Leaf and canopy photosynthesis models for cocksfoot (*Dactylis glomerata* L.) grown in a silvopastoral system

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Abstract of a thesis submitted for the Degree of Doctor of Philosophy.

Leaf and canopy photosynthesis models for cocksfoot (*Dactylis glomerata* L.) grown in a silvopastoral system

By

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The aim of the research reported in this thesis was to construct leaf and canopy photosynthesis models for understorey cocksfoot pasture grown in a 10-11-year old *Pinus radiata* silvopastoral system. From these models, dry matter (DM) production was predicted based on a numerical description of the biological mechanisms involved in canopy photosynthesis.

To do this, a wide range of environmental and management conditions were created through changes in light intensity and regime, temperature, soil moisture, nitrogen (N) and regrowth duration. A unique component of a silvopastoral system is the fluctuating light regime experienced by the understorey plants. The daily photosynthetic photon flux density (PPFD) integral was 55-62% of the open, with periods of full sunlight (1700-1900 $\mu mol\ m^{-2}\ s^{-1}$ PPFD at midday) and severe shade (129-130 $\mu mol\ m^{-2}\ s^{-1}$ PPFD) that changed within 45-120 minutes depending on the solar angle elevation. A similar pattern obtained from artificial slatted structures, also provided a bimodal light regime but with lower light intensity.

The resulting DM growth rates ranged from 2 to 154 kg DM/ha/d. These differences were related to differences in canopy leaf area index (LAI) from 0.5 to 8.2 units caused by a reduced tiller population, canopy etiolation and canopy leaf angle.

Net photosynthesis rate from seven light intensities (0, 100, 250, 500, 750, 1000 and 2000 $\mu mol\ m^{-2}\ s^{-1}$ PPFD) were measured in the field using an open infrared gas analysis system. These were used to construct light curves for the youngest fully expanded intact leaves. The prediction of DM production was based on an integrated leaf photosynthesis model that uses a non-rectangular hyperbola function to estimate the saturated leaf photosynthetic

rate (*Pmax*), the photosynthetic efficiency (α) and the degree of curvature (θ) in the photosynthetic response of individual leaves. The highest *Pmax* was 27.4 μmol CO_2 m^{-2} s^{-1} in non-limited conditions. This decreased to a minimum of -0.5 μmol CO_2 m^{-2} s^{-1} under severe water stress (ψ_{lp}= –16 bar). Values of α ranged from 0.036 μmol CO_2/μmol PPFD in non-limiting conditions to 0.020 μmol CO_2/μmol PPFD at 1.5% N. The degree of curvature of the leaf response curve θ was unaffected by the range of environmental factors and regrowth duration and had a mean value of 0.96 ±0.02. The response of these parameters to different temperature, N, moisture, regrowth and shade conditions were predicted using a multiplicative model for predicting *Pmax*, a 'law of the minimum factor' model for α and a constant for θ.

These parameters were then incorporated into a canopy photosynthesis model with coefficients for respiration, partitioning and the main canopy characteristics that affect light interception (LAI and leaf angle). Based on this model, cocksfoot DM production was predicted for silvopastoral systems in non-limiting situations and where a single, two, three, four or all five factors were limiting for: air temperatures from 2 to 37 °C, water status from ψ_{lp} –0.1 to –16.0 bar (corresponding to a soil volumetric water content to 500 mm depth of 8.5 to 34%), foliage N content from 1.5 to 5.9%, regrowth duration from 20 to 60 days, and time course of shade (severe shade: 5% of open PPFD or moderate shade: 50% of open PPFD) from 1 to 180 minutes and the correspondent induction process (lag in the rise of photosynthesis rate to the maximum value) from 30, 60 and 180 minutes of severe shade.

Using this model, it was shown that the continuous light regime of 50% transmissivity throughout a day had higher canopy photosynthesis than for the same intensity but a fluctuating light regime with periods of 90-120 minutes of full sunlight and severe shade (10.4 vs 8.4 g CO_2 m^{-2} d^{-1}). This was due to (i) a faster decrease in *Pmax* and α for plants experiencing 5% of open PPFD compared with 50% of open PPFD; (ii) the lack of an induction process under continuous shade. These results indicate that artificial shade cloth gives a biased representation of the response of understorey pastures in silvopastoral systems.

Validations from observed DM production data (from 9 to 134 kg DM/ha/d) were obtained from different environmental and management conditions and indicated that approximately 86% of the variation in cocksfoot growth rate was accounted for by using the model proposed. Therefore, the canopy photosynthesis model proposed in this study provides a powerful and useful tool for understanding and predicting DM production of cocksfoot understorey pastures in silvopastoral systems.

Additional key words: canopy temperature, chlorophyll, light quality, light curve, *Pinus radiata*, pre-dawn leaf water potential, transmissivity, water stress.

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LIST OF ABBREVIATIONS

Abbreviation	Description	Units
a	Growth respiration coefficient	dimensionless
b	Maintenance respiration coefficient	day^{-1}
CHL	Chlorophyll content per unit of leaf area	$g\ m^{-2}$
e	Base of natural logarithms (2.718281)	-
Epo	Potential evapotranspiration	mm
F'/F	Ratio between the actual area of a leaf (*F*) and the shadow it would cast (*F'*)	dimensionless
f_w	Correction factor for *b* under water stress	dimensionless
gs	Stomatal conductance	mol $H_2O\ m^{-2}\ s^{-1}$
gs_s	Standardised index value of *gs*	dimensionless
H	Number of daylight	hour
Io	Incident PPFD above the canopy	$W\ m^{-2}$
I(z)	Incident PPFD on the leaf area at the level z in the canopy	$W\ m^{-2}$
I_l	Irradiance incident on leaf	$\mu mol\ m^{-2}\ s^{-1}$ PPFD
Ins_s	Standardised non-stomatal limitation during induction	dimensionless
Is_s	Standardised stomatal limitation during induction	dimensionless
It_s	Standardised total limitation during induction	dimensionless
IS_t	Induction state of the leaf (IS) at any time (t)	dimensionless
k	Extinction coefficient	dimensionless
LAI	Leaf area index	dimensionless
N	Nitrogen content	%
ns_s	Standardised non-stomatal limitation on $Pmax_s$	dimensionless
PAR	Photosynthetically active radiation	-
Pg	Gross photosynthesis rate for each layer of the canopy	mg $CO_2\ m^{-2}\ s^{-1}$
Pmax	Leaf photosynthetic rate at light saturation	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$
$Pmax_s$	Standardised index value of *Pmax*	dimensionless
$Pmax_G$	Saturated gross leaf photosynthetic rate	mg $CO_2\ m^{-2}\ s^{-1}$
Ppmax	Potential or maximum *Pmax* value in non-limiting conditions	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$
Pn	Net canopy photosynthetic rate per day	mg $CO_2\ m^{-2}\ d^{-1}$

