3.4c-e), but from visual observation appeared to increase markedly thereafter at DBG, but not at the other two sites.

Leaves at lower altitudes were significantly larger but had lower SLA, *i.e.* were thinner ($P < 0.001$; Figures 3.5a, 3.6a). LAR therefore increased with altitude ($P < 0.001$), while LWR varied less between the sites (Figures 3.6b, 3.2c). Longest tiller length decreased with altitude ($P < 0.001$), which was probably a function of leaf length increases (Figure 3.5a, b).

There were also changes with development. LWR showed a small but significant decrease with time at the lower altitudes, while highest values of SLA were recorded after five weeks, decreasing thereafter (Figures 3.2c, 3.6a). Thus the product of the two, LAR, also decreased from five weeks (Figure 3.6b). No change with time in LWR, SLA or LAR was apparent at the high altitude site. These trends probably did not reflect changes in water availability because the DBG plants were in mesic compost, maintained by the sprinklers, but still showed a decline in SLA and LAR.

Folding, wet/dry mass ratios and stomata

Apart from the final harvest at DBG, the leaf folding index showed increased folding with altitude ($P < 0.001$; Figure 3.6c). Whole plant wet/dry mass ratios decreased with time and increased with altitude ($P < 0.001$). However, this was complicated by a significant ($P < 0.001$) site-time interaction (Figure 3.6d), which may reflect reduced water content in senescent shoots and reproductive structures.

Stomatal frequency showed no significant changes with time, but very significant increase with altitude ($P < 0.001$; Fig 3.7a, c). Large increases in total numbers of stomata per leaf with time were found ($P < 0.001$; Figure 3.7b, d), probably being a function of leaf length (Figure 3.5a). Trends in total stomatal numbers with altitude were less clear, a small decrease with altitude being found on the adaxial surface ($P < 0.01$).

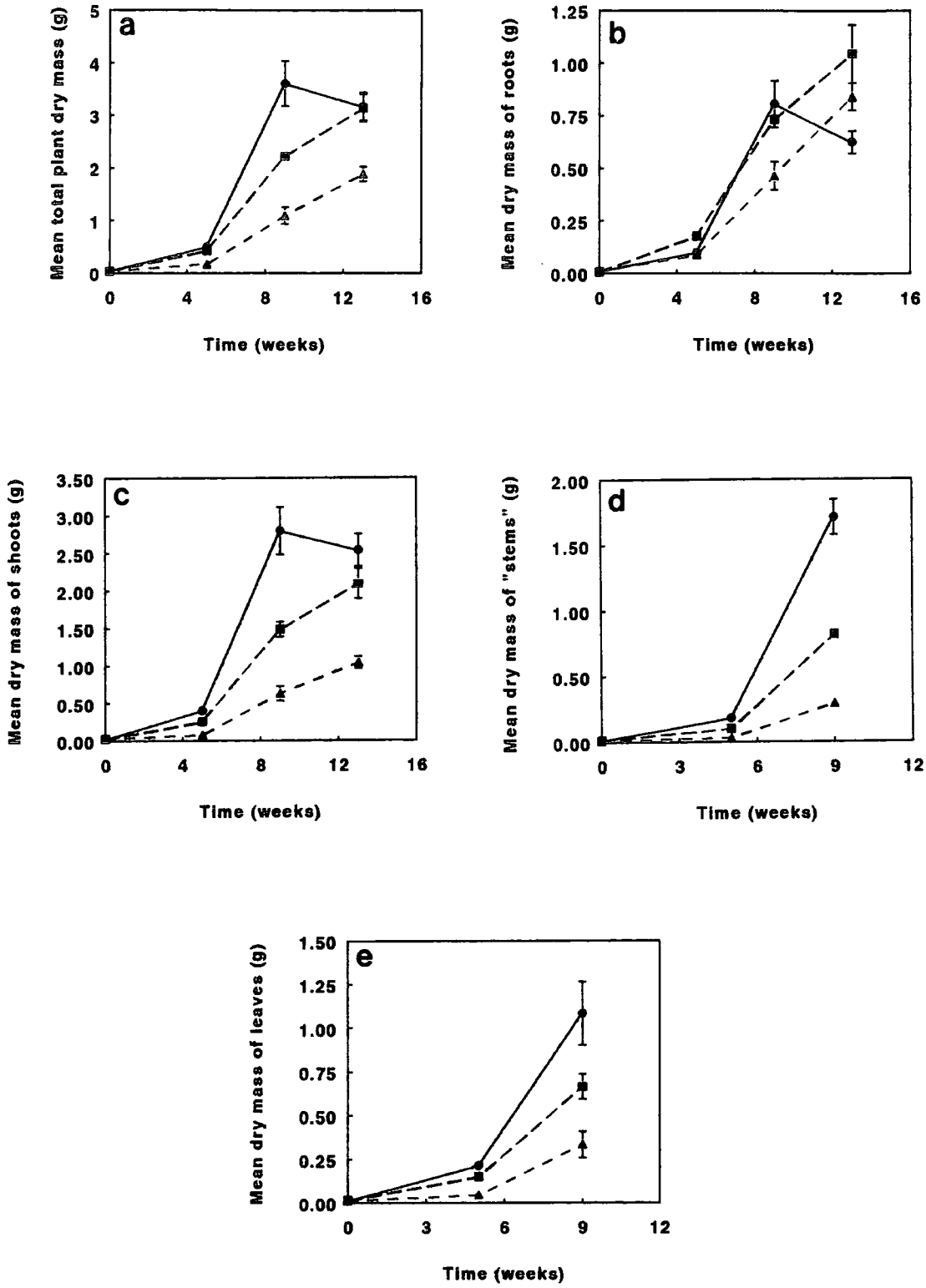


Figure 3.1. Dry mass measures of *Poa annua* grown at different altitudes.

Dry masses of (a) whole plants, (b) roots, (c) shoots, (d) "stems", and (e) leaves of *Poa annua* grown at Durham Botanic Gardens - 100 m (—●—), Widdybank Fell - 513 m (–■–·) and Great Dun Fell - 848 m (– ▲– ·). Data represent the mean ± one standard error of two replicates per site, except for 13 week harvests, which represent 12 replicates for DBG and GDF and three for WBF.

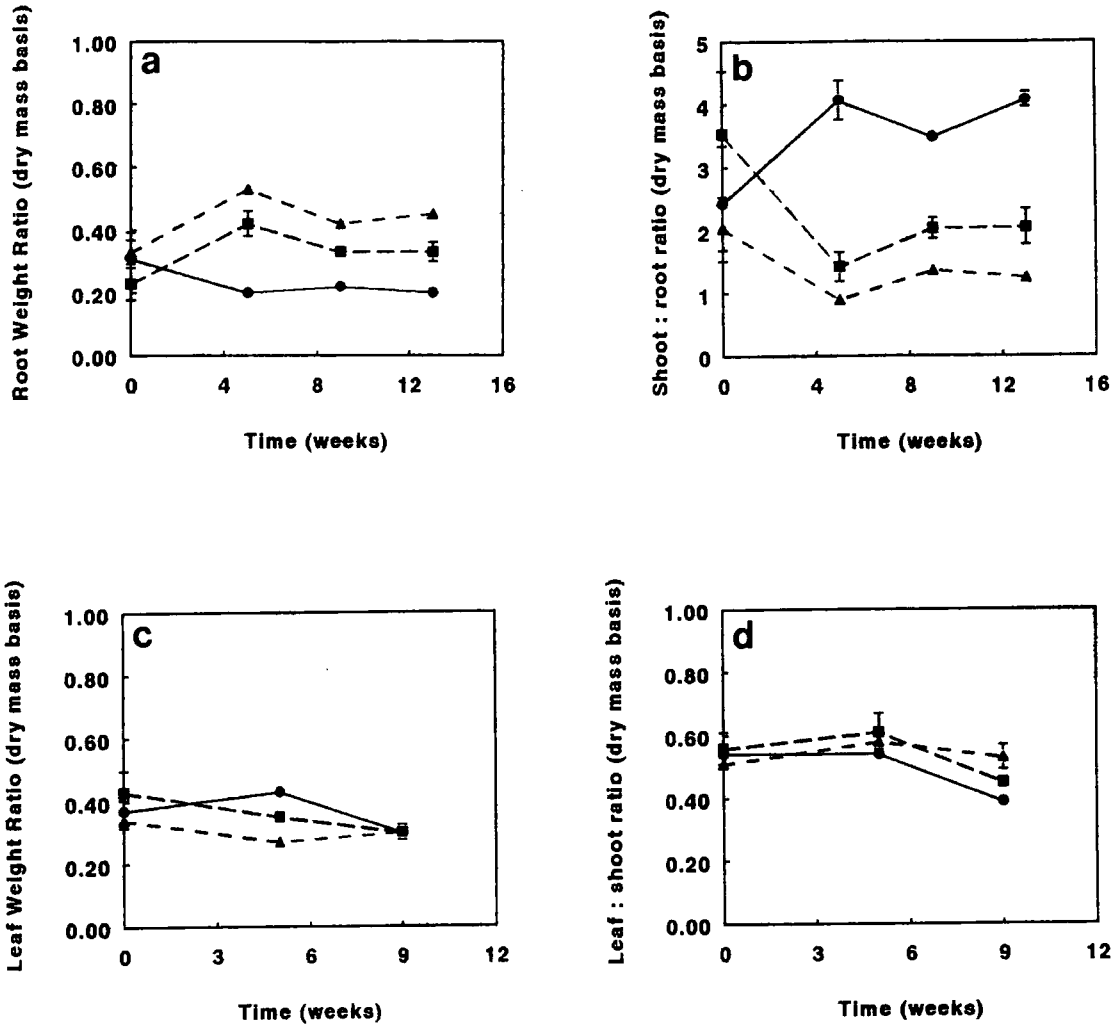


Figure 3.2. Dry matter partitioning in *Poa annua* grown at different altitudes.

(a) RWR, (b) S:R, (c) LWR, and (d) L:S in *Poa annua* grown at Durham Botanic Gardens - 100 m (—■—), Widdybank Fell - 513 m (- -▲- -) and Great Dun Fell - 848 m (—●—). Data represent the mean ± one standard error of two replicates per site, except for 13 week harvests, which represent 12 replicates for DBG and GDF and three for WBF.

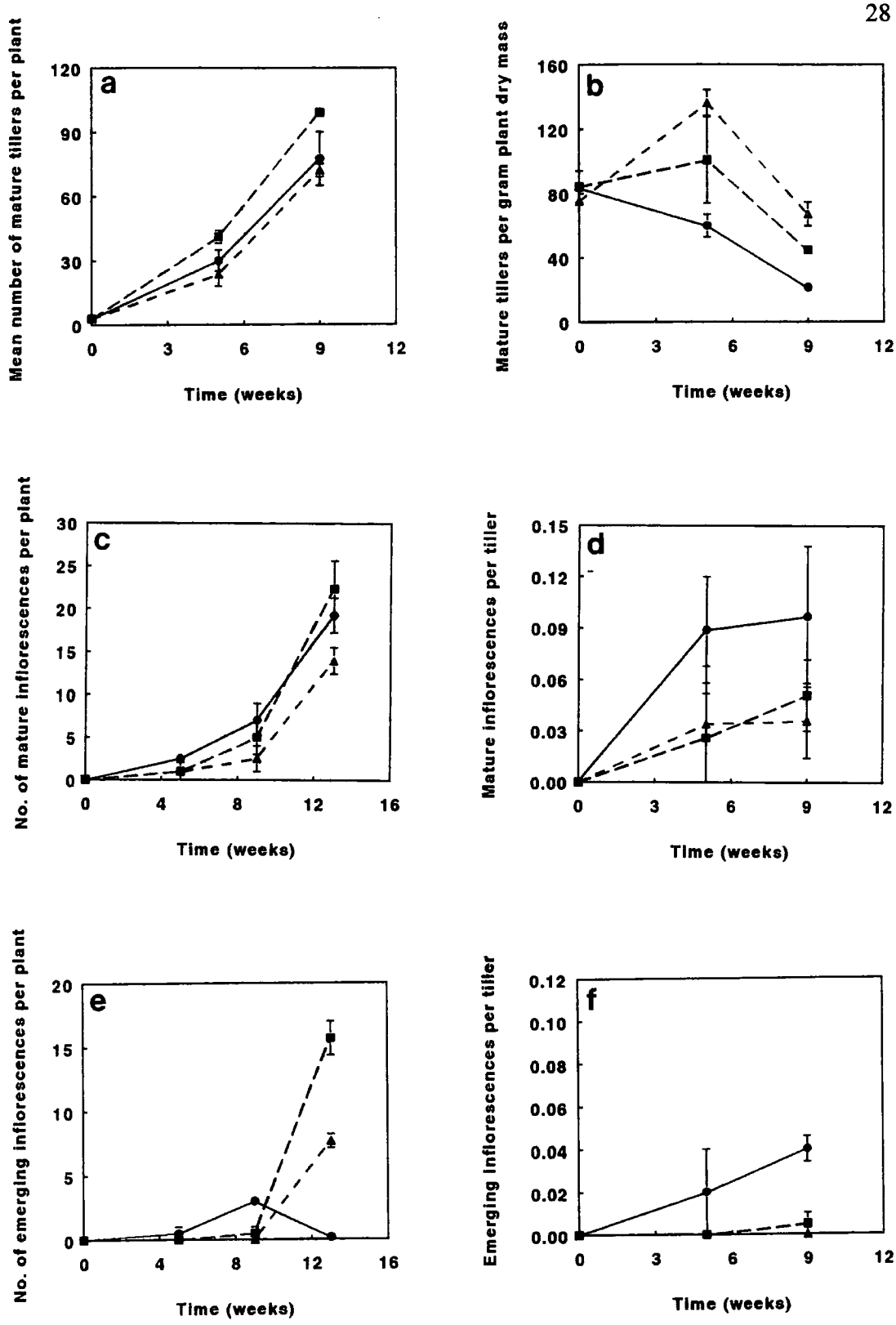


Figure 3.3. Numbers of tillers and inflorescences on *Poa annua* grown at different altitudes.

Numbers of (a) mature tillers, (b) tillers per gram dry mass, (c) mature inflorescences, (d) mature inflorescences per tiller, (e) emerging inflorescences, and (f) emerging inflorescences per tiller on *Poa annua* grown at Durham Botanic Gardens - 100 m (—●—), Widdybank Fell - 513 m (—■—) and Great Dun Fell - 848 m (—▲—). Data represent the mean ± one standard error of two replicates per site, except for 13 week harvests, which represent 12 replicates for DBG and GDF and three for WBF.

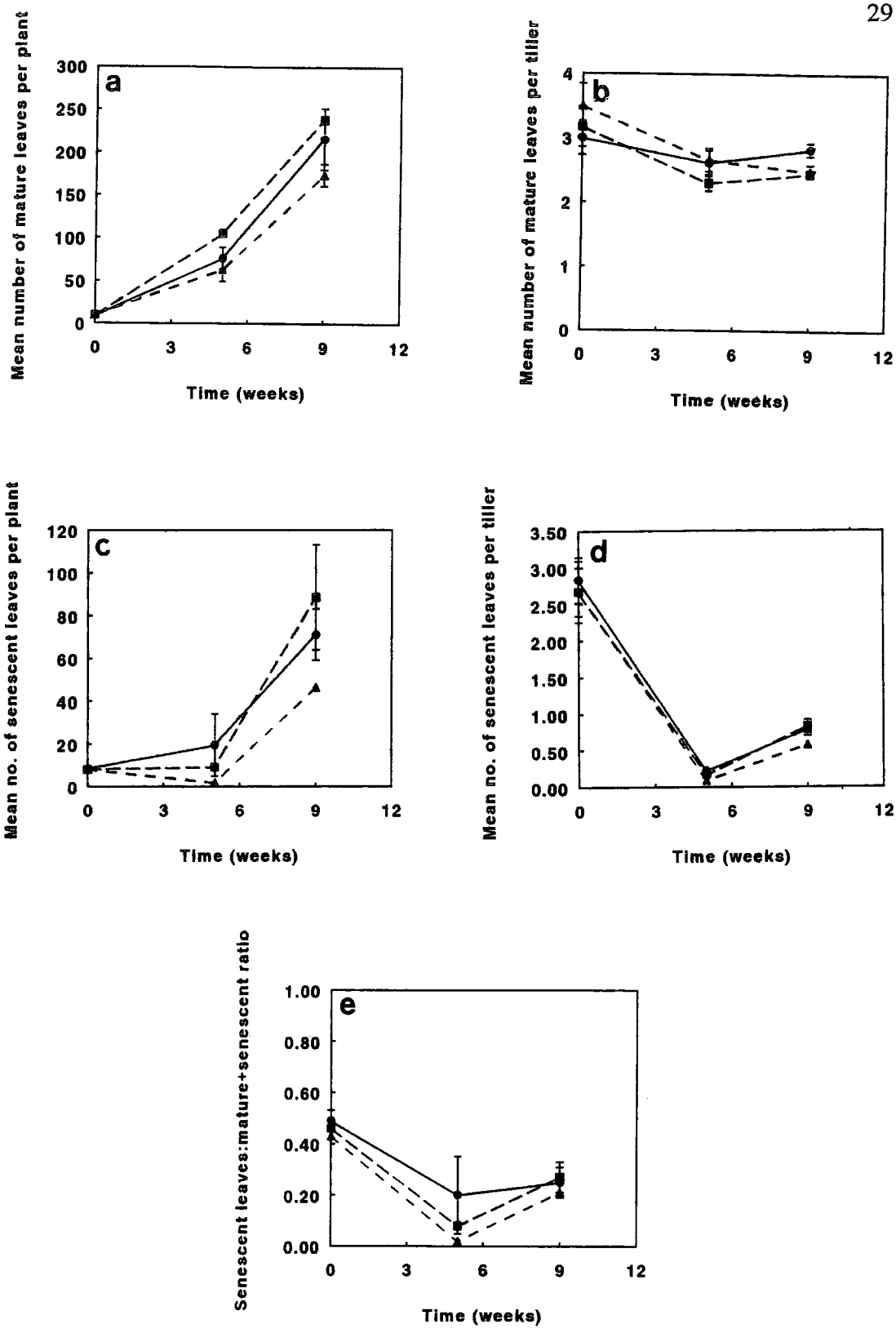


Figure 3.4. Numbers of leaves on *Poa annua* grown at different altitudes.

Numbers of (a) mature leaves, (b) mature leaves per tiller, (c) senescent leaves, (d) senescent leaves per tiller, and (e) the ratio of senescent leaves to all mature and senescent leaves on *Poa annua* grown at Durham Botanic Gardens - 100 m (—●—), Widdybank Fell - 513 m (—■—) and Great Dun Fell - 848 m (—▲—). Data in (a), (c) and (e) represent the mean ± one standard error of two replicates per site. Data in (b) and (d) represent the mean ± one standard error of replicate numbers equal to the number of tillers (see Figure 3.3a).

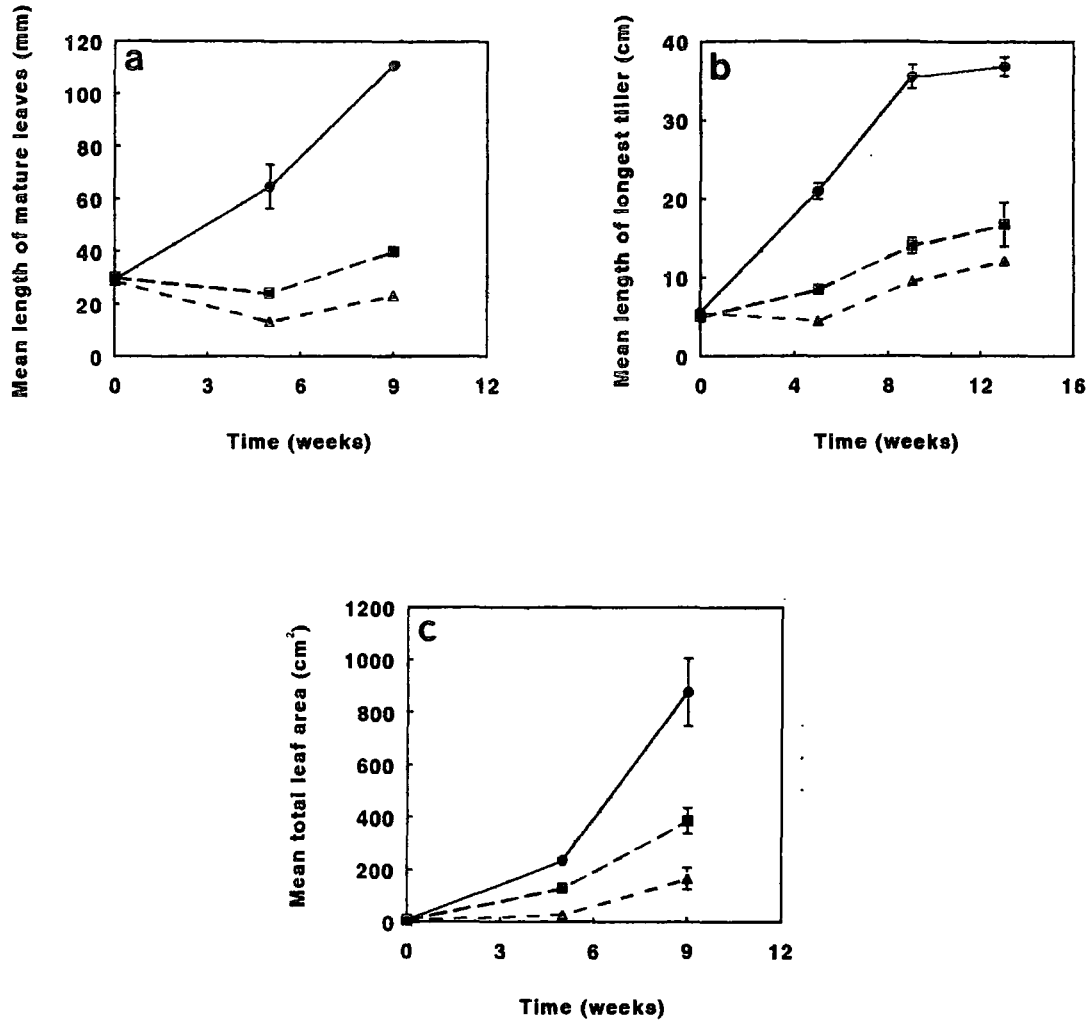


Figure 3.5. Leaf and longest tiller lengths and total leaf area of *Poa annua* grown at different altitudes.

(a) mean length of mature leaves, (b) length of longest tiller, and (c) total leaf area of *Poa annua* grown at Durham Botanic Gardens - 100 m (—●—), Widdybank Fell - 513 m (—■—) and Great Dun Fell - 848 m (- ▲ -). Data represent the mean ± one standard error of two replicates per site, except for 13 week harvests, which represent 12 replicates for DBG and GDF and three for WBF.

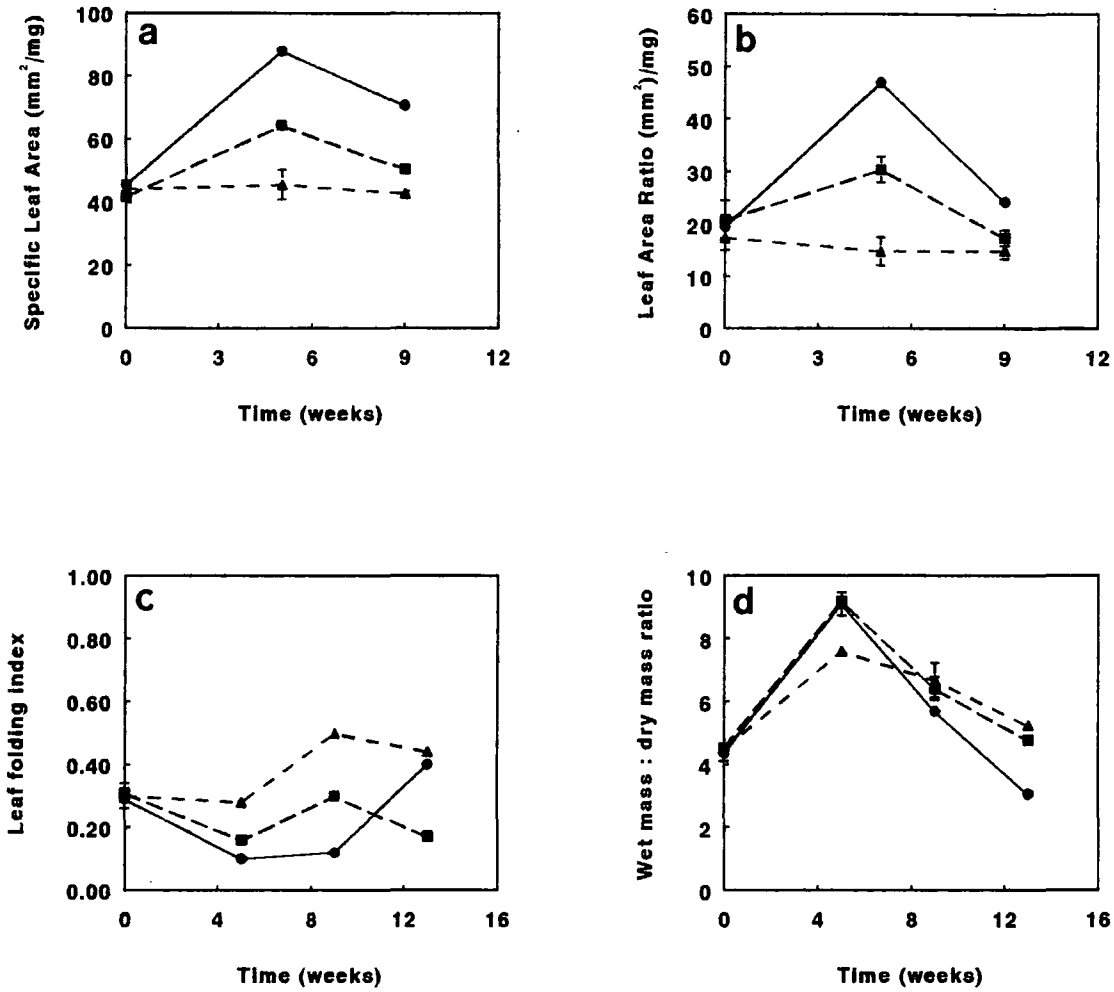


Figure 3.6. SLA, LAR, leaf folding and wet to dry mass ratio of *Poa annua* grown at different altitudes.

(a) SLA, (b) LAR, (c) leaf folding index, and (d) wet to dry mass ratio of *Poa annua* grown at Durham Botanic Gardens - 100 m (—●—), Widdybank Fell - 513 m (–■–·) and Great Dun Fell - 848 m (–▲–·). Data represent the mean ± one standard error of two replicates per site, except for 13 week harvests, which represent 12 replicates for DBG and GDF and three for WBF.

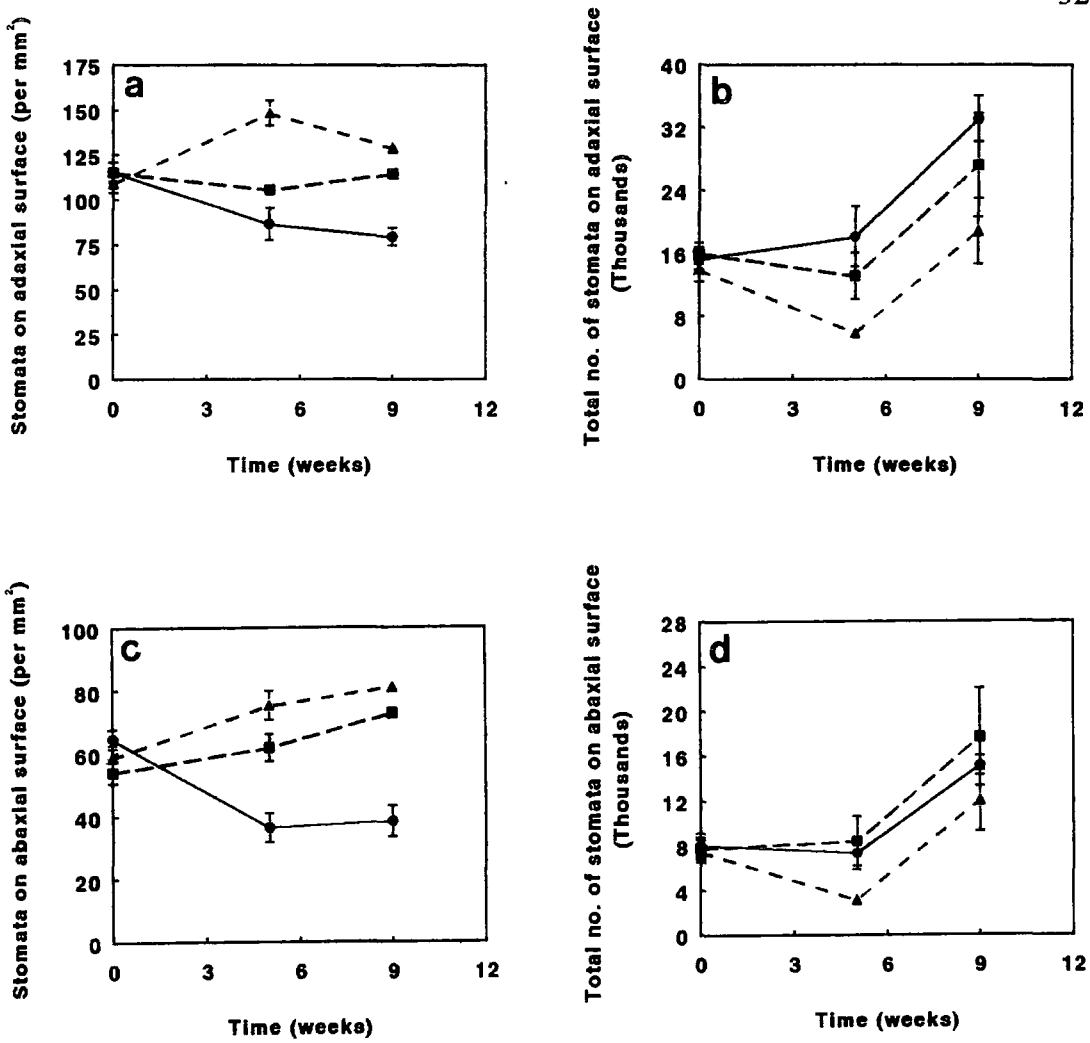


Figure 3.7. Stomatal measures of *Poa annua* grown at different altitudes.