Pn_{max}	Maximum *Pn* value at optimum LAI	mg CO_2 m^{-2} d^{-1}
PPFD	Photosynthetic photon flux density	-
Sm	Senescent material accumulated per unit of LAI	g CH_2O m^{-2}
s_s	Standardised stomatal limitation on $Pmax_s$	dimensionless
R	Rainfall	mm
R_G	Growth respiration rate for each layer of the canopy	mg CO_2 m^{-2} s^{-1}
R_M	Maintenance respiration rate for each layer of the canopy	mg CO_2 m^{-2} s^{-1}
R_T	Total respiration rate for each layer of the canopy	mg CO_2 m^{-2} s^{-1}
R:FR	Red (660 nm) to far-red (730 nm) ratio	dimensionless
RUE	Radiation use efficiency	g MJ^{-1}
T_{air}	Air temperature	°C
T_{canopy}	Canopy temperature	°C
T_{a-c}	Difference between air and canopy temperature	°C
Tmax	Daily maximum temperature	°C
Tmin	Daily minimum temperature	°C
T_s	Standardised total limitation on $Pmax_s$	dimensionless
t_s	time under severe shade (5% of the open PPFD)	minutes
t_m	time under moderate shade (50% of the open PPFD)	minutes
VWC	Soil volumetric water content	%
W	Dry weight of the canopy	g CO_2 m^{-2}
α	Photosynthetic efficiency of the light response curve	µmol CO_2/µmol PPFD or mg CO_2 J^{-1}
α_s	Standardised index value of α	dimensionless
β	Solar elevation above the horizon	radians
γ	Leaf angle	degrees
θ	Degree of curvature or convexity of the light response curve	dimensionless
θ_s	Standardised index value of θ	dimensionless
ψ_{lp}	Pre-dawn leaf water potential	bar

CHAPTER 1

General Introduction

1.1 Background

Silvopastoral systems are integrated land use systems where trees and pastures are grown together. This can provide diversification of farm income, either directly from the sale of timber and animals, and/or indirectly by the provision of stock shelter and beneficial effects on soil conservation. There are ecological and economic interactions (positive and/or negative) between the woody, non-woody and animal components of the systems. The productivity and nutritive value of a pasture in this system is dependent on the interaction of environmental and management factors under the trees, and in turn determine animal performance (Figure 1.1). The main factor responsible for the reduction of pasture production under trees is usually the competition between trees and pasture for solar radiation, water and nutrients. This affects the morphological and physiological processes of the pasture (Figure 1.1). In addition, trees in silvopastoral systems bring about microclimate changes (soil and air temperature, humidity and wind speed) under their canopies. These changes themselves may then indirectly affect pasture growth and thus animal performance.

The input of solar energy as photosynthetically active radiation (PAR) is the main climatic factor limiting the productivity of herbage grasses when other factors such as nutrients, water and temperature are non-limiting (Monteith, 1977). There are two main aspects of incoming PAR which are modified by trees. These are: light intensity and light quality (Figure 1.1). In silvopastoral systems understorey plants experience frequent fluctuations in irradiance from full sun to shade caused by tree canopy shading. The time scale of light/shade fluctuations is dependent on the size of the tree, crown shape, tree planting density, silvicultural practices (e.g. pruning) and the development of foliage area of the trees.

Research with widely spaced radiata pine (*Pinus radiata* D. Don) has suggested that cocksfoot (*Dactylis glomerata* L.) is a suitable grass for silvopastoral systems in temperate climates due to its shade tolerance (Devkota *et al.*, 1997; Joshi *et al.*, 1999). For cocksfoot,

under ambient CO_2 conditions and a defined light regime, the main determinants of growth have been shown to be temperature, water (Barker *et al.*, 1993, Moloney, 1991; Radcliffe and Baars, 1987) and nitrogen (N) status (Donohue *et al.*, 1981; Moloney *et al.*,1993). In addition, several authors have linked dry matter production of cocksfoot to light quantity in silvopastoral systems (Braziotis and Papanastasis, 1995; Devkota *et al.*, 1998; Joshi *et al.*, 1999). However, the influence of each of these factors on cocksfoot has usually been expressed in isolation or by their influence on seasonal production, with limited explanation of the physiological basis for the responses. Therefore, an important research goal is to predict pasture growth rates in silvopastoral systems using a physiological basis and taking into account the host of potential interactions between environmental and management factors.

A physiological-based description of pasture growth operates through estimating the changes in the efficiency of conversion of energy to dry matter and the total amount of energy available for this conversion. This is in turn influenced by the combination of light interception and the photosynthetic activity of individual leaves within the canopy, which are also affected by environmental and management factors (Sheehy and Cooper, 1973). Similarly, canopy photosynthesis models, used to predict growth, are based on the light intercepted by leaf surfaces (dependent upon leaf area index (LAI) and canopy architecture) at different depths in the canopy and the resulting photosynthesis of those leaves (Thornley, 1998).

Leaves are the functional units of pasture photosynthesis and their efficiency of capture and utilization of solar energy determines productivity. Empirical measurements (Acock *et al.*, 1978; Johnson and Thornley, 1983; Johnson *et al.*, 1995) and theoretical models (Rabinowitch, 1951; Marshall and Biscoe, 1980a; Thornley, 1998) have shown that leaf photosynthesis can be described by a non-rectangular hyperbola. This function has subsequently been introduced into canopy photosynthesis models to predict the production of dry matter. Leaf photosynthesis has three parameters: the light-saturated rate which represents the asymptote or maximum saturated leaf photosynthetic rate (*Pmax*), the initial slope of the light response curve or photosynthetic efficiency (α) and a dimensionless parameter indicating the degree of curvature (θ). These parameters have been used to predict growth in pastures and crops using canopy photosynthesis models (Duncan *et al.*, 1967; Loomis and Williams, 1969; Eagles, 1973; Sheehy and Cooper, 1973; Sheehy and

Peacock, 1975; Thornley, 1998). If the three parameters of the leaf photosynthesis (in particular *Pmax*) are affected by temperature, leaf N, water stress, light and management factors such as cutting regime, then *Pmax*, α and θ are physiological variables which can be used in the prediction of pasture growth. These variables may affect pasture growth, and provide a theoretical explanation of a proportion of the variation in growth. To be universally applicable, these variables must then be incorporated into a functional pasture growth model. Presently, the integrated relationships between shade limitation in fluctuating light regimes and other environmental (temperature, N and water stress) and management (regrowth duration) factors affecting photosynthetic rate of cocksfoot leaves in a silvopastoral system have not been defined. Consequently there are currently no known models of pasture growth in a temperate silvopastoral system.

In addition to leaf photosynthetic factors, canopy photosynthesis also varies according to total canopy LAI and the arrangement of the foliage (i.e. the canopy architecture). Together these determine the total interception of solar radiation by a pasture and the distribution of irradiance among individual leaves. Maximum pasture production requires complete capture of incident solar radiation and can only be achieved with supporting levels of water and nutrients and non-limiting temperatures.

LAI, which depends on the rates of leaf appearance, growth and death on individual tillers and leaves and their morphological changes, has also been reported to be dependent on temperature, irradiance, N, water status (Davies, 1988) and light quality (Casal *et al.*, 1987). Change in light quality under trees, mainly the decrease of the red:far red ratio, can also modify LAI because such changes reduce the tiller population and plants become etiolated. Furthermore, total LAI is also dependent on management factors such as the frequency and severity of defoliation. This affects leaf age and light environment, and consequently the photosynthetic capacity of the sward. One of the main canopy architecture parameters which influences light interception is the extinction coefficient (k), a dimensionless parameter that depends on such factors as leaf angle and leaf transmission affecting the light attenuation properties of the pasture. Leaf angle in the canopy may be affected by environment (N, water stress, light), regrowth duration (e.g. lodging) and can vary within layers of a pasture canopy. Philosophically, at best, all of these factors should be capable of amalgamation into a mathematical structure that predicts actual pasture growth in a silvopastoral system.

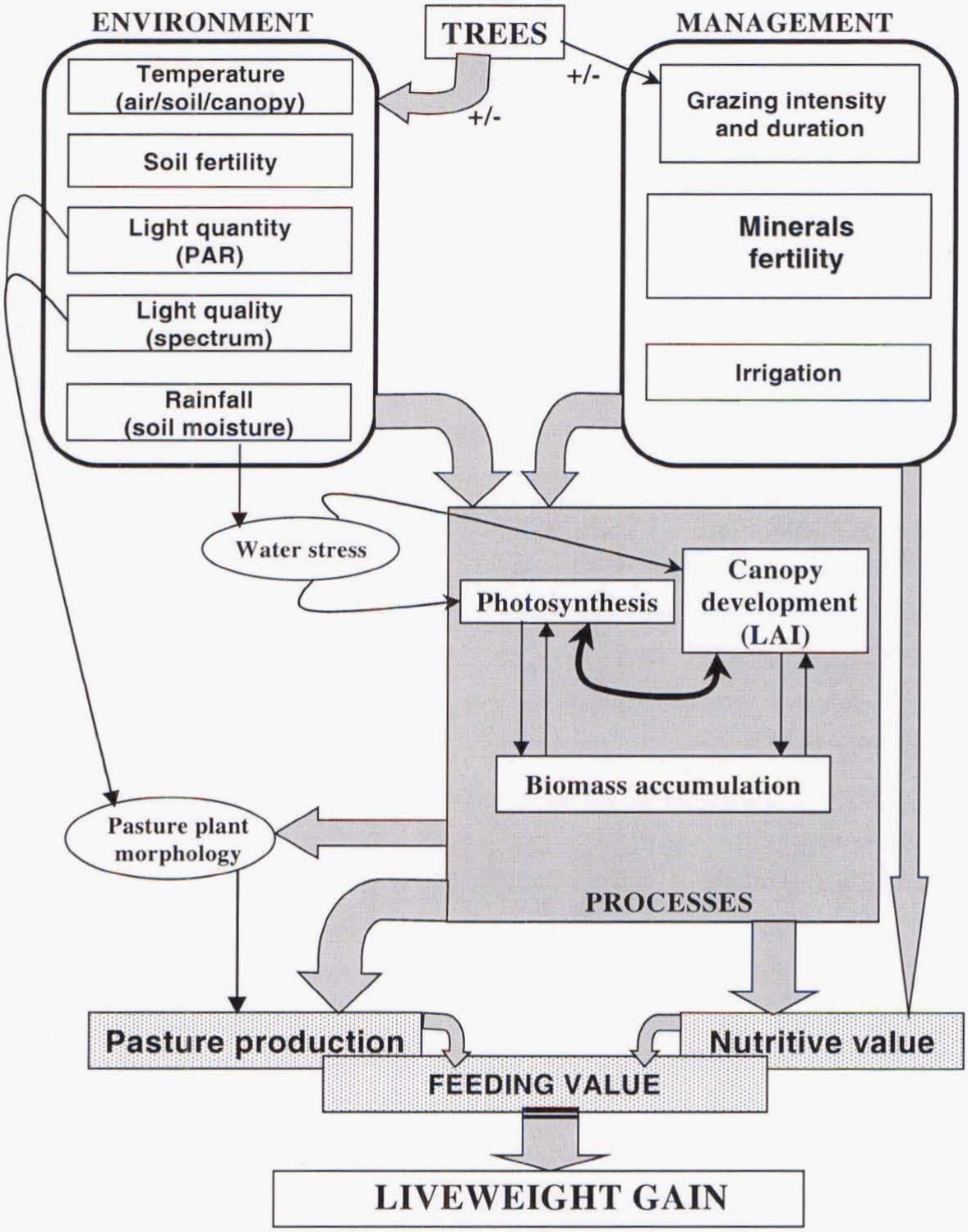

Figure 1.1 Generalised diagram of the main factors affecting pasture and animal production in a silvopastoral system. The figure form ▭ indicates the main environmental and management components of the system; the figure form ▭ indicates the main pasture processes; the figure form ⬭ indicates intermediate components of the main processes; the figure form ▭ indicates the main pasture products of the system; the arrow form ⇨ indicates effects from a whole component or process; the arrow form → indicates a particular effect of some elements of the component or process; the symbol +/- indicates positive and/or negative effects. PAR is the photosynthetically active radiation.