(a) adaxial stomatal density, (b) estimated number of stomata on the adaxial surface, (c) abaxial stomatal density, and (d) estimated number of stomata on the abaxial surface of *Poa annua* grown at Durham Botanic Gardens - 100 m (—●—), Widdybank Fell - 513 m (—■—) and Great Dun Fell - 848 m (– ▲–). Data represent the mean ± one standard error of eight replicates (leaves) per site.

Table 3.1: Summary of statistical significance of the effects of altitude on growth and dry matter partitioning parameters of *Poa annua*.

Dry mass data $\log_e$ transformed; RWR and LWR were angular transformed before analysis. Where necessary, replicate numbers were standardised using general linear modelling. A two-way balanced ANOVA was performed, removing time, altitude (site) and time x altitude interaction effects. All harvests (times) were included in tests marked #, while the first harvest was excluded in the rest. Significance notation: n.s., not significant (P>0.05); * P<0.05; ** P<0.01; *** P<0.001. Sig. is significance; DF is degrees of freedom.

		Source of variation		
Parameter	**Value**	**Time**	**Site**	**Time x site**
# Whole plant dry mass	**Sig.**	***	***	**
(error DF: 12)	F	1010.74	42.82	8.71
	DF	3	2	6
# Dry mass of roots	**Sig.**	***	**n.s.**	*
(error DF: 12)	F	467.15	2.13	3.80
	DF	3	2	6
# Dry mass of shoots	**Sig.**	***	***	***
(error DF: 12)	F	733.07	64.10	9.81
	DF	3	2	6
# Dry mass of stems	**Sig.**	***	***	**
(error DF: 9)	F	429.89	31.10	10.02
	DF	2	2	4
# Dry mass of leaves	**Sig.**	***	***	**
(error DF: 9)	F	485.28	29.66	7.96
	DF	2	2	4
Root weight ratio	**Sig.**	**	***	*
(error DF: 9)	F	8.90	180.40	4.27
	DF	2	2	4
Shoot to root ratio	**Sig.**	**n.s.**	***	**n.s.**
(error DF: 9)	F	2.60	189.56	3.52
	DF	2	2	4
Leaf weight ratio	**Sig.**	**	*	**
(error DF: 6)	F	14.30	10.51	11.52
	DF	1	1	2
Leaf to shoot ratio	**Sig.**	**	**n.s.**	**n.s.**
(error DF: 6)	F	20.12	3.77	1.69
	DF	1	2	2

Table 3.2: Summary of statistical significance of the effects of altitude on numbers of leaves, tillers and inflorescences and derived variables.

Count data were square-root transformed before analysis. Where necessary, replicate numbers were standardised using general linear modelling. A two-way balanced ANOVA was performed, removing time, altitude (site) and time x altitude interaction effects. All harvests (times) were included in tests marked #, while the first harvest was excluded in the rest. Significance notation: n.s., not significant (P>0.05); * P<0.05; ** P<0.01; *** P<0.001. Sig. is significance; DF is degrees of freedom.

		Source of variation		
Parameter	**Value**	**Time**	**Site**	**Time x site**
# Mean number of mature tillers per plant (error DF: 9)	Sig.	***	*	n.s.
	F	316.83	6.48	1.68
	DF	2	2	4
Mean number of tillers per gram plant dry mass (error DF: 6)	Sig.	**	**	n.s.
	F	29.80	12.45	0.79
	DF	1	2	2
# Mean number of mature inflorescences per plant (error DF: 12)	Sig.	***	n.s.	n.s.
	F	77.54	3.52	1.00
	DF	3	2	6
Mean number of mature inflorescences per tiller (error DF: 6)	Sig.	n.s.	n.s.	n.s.
	F	0.60	1.75	0.15
	DF	1	2	2
Mean number of emerging inflorescences per plant (error DF: 9)	Sig.	***	**	***
	F	123.32	16.62	63.52
	DF	2	2	4
Mean number of emerging inflorescences per tiller (error DF: 6)	Sig.	n.s.	*	n.s.
	F	1.73	6.03	0.57
	DF	1	2	2
# Mean number of mature leaves per plant (error DF: 9)	Sig.	***	*	n.s.
	F	268.97	4.68	1.55
	DF	2	2	4
Mean number of mature leaves per tiller (error DF: 6)	Sig.	n.s.	n.s.	n.s.
	F	0.23	3.66	1.27
	DF	1	2	2
Mean number of senescent leaves per plant (error DF: 6)	Sig.	**	n.s.	n.s.
	F	43.95	3.09	0.51
	DF	1	2	2
Mean number of senescent leaves per tiller (error DF: 6)	Sig.	**	n.s.	n.s.
	F	16.21	0.79	0.08
	DF	1	2	2
Ratio of numbers of senescent leaves to all mature and senescent leaves (error DF: 6)	Sig.	*	n.s.	n.s.
	F	8.12	1.65	0.92
	DF	1	2	2

Table 3.3: Summary of statistical significance of the effects of altitude on leaf and tiller lengths, leaf area, SLA, LAR, leaf folding, wet to dry mass ratios and stomatal parameters.

The leaf folding index and the reciprocal of the wet to dry mass ratio were angular transformed before analysis. Where necessary, replicate numbers were standardised using general linear modelling. A two-way balanced ANOVA was performed, removing time, altitude (site) and time x altitude interaction effects. All harvests (times) were included in tests marked #, while the first harvest was excluded in the rest. Significance notation: n.s., not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Sig. is significance; DF is degrees of freedom.

		Source of variation		
Parameter	**Value**	**Time**	**Site**	**Time x site**
Mean length of mature leaves	Sig.	***	***	**
(error DF: 6)	F	63.58	201.55	14.04
	DF	1	2	2
# Mean length of longest tiller	Sig.	***	***	***
(error DF: 12)	F	245.32	404.44	51.34
	DF	3	2	6
# Mean total leaf area	Sig.	***	***	**
(error DF: 9)	F	74.51	30.56	14.95
	DF	2	2	4
Specific leaf area	Sig.	**	***	*
(error DF: 6)	F	40.67	129.89	5.84
	DF	1	2	2
Leaf area ratio	Sig.	***	***	**
(error DF: 6)	F	65.81	68.06	20.46
	DF	1	2	2
Leaf folding index	Sig.	***	***	***
(error DF: 261)	F	87.36	173.42	60.84
	DF	2	2	4
Wet to dry mass ratio	Sig.	***	***	***
(error DF: 9)	F	225.37	31.74	28.02
	DF	2	2	4
Mean stomatal frequency on the	Sig.	**n.s.**	***	*
adaxial surface (error DF: 42)	F	1.64	50.81	3.31
	DF	1	2	2
Mean number of stomata on the	Sig.	***	**	**n.s.**
adaxial surface (error DF: 42)	F	19.05	5.76	0.03
	DF	1	2	2
Mean stomatal frequency on the	Sig.	**n.s.**	***	**n.s.**
abaxial surface (error DF: 42)	F	3.79	59.91	0.67
	DF	1	2	2
Mean number of stomata on the	Sig.	***	**n.s.**	**n.s.**
abaxial surface (error DF: 42)	F	19.57	2.68	0.05
	DF	1	2	2

Chapter 4. The effects of temperature and nutrient availability together and in isolation on the growth, morphology and physiology of *Poa annua.*

4.1 Null Hypotheses

$H_0$1: Temperature had no significant effect on the growth form, physiology and dry matter accumulation of plants from the same population.

$H_0$2: Nutrient availability had no significant effect on the growth form, physiology and dry matter accumulation of plants from the same population.

4.2 Results

Growth

A consistent pattern emerges from the measures of absolute changes in dry matter (Figure 4.1). At low N+P concentration, growth was slow and approximately linear, and no temperature effect was apparent. Without the N+P limitation, increases were much more rapid and exponential, and temperature effects were observed: whole plant, shoot and stem dry masses showing greater increases in the first three weeks in the higher temperature treatment than the lower one (found to be significant in two-sample t-tests comparing these two treatments in the second harvest). After 6 weeks the pattern is not clear, but may reflect the infection by leaf rust at the higher temperature, which probably reduced growth. The situation is complicated by the production of inflorescences, which may largely explain the large dry mass of "stems" in the high temperature high nutrient treatment at the final harvest (Figures 4.1d, 4.3c). There was no significant increase in leaf or root dry mass between weeks 3 and 6 in this treatment, suggesting that the increase in total plant mass was almost entirely due to the increase in "stem" dry mass.

Dry matter partitioning

The amount of root per unit shoot was significantly greater at the lower temperature, especially at high N+P ($P < 0.001$; Figure 4.2a, b; Table 4.1). A similar nutrient effect is observed ($P < 0.001$). A temperature effect ($P < 0.001$) was found in above-ground dry matter partitioning between leaves and "stems", with greater relative investment of dry matter in the "stems" at the higher temperature (Figure 4.2d). Similarly, LWR decreased with temperature (Figure 4.2c).

Tillers, inflorescences and leaves

There were significant increases in numbers of mature inflorescences with time, and significant differences between treatments ($P < 0.001$; Figure 4.3c, e; Table 4.2). Greater numbers were recorded at the higher nutrient concentration and at the higher temperature. This temperature effect was only observed without N+P limitation. Greater numbers of mature inflorescences per tiller were recorded at the higher temperature at both nutrient concentrations (Figure 4.3d).

Once the plants had become established, neither significant variation between treatments nor change with plant development was found in the mean number of leaves per tiller (Figure 4.4b). Variation in total leaf numbers per plant was thus almost entirely attributable to covariation in tiller numbers (Figures 4.3a, 4.4a). Leaf and tiller numbers per plant both showed little increase and no significant temperature effect at low nutrient levels. Without N+P limitation, however, significant increases and a marked temperature effect were observed, with greater numbers of both leaves and tillers at the higher temperature after three weeks ($P < 0.001$ from two-sample t-tests). These differences were not found at the final harvest.

Apart from the large number of senescent leaves in the high temperature high nutrient treatment in week 6 (probably caused by the rust), greater relative leaf senescence was generally found at lower nutrient levels ($P < 0.001$; Figure 4.4d, e). This may be a function of slower leaf production.

Leaf and longest tiller lengths were both greater at high N+P ($P < 0.001$; Figure 4.5a, b). At the higher nutrient concentration, no temperature effect was apparent for longest tiller lengths, but mean leaf length was greater at the lower temperature ($P < 0.001$). This cancelled out the temperature effect on leaf numbers to give no difference in mean total leaf area between the two temperatures after three weeks (Figure 4.5). Between weeks 3 and 6 total leaf area for the high nutrient high temperature treatment showed no increase (the mean value actually fell, though not significantly) while the number of leaves rose substantially.

Measures of leaf morphology and relative dry matter allocation to the leaves at low nutrient levels suggest little or no temperature effect, and little change in these variables with plant development (Figures 4.2c, 4.6a, b). At high N+P the combined effects of small differences in SLA, LWR and total leaf area between the two temperatures caused a large and significant ($P < 0.001$) reduction in LAR with temperature.

With development there was little change in SLA (Figure 4.6a), and the decreases in LAR at the higher nutrient concentration were therefore largely due to the decreases in LWR.

Folding, wet/dry mass ratios and stomata

Leaf folding exhibited strong temperature and nutrient effects: folding was greater at both lower temperature and lower N+P ($P < 0.001$; Figure 4.6c; Table 4.3). The wet/dry mass ratio showed significant increases with N+P concentration ($P < 0.001$), and a temperature effect at the high nutrient level only $P < 0.001$), with greater values at the lower temperature (Figure 4.6d). This, and the decrease in the ratio with development, may again be associated with greater allocation to "stems" in the high temperature high nutrient treatment.

Stomatal counts on both surfaces indicated a nutrient effect, in which the stomatal frequency is greater for the lower N+P treatments (Figure 4.7a, c). The effects of leaf size, however, more than compensated, to give significantly greater numbers of stomata per leaf on both surfaces at higher N+P concentrations ($P < 0.001$; Figure 4.7c, d).

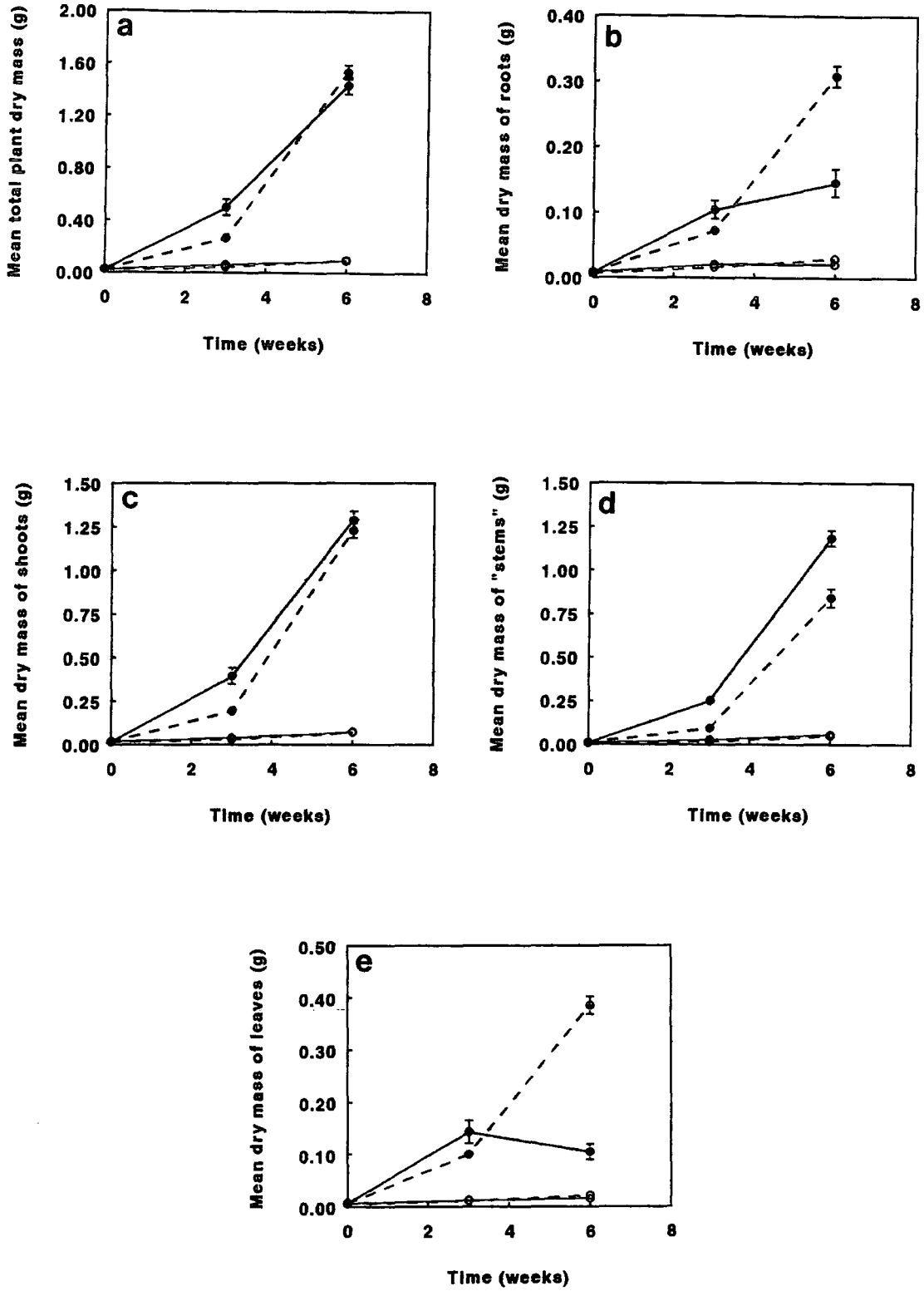


Figure 4.1. Dry mass measures of *Poa annua* grown at different temperatures and nitrate-phosphate concentrations.

Dry masses of (a) whole plants, (b) roots, (c) shoots, (d) "stems", and (e) leaves of *Poa annua* grown at 15°C with high N+P concentration (– ● – ·), 15°C with low N+P concentration(– ○ – ·), 21°C with high N+P concentration (—●—) and 21°C with low N+P concentration (—○—). Data represent the mean ± one standard error of five replicates per treatment.

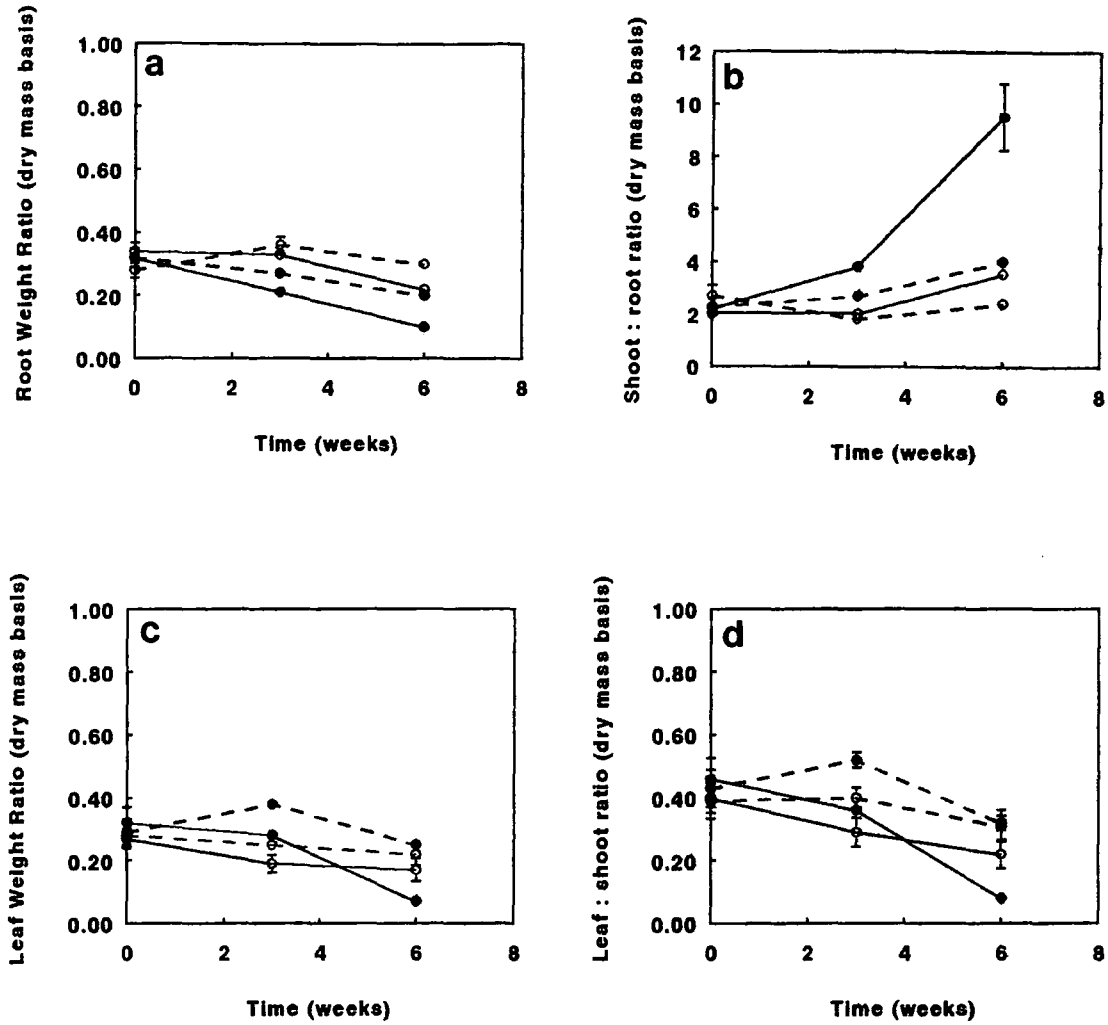


Figure 4.2. Dry matter partitioning in *Poa annua* grown at different temperatures and nitrate-phosphate concentrations.

(a) RWR, (b) S:R, (c) LWR, and (d) L:S in *Poa annua* grown at 15°C with high N+P concentration (- ●- ·), 15°C with low N+P concentration (· -○- -), 21°C with high N+P concentration (—●—) and 21°C with low N+P concentration (—○—). Data represent the mean ± one standard error of five replicates per treatment.

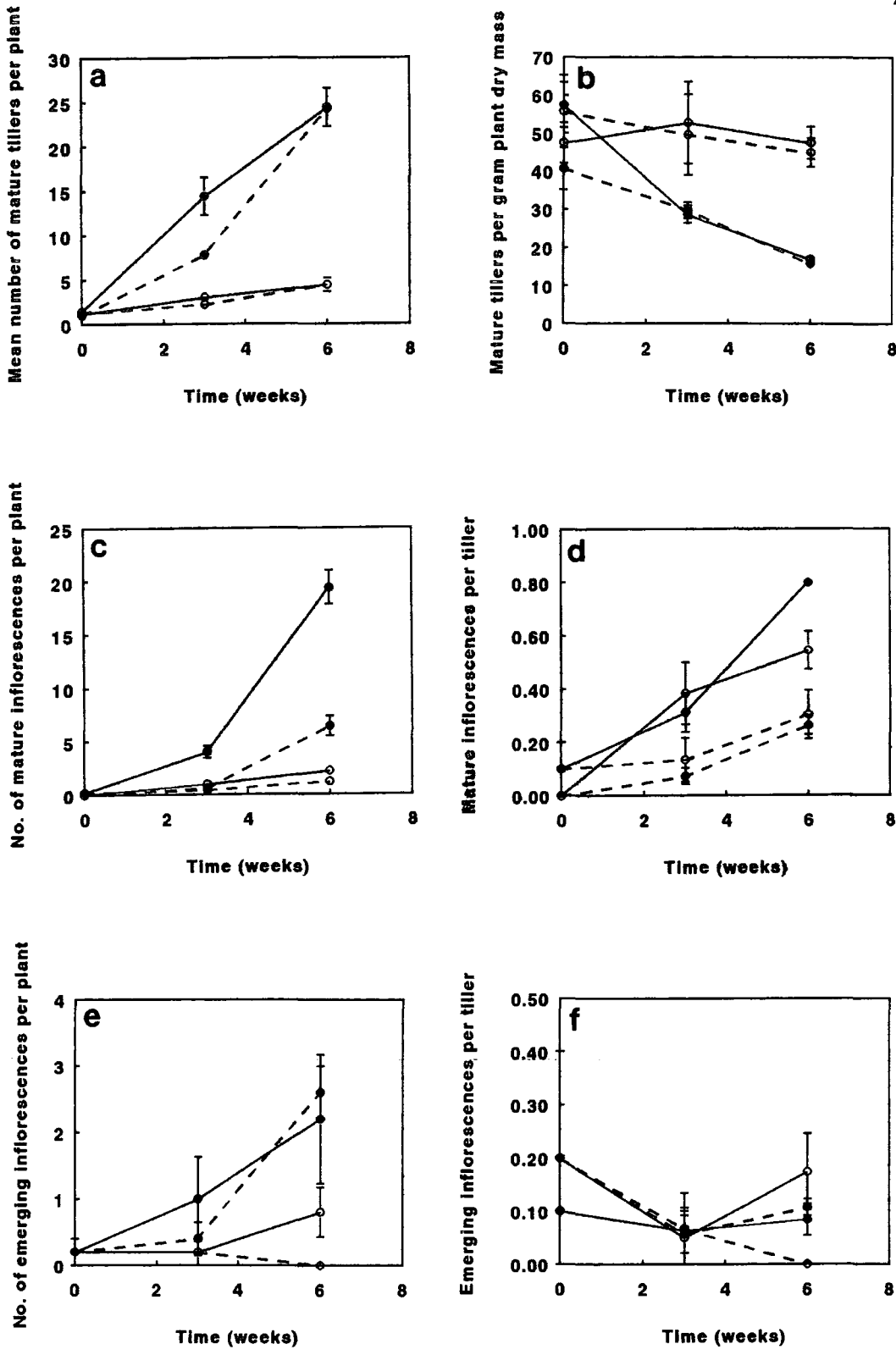


Figure 4.3. Numbers of tillers and inflorescences on *Poa annua* grown at different temperatures and nitrate-phosphate concentrations.

Numbers of (a) mature tillers, (b) tillers per gram dry mass, (c) mature inflorescences, (d) mature inflorescences per tiller, (e) emerging inflorescences, and (f) emerging inflorescences per tiller on *Poa annua* grown at 15°C with high N+P concentration (– ● – ·), 15°C with low N+P concentration (– ○ – ·), 21°C with high N+P concentration (—● —) and 21°C with low N+P concentration (—○—). Data represent the mean ± one standard error of five replicates per treatment.

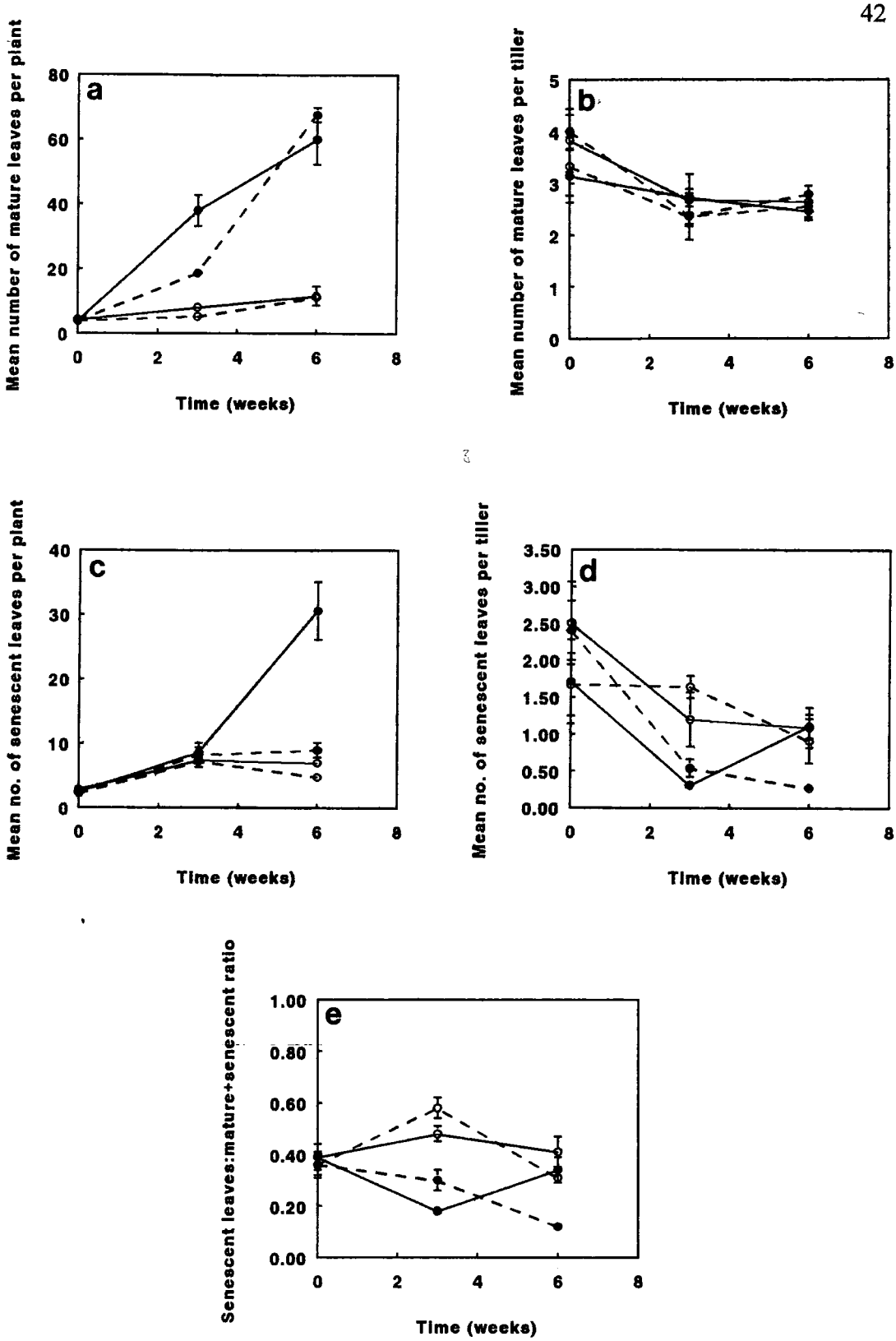


Figure 4.4. Numbers of leaves on *Poa annua* grown at different temperatures and nitrate-phosphate concentrations.