A mathematical model can be thought of as a concise mechanism for providing a numerical description of a process or an object. If the model is sufficiently accurate, it may be used to mimic the actual growth of a pasture and simulate growth under a greater diversity of conditions than is possible in the 'real world'. For improvements to be made in the efficiency of silvopastoral systems an understanding of the pasture understorey is essential. One benefit derived from modelling is the exposure of gaps in knowledge at the sub-model level (i.e. the individual processes), such as photosynthesis, which contribute to general pasture growth models. Another aspect is that many data points, relevant to complex processes, can be described concisely in terms of model parameters. Thus, data describing the relationship between two sets of variables can be described in terms of regression coefficients and constants. In addition, models have the capacity for prediction, which makes them powerful and valuable tools. This power to predict the effects of changes at sub-system level may also have immediate application in pasture management or in assisting agronomists to improve practices in silvopastoral systems. Furthermore, the performance of a model in relation to the behaviour of the actual system that is being simulated, can be evaluated by comparing the results obtained from the model in a well-defined situation with experimental data under similar conditions. Thus, a critical evaluation or a quantitative validation using an independent set of data (not used during model development) is an important aspect in modelling which gives the conceptual constraints and the accuracy of the model.

1.2 Aim and objectives

The primary aim of the research was to construct leaf and canopy photosynthesis models for cocksfoot pasture in a silvopastoral system. An attempt was made, using a semi-mechanistic mathematical model, to predict actual growth rates of a cocksfoot understorey pasture in a *Pinus radiata* silvopastoral system. To do this, predictions need to be valid under a wide range of environmental (seasonal) and management situations. Achieving this implies that the model is based on biological mechanisms and the processes represented in the model are important in the silvopastoral system. In this study, prediction of canopy photosynthesis was considered the primary process required for the prediction of pasture understorey growth (Figure 1.1) and statistical techniques were used to establish the most satisfactory numerical description for the processes. Thus, to develop a predictive model of the silvopastoral system, several complementary objectives were developed.

1) To create a range of environmental (temperature, N, water) and management (regrowth duration) situations in the field under different light regimes and to grow cocksfoot pastures in these situations.

2) To measure cocksfoot dry matter growth rates and the main canopy characteristics affecting light interception for the range of environmental and management situations created.

3) To provide an intermediate step whereby net leaf photosynthesis (*Pmax*, α and θ), the key driver of canopy photosynthesis models, was related to the main environmental variables that affect cocksfoot growth in silvopastotal systems. Specifically, the effects of temperature, moisture, nitrogen, regrowth duration and shade (intensity and regime) on photosynthetic functions of individual leaves of cocksfoot in field conditions are examined and biological explanations for each of the derived functions are proposed.

4) To integrate the individual functions of leaf photosynthesis into one model, which incorporates any interactions among the factors.

5) To validate the leaf photosynthesis models developed in objective 4 against actual data obtained from objectives 1-3.

6) To incorporate the leaf photosynthesis model into a canopy photosynthesis model that includes responses to the main environmental and management factors under a range of light intensities and regimes.

7) To validate prediction of dry matter production from the integrated canopy photosynthesis model by comparison with data collected from field conditions.

The success of this approach for predicting pasture growth, using leaf photosynthesis parameters and canopy characteristics affecting light interception, is reliant on the relationships holding in environments outside those from which they were derived. To confer such repeatability, the relationships used must have a biologically meaningful basis and should be consistent with previous reports based on single factor analysis for cocksfoot.

1.3 Thesis structure

A diagrammatic representation of the relationship of the main result chapters of the thesis is given in Figure 1.2. Chapter 2 is a review of the literature related to the effects of the main environment factors affecting the production of pastures in silvopastoral systems. Particular reference is made to the physiological causes of variation in dry matter production. Chapter 3 outlines the field experiment layout related to objective 1 whereby a wide range of environmental and management conditions were created. Variation in dry matter production and the main canopy structure variables, which have an influence on radiation interception, are presented for objective 2 in relation to the combination of environmental and management factors measured. Chapter 4 provides the physiological basis for objective 3 of a multiplicative model for *Pmax* prediction against N, water and temperature for individual cocksfoot leaves. Biological explanations for each of the derived functions and interactions are given. In Chapter 5 the effect of regrowth duration as a management factor on *Pmax* for objective 3 is assessed by modelling an individual function with biological explanations and integrating this function with temperature, N and water status factors. To complete objective 3, in Chapter 6, the response of *Pmax* to sunlight fluctuations experienced in a silvopastoral system are modelled. The relationship of *Pmax* with environment (temperature, water and nitrogen) and management (regrowth

duration) factors is discussed. In this chapter *Pmax* is integrated in a single model and validated for objectives 4 and 5. This provides a framework to develop quantitative predictions of cocksfoot growth in these environments. To complete objectives 3-5, analyses of the effect of the five factors, described previously, on α and θ are integrated into a single model in Chapter 7. In Chapter 8 a canopy photosynthesis model is proposed to meet objective 6 based on incorporating *Pmax*, α and θ responses to different light regimes and canopy structures. The outputs of this model are then compared with the actual growth rate and dry matter data presented in Chapter 3. Finally, in Chapter 9 the results are drawn together and compared with those previously reported in the literature. Practical implications for predicting cocksfoot production in silvopastoral systems are discussed and future directions for model improvement are proposed.

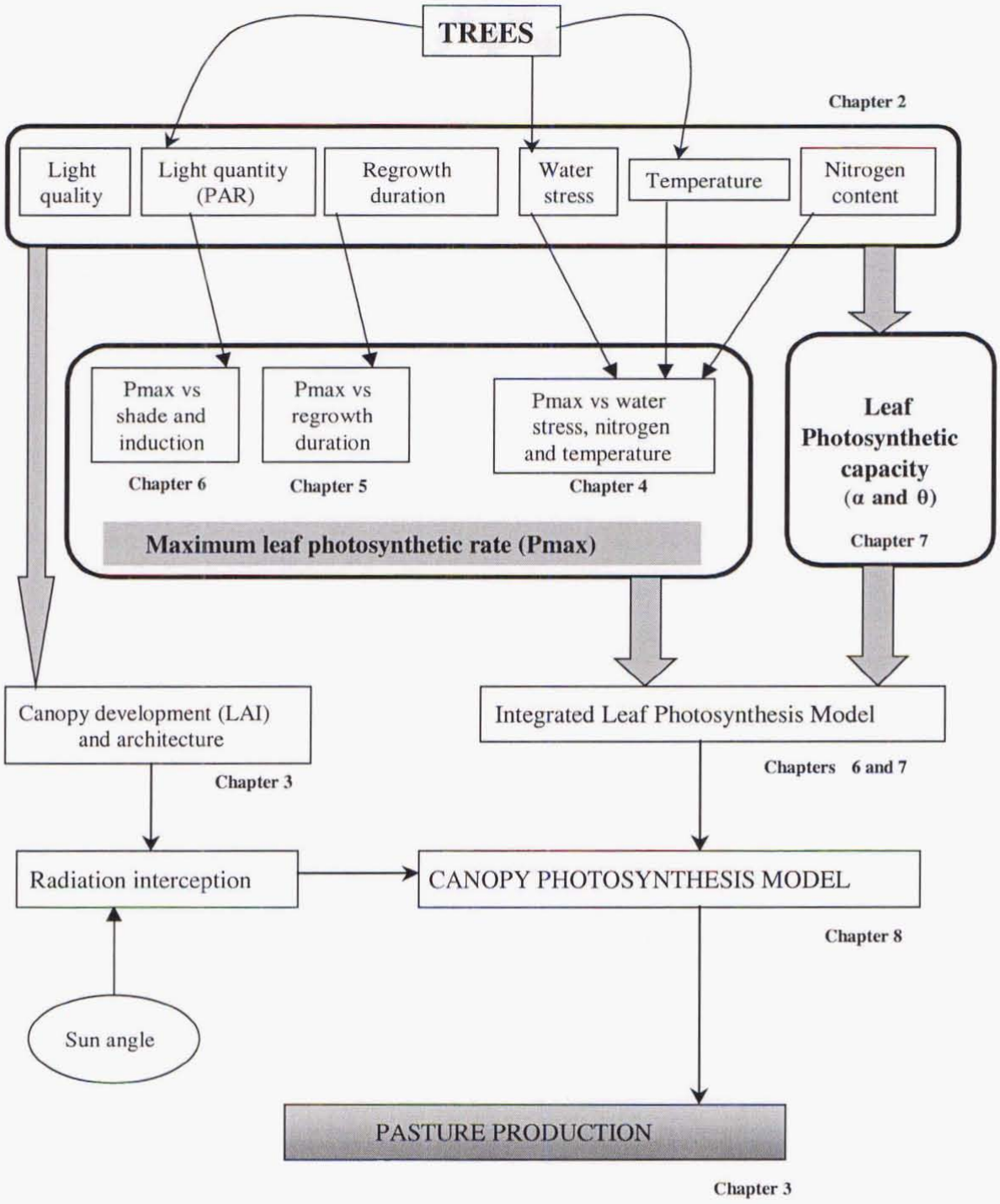

Figure 1.2 Diagrammatic representation of the relationship of the chapters of this thesis.

CHAPTER 2

Literature review

In this chapter the main environmental and management factors that affect the dry matter (DM) production of cocksfoot in temperate silvopastoral systems are reviewed. The emphasis is on New Zealand conditions and in the absence of data for cocksfoot, examples from other temperate grasses, particularly perennial ryegrass are given. Initially the agronomic impacts of the main environmental (temperature, nitrogen, water and shade) and management (regrowth duration) factors are presented. This is followed by a review of how DM production could be predicted from a canopy photosynthesis model based on the photosynthetic capacity of leaves, the light intercepted by leaf surfaces (dependent upon canopy architecture and leaf area index, (LAI)) and partitioning of photosynthates to respiration. Primarily, the focus of this review is on the leaf and canopy photosynthesis level in accordance with the study objectives (Section 1.2). However, a description of enzymatic and biochemical activity at the leaf photosynthesis level, related to changes in environmental and management factors, is also given.

2.1 Silvopastoral systems

Silvopastoral systems are integrated land use systems where trees and pastures are grown together. This can provide diversification of farm income, either directly from the sale of timber and animals, and/or indirectly by the provision of stock shelter and beneficial effects on soil conservation. In New Zealand, a wide-spaced tree system was first formally recognised in 1969, as a result of developments in plantation forestry with 'direct sawlog' regimes for radiata pine (Hawke and Knowles, 1997). Grazing these systems was considered a good option for utilising pasture growth under the trees to provide an additional, and earlier, financial return. In addition, Wilkinson (1997) reported that 32% of the North Island and 25% of the South Island of New Zealand pastoral lands require farm woodlots and wide-spaced tree planting for soil conservation.

Some pasture species may adapt to shaded environments more successfully than others in silvopastoral systems. The term "shade tolerance" is used extensively in the discussion of

forage for plantation crops. It is normally used to describe those species that produce relatively more than other species in shaded habitats (Stur, 1991). A common characteristic is that their DM productivity and persistence are maintained under decreased light compared with less shade-tolerant species. Research with widely spaced radiata pine has suggested that cocksfoot is suitable for silvopastoral systems due to its shade tolerance (Devkota *et al.*, 1997, 2001) and it is the most persistent grass species in the silvopastoral experiment used as the focus of the present study (Joshi *et al.*, 1999).

2.2 Effect of environmental and management factors on pasture dry matter production in silvopastoral systems

In a silvopastoral system, the productivity of a pasture is dependent on the interaction of environmental and management factors that affect the photosynthetic capacity and morphological aspects of the understorey sward (Ong *et al.*, 1991; Nair, 1993). This can be expressed quantitatively as a function of the interrelationships between a multitude of biotic and abiotic factors (Equation 2.1).