Numbers of (a) mature leaves, (b) mature leaves per tiller, (c) senescent leaves, (d) senescent leaves per tiller, and (e) the ratio of senescent leaves to all mature and senescent leaves on *Poa annua* grown at 15°C with high N+P concentration (– ●– ·), 15°C with low N+P concentration (– ○– ·), 21°C with high N+P concentration (—●—) and 21°C with low N+P concentration (—○—). Data in (a), (c) and (e) represent the mean ± one standard error of five replicates per treatment. Data in (b) and (d) represent the mean ± one standard error of replicate numbers equal to the number of tillers (see Figure 4.3a).

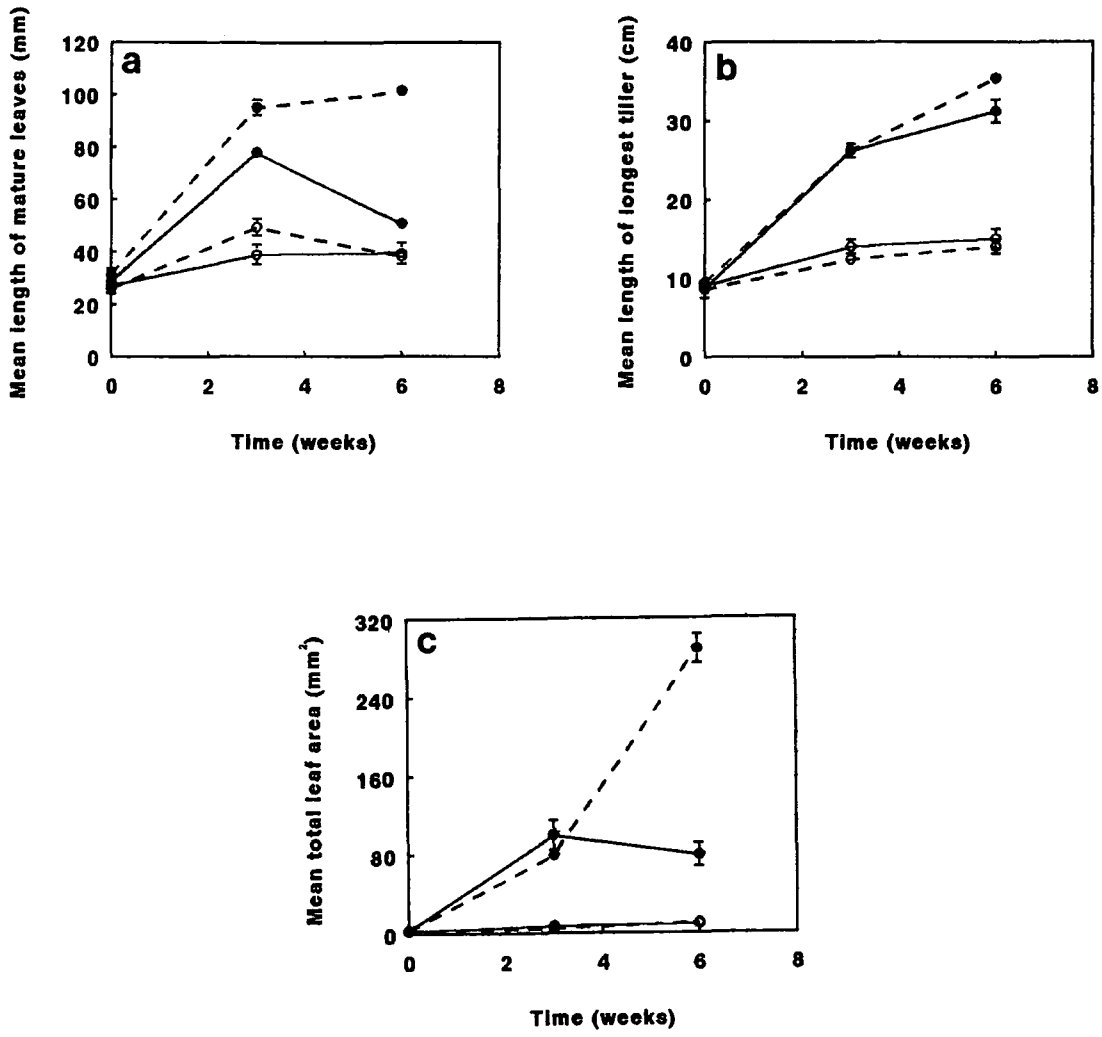


Figure 4.5. Leaf and longest tiller lengths and total leaf area of *Poa annua* grown at different temperatures and nitrate-phosphate concentrations.

(a) mean length of mature leaves, (b) length of longest tiller, and (c) total leaf area of *Poa annua* grown at 15°C with high N+P concentration (- ●- ·), 15°C with low N+P concentration (- ⊖- ·), 21°C with high N+P concentration (—●—) and 21°C with low N+P concentration (—⊖—). Data represent the mean ± one standard error of five replicates per treatment.

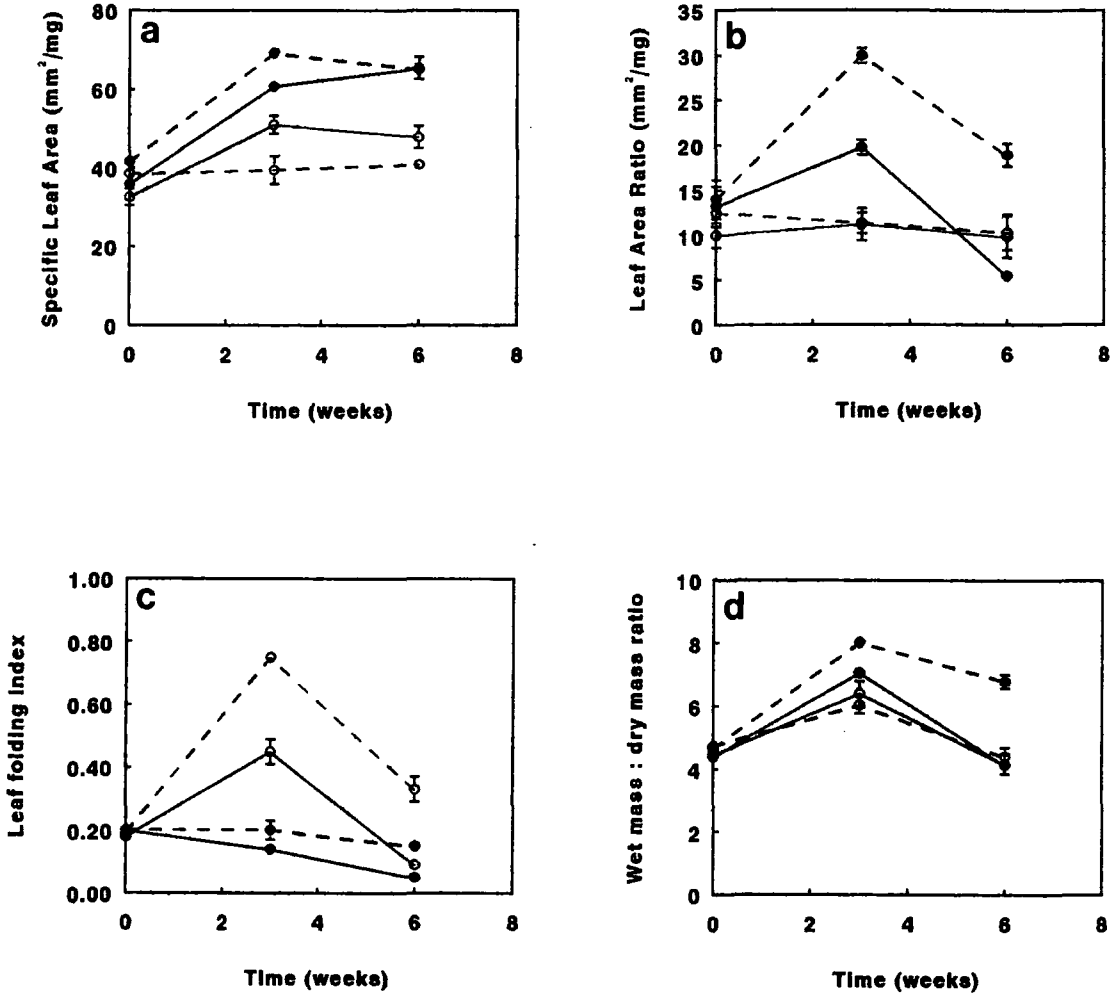


Figure 4.6. SLA, LAR, leaf folding and wet to dry mass ratio of *Poa annua* grown at different temperatures and nitrate-phosphate concentrations.

(a) SLA, (b) LAR, (c) leaf folding index, and (d) wet to dry mass ratio of *Poa annua* grown at 15°C with high N+P concentration (– ●– ·), 15°C with low N+P concentration (– ○– ·), 21°C with high N+P concentration (—●—) and 21°C with low N+P concentration (—○—). Data represent the mean ± one standard error of five replicates per treatment.

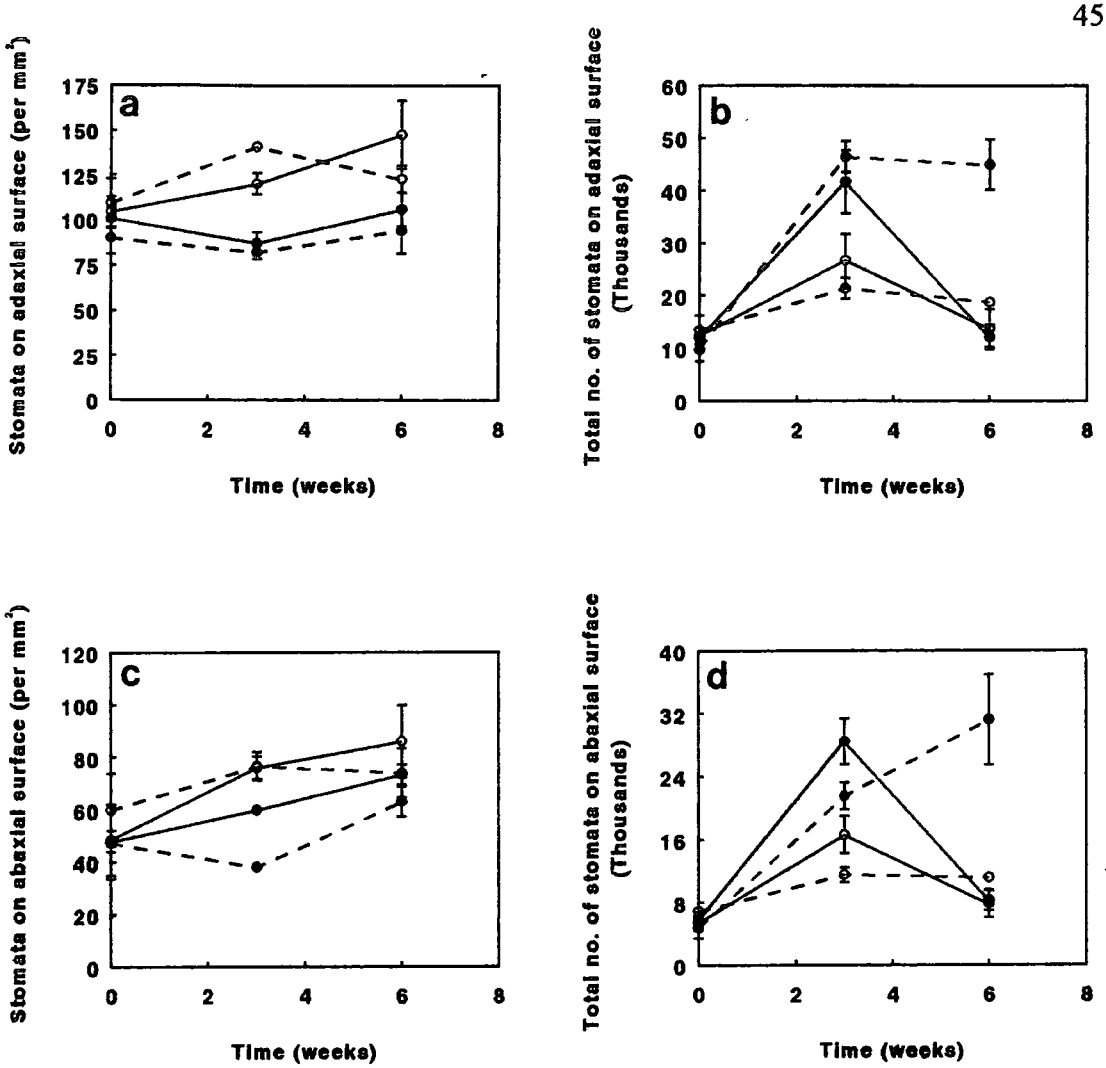


Figure 4.7. Stomatal measures of *Poa annua* grown at different temperatures and nitrate-phosphate concentrations.

(a) adaxial stomatal density, (b) estimated number of stomata on the adaxial surface, (c) abaxial stomatal density, and (d) estimated number of stomata on the abaxial surface of *Poa annua* grown at 15°C with high N+P concentration (– ●– ·), 15°C with low N+P concentration (– ○– ·), 21°C with high N+P concentration (—●—) and 21°C with low N+P concentration (—○—). Data represent the mean ± one standard error of three replicates (leaves) per treatment.

Table 4.1: Summary of statistical significance of the effects of temperature and nutrient (N+P) concentration on growth and dry matter partitioning parameters of *Poa annua*.

Dry mass data $\log_e$ transformed; RWR and LWR were angular transformed before analysis. A three-way balanced ANOVA was performed, removing time, N+P, temperature (temp), time x N+P interaction, time x temperature interaction and N+P x temperature interaction effects. All harvests (times) were included in tests marked #, while the first harvest was excluded in the rest. Significance notation: n.s., not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Sig. is significance; DF is degrees of freedom.

		Source of variation					
Parameter	**Value**	**Time**	**N+P**	**Temp**	**Time x N+P**	**Time x Temp**	**N+P x Temp**
# Whole plant dry mass	**Sig.**	***	***	**n.s.**	***	**	**n.s.**
(error DF: 50)	F	538.92	531.11	3.74	136.06	5.83	0.05
	DF	2	1	1	2	2	1
# Dry mass of roots	**Sig.**	***	***	**n.s.**	***	***	**n.s.**
(error DF: 50)	F	359.23	321.29	0.66	76.14	14.03	2.79
	DF	2	1	1	2	2	1
# Dry mass of shoots	**Sig.**	***	***	*	***	**	**n.s.**
(error DF: 50)	F	512.52	514.27	5.78	132.19	5.58	0.26
	DF	2	1	1	2	2	1
# Dry mass of stems	**Sig.**	***	***	**	***	**	**n.s.**
(error DF: 50)	F	359.84	280.63	10.21	84.18	6.04	0.64
	DF	2	1	1	2	2	1
# Dry mass of leaves	**Sig.**	***	***	*	***	***	**n.s.**
(error DF: 50)	F	174.04	281.18	4.88	58.88	10.18	1.59
	DF	2	1	1	2	2	1
Root weight ratio	**Sig.**	***	***	***	**n.s.**	**	*
(error DF: 33)	F	109.13	156.37	64.73	0.98	8.79	6.34
	DF	1	1	1	1	1	1
Shoot to root ratio	**Sig.**	***	***	***	**	**	**
(error DF: 33)	F	40.31	50.63	30.47	11.65	13.56	13.71
	DF	1	1	1	1	1	1
Leaf weight ratio	**Sig.**	***	**n.s.**	***	***	**n.s.**	*
(error DF: 33)	F	33.71	3.06	32.21	16.71	2.47	6.63
	DF	1	1	1	1	1	1
Leaf to shoot ratio	**Sig.**	***	**n.s.**	***	**	**n.s.**	*
(error DF: 33)	F	45.74	0.04	39.96	12.26	1.36	5.41
	DF	1	1	1	1	1	1

Table 4.2: Summary of statistical significance of the effects of temperature and nutrient (N+P) concentration on numbers of leaves, tillers and inflorescences and derived variables.

Count data were square-root transformed before analysis. A three-way balanced ANOVA was performed, removing time, N+P, temperature (temp), time x N+P interaction, time x temperature interaction and N+P x temperature interaction effects. All harvests (times) were included in tests marked #, while the first harvest was excluded in the rest. Significance notation: n.s., not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Sig. is significance; DF is degrees of freedom.

		Source of variation					
Parameter	**Value**	**Time**	**N+P**	**Temp**	**Time x N+P**	**Time x Temp**	**N+P x Temp**
# Mean number of mature tillers per plant (error DF: 50)	Sig.	***	***	**	***	**	n.s.
	F	263.50	318.83	7.14	96.13	5.24	3.00
	DF	2	1	1	2	2	1
Mean number of tillers per gram plant dry mass (error DF: 33)	Sig.	*	***	n.s.	n.s.	n.s.	n.s.
	F	4.78	40.45	.013	0.94	0.01	.012
	DF	1	1	1	1	1	1
# Mean number of mature inflorescences per plant (error DF: 50)	Sig.	***	***	***	***	***	***
	F	177.73	119.76	58.32	61.94	16.65	28.82
	DF	2	1	1	2	2	1
Mean number of mature inflorescences per tiller (error DF: 33)	Sig.	***	n.s.	***	n.s.	n.s.	n.s.
	F	23.60	0.37	29.78	1.07	0.11	1.28
	DF	1	1	1	1	1	1
Mean number of emerging inflorescences per plant (error DF: 33)	Sig.	**	***	n.s.	*	n.s.	n.s.
	F	9.71	16.91	0.74	5.58	0.00	0.65
	DF	1	1	1	1	1	1
Mean number of emerging inflorescences per tiller (error DF: 33)	Sig.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	F	2.31	1.81	1.09	0.45	1.27	1.88
	DF	1	1	1	1	1	1
# Mean number of mature leaves per plant (error DF: 50)	Sig.	***	***	*	***	**	n.s.
	F	182.91	244.68	4.23	80.11	8.17	0.31
	DF	2	1	1	2	2	1
Mean number of mature leaves per tiller (error DF: 33)	Sig.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	F	0.10	0.00	0.27	0.27	2.62	0.38
	DF	1	1	1	1	1	1
Mean number of senescent leaves per plant (error DF: 33)	Sig.	***	***	***	***	***	*
	F	60.95	23.96	17.95	19.63	12.06	4.94
	DF	1	1	1	1	1	1
Mean number of senescent leaves per tiller (error DF: 33)	Sig.	n.s.	***	n.s.	**	**	n.s.
	F	0.71	42.62	1.43	10.04	13.80	2.48
	DF	1	1	1	1	1	1
Ratio of numbers of senescent leaves to all mature and senescent leaves (error DF: 33)	Sig.	**	***	n.s.	*	***	n.s.
	F	11.60	58.74	1.29	6.71	25.69	1.27
	DF	1	1	1	1	1	1

Table 4.3: Summary of statistical significance of the effects of temperature and nutrient (N+P) concentration on leaf and tiller lengths, leaf area, SLA, LAR, leaf folding, wet to dry mass ratios and stomatal parameters.

The leaf folding index and the reciprocal of the wet to dry mass ratio were angular transformed before analysis. A three-way balanced ANOVA was performed, removing time, N+P, temperature (temp), time x N+P interaction, time x temperature interaction and N+P x temperature interaction effects. All harvests (times) were included in tests marked #, while the first harvest was excluded in the rest. Significance notation: n.s., not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Sig. is significance; DF is degrees of freedom.

		Source of variation					
Parameter	**Value**	**Time**	**N+P**	**Temp**	**Time x N+P**	**Time x Temp**	**N+P x Temp**
Mean length of mature leaves	**Sig.**	**	***	***	**n.s.**	**n.s.**	***
(error DF: 33)	F	8.14	210.45	49.25	0.83	3.93	28.42
	DF	1	1	1	1	1	1
# Mean length of longest tiller	**Sig.**	***	***	**n.s.**	***	**n.s.**	*
(error DF: 50)	F	301.61	429.82	0.42	114.05	1.83	6.72
	DF	2	1	1	2	2	1
# Mean total leaf area	**Sig.**	***	***	***	***	***	***
(error DF: 33)	F	42.43	106.99	14.30	36.22	19.88	14.47
	DF	2	1	1	2	2	1
Specific leaf area	**Sig.**	**n.s.**	***	**n.s.**	**n.s.**	**n.s.**	***
(error DF: 33)	F	0.03	154.54	2.53	0.12	0.39	16.64
	DF	1	1	1	1	1	1
Leaf area ratio	**Sig.**	***	***	***	***	**n.s.**	***
(error DF: 33)	F	45.53	58.48	34.61	30.83	0.75	30.99
	DF	1	1	1	1	1	1
Leaf folding index	**Sig.**	***	***	***	***	**n.s.**	***
(error DF: 113)	F	161.26	207.01	102.84	52.83	0.89	17.71
	DF	1	1	1	1	1	1
Wet to dry mass ratio	**Sig.**	***	***	***	**n.s.**	**	**
(error DF: 33)	F	93.22	26.75	17.59	0.52	11.93	14.69
	DF	1	1	1	1	1	1
Mean stomatal frequency on the	**Sig.**	**n.s.**	***	**n.s.**	**n.s.**	**n.s.**	**n.s.**
adaxial surface (error DF: 17)	F	2.10	33.04	0.56	0.58	3.43	0.21
	DF	1	1	1	1	1	1
Mean number of stomata on the	**Sig.**	**	***	**	**n.s.**	**	**
adaxial surface (error DF: 17)	F	16.97	32.70	10.83	1.89	11.60	11.32
	DF	1	1	1	1	1	1
Mean stomatal frequency on the	**Sig.**	*	**	*	**n.s.**	**n.s.**	**n.s.**
abaxial surface (error DF: 17)	F	5.30	15.56	4.87	2.48	0.00	1.10
	DF	1	1	1	1	1	1
Mean number of stomata on the	**Sig.**	*	***	**n.s.**	**n.s.**	***	**n.s.**
abaxial surface (error DF: 17)	F	4.89	22.60	2.57	0.02	18.46	3.99
	DF	1	1	1	1	1	1

Chapter 5. The effect of light intensity on the growth, morphology and physiology of *Poa annua.*

5.1 Null Hypothesis

Light intensity had no significant effect on the growth form, physiology and dry matter accumulation of plants from the same population.

5.2 Results

Growth

Exponential increases were recorded in all the dry mass measures with time (Figure 5.1). Significantly greater increases in these variables occurred in the high light intensity treatment than in the low, except for leaf dry mass. In most cases the difference did not appear until the final harvest. In all cases, where measured, the medium light intensity produced intermediate values.

Dry matter partitioning

Measures of dry matter partitioning between shoots and roots showed significantly greater relative allocation to roots with higher light intensity (Figure 5.2a, b; Table 5.1). Leaf/shoot ratio, in which there were no differences between treatments, declined with development, as did LWR.

Tillers, inflorescences and leaves

The number of mature inflorescences per plant increased with light intensity, but expressed on a per tiller basis no difference was found (Figure 5.3c, d; Table 5.2). This may therefore reflect faster growth rather than more rapid development with increased light intensity.

Significantly greater numbers of leaves and tillers were produced at the higher light intensity (Figures 5.3a, 5.4a). As in the experiments reported above there was no difference in the number of leaves per tiller (Figure 5.4b). Unlike in those experiments there was a small but significant increase in this number with time ($P < 0.001$).

There was no difference between the treatments in mean leaf length or length of longest tiller (Figure 5.5a, b). The increase in leaf area with light intensity is therefore a function of the greater number of leaves (Figures 5.5c, 5.4a). A greater number of senescent leaves was recorded at the higher light intensity at the final harvest (Figure 5.4c).

SLA showed no significant differences between treatments, and the small but significant decrease in LAR with light intensity was therefore due to an equivalent decrease in LWR (Figures 5.6a, b, 5.2c). SLA also remained approximately constant over time, the decline in LAR being due to the decline in LWR.

Folding, wet/dry mass ratios and stomata

The leaf folding index showed no significant differences with treatment, and no change with development (Figure 5.6c; Table 5.3). Whole plant wet/dry mass ratios were not affected by treatment, but gradually decreased with time, reflecting an increased proportion of structural matter with plant age and size (Figures 5.6d, 5.2d).

Stomatal counts on both leaf surfaces showed no differences with treatment or time in stomatal numbers or frequencies (Figure 5.7).

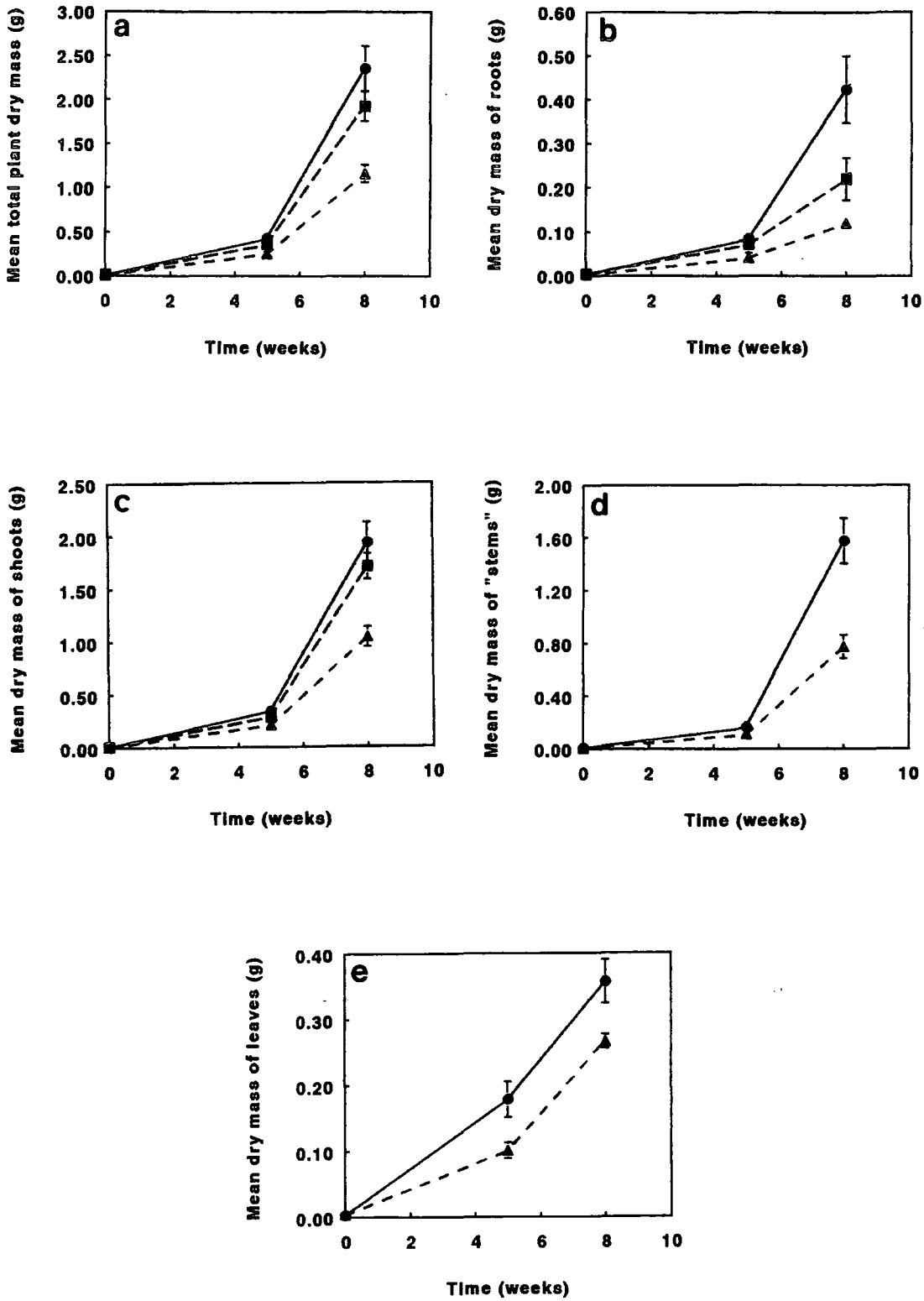


Figure 5.1. Dry mass measures of *Poa annua* grown at different light intensities.