Growth= f [Radiation (R), Temperature (T), Nutrients (Nu), Water (W), Regrowth duration (M), Canopy architecture (C), Grazing regime (G)..... n] **Equation 2.1**

2.2.1 Solar radiation and shade

For all plants, the seasonal input of solar energy as photosynthetically active radiation (PAR) is the main determinant of growth when factors such as nutrients, water and temperature are non-limiting (Monteith, 1977). In such conditions the conversion of PAR to DM is conservative among C_3 species at about 1.4 g MJ^{-1} (Sinclair and Muchow, 1999). This concept has been utilised for the development of predictive models, particularly for annual crops (Sheehy and Cooper, 1973).

However, trees modify both the intensity and quality of the incoming radiation. Specifically, in silvopastoral systems understorey plants experience frequent fluctuations in irradiance from full sun to shade caused by tree canopy shading. The time scale of full sunlight/shade periods is dependent on the size of the tree, crown shape, tree planting

density, silvicultural practices and the development of foliage area of the trees (Kellomäki *et al.*, 1985; Miah *et al.*, 1995). Thus, as expected there is a negative linear relationship between tree population and light transmission (Anderson *et al.*, 1978). In addition, when solar radiation passes through the canopy the tree leaves absorb light in the 400-700 nm waveband, which alters the light quality for the understorey species. Holmes (1981) reported that under tree shade the blue and red light are reduced compared with green and far-red which decreases the red to far- red (R:FR) ratio. For example, Devkota *et al.* (1998) reported that R:FR under 11-year old alder trees declined from 1.24 for light shade (77% of full sunlight, pruning to 7.0 m) to 0.96 for heavy shade (17% of full sunlight, pruning to 2.5 m).

This decrease in light transmission also reduces pasture yield. For example, Joshi *et al.* (1999) reported that yield of irrigated cocksfoot pasture under 650 trees/ha (18% of the open PPFD) was reduced by 55% and by 16% under 300 trees/ha (40% of the open PPFD) compared with open pasture. Similarly, in northern Greece, Braziotis and Papanastasis (1995) reported that cocksfoot DM production during spring was reduced by 55% under a 20-year-old maritime pine (*Pinus pinaster* Aiton.) plantation thinned at 1750 trees/ha (mean light intensity of 31% of the open area) compared with pastures under 1000 trees/ha (mean light intensity of 41% of the open area). Hawke and Knowles (1997) reported that DM production of ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens* L.) pastures at Tikitere (Rotorua, North Island, New Zealand) was 25% of the open pasture production at age 13 years for 200 trees/ha and at 19 years for 100 trees/ha. Similar results have been reported for perennial ryegrass in South Otago (New Zealand) (Cossen, 1984) and in nine silvopastotal environments in the United Kingdom (Sibbald *et al.*, 1991).

2.2.2 Temperature

Temperatures below and above the optimum for a plant affect phenological, morphological and physiological processes and therefore its DM production. The optimum temperature for growth of most temperate grasses is 20 to 25 °C (Robson *et al.*, 1988). Under a controlled environment, Mitchell and Lucanus (1960) reported that cocksfoot growth at 7 °C during the day decreased by 78% compared with 15.5 °C. Knievel and Smith (1973) showed that temperatures above 28 °C greatly reduce cocksfoot growth. Trees may modify the air temperature and therefore alter the potential DM production of the understorey species. Garnier and Roy (1988) reported that tree cover acted as a buffer for the

understorey environment compared with open swards. Thus, the monthly mean temperature under tree shade for a cocksfoot pasture in France was 0.6 °C higher in winter but 1.6 °C lower in summer than in an adjacent open sward.

Understorey canopy temperature may also be reduced by tree shade. These differences can be explained from the energy balance of leaves. The complete energy-balance equation suggests that canopy temperature depends mainly on variations of air temperature, net radiation, latent heat (factors associated with transpiration) and sensible heat (factors related to heat conduction and convection) (Gates, 1980; Nobel, 1999). The main variable in this equation, which may differ between full sunlight and shade situations within the silvopastoral site, is the radiation. Hatfield (1985) reported for irrigated wheat (*Triticum aestivum* L.) that the effect of shading (50% of total radiation) compared with full sunlight on air temperature was minimal but the canopy temperature was reduced by 6 °C. These results suggest that to accurately asses DM production in a silvopastoral system air and canopy temperatures need to be monitored for understorey plants in both full sunlight and under shade.

2.2.3 Water

Cocksfoot is a widespread perennial grass, which is well-adapted to dry conditions and can survive a soil water deficit more effectively than most temperate forage grasses (Volaire and Thomas, 1995). Stevens *et al.* (1992) reported that under a lax grazing system in a dry Canterbury site, 'Kara' cocksfoot produced 55% more DM than 'Nui' ryegrass during summer. Similarly, Lancashire and Brock (1983) reported that 'Grasslands Wana' cocksfoot pasture produced 62 kg DM/ha/d compared with 42 kg DM/ha/d from 'Nui' ryegrass during summer on a dry hill country site. This is assumed to be because of the deep rooting system of cocksfoot which can withdraw moisture from a greater soil depth and also for the more effective dehydration delay associated with slower decrease in photosynthesis activity and improved carbon balance (Volaire and Thomas, 1995).

On other hand, cocksfoot is also responsive to irrigation. Volaire and Thomas (1995) reported that irrigated cocksfoot plants (watered to field capacity every 2 days) produced 3 times more aerial biomass than stressed plants (80 days droughted plants with a leaf water potential of –30 bar) after 60 days regrowth. Penman (1962) reported that irrigated 'S37'

cocksfoot produced a mean of 25% more DM than controls (soil moisture deficit > 50 mm) over six years evaluation at Woburn (England).

Trees in silvopastoral systems may reduce soil moisture by creating a rain shadow, direct interception of rainfall and root competition. Gautam (1998) reported that the proportion of fine roots (≤ 2 mm diameter) of radiata pine trees are mostly concentrated in the 10-30 cm soil depth which is also where 88% of cocksfoot roots are distributed (Evans, 1978). Thus, competition between tree and pasture roots for water will occur whenever soil moisture drops below field capacity. However, shade may also conserve soil moisture through a reduction in evapotranspiration from pastures under shade through a reduction in canopy temperature and stomatal closure (Knapp and Smith, 1988).