Dry masses of (a) whole plants, (b) roots, (c) shoots, (d) "stems", and (e) leaves of *Poa annua* grown at high (—●—), medium (·—■—) and low (– –▲– ·) light intensities. Data represent the mean ± one standard error of five replicates per treatment.

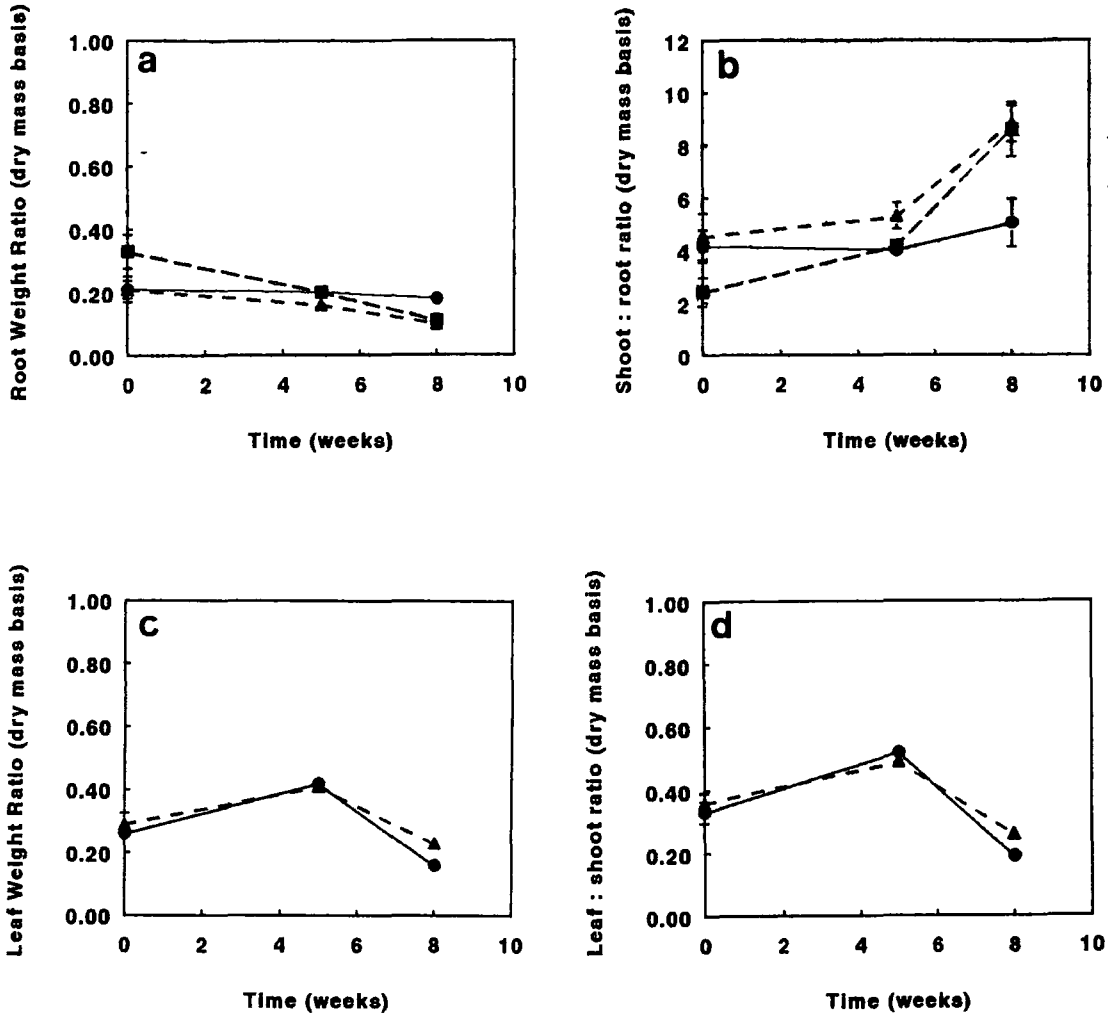


Figure 5.2. Dry matter partitioning in *Poa annua* grown at different light intensities. (a) RWR, (b) S:R, (c) LWR, and (d) L:S in *Poa annua* grown at high (—●—), medium (–■–·) and low (– ▲– ·) light intensities. Data represent the mean ± one standard error of five replicates per treatment.

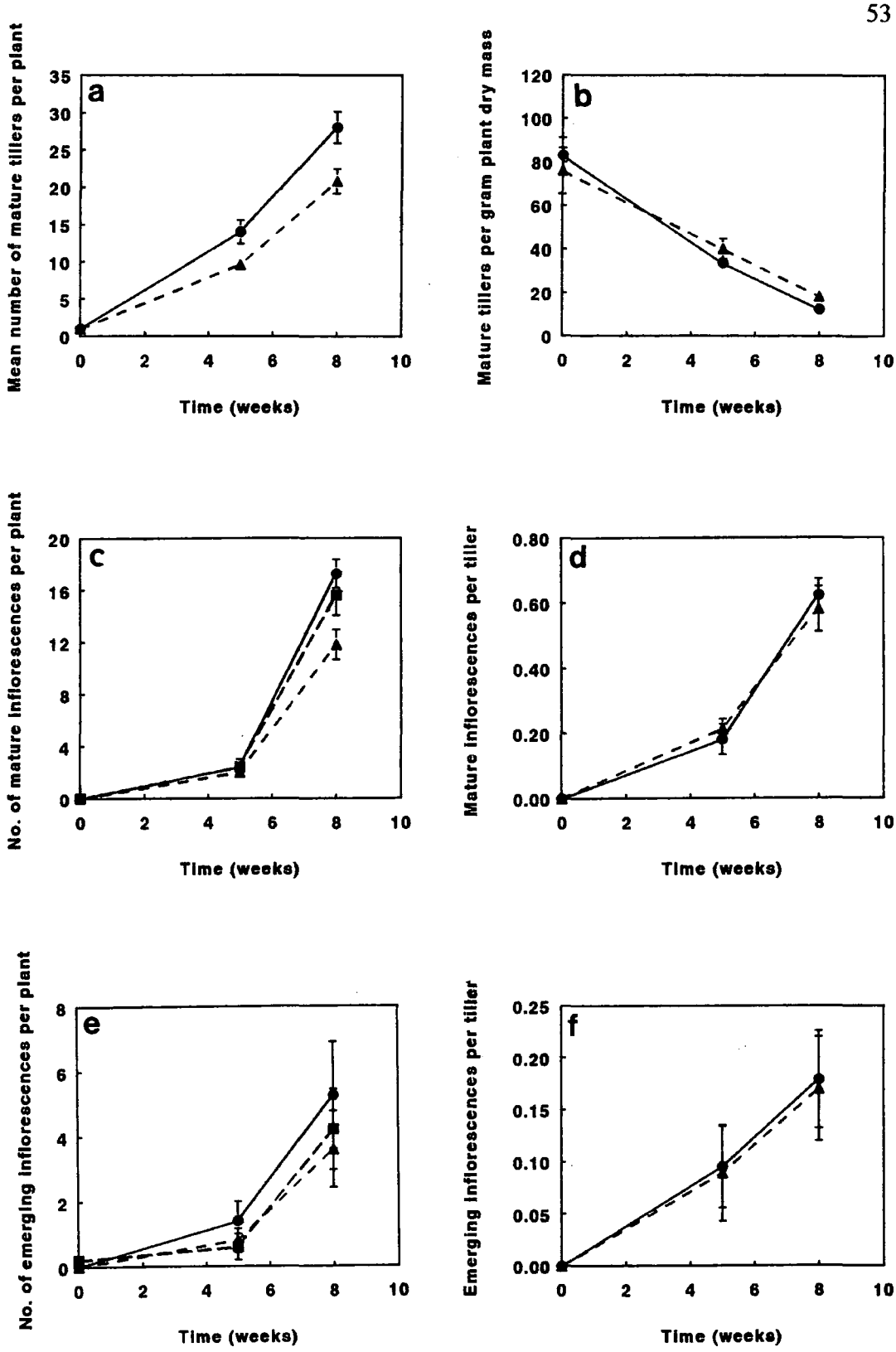


Figure 5.3. Numbers of tillers and inflorescences on *Poa annua* grown at different light intensities.

Numbers of (a) mature tillers, (b) tillers per gram dry mass, (c) mature inflorescences, (d) mature inflorescences per tiller, (e) emerging inflorescences, and (f) emerging inflorescences per tiller on *Poa annua* grown at high (—●—), medium (—■—) and low (—▲—) light intensities. Data represent the mean ± one standard error of five replicates per treatment.

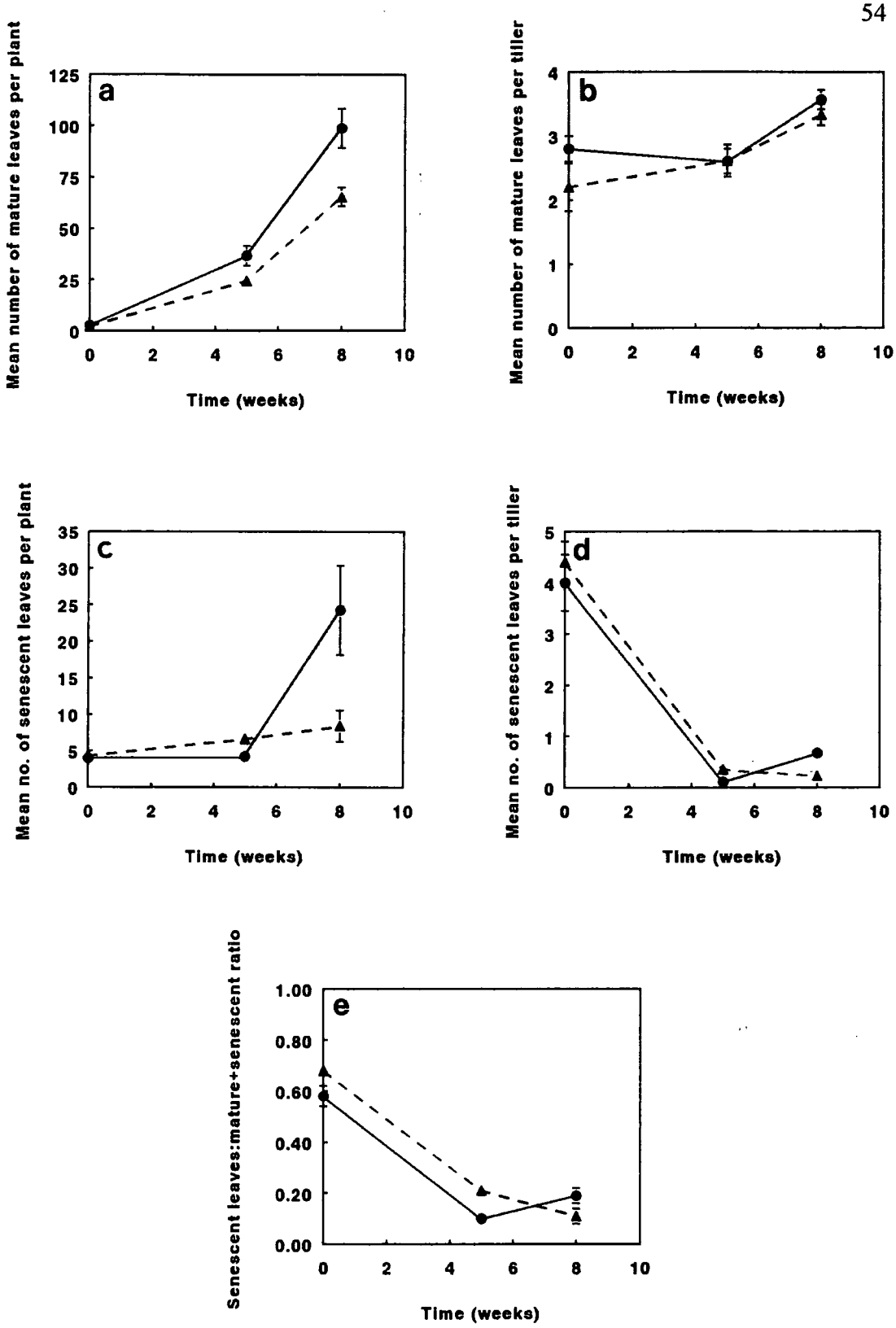


Figure 5.4. Numbers of leaves on *Poa annua* grown at different light intensities.

Numbers of (a) mature leaves, (b) mature leaves per tiller, (c) senescent leaves, (d) senescent leaves per tiller, and (e) the ratio of senescent leaves to all mature and senescent leaves on *Poa annua* grown at high (—●—) and low (- -▲- -) light intensities. Data in (a), (c) and (e) represent the mean ± one standard error of five replicates per treatment. Data in (b) and (d) represent the mean ± one standard error of replicate numbers equal to the number of tillers (see Figure 5.3a).

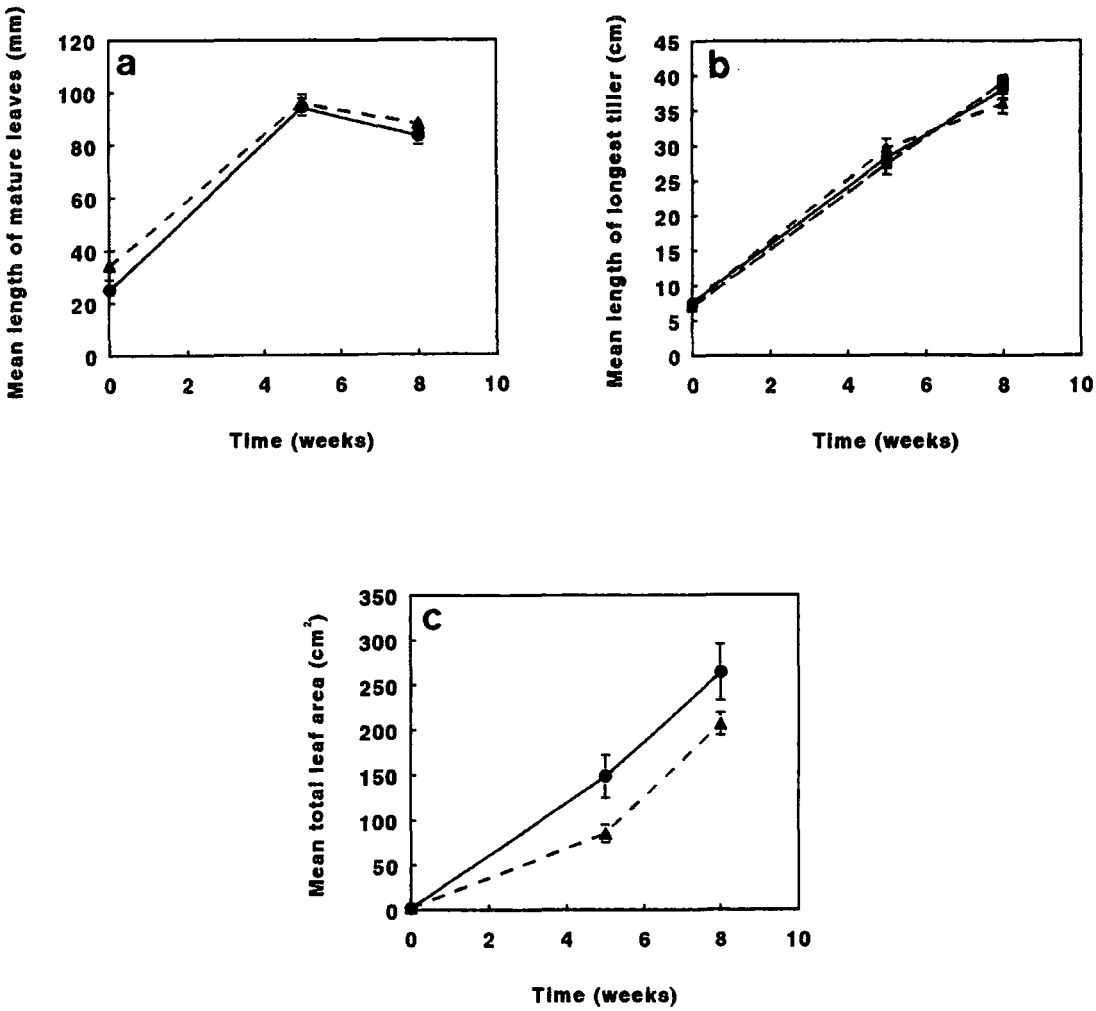


Figure 5.5. Leaf and longest tiller lengths and total leaf area of *Poa annua* grown at different light intensities.

(a) mean length of mature leaves, (b) length of longest tiller, and (c) total leaf area of *Poa annua* grown at high (—●—), medium (·—■—) and low (– –▲– ·) light intensities. Data represent the mean ± one standard error of five replicates per treatment.

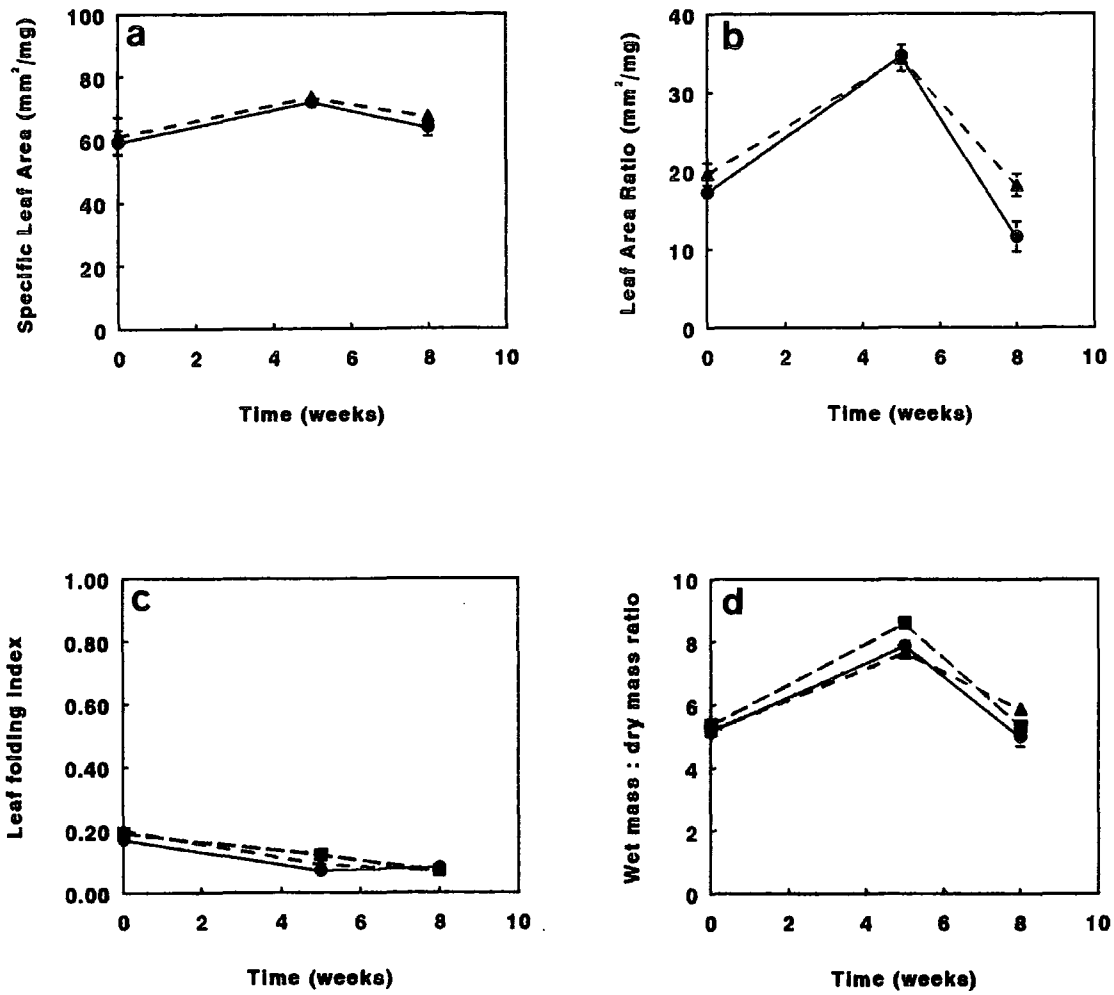


Figure 5.6. SLA, LAR, leaf folding and wet to dry mass ratio of *Poa annua* grown at different light intensities.

(a) SLA, (b) LAR, (c) leaf folding index, and (d) wet to dry mass ratio of *Poa annua* grown at high (—●—), medium (—■—) and low (—▲—) light intensities. Data represent the mean ± one standard error of five replicates per treatment.

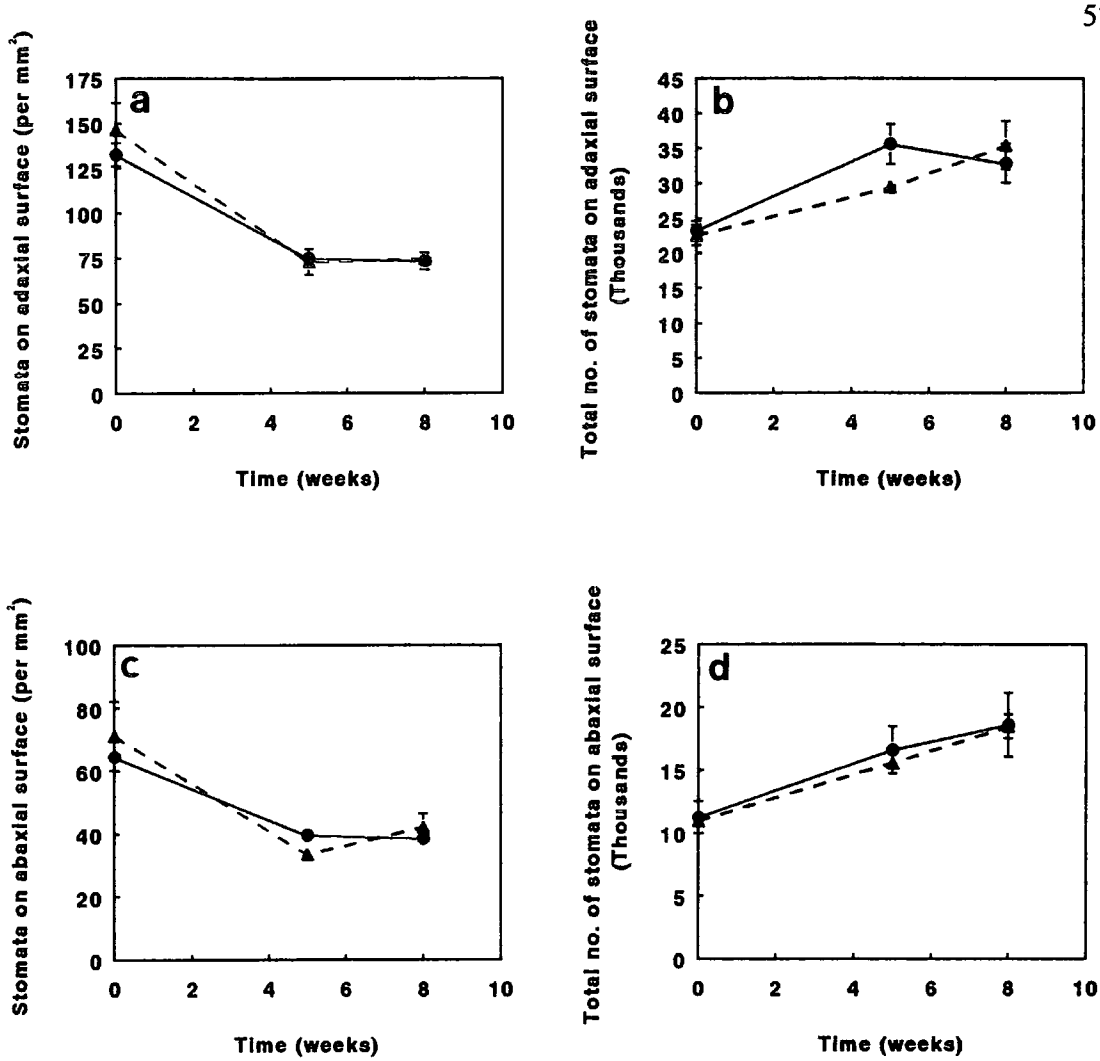


Figure 5.7. Stomatal measures of *Poa annua* grown at different light intensities.

(a) adaxial stomatal density, (b) estimated number of stomata on the adaxial surface, (c) abaxial stomatal density, and (d) estimated number of stomata on the abaxial surface of *Poa annua* grown at high (—●—) and low (- -▲- - ·) light intensities. Data represent the mean ± one standard error of three replicates (leaves) per treatment.

Table 5.1: Summary of statistical significance of the effects of light intensity on growth and dry matter partitioning parameters of *Poa annua*.

Dry mass data $\log_e$ transformed; RWR and LWR were angular transformed before analysis. A two-way balanced ANOVA was performed, removing time, light intensity (light) and time x light intensity interaction effects. All harvests (times) were included in tests marked #, while the first harvest was excluded in the rest. Significance notation: n.s., not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Sig. is significance; DF is degrees of freedom.

		Source of variation		
Parameter	**Value**	**Time**	**Light**	**Time x light**
# Whole plant dry mass	**Sig.**	***	**	**n.s.**
(error DF: 36)	F	961.73	5.44	2.60
	DF	2	2	4
# Dry mass of roots	**Sig.**	***	**	*
(error DF: 36)	F	433.33	9.42	3.67
	DF	2	2	4
# Dry mass of shoots	**Sig.**	***	*	*
(error DF: 36)	F	1009.81	4.22	2.74
	DF	2	2	4
# Dry mass of stems	**Sig.**	***	**	*
(error DF: 24)	F	655.32	9.54	3.77
	DF	2	1	2
# Dry mass of leaves	**Sig.**	***	**n.s.**	*
(error DF: 24)	F	579.58	3.44	3.92
	DF	2	1	2
Root weight ratio	**Sig.**	***	***	**n.s.**
(error DF: 24)	F	35.40	11.01	3.39
	DF	1	2	2
Shoot to root ratio	**Sig.**	***	**	*
(error DF: 24)	F	34.12	8.67	3.90
	DF	1	2	2
Leaf weight ratio	**Sig.**	***	*	**
(error DF: 16)	F	208.42	6.70	11.29
	DF	1	1	1
Leaf to shoot ratio	**Sig.**	***	**n.s.**	**
(error DF: 16)	F	247.71	1.72	11.29
	DF	1	1	1

Table 5.2: Summary of statistical significance of the effects of light intensity on numbers of leaves, tillers and inflorescences and derived variables.

Count data were square-root transformed before analysis. A two-way balanced ANOVA was performed, removing time, light intensity (light) and time x light intensity interaction effects. All harvests (times) were included in tests marked #, while the first harvest was excluded in the rest. Significance notation: n.s., not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Sig. is significance; DF is degrees of freedom.

		Source of variation		
Parameter	**Value**	**Time**	**Light**	**Time x light**
# Mean number of mature tillers per plant (error DF: 24)	**Sig.**	***	**	*
	F	379.82	16.01	4.08
	DF	2	1	2
Mean number of tillers per gram plant dry mass (error DF: 16)	**Sig.**	***	*	**n.s.**
	F	57.61	5.06	0.01
	DF	1	1	1
# Mean number of mature inflorescences per plant (error DF: 36)	**Sig.**	***	*	**n.s.**
	F	517.53	3.69	2.45
	DF	2	2	4
Mean number of mature inflorescences per tiller (error DF: 24)	**Sig.**	***	**n.s.**	**n.s.**
	F	60.24	0.01	0.63
	DF	1	1	1
Mean number of emerging inflorescences per plant (error DF: 24)	**Sig.**	***	**n.s.**	**n.s.**
	F	25.70	0.90	0.12
	DF	1	2	2
Mean number of emerging inflorescences per tiller (error DF: 24)	**Sig.**	*	**n.s.**	**n.s.**
	F	4.97	0.03	0.00
	DF	1	1	1
# Mean number of mature leaves per plant (error DF: 24)	**Sig.**	***	***	*
	F	381.66	22.99	4.89
	DF	2	1	2
Mean number of mature leaves per tiller (error DF: 16)	**Sig.**	***	**n.s.**	**n.s.**
	F	50.03	0.66	1.10
	DF	1	1	1
Mean number of senescent leaves per plant (error DF: 16)	**Sig.**	***	*	**
	F	21.03	5.16	15.02
	DF	1	1	1
Mean number of senescent leaves per tiller (error DF: 16)	**Sig.**	*	**n.s.**	**
	F	4.71	1.17	10.50
	DF	1	1	1
Ratio of numbers of senescent leaves to all mature and senescent leaves (error DF: 16)	**Sig.**	**n.s.**	**n.s.**	**
	F	0.08	0.27	17.46
	DF	1	1	1

Table 5.3: Summary of statistical significance of the effects of light intensity on leaf and tiller lengths, leaf area, SLA, LAR, leaf folding, wet to dry mass ratios and stomatal parameters.