2.2.4 Nitrogen (N)

The response of a grass sward to fertiliser N applied at a range of different rates has been examined in numerous field trials. In a review, Whitehead (1995) reported that as the rate of applied N increased, there was an almost linear increase in herbage yield of 15-20 kg DM/kg N applied until application rates reached 250-400 kg N/ha/yr. High values of DM production for cocksfoot has been reported in other countries with the application of N fertiliser. In France, Lemaire *et al.* (1983) reported for cocksfoot a potential growth rate of 154 kg DM/ha/d with the application of 210 kg N/ha. In Finland, Rinne (1978) reported for cocksfoot pastures fertilised with 300 kg N/ha and grazed with dairy cows a mean DM production of 139±14 kg DM/ha/d during 34 days of regrowth.

Under *P. radiata* trees, increased rates of mineralisation of N in soils under pasture have been reported (Davis and Lang, 1991). In addition, according to Steele and Percival (1984), N fixation studies indicate that the proportion of N fixated is unaffected by the presence of trees at either 200 or 400 stems per hectare. However, as the yield of white clover declined with increasing tree density, the reduction in total N fixation was expected to be proportionately greater than the effects of the trees in reducing pasture yield. This suggests that if clover is the major source of nitrogen in a silvopastoral system there may be a long term decline in the labile soil organic N pool under the trees, reducing plant available N. However, intensive farming systems rely on the provision of additional N through fertiliser. In the Tikitere silvopastoral trial, at each rate of N application the total recovery of fertiliser N in pasture plants and soil decreased as the tree numbers increased

(Steele and Percival, 1984). This suggests increasing competition for fertiliser N by the trees, which was supported by an elevated N content in fresh pine needles (up to ^{15}N= 9.1%).

A lack of clover and presence of obvious green urine patches in cocksfoot pastures can be used to indicate that they are nitrogen stressed. The impact of N on cocksfoot was reported by Joshi *et al.* (1999) who showed a 42% increase in cocksfoot DM production under both moderate shade (67% of the open PPFD) and full sunlight in urine patches compared with adjacent non-urine patches.

2.2.5 Regrowth duration

The productivity of a pasture is also dependent on management factors that affect the growth of the sward (Equation 2.1). Regrowth duration is a management factor that can be modified through the frequency and severity of defoliation (e.g. infrequent cutting for hay or silage, rotational or continuous grazing).

In each growth period following cutting there is an initial lag phase, succeeded by a period of nearly constant linear growth and finally an asymptotic phase where leaf area exceeds optimal values (when 95% of light is intercepted) for the particular species (Brougham, 1956). According to Davies (1988), the decrease in growth rate leading to a ceiling yield may be due to: (i) net carbon fixation falling to zero due either to an increase in the rate of respiration, or a decrease in the rate of photosynthesis, or both; (ii) a change in the partitioning of carbon between competing 'sinks' so that none goes into leaf growth but a greater proportion enters roots; (iii) carbon continuing to enter the harvestable fraction but being simultaneously, and in equal quantity, lost from leaf and tiller death.

Pearce *et al.* (1965) reported that, from irrigated and fertilised cocksfoot sward, the maximum growth was at LAI= 5.5 after approximately 20 days regrowth and then declined by 35% at an LAI of about 8.5. Robson (1973) reported for a perennial ryegrass sward grown in controlled conditions that total DM production reached a ceiling at about 200 kg DM/ha/d after 10 weeks of regrowth (equivalent to 10 t DM/ha) when the rate of lamina death equalled the rate of production. Alberda and Sibma (1968) reported similar results for field perennial ryegrass swards grown under optimum conditions.

A criticism of much of the agronomic work reported for cocksfoot and silvopastoral systems (Section 2.2) is that the influence of each of these factors (shade, temperature, N, water and regrowth duration) has usually been expressed in isolation or by their influence on seasonal production. There is limited explanation of the physiological basis for the responses, and consequently no predictive capacity for DM production. This limits the application of results to environments, sites and seasons outside those in which they were measured. To overcome this, an important research goal must be to predict pasture growth rates in silvopastoral systems using a physiological basis and taking into account potential interactions between environmental and management factors.

2.3 Modelling pasture growth based on canopy photosynthesis

For prediction of understorey pasture production in silvopastoral systems an understanding of the factors, and their interactions, that impact on the pasture understorey is essential. They can then be combined into a predictive framewok through computer simulation models. This predictive capacity makes models powerful and valuable tools for pasture management or in assisting agronomists to improve practices in silvopastoral systems. A further benefit derived from modelling is that it exposes gaps in knowledge at the sub-model level (i.e. the individual processes), such as photosynthesis, which contribute to general pasture growth models.

In general, pasture simulation models are classified as; (i) empirical, which is essentially direct descriptions of observed data through mathematical relationships with no assumptions about the components of a system; (ii) mechanistic, which provides a quantitative description based on assumptions about the mechanisms of processes represented in the model and their interactions. In mechanistic models any predictions can be traced back to what these processes are doing. In reality, most models are a combination of both approaches. For example, the grassland ecosystem model proposed by Thornley (1998) is a mechanistic model at the whole-system level, but the component of the plant sub-model describing leaf photosynthesis is empirical. An important feature of models is the dynamics, which describe the relationship between various state variables, such as nitrogen content, or leaf water potential and driving environmental variables such as temperature, rainfall or radiation over time. The dynamic properties of a model can then be

analysed either on a (i) *diurnal time-scale*, for predictions of DM production, which arise from the diurnally varying components of the environment and shortest turnover pools (N, plant water status); or a (ii) *seasonal time-scale*, in which predictions are determined by the average values of the fast pools and slower pools such as plant structural pools, metabolic and cellulose litter pools and soil biomass (Thornley, 1998).

A physiologically-based description of pasture growth operates through changes in the efficiency of conversion of energy to DM, and the total amount of energy available for this conversion. This is in turn influenced by the combination of light interception and the photosynthetic activity of individual leaves within the canopy, which are affected by environmental and management factors (Monteith, 1965; Sheehy and Cooper, 1973). Thus, when a factor is limited (e.g. nitrogen), canopy photosynthesis may be limited by both leaf area development and leaf capacity for photosynthesis as initially proposed by Blackman (1919).