The leaf folding index and the reciprocal of the wet to dry mass ratio were angular transformed before analysis. A two-way balanced ANOVA was performed, removing time, light intensity (light) and time x light intensity interaction effects. All harvests (times) were included in tests marked #, while the first harvest was excluded in the rest. Significance notation: n.s., not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Sig. is significance; DF is degrees of freedom.

		Source of variation		
Parameter	**Value**	**Time**	**Light**	**Time x light**
Mean length of mature leaves	**Sig.**	**	**n.s.**	**n.s.**
(error DF: 16)	F	9.92	1.12	0.26
	DF	1	1	1
# Mean length of longest tiller	**Sig.**	***	**n.s.**	**n.s.**
(error DF: 36)	F	607.98	0.03	1.54
	DF	2	2	4
# Mean total leaf area	**Sig.**	***	**	**n.s.**
(error DF: 24)	F	117.77	10.42	2.71
	DF	2	1	2
Specific leaf area	**Sig.**	**	**n.s.**	**n.s.**
(error DF: 16)	F	16.92	2.17	0.29
	DF	1	1	1
Leaf area ratio	**Sig.**	***	*	*
(error DF: 16)	F	196.43	4.99	6.04
	DF	1	1	1
Leaf folding index	**Sig.**	**n.s.**	**n.s.**	**n.s.**
(error DF: 84)	F	3.79	1.51	2.34
	DF	1	2	2
Wet to dry mass ratio	**Sig.**	***	*	**
(error DF: 24)	F	195.14	3.51	6.11
	DF	1	2	2
Mean stomatal frequency on the	**Sig.**	**n.s.**	**n.s.**	**n.s.**
adaxial surface (error DF: 8)	F	0.00	0.00	0.09
	DF	1	1	1
Mean number of stomata on the	**Sig.**	**n.s.**	**n.s.**	**n.s.**
adaxial surface (error DF: 8)	F	0.36	0.44	2.64
	DF	1	1	1
Mean stomatal frequency on the	**Sig.**	**n.s.**	**n.s.**	**n.s.**
abaxial surface (error DF: 8)	F	1.78	0.19	2.95
	DF	1	1	1
Mean number of stomata on the	**Sig.**	**n.s.**	**n.s.**	**n.s.**
abaxial surface (error DF: 8)	F	2.13	0.12	0.08
	DF	1	1	1

Chapter 6. The growth, morphology and physiology of *Poa annua* from populations found at different altitudes, when grown under the same environmental conditions.

6.1 Null Hypothesis

There were no significant differences in the growth form, physiology and dry matter accumulation of plants from the different populations.

6.2 Results

As noted above (Chapter 2), this experiment was not run along exactly the same lines as the others, and only the first and last harvests are reported here. In this chapter, and subsequent discussion of the experiment, plants taken from the different source populations will be referred to as the "WBF plants", "Alston plants" and "Durham plants".

Growth

For all measures of dry matter, increases in the Alston plants were greater than for the plants from the other two populations ($P < 0.001$, Figure 6.1). No significant difference was found between the plants from the other two sites in terms of total plant dry mass and root dry mass, but for above-ground dry matter the values for the WBF plants were larger. In all cases the increase in dry mass between the harvests was significant.

Dry matter partitioning

The Durham plants showed the greatest relative investment in roots at the final harvest, and the Alston plants the least (Figure 6.2a, b). No differences were found at the final harvest between the populations in the partitioning of above-ground dry matter to leaves and "stems" (Figure 6.2c). This ratio declined in the Durham plants between the harvests, but increased in the WBF plants, with no change in the Alston ones. The order of LWR values at the final harvest was again Alston plants > WBF > Durham (Figure 6.2d).

Tillers, inflorescences and leaves

The Alston plants showed the greatest increase in leaf and tiller numbers per plant (Figures 6.3a, 6.4a). The latter probably caused the former as there was no difference in leaf numbers per tiller (Figure 6.3b). Lengths of leaves and longest tillers, and area of leaves showed significant differences between all three populations (Figure 6.4c-e). In each case, the order of values was Alston plants > WBF > Durham. These measures

were probably closely linked, mean total leaf area (like leaf dry mass - Figure 6.1) showing the greatest differences because it is a function of leaf length and leaf numbers per plant. The values of these measures increased between the harvests, though not significantly for mean leaf length in the Durham plants.

There were no significant differences between the populations in number of senescent leaves per plant at the final harvest (Figure 6.3c). There were differences, however, in number of senescent leaves per tiller at the final harvest, when Durham plants had significantly more, despite having had fewer at the initial harvest (Figure 6.3d).

SLA (final harvest) did not differ between the Alston and WBF plants, for both of which it increased between harvests; but SLA was lower in the Durham plants, which showed no change with time (Figure 6.5a). The lower SLA of the Durham plants cancelled out their higher RWR to give similar patterns of LAR to LWR results at the final harvest: again Alston plants > WBF > DBG (Figures 6.5a, b, 6.2a, c).

Only two inflorescences were recorded throughout the experiment. Inflorescence results are therefore not presented here.

Folding, wet/dry mass ratios and stomata

Decreased folding of leaves with altitude of source population was found at the initial harvest (Figure 6.5c). The degree of leaf folding did not change between the two harvests for the Durham plants, but showed a significant decrease in the other two cases, magnifying the differences between the Durham plants and the others.

Wet/dry mass ratios tended to increase between the harvests (Figure 6.5d). In both harvests the ratio was less for the Durham plants than the Alston ones. This might reflect the less "leafy" (lower SLA) nature of the Durham plants.

No differences in stomatal frequencies were found, despite some differences in the initial harvest (Figure 6.6a, c). All, however, showed significant reduction between harvests, except for the abaxial surface of the Durham plants. No differences were found in stomatal numbers per leaf surface between the source populations (initial harvest), except that the adaxial surface in the Durham population had more than that in the WBF population (Figure 6.6b, d). Only in the case of the Alston plants did these totals change significantly between the harvests: they showed large increases, and had significantly more stomata per leaf (both surfaces) than the Durham and WBF plants at the final harvest.

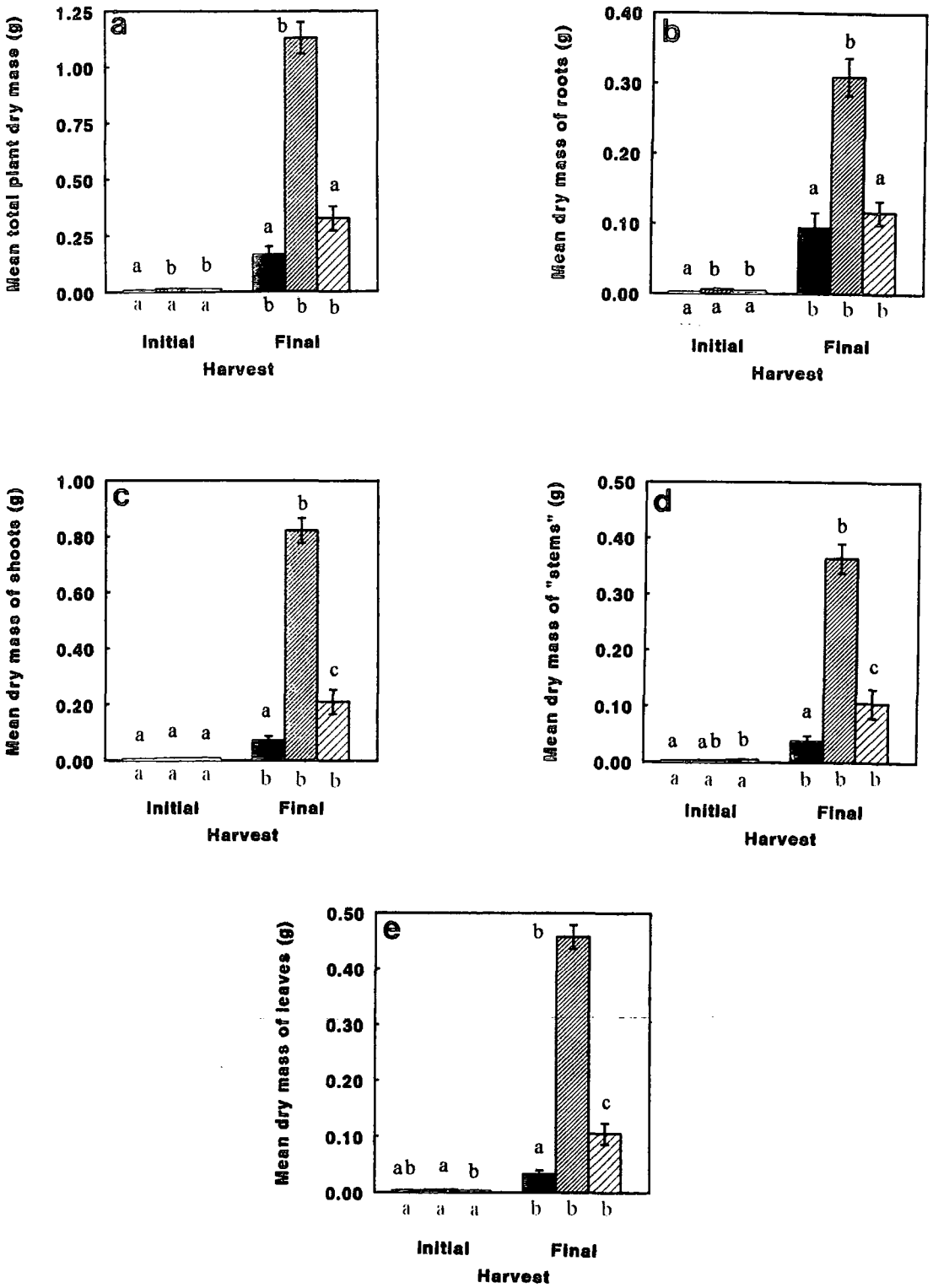


Figure 6.1. Dry mass measures of *Poa annua* taken from different altitudes and grown under the same conditions, with significance shown.

Dry masses of (a) whole plants, (b) roots, (c) shoots, (d) "stems", and (e) leaves of *Poa annua* taken from populations growing at Durham Botanic Gardens - 100 m ( ), Alston - 330 m ( ) and Widdybank Fell - 513 m ( ) and grown under the same conditions, in Durham Botanic Gardens. Data represent the mean ± one s.e. of five replicates per treatment. Significance: the letters above the graphs indicate differences between the populations of *Poa annua* within each harvest (from one-way ANOVA with Tukey's multiple comparison of means); the letters under the x-axis indicate differences between the first and final harvests for each treatment (from two-sample t-tests). No common letters indicate a significant difference (5% level).

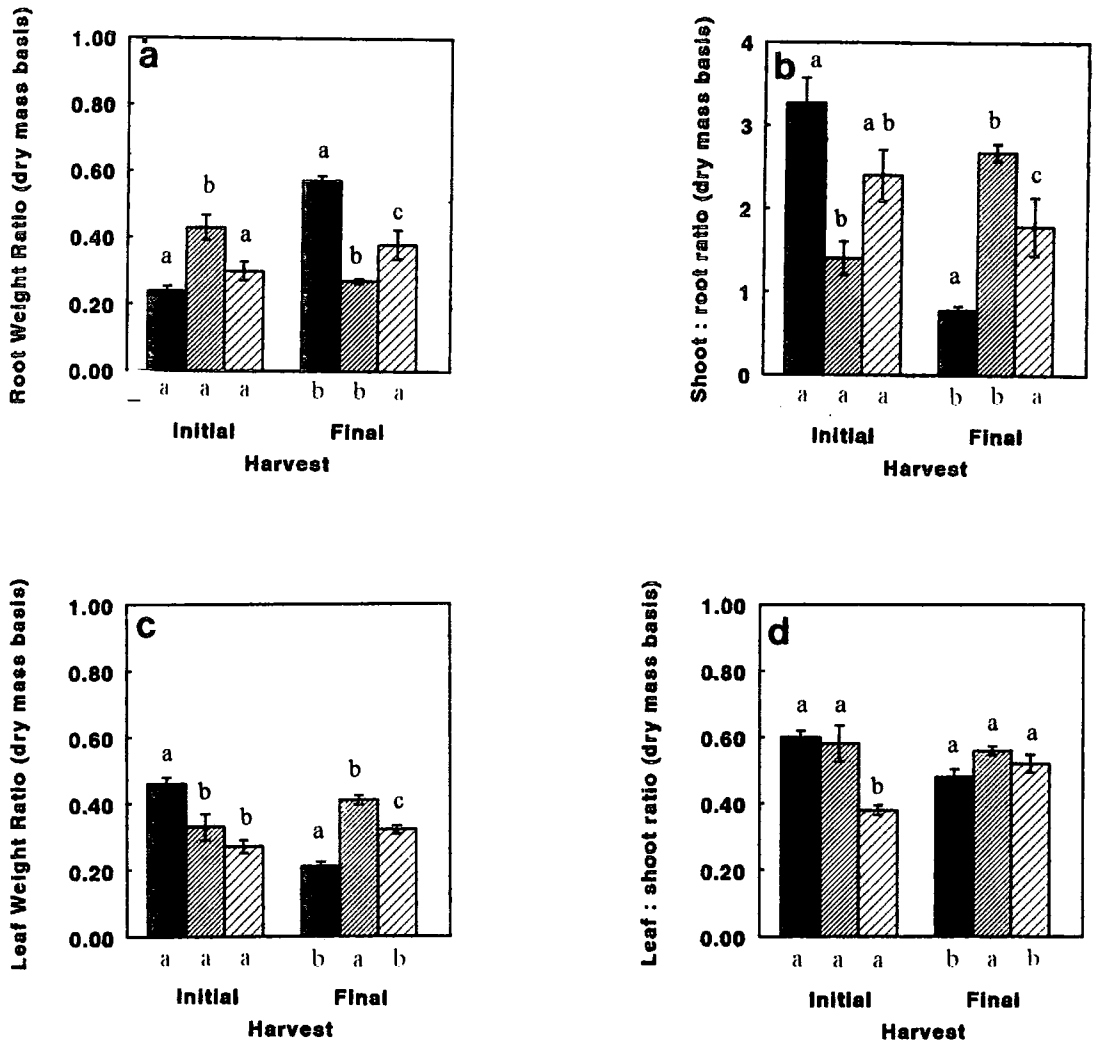


Figure 6.2. Dry matter partitioning in *Poa annua* taken from different altitudes and grown under the same conditions, with significance shown.

(a) RWR, (b) S:R, (c) LWR, and (d) L:S in *Poa annua* taken from populations growing at Durham Botanic Gardens - 100 m ( ), Alston - 330 m ( ) and Widdybank Fell - 513 m ( ) and grown under the same conditions, in Durham Botanic Gardens. Data represent the mean ± one s.e. of five replicates per treatment. Significance: the letters above the graphs indicate differences between the populations of *Poa annua* within each harvest (from one-way ANOVA with Tukey's multiple comparison of means); the letters under the x-axis indicate differences between the first and final harvests for each treatment (from two-sample t-tests). No common letters indicate a significant difference (5% level).

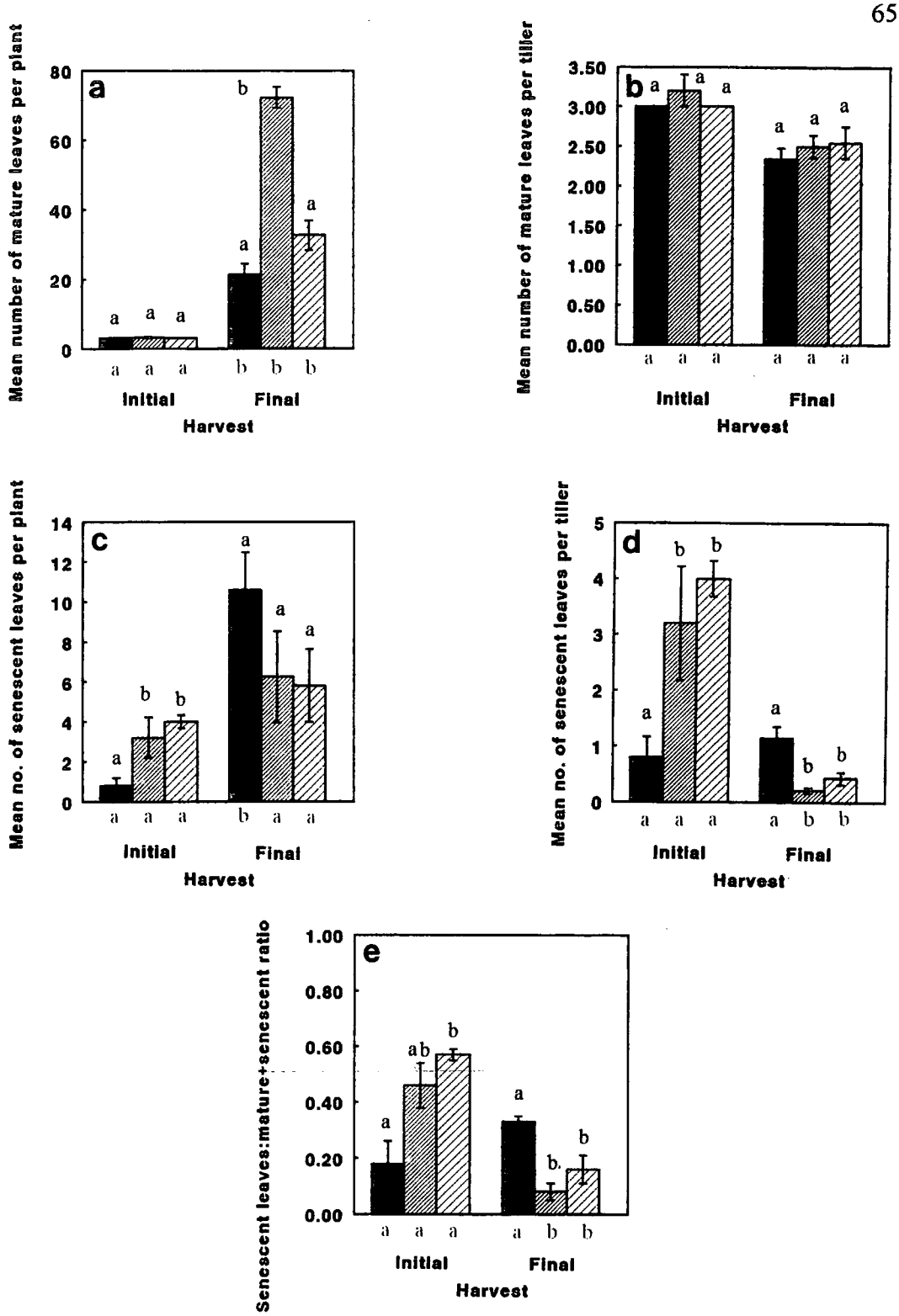


Figure 6.3. Numbers of leaves on *Poa annua* taken from different altitudes and grown under the same conditions, with significance shown.

Numbers of (a) mature leaves, (b) mature leaves per tiller, (c) senescent leaves, (d) senescent leaves per tiller, and (e) the ratio of senescent leaves to all mature and senescent leaves on *Poa annua* taken from populations growing at Durham Botanic Gardens - 100 m (■), Alston - 330 m (▨) and Widdybank Fell - 513 m (▨) and grown under the same conditions, in Durham Botanic Gardens.. Data in (a), (c) and (e) represent the mean ± one s.e of five replicates per treatment. Data in (b) and (d) represent the mean ± one s.e. of replicate numbers equal to the number of tillers (see Figure 6.4a). Significance: the letters above the graphs indicate differences between the populations of *Poa annua* within each harvest (from one-way ANOVA with Tukey's multiple comparison of means); the letters under the x-axis indicate differences between the first and final harvests for each treatment (from two-sample t-tests). No common letters indicate a significant difference (5% level).

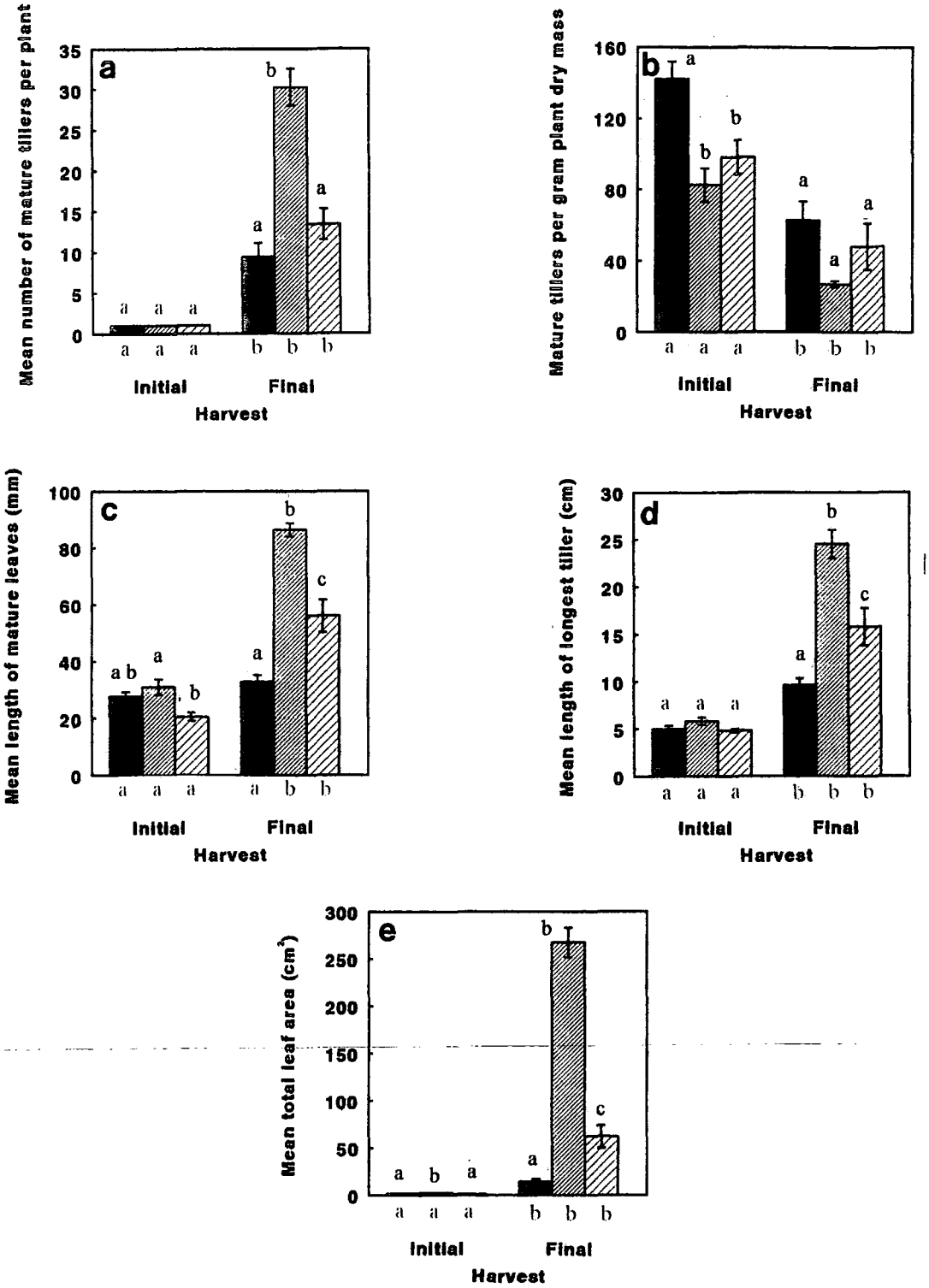


Figure 6.4. Numbers of tillers, leaf and longest tiller lengths and total leaf area of *Poa annua* taken from different altitudes and grown under the same conditions, with significance shown.

Numbers of (a) mature tillers, (b) tillers per gram dry mass; mean lengths of (c) mature leaves, (d) longest tiller; and (e) total leaf area of *Poa annua* taken from populations growing at Durham Botanic Gardens - 100 m (■), Alston - 330 m (▨) and Widdybank Fell - 513 m (▨) and grown under the same conditions, in Durham Botanic Gardens. Data represent the mean ± one s.e. of five replicates per treatment. Significance: the letters above the graphs indicate differences between the populations of *Poa annua* within each harvest (from one-way ANOVA with Tukey's multiple comparison of means); the letters under the x-axis indicate differences between the first and final harvests for each treatment (from two-sample t-tests). No common letters indicate a significant difference (5% level).

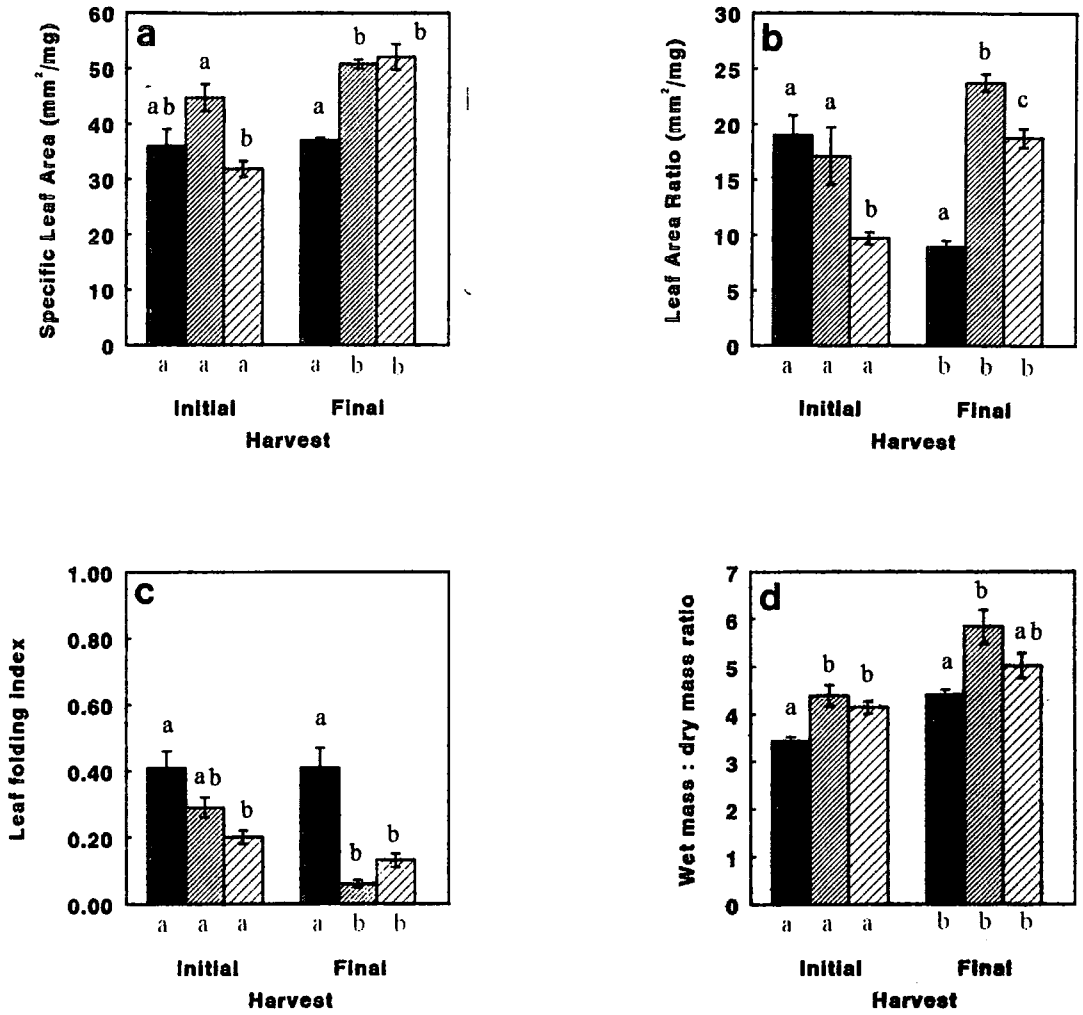


Figure 6.5. SLA, LAR, leaf folding and wet to dry mass ratio of *Poa annua* taken from different altitudes and grown under the same conditions, with significance shown.

(a) SLA, (b) LAR, (c) leaf folding index, and (d) wet to dry mass ratio of *Poa annua* taken from populations growing at Durham Botanic Gardens - 100 m ( ), Alston - 330 m ( ) and Widdybank Fell - 513 m ( ) and grown under the same conditions, in Durham Botanic Gardens. Data represent the mean ± one s.e. of five replicates per treatment. Significance: the letters above the graphs indicate differences between the populations of *Poa annua* within each harvest; the letters under the x-axis indicate differences between the first and final harvests for each treatment. No common letters indicate a significant difference (5% level).

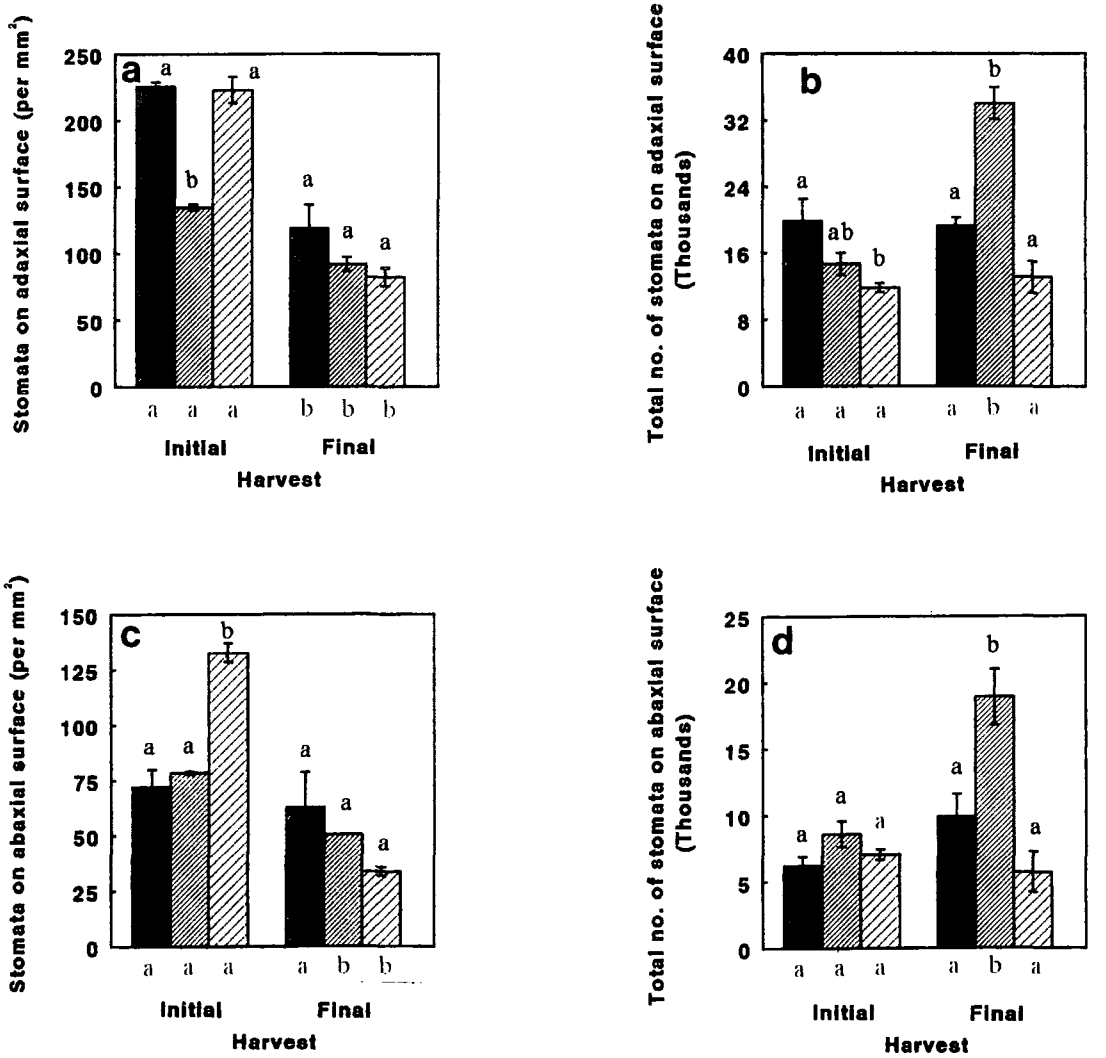


Figure 6.6. Stomatal measures of *Poa annua* taken from different altitudes and grown under the same conditions, with significance shown.

(a) adaxial stomatal density, (b) estimated number of stomata on the adaxial surface, (c) abaxial stomatal density, and (d) estimated number of stomata on the abaxial surface of *Poa annua* taken from populations growing at Durham Botanic Gardens - 100 m ( ), Alston - 330 m ( ) and Widdybank Fell - 513 m ( ) and grown under the same conditions, in Durham Botanic Gardens. Data represent the mean ± one s.e. of three replicates (leaves) per treatment. Significance: the letters above the graphs indicate differences between the populations of *Poa annua* within each harvest; the letters under the x-axis indicate differences between the first and final harvests for each treatment. No common letters indicate a significant difference (5% level).

Chapter 7. Discussion

This set of experiments, as is commonly the case in ecology, suffers from pseudoreplication (*sensu* Hurlbert, 1984). In other words, although there is randomisation within the plots used, the plots themselves are not randomised (this would involved multiplication of the numbers of plants to be sampled and analysed). Therefore only sub-sampling of areas is performed. From this one cannot conclude on statistical grounds that a factor or treatment such as temperature or altitude has had an absolute effect; only that the plots are different. As such, one can only infer subjectively that part or most of the effect may be due to the factor or treatment. The design of the light intensity experiment (Chapter 5) avoids this problem to some extent, sampling from five different compartments beneath the shading frame for each light regime.

Despite these limitations, inferences from the results found to be significant in this study are useful. First they are interesting in themselves. Second, if there is agreement with similar studies in the literature (studies on the effects of altitude or temperature on similar variables in grasses, for example), then the separate studies may approximate to replication which, though inevitably involving differences, get round the problem of pseudoreplication to some extent, and start to allow generalisations to be made.

In this chapter, the phenotypic plasticity experiments are discussed on their own and in relation both to altitude and to the literature. The population difference experiment is then considered.

7.1 Altitude experiment (Chapter 3)

Growth

Reduced growth with higher altitude may relate to reduced rates of various physiological processes with reduced temperature, which is generally recognised as a dominant factor associated with altitude in relation to plants (Friend and Woodward, 1990). But it may also reflect many other factors such as exposure, nutrient availability, light quality and intensity, *etc*. This is what this study was designed to investigate.

Dry matter partitioning

Though there may have been some degree of pot-binding, greater relative allocation to roots with altitude is a result which has been found many times in different species, and

often appears to have a genetic component with adaptive value (Woodward, 1979; Wardlaw *et al.*, 1983; Graves and Taylor, 1986; Körner and Renhardt, 1987). Reasons for this may include greater exposure to wind at higher elevation (reducing shoot allocation), or other factors such as temperature, nutrient status and light intensity (see Chapters 4 and 5). It has been suggested that greater allocation to roots, and morphologically-reduced shoots, with increased altitude improves nutrient relations (Chapin, 1980), reduces wind damage (Woodward, 1986), and increases plant temperature because leaves are nearer the warmer soil surface (Fitter and Hay, 1987). Wilson *et al.* (1987) have suggested that dwarfism in alpine plants can be a plastic response to the lower temperatures associated with increased altitude.

Water-stress induced investment in roots probably does not explain the observed increase in RWR at the higher sites because it was associated with an increase in rainfall (Appendix 3), though wind speed also increased (Appendix 3), which may increase water loss through evapotranspiration. Assimilate partitioning determines both the efficiency of substrate use by the plant and the degree of its productive investment in growth, which in turn influences subsequent photosynthetic and growth potential (Farrar and Williams, 1991). Thus the reduced allocation to shoots with altitude may partly explain the smaller total plant dry mass increases.

Tillers, inflorescences and leaves

The lack of an altitudinal trend in measures of leaf and tiller numbers (Figures 3.4a, b, 3.3a) is interesting in the light of the statement by Baxter *et al.* (1994a):

> "Increased shoot dry weight in grasses is usually associated with a significant increase in tiller production and numbers of leaves, associated with changes in morphology of the shoot."

Here the morphological component appears dominant, the reduction with altitude of shoot, leaf and stem dry masses probably being explained largely by greater leaf lengths and areas and greater tiller lengths at lower altitudes (Figure 3.5), rather than numbers of these structures. The increases in leaf area and length, LAR and tiller length at lower sites are in line with the results of other studies (*e.g.* Williams and Black, 1993), and may be associated with reduced leaf expansion at higher altitudes (Williams and Black, 1993).

Greater increases in dry mass (from similar starting points) were associated with higher values of SLA and LAR. Similar effects have been found in other studies. Baxter *et al.* (1994a), for example, found even short-lived changes in SLA and LAR to be major components of relative growth rate contributing to changes in the whole plant dry mass

of three grass species grown at different levels of atmospheric CO_2. Effects on photosynthesis of the reduced SLA and LAR are important. If light intensity within thicker leaves is sufficient not to hinder photosynthesis, then the photosynthetic potential may be more a function of leaf dry mass than of leaf area. Factors such as gas exchange potential (in which the role of stomata is crucial - Jones, 1992) and chlorophyll content per unit leaf mass then assume great importance. Williams and Black (1993) suggested that reduced leaf expansion at higher altitudes tends to be accompanied by increased mesophyll thickness (and therefore reduced SLA), and greater nitrogen contents per unit leaf area. Thus plants at higher altitudes can have greater photosynthetic capacity on a leaf area basis (Körner, Farquhar and Roksandic, 1988; Friend, Woodward and Switsur, 1989).

The reduced growth rates with altitude suggest that photosynthesis also declines with altitude in *Poa annua*, but may reflect greater efficiencies of use and incorporation of nutrients and/or photosynthetic products (though no relationship was found by Williams and Black, 1993, in nitrogen use efficiency between different temperature regimes). Poorter and Pothmann (1992) found that differences between slow- and fast-growing grass species were mainly due to the higher LAR of the faster-growing species. They measured photosynthetic rate, and found that it differed greatly when expressed on a leaf mass basis, but differed only slightly on a leaf area basis. This suggests that photosynthesis tends, at least in those species, to increase with SLA and LAR rather than with leaf volume. Garnier (1992) found relative growth rate to be significantly correlated with SLA, LAR and unit leaf rate (but not with dry mass allocation parameters), the single factor best explaining the RGR variation being SLA. The results of those studies appear to support the suggestion of reduced photosynthesis with altitude in this experiment, caused at least in part by reduced SLA, and with it LAR.

The later production of inflorescences with altitude, and the reduction in emerging inflorescences nearly to zero at the final DBG harvest, support the visual observations of leaf senescence at this time to suggest reduced development and maturation rates with altitude. These findings also fit in with the suggestion by St Omer and Horvath (1983) that increased leaf senescence may be linked to faster development and earlier production of inflorescences.

Folding, wet/dry mass ratios and stomata

The increase in leaf folding with altitude may indicate increased stress from greater exposure or reduced temperature at higher altitudes.

The lower wet/dry mass ratio at lower sites in the last harvests may reflect reduced water content in the senescent shoots and reproductive structures. Wet/dry mass ratio also decreased with development. A possible reason is an increased proportion of structural matter (included in "stems") with plant age and size. Tentative support for this conclusion, if one assumes a greater relative amount of structural material in perennials than annuals, comes from the finding by Garnier (1992) that the wet/dry mass ratio was higher for annuals than perennials for seven annual/perennial pairs of grass species (six congeneric and one random pair).

Increases in the total number of stomata per leaf with time may reflect the higher insertion of the leaves at later harvests. Tichà (1982), summarising observations from many studies, concluded that total stomatal numbers per leaf peak in mid-insertion leaves, which is compatible with the findings of the present study. However, Tichà (1982) also concluded that stomatal frequency increases with height of insertion, an effect not found here. The large increase in stomatal frequency with altitude can largely be explained by reduction in leaf length/area, especially in the abaxial surface, which showed no altitude effect. This agrees with the idea that stomatal numbers per leaf are under a genetic programme (Schoch, Zinsou and Sibi, 1980), the stomatal index varying little within any given species (Salisbury, 1927; Meidner and Mansfield, 1968). However, significantly fewer stomata per leaf were found with altitude on the adaxial surface, in which the reduced area of each leaf more than compensated for the increase in stomatal frequency. This suggests a plastic response to altitude on this surface, challenging the idea of genetic programming of leaf stomatal numbers. But it may instead result from hindered stomatal cell differentiation at higher altitudes.

Overview

With altitude there were smaller changes in morphology as well as smaller increases in dry mass. The greater investment of dry matter in leaves per unit leaf area (*i.e.* reduced SLA) with altitude agrees with the findings of Williams and Black (1993). Exposure may be one reason: wind speeds increased with altitude ($P < 0.001$, Table 2.2; Appendix 3), and large thin leaves are likely to increase water loss compared with small thick ones. It may be hypothesised that the leaves growing at higher altitudes have thicker cuticles for greater protection from exposure. This would have been important in the warm, dry weather experienced during the study period. The folding results back this up: increased folding (found at the higher sites) serves to decrease the area of leaf exposed, affecting the adaxial surface on which more stomata are found. Relative humidity at this surface may be increased by the pockets of air created, further reducing water loss.

It is unclear how permanent are the variations found here. Baxter *et al.* (1994), working on three montane grass species, found that several of the observed changes with doubling of ambient CO_2 (*e.g.* SLA, LAR and LWR) were transient. It could be that some of the variation found in the current study was also of a temporary nature, possibly resulting from the effects of transplantation of the grass. However, 13 weeks is a relatively long time for a short-lived annual, and the DBG plants seemed to have achieved their full lifespan. In this context, any changes still observed after 9 or 13 weeks may be regarded as permanent.

7.2 Temperature and nutrient experiment (Chapter 4)

Comparability with the altitude experiment

The main assumption being made is that temperature decreases with altitude, which follows from the effects of adiabatic lapse rates. However the intricacies of topography and weather systems can invalidate simple altitude-temperature relationships, and to confirm the validity of the assumption the temperature measures (Appendix 3) recorded for the sites used in the altitude experiment were compared. The inverse temperature-altitude relationship was found to hold for all measures of temperature (Table 2.2).

Comparison between this experiment and the altitude one is limited by the differences in temperature regime, and the different conditions for growth (in terms of rooting substrates, light regimes, *etc.*). Although mean daily maximum temperatures in the altitude experiment (Appendix 3) were of the same order as in this one, the growth room temperatures were constant, while those in the field varied both on an inter-day and an intra-day (diurnal rhythm) basis (Appendix 3).

It is unlikely that temperature works in isolation from other environmental factors; it probably acts in combination with other variables to affect physiological processes within the plant. Several other experiments have found that interactions between various environmental factors are important in determining growth and assimilate partitioning. These include interactions between nutrient availability and CO_2 level (*e.g.* Tissue and Oechel, 1987; Cure, Israel and Rufty, 1988; Bowler and Press, 1993; Hunt *et al.*, 1995), and between temperature and CO_2 level (*e.g.* Ackerly *et al.*, 1992). Therefore any conclusions drawn, when comparing the effects of contrasting temperature regimes in a controlled, artificial environment with the effects of contrasting temperature regimes in the field, must be tentative.

Because the altitude experiment plants were growing in nutrient-rich compost it is assumed that nutrient limitation does not apply to that experiment. Therefore nutrient effects found in this investigation should not explain any of the variation with altitude in Chapter 3. But it is instructive to establish any apparent nutrient effects when considering "real" conditions in the field.

Discussion

Growth

Because dry mass increases were linear when plants were subjected to N+P limitation and exponential at higher concentrations, the exponential growth observed in the altitude experiment backs up the assumption that those plants were not nutrient limited. The temperature effect observed in the first 3 weeks of the current experiment suggests that at least part of the altitudinal variation in growth in Chapter 3 was due to temperature. Reduced growth at lower temperature was also found by Williams and Black (1993), who suggested (after Berry and Raison, 1981) that the causes included the combined effects of low temperature on metabolism, cell division, meristem growth, leaf development and soil nutrient uptake.

Reduced growth, photosynthesis and respiration rates are often found at lower nutrient levels (*e.g.* Poorter *et al*, 1995), but the interactions between nutrient availability and processes leading to plant growth are complex (Baxter *et al.*, 1994b). Carbohydrate status, for instance, interacts with other nutrient factors such as nitrogen availability (Baxter *et al.*, 1995), in affecting photosynthesis and nutrient assimilation and allocation (Kaiser and Forster, 1989). Maximum photosynthetic capacity and leaf nitrogen concentrations are linked for many species (Morita and Kono, 1974; Field and Mooney, 1986), because 70-75% of leaf nitrogen tends to be chloroplastic (Baxter *et al.*, 1995). No measurements of leaf nutrient status were undertaken in this experiment, and it is therefore difficult to draw conclusions about nutrient effects on growth. However, only nitrate-phosphate availability was varied, and it seems plausible that when this was limiting, reduction in photosynthesis resulted. If low availability of N+P was associated with low levels of leaf nitrogen concentration, then leaf photosynthesis may have been reduced because of lower leaf protein contents, including Rubisco (Baxter *et al.*, 1995).

Dry matter partitioning

Reduced allocation of dry matter to shoot relative to root at lower temperature has also been found in many other studies (*e.g.* Williams and Black, 1993). It appears to go some way towards explaining the altitude effect in Chapter 3. A similar nutrient effect was observed ($P < 0.001$). Nutrient availability is known to change source to

sink balances in tissue allocation (Wong, 1979; Farrar and Williams, 1991), which may be important here.

The nutrient effect also agrees with the literature: Bushby, Vallis and Myers (1992) concluded that the C_4 grass *Panicum maximum* responds to low availability of soil nitrogen by allocating a large proportion of their resources below-ground to maximise soil exploration. A similar result was obtained by Belanger, Gastal and Warembourg (1994), who found that N deficiency in *Festuca arundinacea* was associated with reduced allocation of carbon to shoots, resulting in decreased shoot growth. Increased RWR with reduction of N availability (below optimality) has also been found, in various grasses and other plants, by Aerts, Boot and Van der Aart (1991), Smolders and Merckx (1992), Van de Vijver *et al.* (1993), Bowler and Press (1993). Li and Redmann (1992), found the same when nitrogen was supplied as NH_4 but no effect when supplied as NO_3. All these results fit in with the "balanced growth hypothesis" of Thornley (1972a, b), which postulates that the plant allocates greater resources to the part which absorbs the most limiting resource at the time.

The increase in S:R with plant age at non-limiting N+P concentration agrees with the same trend noted by Wilson (1988) for herbaceous plants in general.

Tillers, inflorescences and leaves

The trends in numbers of inflorescences suggest both hindered development in the low nutrient regime, and faster development at the higher temperature when nutrients were abundant. It is possible, though, that the large investment in reproductive structure in the high temperature high nutrient regime may have been a response to the stress of the rust.

The rust may also have reduced the number of leaves and tillers produced in the last three weeks of the experiment. However, Williams and Black (1993), who found a greater number of tillers in the higher temperature treatment than the lower after 3 weeks in *Pennisetum setaceum*, also found fewer after 6 weeks. This suggests that ontogenetic effects may have been important in both studies.

In the altitude experiment, factors other than temperature (and nutrients) may have been more important in determining tiller numbers. It is possible that exposure reduced the lengths of leaves and heights of crowns at higher altitude and caused greater allocation of plant resources to tiller production: greater tiller production per gram dry mass was found with altitude ($P < 0.01$; Figure 4.3b).

Leaf and longest tiller length measures for the low temperature high nutrient treatment reach approximately the same maxima as those for DBG in the altitude experiment (Figure 3.5a, b). Again this may support the suggestion that leaf lengths at the higher-altitude sites were kept low by exposure to wind (neither DBG nor the growth rooms experienced much wind), an effect which may outweigh the apparent length reductions with temperature found here.

Williams and Black (1993) and Ackerly *et al.* (1992) found increased leaf area with temperature, an effect not found here. The reason for this difference may be related to the species, or to experimental conditions, but may also be ontogenetic, with faster development and/or greater allocation to reproduction in the higher temperature treatment. Greater inflorescence production at the higher temperature (Figure 4.3c) may have been important: leaf lengths always increased with insertion, before decreasing again towards an inflorescence. This may also explain the reduction in total leaf area, while leaf number increased, between the second and final harvests in the high temperature high nutrient treatment. Leaf area concerned only living leaves, and leaf turnover was observed to be high throughout the experiments(see Section 2.4). Many senescent leaves were present in this treatment at the final harvest (Figure 4.4c), and if these were on average much larger than the greater number of new leaves replacing them, the decrease in total leaf area can be explained. Experimental error due to the necessity for destructive sampling, and therefore the use of different plants at each harvest, may also have been a reason.

The greater low temperature treatment values of LAR and LWR, as well as SLA (which contrasts with the findings of Wardlaw *et al.*, 1983, and Williams and Black, 1993) may be associated with the greater allocation of dry matter to stems and reproductive organs (included in "stems") in the high temperature treatment (Figure 4.2d). In the altitude experiment increases in SLA and LAR were both associated with warmer temperatures (lower altitudes), the opposite effect to that found here. This suggests that a factor like exposure may be more important in determining SLA in that experiment. That would agree with the findings of Delucia, Heckathorn and Day (1992) that increased soil (only) temperature, up to 25°C, was associated with higher rates of growth in the grass *Andropogon gerardii*, but had no effect on LAR, and that the effect of soil temperature on growth was primarily through its influence on unit leaf rate. In both this and the altitude experiment, LAR decreased with time, which may reflect the increased proportion of structural and reproductive matter (Figure 4.2d).

Folding, wet/dry mass ratios and stomata

The temperature effect on folding may partly explain the increased folding with elevation in the altitude experiment. It is interesting that the rust appeared not to affect this variable.

The nutrient effect on stomatal frequency indicates higher density of stomata per unit area on the smaller leaves. But greater total numbers of stomata were found on the larger leaves of the high N+P treatment at both temperatures. Again this could represent a plastic response to low N+P availability, or could reflect hindered stomatal cell production at the lower nutrient status.

Overview

Temperature and nutrient status appear to work in combination, the most striking aspect of which, in this investigation, concerned the dry mass variables: in almost every case, no temperature effect was found at low N+P, but a marked one found without that limitation. The importance of providing sufficient nutrients in investigations of the effects of temperature is therefore stressed. Nutrient availability and other environmental factors may interact in very different ways; for example Bowler and Press (1993) found a proportionally greater increase in total plant dry mass with increased CO_2 at low N than at high N.

Differences between the treatments in terms of morphological measures and dry matter partitioning were less clear-cut. It is therefore suggested that the relationships found between those variables and altitude were less related to temperature than were increases in dry matter.

7.3 Light intensity experiment (Chapter 5)

Comparability with the altitude experiment

The comparisons between this and the altitude experiment are less obvious than for the temperature and nutrient investigation. No single overriding factor like adiabatic lapse rates applies to comparisons between altitude and light intensity. Several factors may be important. Cloud cover is often greater at higher elevation, largely because of orographic effects, and would particularly apply to the high ridge of which GDF is a part. Local knowledge backs this up: people from the area say that cloud over GDF is the norm. Countering the cloud cover effect may be the influence of the atmosphere, which is thinner at higher elevation, and may therefore absorb and scatter less incoming photosynthetically active radiation (PAR), tending to increase PAR intensity (as well as

UV) with altitude. Such an effect is probably minor, though, over altitudinal differences of the order of 700 metres.

Light intensity was not measured at the different sites used in the altitude experiment, because differences would be swamped by the large temporal heterogeneity characteristic of this variable. The method of quantification used, in the absence of light meters connected to dataloggers, was the number of sunshine hours, which gives an indication of cloud cover. Unfortunately these data were not available for GDF, but were collected for the other two sites (Appendix 3). No significant difference was found (Table 2.2). It may be, however, that the light intensity in the altitude experiment was relatively low at DBG, because of shading from nearby trees and a shed, and green netting overhead (Plate 2.1). There was also considerable shading by surrounding plants at WBF towards the end of the altitude experiment (Plate 2.5). This makes the tasks of quantifying light differences between the sites, and of comparing between the two experiments, even more difficult. However, one advantage is that most of the other environmental variables were quite consistent between the light intensity and altitude experiments, because both were outdoors and the former was only five metres from the DBG altitude site. Thus the most valid comparisons which can be made are those with the DBG results. There were still differences, though, particularly that the DBG site was to the north of the shed (shaded from the midday and afternoon sun), while the light intensity experiment was to the south, fully exposed to the midday and afternoon sun.

Discussion

Error bars are generally larger in this experiment than the others because there were differences in light intensity from one end of the shading frame to the other, caused by shading from nearby trees and a shed (Plate 2.1).

Growth

The large increases in dry mass measures with light intensity agree with similar findings in several studies (*e.g.* Jeangros and Noseberger, 1992). The parsimonious explanation is that photosynthesis increased with light intensity, which suggests that, at least during significant portions of the day, the plants were not experiencing light saturation.

Dry matter partitioning

Greater relative dry matter allocation to roots with higher light intensity fits in with the balanced growth hypothesis (Thornley, 1972a, b - see Section 7.2). If leaves can photosynthesise more efficiently per unit leaf mass or area (as would be expected at higher light intensities) then water and nutrient uptake become more limiting than light.

According to the hypothesis, this would cause greater relative allocation to the part of the plant absorbing these resources: the roots.

Other variables

Few differences were found between the treatments in terms of the other variables measured, suggesting that the main effect of light intensity was on growth rates and dry matter partitioning. Notable exceptions were increases with light intensity in numbers of tillers per plant (despite significantly fewer tillers per gram dry mass), and in total leaf area. Numbers of mature leaves and mature tillers per plant were probably functions of tiller numbers, because neither varied significantly on a per tiller basis. Increased total leaf area with light intensity was also found by Kubínová (1991) in *Hordeum vulgare* (barley), but the opposite was reported by Knecht and O'Leary (1972) for *Phaseolus vulgaris* and Rahim and Fordham (1991) for *Allium sativum* (garlic).

The measured light transmission values may represent upper limits to the differences in light intensity between the treatments. Light tended to disperse between the compartments below the frame, especially in late afternoon when the angle of the sun was low. Thus the variation in light intensity was probably smaller than that used in other experiments (*e.g.* Knecht and O'Leary, 1972; Dale, 1982; Rahim and Fordham, 1991; Kubínová, 1991; Schmitt, 1993). However, significant differences were found for some variables despite this, and it may be that greater light differentials would have produced significant differences in variables such as stomatal measures (as in Friend and Pomeroy, 1970; Dale, Felippe and Fletcher, 1972; Knecht and O'Leary, 1972; Gay and Hurd, 1975; Lichtenthaler, 1985; Rahim and Fordham, 1991).

There are also implications for self-shading within plants: the current study's results are compatible with those of Golovko & Lavrinenko (1994), who studied the effects of stand density in annual ryegrass, and found a 15-25% reduction in effectiveness of crop growth in closed canopies compared with open canopies.

Overview

Overall these and the other measures seem to provide results which have little or no consistency of comparison with those of the altitude experiment. Trends with high light intensity sometimes mirror those with increasing altitude (*e.g.* for shoot to root dry matter partitioning, while for other variables (*e.g.* growth measures, wet/dry mass ratios) it is the other way round. In many cases, significant differences for a given variable are found in one of the experiments but not the other (*e.g.* numbers of tillers

and leaves; and mean leaf and longest tiller lengths). This suggests that the effects of light intensity explain little (if any) of the variation in the altitude experiment. Such a conclusion is not surprising as there was no evidence for significant differences between those sites in terms of light intensity, and little reason to suggest *a priori* that large differences exist. However, it is useful to have established some of the trends in growth, morphology and physiology associated with light intensity, and to have established that this factor was not of major importance in the altitudinal variations being investigated.

7.4 Collation of the results from the phenotypic plasticity experiments

The null hypothesis was rejected in each case, indicating that each of the environmental factors measured had significant effects on some or all of the variables measured.

1. Can any of the altitudinal variations be attributed to any of the environmental factors investigated?

Temperature (without N+P limitation) seems to explain much of the altitudinal variation in growth measures and root to shoot allocation, but still leaves a lot unexplained, especially variation in morphological measures and above-ground dry matter partitioning. The main effect of temperature may therefore be on the rate of operation of many physiological processes. This analysis would fit in with the findings of other researchers that altitudinal changes in temperature affect many physiological processes to the extent that population differentiation may result (Berry and Björkman, 1980; Friend and Woodward, 1990). For example, reduced plant growth and stature may be associated with higher altitudes because of the inhibitory effects of lower temperatures on leaf extension and expansion (Woodward, 1979; Graves and Taylor, 1986, 1988; Körner and Woodward, 1987; Woodward and Friend, 1988).

2. Can any of the environmental factors tested definitely be rejected as being important in explaining the altitudinal variations found?

Nutrient availability was standardised in the altitude experiment, and was therefore not important in this context. It may, however, be influential with altitude in field situations (Friend and Woodward, 1990). This would require further investigation. Light intensity appears to have had little influence on the altitudinal results, though as discussed in the previous section, it was difficult to quantify at the three sites.

3. Are there any environmental factors not studied which are likely to be important in explaining the variations found with altitude?

Exposure is probably important, especially in determining morphological and physiological aspects of the leaves, and dry matter partitioning within the plant. Wind speed (at 10 a.m.) was recorded at the three sites, and found to increase with altitude ($P < 0.001$, Table 2.2; Appendix 3). If wind speed correlates with exposure, then one could postulate that it may be related to some of the trends found with altitude, such as decreased leaf and tiller lengths, reduced whole plant growth, reduced relative investment in leaves, greater leaf folding and greater density of stomata on leaf surfaces. Note that the wind speed at the sheltered DBG site was probably less than that recorded at the Observatory.

The effects of exposure require further study. Those of relative humidity and water stress may also affect plant growth, physiology and morphology, but in general are probably less well-correlated with altitude, though relative humidity was found to be significantly less at Durham than the higher-altitude sites (Table 2.2).

4. Was there anything common to all the experiments?

The number of leaves per tiller varied little. This may reflect the way *Poa annua* grows, and the balance between new leaf production and old leaf senescence on any given tiller. Growth seemed primarily to be associated with increased leaf (and tiller) length, and with increased tiller numbers, which were responsible for increases in leaf numbers.

Many environmental factors and their relationships with altitude are very site-dependent. For instance, while exposure tends to increase with elevation, it happens that in this study the two higher-altitude sites were very exposed fells, while the low elevation site was a very sheltered spot. Thus the differences are amplified. Similarly, the degree of shading in DBG was probably affected by local conditions, as discussed in Section 7.3.

7.5 Population difference experiment (Chapter 6)

Growth and dry matter partitioning

The measures of dry mass increase suggest that the Alston plants were the most vigorous under these conditions. The Durham plants invested the most (relatively) in roots and the Alston plants the least. This suggests that the Alston plants may have

been the most suited to the new conditions, and the Durham ones the least, or may simply reflect different growth habits of the populations. For plants growing on the edge of a regularly-mown lawn (the Durham source population), relatively high investment in roots would probably be advantageous. It could also reflect nutrient availability to the source populations, if a degree of local adaptation is assumed: Elberse and Berendse (1993), studying eight grass species from habitats of contrasting soil fertility growing in controlled conditions, found that species from nutrient-poor habitats allocated less dry matter to the roots than the species from nutrient-rich conditions. It is likely that nutrient availability to the Alston source population was less than that to the Durham one, though no measurements were taken.

Tillers and leaves

Mean leaf length increased between the harvests in the plants from Alston and WBF. This is to be expected when, as here, plants are removed from relatively adverse conditions, and allowed to grow in good soil, free from competition. The Durham plants, however, showed no increase in mean leaf length. This and the relatively high number of senescent leaves per tiller may suggest relatively low suitability of these plants to the new conditions, and/or a different growth habit, but it may also reflect greater responsiveness of the Alston plants and (to a lesser extent) the Widdybank ones, to changes in conditions. The degree of response may be a function of the magnitude of change in growing conditions. It was not possible to test this hypothesis with the data collected.

The only major difference found between different-altitude genotypes of *Pennisetum setaceum*, grown under uniform conditions, was in total leaf area: highest in the high-altitude genotype (Williams and Black, 1993). In the current study, too, significant differences in total leaf area were found between the plants from the different-altitude populations at the final harvest, but the highest values were for the mid-altitude (Alston) plants. This suggests that factors other than altitude of origin were more important in determining total leaf area.

The leaf length, SLA, L:S and LWR results fit in with the idea of adaptation of the Durham population to a regime of frequent mowing, in which investment of dry matter in large, thin leaves would be disadvantageous in comparison with smaller, thicker leaves. At the initial harvest, SLA for the WBF population was lower than for Alston. Grazing at WBF may partly explain this - there is a high density of sheep there, which suggests that, there as well, short, thick leaves may be more suitable than large, thin ones. Both leaf length and SLA increased for the WBF plants when transplanted into conditions where the stress of removal of above-ground biomass was no longer

present, but remained constant for the Durham plants. This suggests greater responsiveness and plasticity of the WBF plants than the Durham population. The conditions in the car park at Alston may have favoured short but thin leaves: there was little or no change in SLA but a large increase in length between the first and last harvests. One could therefore postulate that removal of leaves (*e.g.* by grazing) was less of a problem at Alston than at the locations of the other two source populations. This could explain the apparently high responsiveness of these plants: with improved growing conditions, benefits from rapid increase in leaf length and area are less likely to be lost via removal of the new leaf tissue at the next cut or graze.

Folding, wet/dry mass ratios and stomata

The decrease in leaf folding with altitude of origin at the initial harvest probably relates more to differences between the populations or the specific growing conditions than to altitude itself, as the opposite relationship was found in Chapter 3. The fact that the degree of folding did not change between the two harvests for the Durham plants but did for the others suggests that the differences may have been a function of the magnitude of change in atmospheric growing conditions. At the final harvest, the same pattern of response was found as for other variables: the plants from WBF having values in between the other two populations. This again suggests the order of responsiveness: Alston plants > WBF > Durham.

The general trend of increasing wet/dry mass ratios between the first and final harvests may reflect the watering regimes: some water stress was probably experienced by the wild plants of all three sites in the warm, dry summer of 1995, but the watering regime of the transplants ensured mesic conditions.

The reduction in stomatal frequency between harvests in the WBF and Alston plants and the lack of change for the abaxial surface of the Durham plants may be explained by increasing and unchanging leaf lengths respectively. The reduction for the adaxial surface of the Durham plants may indicate a plastic response, though mean leaf length did actually increase slightly. This increase was not significant given the replicate number and degree of change relative to the variability, but may have been biologically meaningful in that it could have caused the change in one leaf surface, but not the other, to come out as significant in the t-tests. The lack of change between harvests in numbers of stomata per leaf in the Durham and WBF plants agrees with the idea of genetic predetermination of leaf stomatal numbers (Schoch *et al.*, 1980). The large increase for the Alston plants, however, suggests a plastic response, though it is possible that this merely represents release from conditions in which stomatal differentiation was hindered - perhaps by nutrient status, as suggested in Chapter 4.

Overview

Why should the Durham plants be less suited to apparently good growing conditions only about 15 metres from the source population, than plants from higher altitude, when the other source populations were located within the range of dispersal (by wind) of this species, with no obvious intervening barriers? It could be that the relevant seed just happened to arrive and establish first where the source population was, and once established, excluded apparently "fitter" plants (or different species). Or it may be that the particular conditions in which the Durham source population was located were highly suited to the characteristics of its plants - senescence levels at the initial harvest were, after all, low relative to the other populations. The growth habit of relatively short leaves and high investment in roots, as well as low responsiveness to improved growing conditions (in the above-ground parts of the plant), would be advantageous on the edge of a lawn subject to both grazing and mowing, as discussed above.

Significant differences were found between the plants according to population of origin for most of the variables measured. This suggests genotypic differences, with the Alston plants being the most vigorous and responsive to change under these conditions, and the Durham population the least. It may be that the Alston plants were of a more vigorous, "fitter" genotype than the others. In terms of local adaptation this could be interpreted as arising from the conditions under which the source populations were growing. Because, in Chapter 3, altitude appeared to be associated with decreased growth, increasing elevation may be considered as a stress constraining growth, suggesting that the greatest stresses were experienced by the WBF population, followed by the Alston and Durham populations respectively. However, the WBF population was growing in peat in a relatively sheltered location by the road, which may be regarded as relatively good growing conditions. The Alston population was growing in thick gravel in the middle of a car park, where high levels of disturbance, water and nutrient stresses were likely. The Durham population was growing mainly in gravel, but with loamy soil from the adjacent lawn also present; these conditions may be considered reasonably good. When both atmospheric (altitudinal) and rooting conditions are taken into account, a subjective ranking would therefore suggest greatest stress on the Alston source population and least on the Durham one. This would fit with the "fitness of genotypes" hypothesis. No analyses of nutrient or water conditions were undertaken on the source population to allow more objective ranking. The effects of grass cuttings routinely left on the lawn after mowing were unknown, but would only have affected the Durham source population. The adaptationist arguments of this paragraph can be fitted in with those used in relation to grazing and mowing regimes in the discussions above.

However, it is tempting to try to think of adaptive explanations for any set of results, and one risks predetermination of conclusions. As Gould and Lewontin (1979) put it "the range of adaptive stories is as wide as our minds are fertile ... plausible stories can always be told." An alternative hypothesis is that the growth results are simply an extension of phenotypic plasticity. It could be that the same genotype, when highly stressed over a period of time, displays more vigorous growth and greater responsiveness when suddenly released from these stresses and placed in good growing conditions, than when it has been growing in less stressful conditions. The same arguments about levels of stress would apply as in the local adaptation hypothesis. In other words, the apparent genotypic differences could be a result of the peculiarities of the experiment. Many of the arguments put forward so far focus on postulated adaptive traits, and therefore assume a degree of local adaptation (and therefore different genotypes). If this alternative hypothesis of extended phenotypic plasticity is correct, then such explanations are invalid.

A third hypothesis is that the genotypes are different, but that their differences are purely chance and have no adaptive significance. In this case one would have either to assume no major differences in "fitness" between the genotypes, or to explain why less "fit" plants were growing well within dispersal range of the more "fit" plants. As suggested above, the relevant seed may just have happened to arrive and establish first, and thereafter excluded apparently "fitter" individuals (and other species).

An objective method of differentiating between genotypes, such as PCR, would be useful in trying to determine which of these hypotheses is more likely to be valid.

7.6 Ontogeny

Changes in the magnitude of variables such as those measured here have been found in many studies to vary significantly according to stage of plant development (*e.g.* Cure *et al.*, 1988; Garnier, 1992; Pettersson, 1993; Bowler & Press, 1993; Golovko & Lavrinenko, 1994; Hunt *et al.*, 1995). Eamus and Jarvis (1989) suggested that the main effect of increased atmospheric CO_2 is on growth, with observed changes in dry mass allocation merely reflecting changed development times. This seems unlikely in the current study, as most of the variables showed no sign of sequential changes between the sites.

One way of dealing with this problem is to use the allometric growth coefficient, K, in analysis of the data. This represents the ratio of the logarithms of root and shoot growth rates, and is a powerful tool for dealing with ontogenetic effects (Pearsall, 1927; Brouwer, 1983). However, as explained above, the root dry mass values obtained for the first harvests have little biological meaning. Also the constant K does not hold when, as here, inflorescences are produced (Troughton, 1956, 1960). Therefore the use of K is precluded. Instead, an analogy has been drawn, in this study, between the effects of ontogeny and the interaction between time and tested environmental factors. These interactions have been quantified and assigned significance values by two-way and three-way ANOVA tests. In addition, the leaves used for epidermal peels, SLA calculation and preservation in FAA, were determined according to a plastochron index (Erickson and Michelini, 1957), to try to standardise for the effects of ontogeny between the different treatments (Section 2.4).

It may be that ontogenetic effects are less of a problem than is sometimes considered. There is some evidence that differences in many of the variables of interest persist during development, as Poorter and Pothmann (1992) found. In a study of selected grass species they concluded that, although most measured variables showed some ontogenetic drift, differences found for young seedlings persisted at least until plants reached a dry weight of about 3 grams.

Chapter 8. Conclusions

In all the experiments undertaken, significant differences were found between the treatments in at least some of the variables measured, and therefore the null hypothesis was rejected in each case. It has not been proved that the plants used in the phenotypic plasticity experiments were genetically uniform, or that the plants in the population difference experiment were genetically different. However, the results suggest that at least some specimens of *Poa annua* growing in County Durham show considerable phenotypic plasticity with respect to altitudinal variations, and to differences in temperature, nutrient availability and light intensity. The degree of plasticity appears to vary according to the population from which the stock is taken, which suggests that there is genetic variation in the *Poa annua* of the area. Whether the genetic variation is adaptive, neutral, or even counter-adaptation arising by chance is unclear, though (as ever) adaptive explanations can be thought up to explain the results.

8.1 Improvements

Improvements not feasible with the time and resources available

* Use of objective methods of determining genetic differences.
* Multiple replication of the entire experiments, to avoid problems of pseudoreplication.
* Transplanting of source populations to identical rooting conditions for long enough to equilibrate, prior to transplanting from each source population to each site in the population difference experiment.
* Frequent sampling throughout the entire lifespans of the plants concerned. This would allow more detailed assessment of development and the effects of ontogeny. It would, however, be difficult to standardise for different weather conditions during the equivalent stages of plant development in the experiments conducted outdoors.
* Use of advanced statistical techniques, and packages such as Genstat and SAS.

More feasible improvements

* A greater number of replicates per treatment, standardised across all the experiments. This would have to be coupled with sampling techniques to reduce the time taken in analysis, *e.g.* random selections of leaves for estimation of total leaf area, mean leaf length, and mean leaf number per tiller, rather than measuring all the leaves on every plant (up to 400). Such sampling and estimation procedures were used by Williams and Black (1993).

* Analysis of plants when they reached certain pre-determined sizes (c.f. Poorter and Pothmann, 1992), rather than harvesting at a pre-determined time, to account for ontogenetic effects. Analyses of growth rates would not be precluded if the dates of harvesting were always recorded. The time method is more common in the literature, but can usually be combined with the use of the allometric growth coefficient K.

8.2 Suggestions for further investigation

* Investigation of other environmental factors related to altitude. Not all the variability in Chapter 3 has been explained by the factors tested in Chapters 4 and 5. Differences in exposure to wind and water availability may be important. So may variations in light quality, such as UV content and red/far red ratio, which is thought to affect leaf growth and stomatal differentiation (Mitchell and Woodward, 1988). The factors considered here could also be investigated in different ways, *e.g.* the effects of diurnal variations or random fluctuations in temperature, or of variations in nutrient combinations other than N+P; or of changes in the photoperiod of light. Different combinations of factors might reveal interesting interactions.

* Analyses of changes in leaf internal structure with changes in altitude and the other factors investigated. This might itself produce interesting results, and might elucidate some of the questions raised in this study, such as whether the reductions in SLA with altitude are related to leaf thickness or other factors like more dense internal leaf structure. The leaves preserved in FAA were taken for this purpose, and would be available from the author had they not been destroyed without his knowledge or consent in a laboratory clearout.

* Measurement of chlorophyll levels, nutrient content of leaves and rates of photosynthesis, to determine whether decreased growth rates of *Poa annua* with altitude reflect greater photosynthesis, or other factors such as nutrient use efficiency.

* Investigation of rates of operation of plant physiological processes in *Poa annua* in relation to temperature, to determine whether this is the main mechanism by which temperature affects growth rates (and other variables). One possibility is nutrient transport from the roots to the shoots: Delucia *et al.* (1992) found that soil temperatures below 20°C caused significant reductions in foliar N and P concentrations of the grass *Andropogon gerardii*, while concentrations of these nutrients in the roots were high. They suggested that this effect may have contributed to reduced photosynthesis observed at lower soil temperatures.

* Measurement of variables such as stomatal index and area, guard cell size and stomatal aperture width, in relation to changes in altitude and the other factors

investigated in this study. This would allow much more useful discussion and analysis of the stomatal responses. For example, anatomical measurements can be used to derive estimates of stomatal diffusion resistance using diffusion theory (Penman and Schofield, 1951; Jones, 1992).

* Investigation of the factors with which leaf folding tends to correlate. In this study, higher altitude, lower temperature and lower nutrient status were associated with increased folding in *Poa annua* from the same stock. The mechanisms probably involve the pulvini in the vein, which may have reduced turgor as a response to environmental factors such as water stress and/or exposure, or may never have expanded fully because of a constraint such as low nutrient availability. The effect of folding is to reduce the exposed leaf surface area on the adaxial surface, which has been shown here to contain more stomata, and it may therefore aid in water conservation. Measurement of this variable does not appear to be reported in the literature. If degree of leaf folding can be related to factors such as water stress and exposure, as well as temperature and nutrient availability, and is applicable also to other Poaceae such as cereal crops, it is potentially very useful because it is relatively easy to measure, requiring no expensive specialist equipment.

This has been an autecological study of responses of *Poa annua* to altitude and related environmental factors. As such it has not addressed the community or ecosystem level of organisation. Once altitudinal effects on plants in isolation are better understood, the next logical step is to consider altitudinal effects on, and in the context of, intra- and inter-specific interactions between plants.

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Appendices

Appendix 1. Glossary

Most of the following terms are in widespread usage, but some have been defined specifically for the purposes of this study. These are labelled *.

Abaxial leaf surface - the surface facing away from the stem, *i.e.* the lower surface.

Adaxial leaf surface - the surface facing the stem, *i.e.* the upper surface.

Allometric growth coefficient (K) - the ratio between the mean relative growth rates of root and shoot. It is a powerful tool for dealing with ontogenetic effects (Pearsall, 1927; Brouwer, 1983). However, it is invalid in grasses when inflorescences are produced (Troughton, 1956, 1960).

Emerging inflorescence - an inflorescence which is emerging from the leaf sheath.

Lamina - the flattened, bladelike part of the leaf.

Leaf area ratio (LAR) - a morphological index of the "leafiness" of the plant (Hunt, 1990). It is the ratio (or more strictly quotient) between the total plant leaf area and the total plant dry mass.

* *Leaf folding* - the degree to which the leaf is folded in cross-section. The leaf folding index was defined as:

1 - (width of unflattened leaf / width of flattened leaf)

Thus a leaf with no folding has an index of zero, and a completely folded leaf an index of one.

* *Leaf to shoot ratio (L:S)* - an index of the "leafiness" of the shoots on a dry mass basis. It is the ratio (or more strictly fraction) between total leaf dry mass per plant and total shoot dry mass per plant.

Leaf weight ratio (LWR) - an index of the "leafiness" of the plant on a dry mass basis (Hunt, 1990). It is the ratio (or more strictly fraction) between total leaf dry mass per plant and total plant dry mass.

Ligule - scalelike flap of tissue growing out from the top of the leaf sheath, at the base of the lamina..

* *Mature inflorescence* - Any inflorescence which has emerged from the leaf sheath, including senescent inflorescences.

* *Mature leaf* - Any leaf which has developed a full ligule, which was taken to indicate full expansion.

* *Mature tiller* - Any tiller with at least one mature or senescent leaf and/or at least one mature inflorescence.

Photosynthetically active radiation (PAR) - the part of the electromagnetic spectrum in which plant photosynthesis tends to be most active. It approximately corresponds to the human visual spectrum.

Pulvinus - thickened region in the stem or leaf sheath, containing an intercalary meristem.

Root weight ratio (RWR) - the ratio (or more strictly fraction) between total root dry mass per plant and total plant dry mass.

* *Shoot* - any above-ground part of the plant, *i.e.* leaves and structural material. Dead material was also included.

* *Shoot : root ratio (S:R)* - the ratio between total shoot dry mass per plant and total root dry mass per plant.

Specific leaf area (SLA) - an index of the "leafiness" of the leaf (Hunt, 1990). It is the ratio between total leaf area per plant and total leaf dry mass per plant.

* *Stem* - Any above-ground part of the plant which is not live leaf tissue. Thus dead leaves were included as "stem".

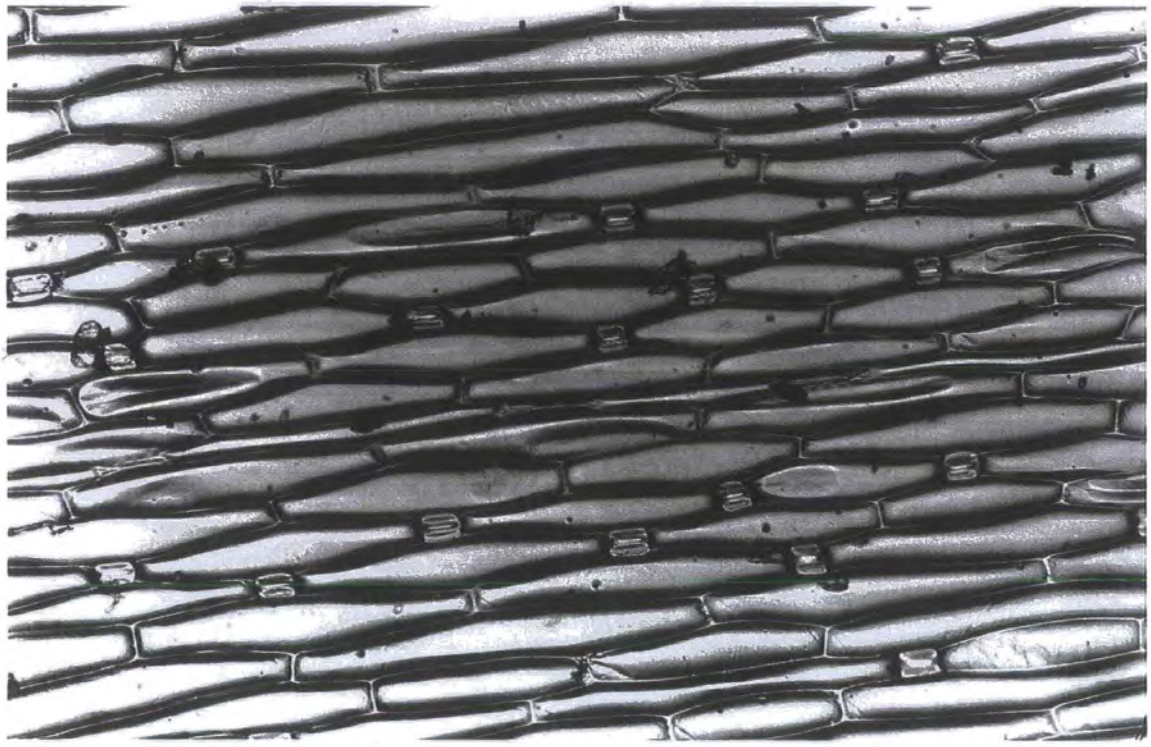
Stomatal frequency - the number of stomata per unit area of leaf surface.

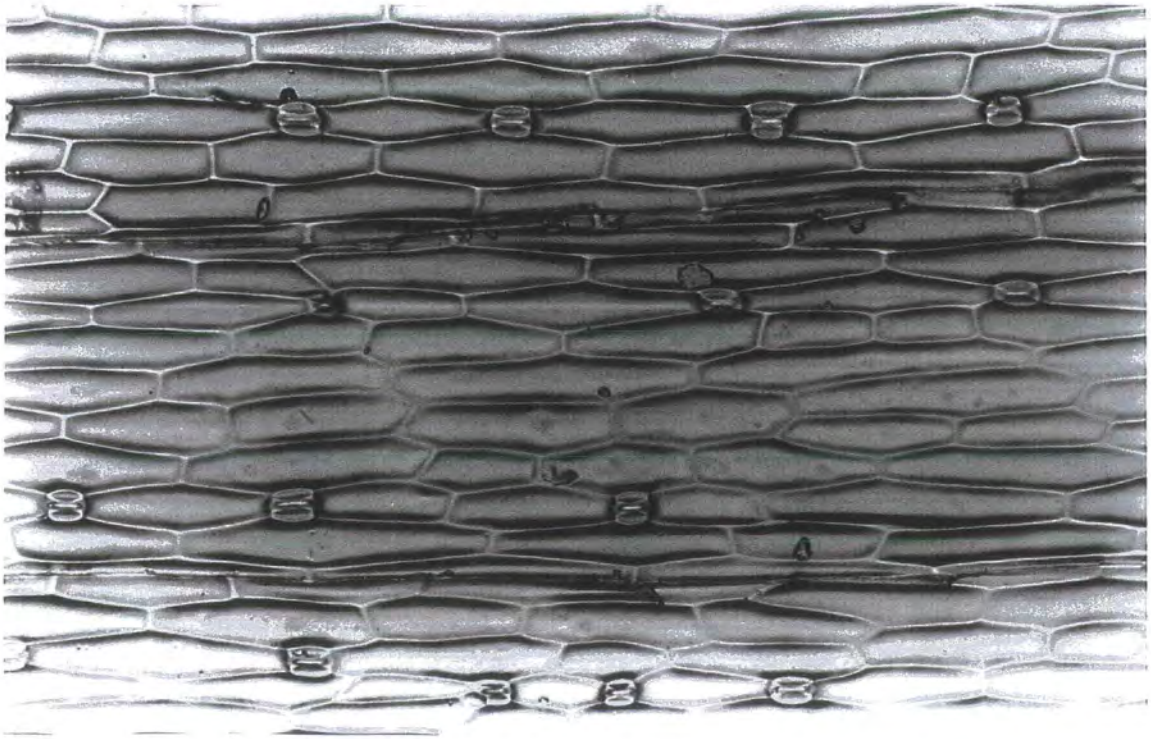
Stomatal index - the ratio (or more strictly fraction) between the number of stomata and the sum of the number of stomata and the number of epidermal cells, on a unit area basis.

Unit leaf rate - the rate of dry mass production of a plant expressed per unit ot total leaf area. Also known as net assimilation rate (NAR).

Appendix 2. Photographs of nail varnish peels from leaf surfaces

Plate A1: Photographs of nail varnish peels from leaf surfaces, viewed through a microscope. The top picture shows an example of a peel from the adaxial surface, and the bottom picture a peel from the abaxial surface. Note the greater number of stomata on the adaxial surface. Both photographs are from the second WBF harvest.





Appendix 3. Graphs of weather data covering the study period

The weather data collected for the sites are presented as separate graphs for each variable:

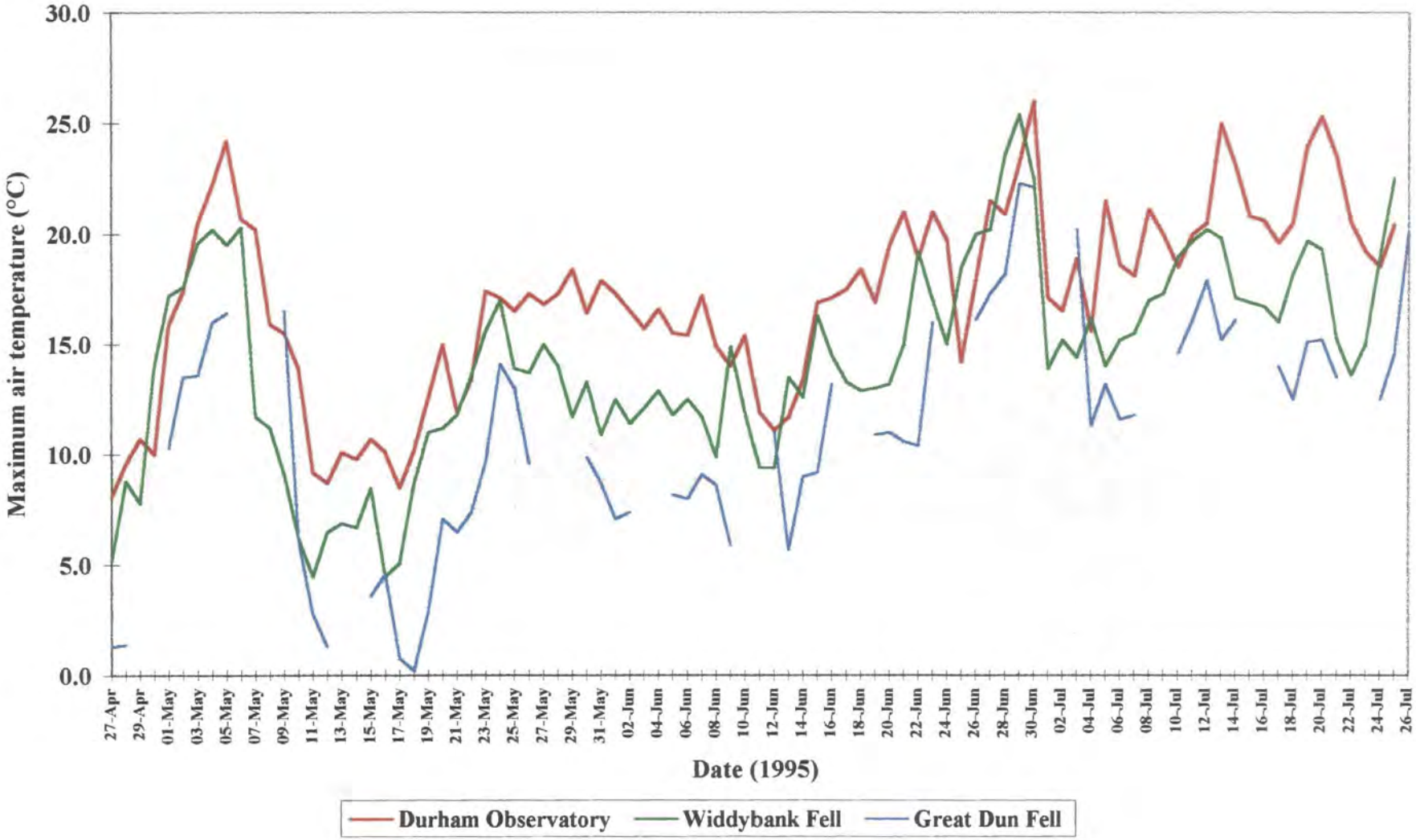
All three sites:

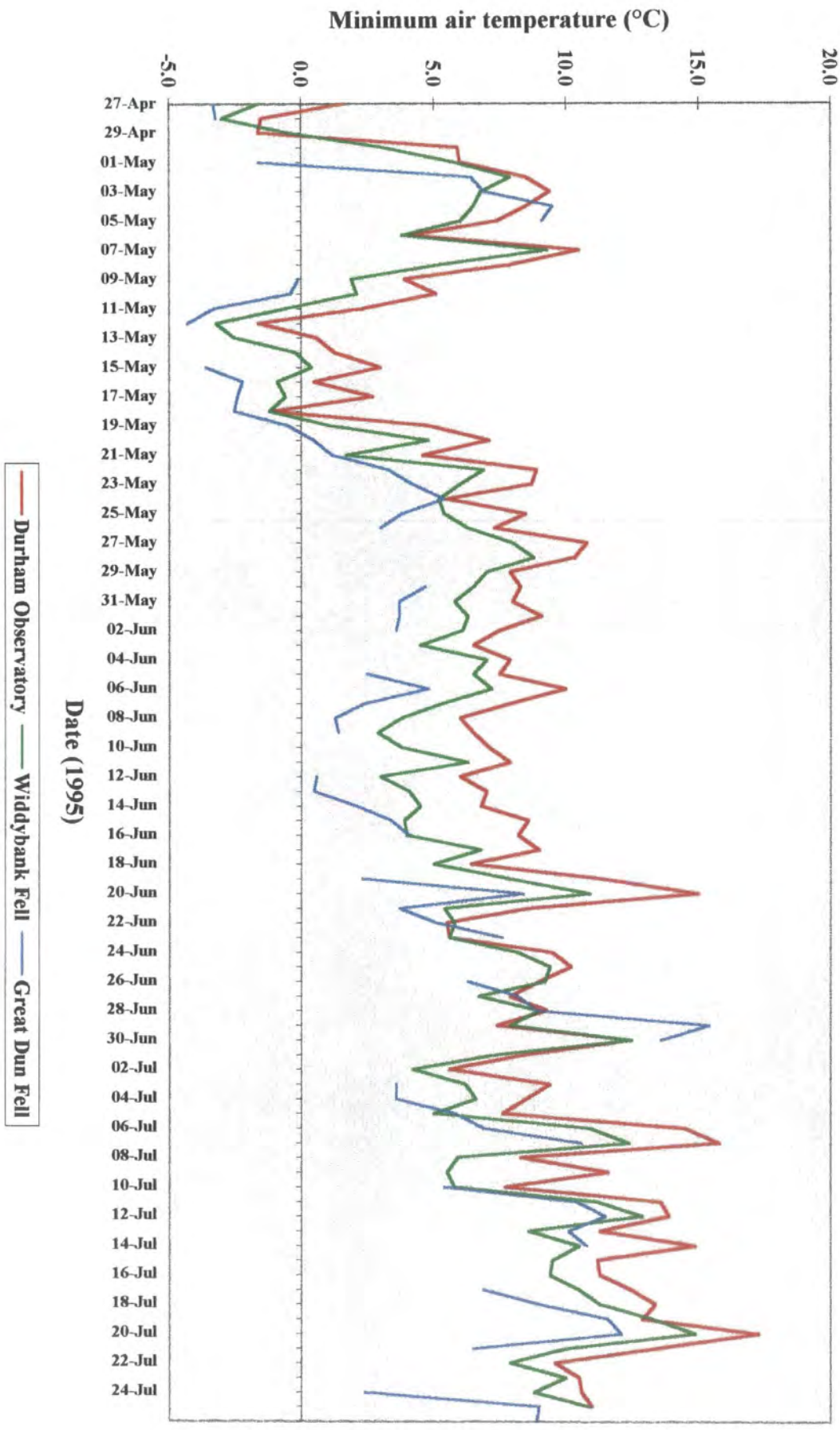
1. Maximum air temperature (10 a.m.-10 a.m.).
2. Minimum air temperature (10 a.m.-10 a.m.).
3. Daily rainfall (10 a.m.-10 a.m.).
4. Wind speed at 10 a.m..
5. Relative humidity at 10 a.m. (via wet bulb and dry bulb air temperatures).

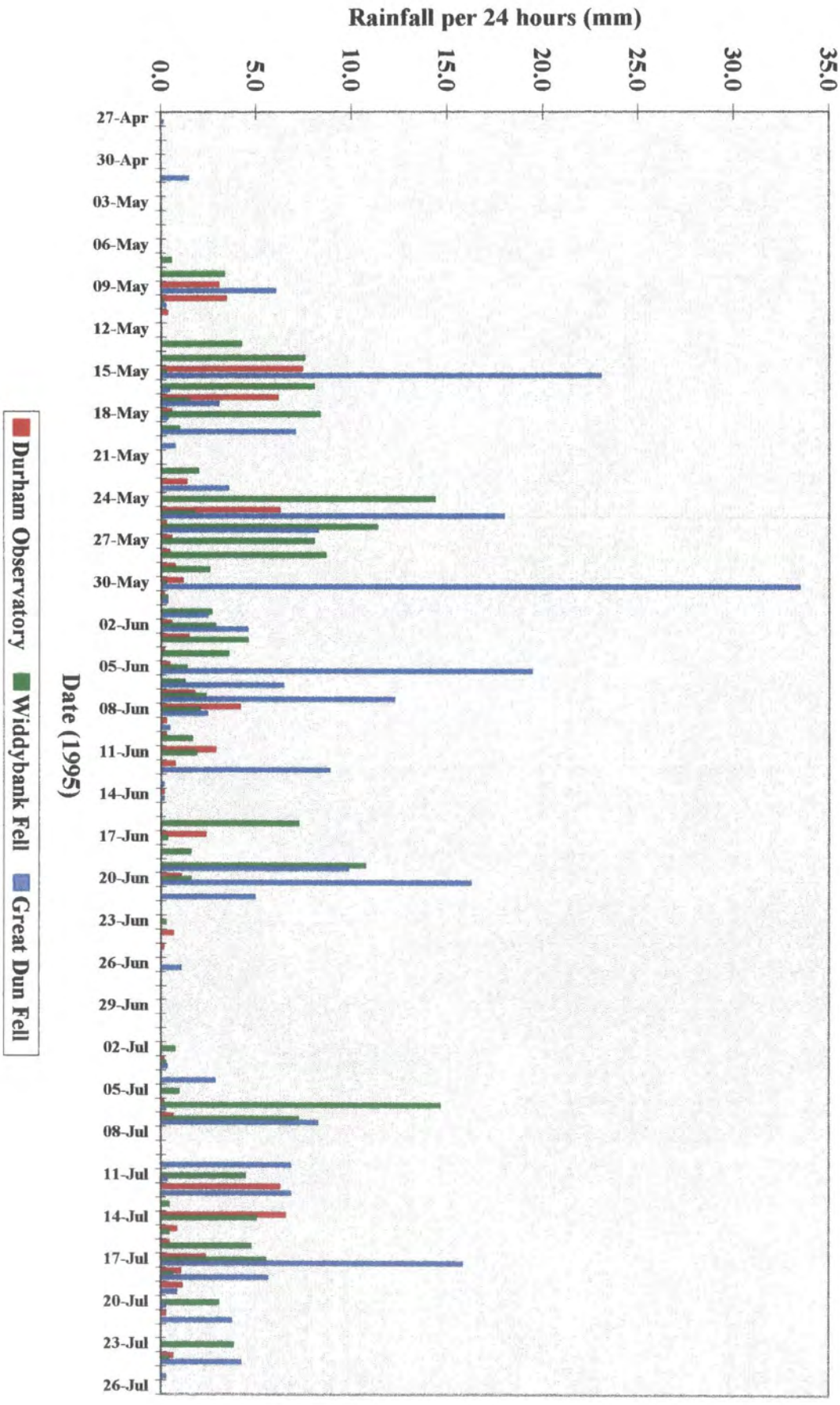
Durham and WBF only:

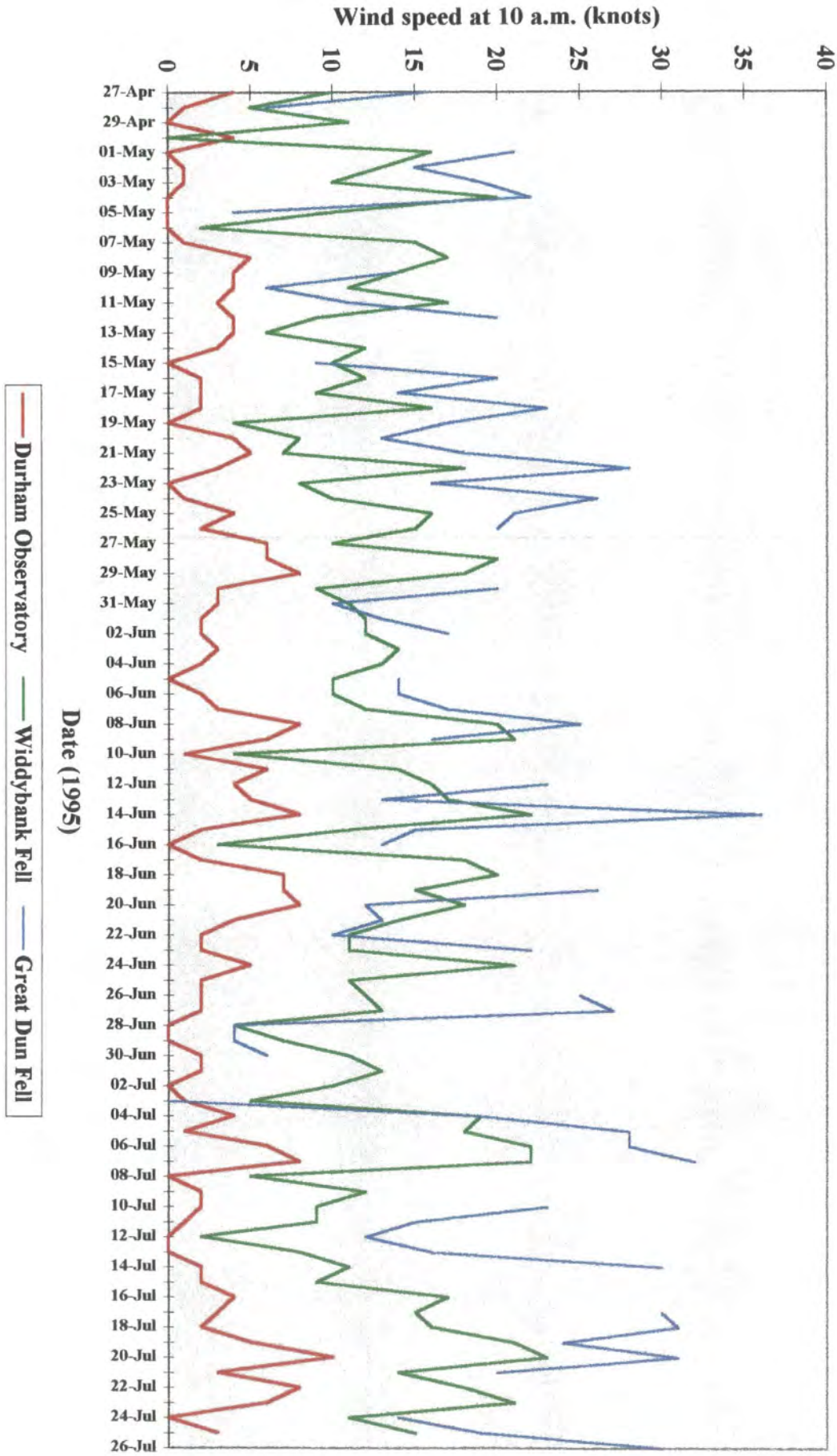
6. Number of sunshine hours (10 a.m.-10 a.m.).
7. Grass minimum temperature (10 a.m.-10 a.m.).

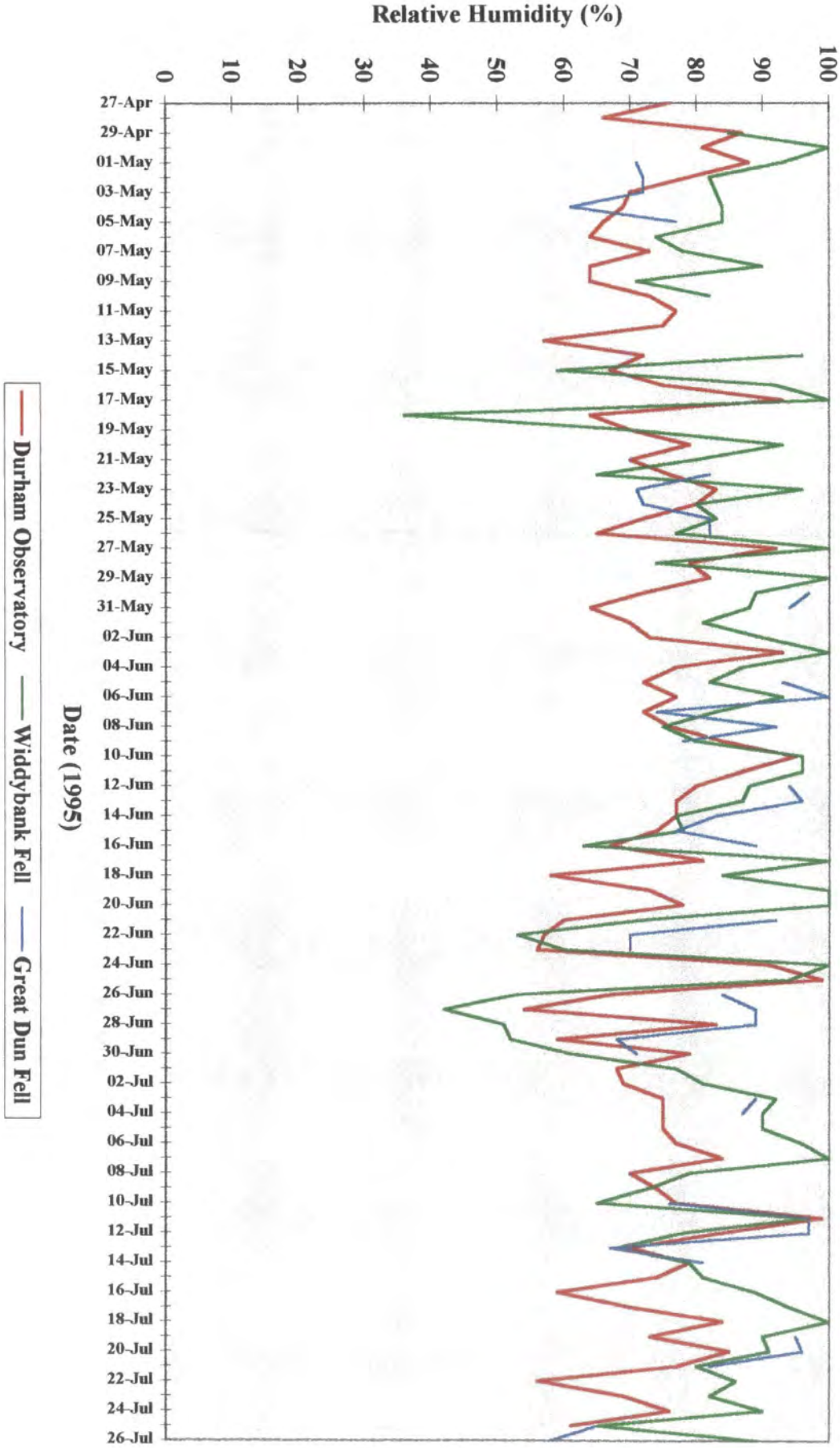
Durham data were collected from the Observatory, and were assumed to be equivalent to DBG. There are gaps in the Great Dun Fell graphs because data are not routinely collected there during weekends and bank holidays.

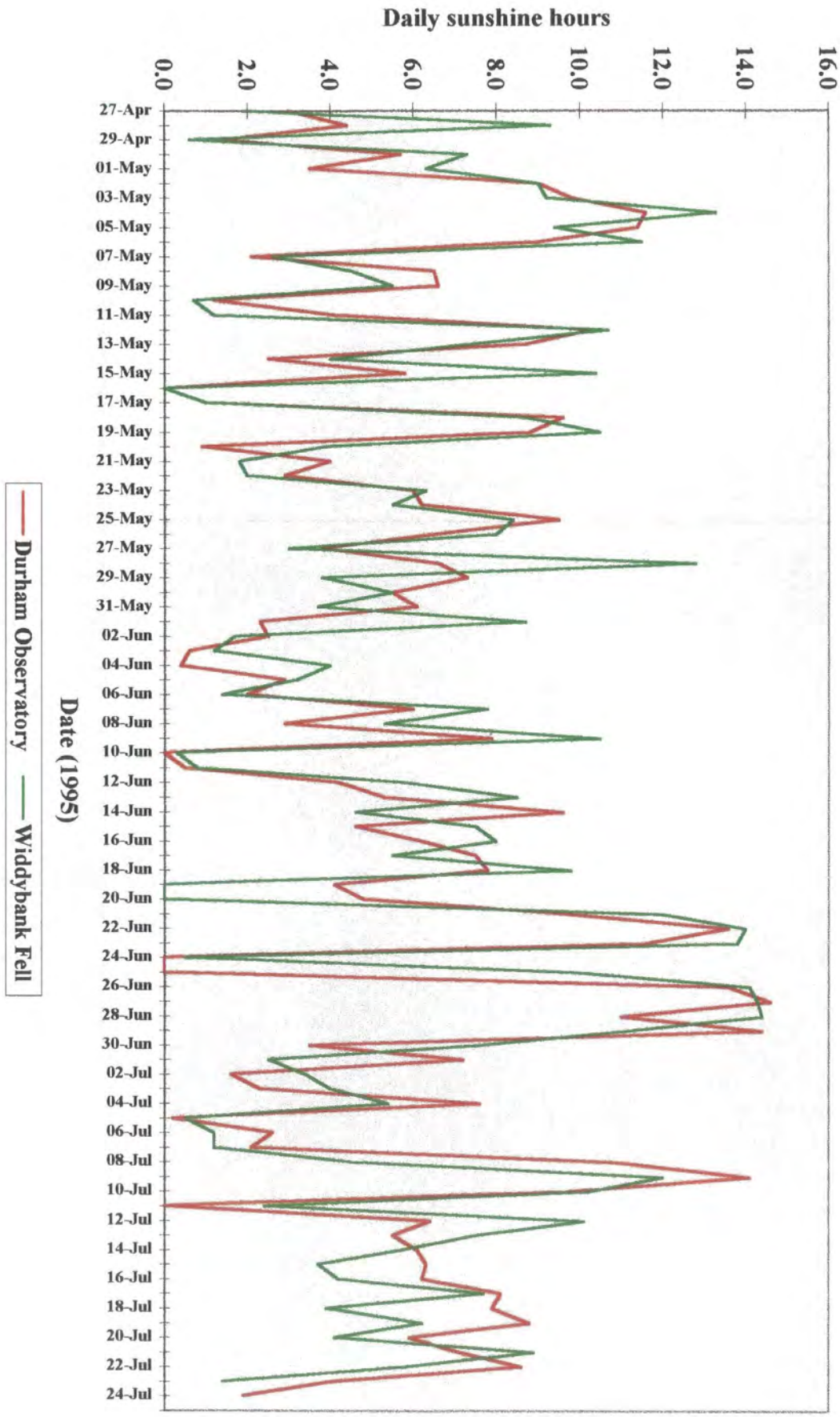

Maximum air temperature (°C)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
27-Apr
29-Apr
01-May
03-May
05-May
07-May
09-May
11-May
13-May
15-May
17-May
19-May
21-May
23-May
25-May
27-May
29-May
31-May
02-Jun
04-Jun
06-Jun
08-Jun
10-Jun
12-Jun
14-Jun
16-Jun
18-Jun
20-Jun
22-Jun
24-Jun
26-Jun
28-Jun
30-Jun
02-Jul
04-Jul
06-Jul
08-Jul
10-Jul
12-Jul
14-Jul
16-Jul
18-Jul
20-Jul
22-Jul
24-Jul
26-Jul
Date (1995)
Durham Observatory
Widdybank Fell
Great Dun Fell

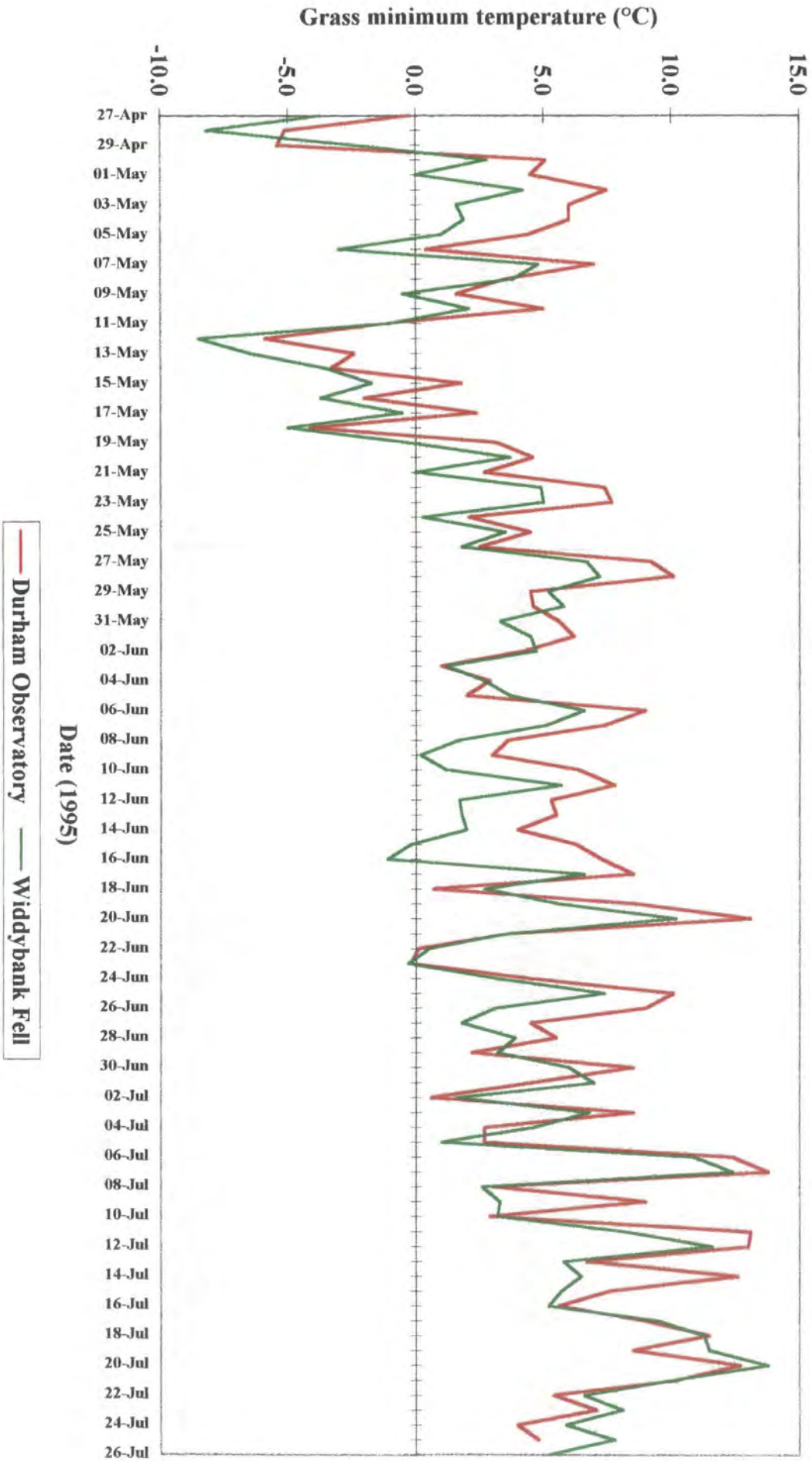

Minimum air temperature (°C)
-5.0
0.0
5.0
10.0
15.0
20.0
27-Apr
29-Apr
01-May
03-May
05-May
07-May
09-May
11-May
13-May
15-May
17-May
19-May
21-May
23-May
25-May
27-May
29-May
31-May
02-Jun
04-Jun
06-Jun
08-Jun
10-Jun
12-Jun
14-Jun
16-Jun
18-Jun
20-Jun
22-Jun
24-Jun
26-Jun
28-Jun
30-Jun
02-Jul
04-Jul
06-Jul
08-Jul
10-Jul
12-Jul
14-Jul
16-Jul
18-Jul
20-Jul
22-Jul
24-Jul
Date (1995)
Durham Observatory
Widdybank Fell
Great Dun Fell


Rainfall per 24 hours (mm)
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
Date (1995)
27-Apr
30-Apr
03-May
06-May
09-May
12-May
15-May
18-May
21-May
24-May
27-May
30-May
02-Jun
05-Jun
08-Jun
11-Jun
14-Jun
17-Jun
20-Jun
23-Jun
26-Jun
29-Jun
02-Jul
05-Jul
08-Jul
11-Jul
14-Jul
17-Jul
20-Jul
23-Jul
26-Jul
Durham Observatory
Widdybank Fell
Great Dun Fell


Wind speed at 10 a.m. (knots)
0
5
10
15
20
25
30
35
40
27-Apr
29-Apr
01-May
03-May
05-May
07-May
09-May
11-May
13-May
15-May
17-May
19-May
21-May
23-May
25-May
27-May
29-May
31-May
02-Jun
04-Jun
06-Jun
08-Jun
10-Jun
12-Jun
14-Jun
16-Jun
18-Jun
20-Jun
22-Jun
24-Jun
26-Jun
28-Jun
30-Jun
02-Jul
04-Jul
06-Jul
08-Jul
10-Jul
12-Jul
14-Jul
16-Jul
18-Jul
20-Jul
22-Jul
24-Jul
26-Jul
Date (1995)
Durham Observatory
Widdybank Fell
Great Dun Fell


Relative Humidity (%)
0
10
20
30
40
50
60
70
80
90
100
27-Apr
29-Apr
01-May
03-May
05-May
07-May
09-May
11-May
13-May
15-May
17-May
19-May
21-May
23-May
25-May
27-May
29-May
31-May
02-Jun
04-Jun
06-Jun
08-Jun
10-Jun
12-Jun
14-Jun
16-Jun
18-Jun
20-Jun
22-Jun
24-Jun
26-Jun
28-Jun
30-Jun
02-Jul
04-Jul
06-Jul
08-Jul
10-Jul
12-Jul
14-Jul
16-Jul
18-Jul
20-Jul
22-Jul
24-Jul
26-Jul
Date (1995)
Durham Observatory
Widdybank Fell
Great Dun Fell


Daily sunshine hours
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
27-Apr
29-Apr
01-May
03-May
05-May
07-May
09-May
11-May
13-May
15-May
17-May
19-May
21-May
23-May
25-May
27-May
29-May
31-May
02-Jun
04-Jun
06-Jun
08-Jun
10-Jun
12-Jun
14-Jun
16-Jun
18-Jun
20-Jun
22-Jun
24-Jun
26-Jun
28-Jun
30-Jun
02-Jul
04-Jul
06-Jul
08-Jul
10-Jul
12-Jul
14-Jul
16-Jul
18-Jul
20-Jul
22-Jul
24-Jul
Date (1995)
Durham Observatory
Widdybank Fell


Grass minimum temperature (°C)
-10.0
-5.0
0.0
5.0
10.0
15.0
27-Apr
29-Apr
01-May
03-May
05-May
07-May
09-May
11-May
13-May
15-May
17-May
19-May
21-May
23-May
25-May
27-May
29-May
31-May
02-Jun
04-Jun
06-Jun
08-Jun
10-Jun
12-Jun
14-Jun
16-Jun
18-Jun
20-Jun
22-Jun
24-Jun
26-Jun
28-Jun
30-Jun
02-Jul
04-Jul
06-Jul
08-Jul
10-Jul
12-Jul
14-Jul
16-Jul
18-Jul
20-Jul
22-Jul
24-Jul
26-Jul
Date (1995)
Durham Observatory
Widdybank Fell