Canopy photosynthesis models, used to predict growth, are based on three main integrated components or sub-models: (i) the light intercepted by leaf surfaces (dependent upon LAI and canopy architecture) at different depths in the canopy, (ii) the resulting photosynthesis of those leaves, and (iii) partitioning of photosynthates to respiration (Wilson, 1960; Monteith, 1965; Duncan *et al.*, 1967; Marshall and Biscoe, 1980a, 1980b; Charles-Edwards, 1981; Weir *et al.*, 1984; Loomis and Connor, 1992; Thornley, 1998). The rate of canopy photosynthesis is derived by integrating the leaf photosynthetic rate throughout the depth of the canopy as it varies in response to the light attenuation by the canopy. The above approach can lead to a model for canopy photosynthesis in which the integration of leaf photosynthesis over the canopy is simple, and such that the integration over time leads to simple analytical expressions for daily photosynthesis by canopies (Thornley, 1998).

At present, numerous net canopy photosynthesis models have been used for different crops and for grasslands under full sunlight regimes. For example, Duncan *et al.* (1967) simulated net canopy photosynthesis for various fully illuminated plant communities with different LAI and leaf angles. Weir *et al.* (1984) using the ARCWHEAT model, which includes a light interception and photosynthesis sub-model, predicted growth for winter wheat in non-limiting conditions. However, there is no canopy photosynthesis model for

predicting DM production of understorey pasture species under fluctuating light regimes in silvopastoral systems.

2.3.1 Leaf photosynthesis

Leaves are the functional units of pasture photosynthesis and their efficiency of capture and utilisation of solar energy is the main determinant of productivity. Empirical measurements (Acock *et al.*, 1978; Johnson and Thornley, 1983; Johnson *et al.*, 1995) and theoretical models (Rabinowitch, 1951; Marshall and Biscoe, 1980a; Thornley, 1998) have shown that leaf photosynthesis, as a function of PPFD, can be described by a non-rectangular hyperbola. This leaf photosynthesis function has three parameters: the light-saturated rate which represents the asymptote or maximum saturated leaf photosynthetic rate (*Pmax*), the initial slope of the light response curve or photosynthetic efficiency (α) and a dimensionless parameter indicating the degree of curvature (θ).

The non-rectangular hyperbola provides a useful framework for analysing the effects of environmental factors on the light response of leaf photosynthesis. For $\theta = 0$ the non-rectangular hyperbola equation is reduced to the rectangular hyperbola. The rectangular hyperbola for a single leaf has been used to predict canopy photosynthesis in crops (Monteith, 1965) and grasses (Sheehy and Cooper, 1973), but this function overestimates the rate of photosynthesis at low and high irradiances and underestimates it at intermediate irradiance (Marshall and Biscoe, 1980a).

The response of *Pmax*, α and θ to environmental variables has been used to predict growth in pastures and crops incorporating these parameters into canopy photosynthesis models (Duncan *et al.*, 1967; Loomis and Williams, 1969; Eagles, 1973; Sheehy and Cooper, 1973; Sheehy and Peacock, 1975; Thornley, 1998).

2.3.1.1 Effect of shade on *Pmax*

The extent of overstorey shading can alter the efficiency of energy conversion to DM by affecting light interception and the photosynthetic activity of individual leaves (Sheehy and Cooper, 1973). In field environments plants can experience frequent fluctuations in irradiance from full sun to shade caused by cloud cover, overstory shading (e.g. silvopastoral systems) and within canopy shading (Chazdon and Pearcy, 1986; Knapp and Smith, 1987). Therefore, to quantify changes in carbon gain (or DM production) of a

canopy experiencing fluctuating light regimes, responses of photosynthetic activity of individual leaves under this regime must be understood.

2.3.1.1.1 Continuous light regime

The effects of different uniform light energy levels on leaf photosynthesis has been reported for cocksfoot. In controlled environment conditions, Frank and Barker (1976) reported an increment in the rate of net photosynthesis of about 80% from 200 to 1160 μmol m^{-2} s^{-1} PAR. Similarly, Eagles and Treharne (1969) reported that the photosynthetic rate on a chlorophyll basis was 60% higher as light intensity increased from 48 to 144 W m^{-2} for a natural Norwegian population of cocksfoot. In contrast, Singh *et al.* (1974) found that photosynthesis per unit leaf area (21 mg CO_2 dm^{-2} hr^{-1}) and RuDP carboxylase activity of cocksfoot did not respond to different light intensities from 30 to 100% of full sunlight, but no explanation for this anomaly compared with previous literature was given.

Woledge (1972) found that the decrease of the rate of net photosynthesis of young *Lolium temulentum* L. leaves grown in severe shade (20 W m^{-2} or less), compared with those grown in bright light (119 W m^{-2}), was due mainly to an increment in both mesophyll and stomatal diffusion resistances. In contrast, according to Frank and Barker (1976) stomata diffusion resistance for water vapour of cocksfoot growing in a controlled environment did not respond to different light levels (2.5-3 s cm^{-1} between 200 and 1160 μmol m^{-2} s^{-1} PAR) indicating that leaf photosynthesis was limited by the mesophyll resistance. This indicates that measurement of stomatal conductance (or stomatal resistance) in cocksfoot plants exposed to shade is important for understanding causes of the reduction in *Pmax*.

Unfortunately, a continuous light regime does not reflect the fluctuating light, with periods of full sunlight and shade, that understorey plants experience in a silvopastoral system.

2.3.1.1.2 Fluctuating light regime

Rabinowitch (1956) stated that photosynthesis production can be expected to be higher in alternating light (defined as a fluctuating light regime with equal periods of light and dark) compared with continuous light of equal mean light flux densities if the periods of light are very long or very short. Long intervals (>10 hours) can improve the utilisation of light energy because during the dark period the plant can recuperate from exhaustion that usually follows a period of intense photosynthesis. Very short periods (< 1 second) may

also cause an improvement of the energy conversion yield, since they allow the dark reactions of photosynthesis to reach completion, restoring the photosynthetic apparatus to its full efficiency at the beginning of each new light period. Garner and Allard (1931) also reported this trend in an early work for seven higher plants. In contrast, if the frequencies of light/dark periods ranges from > 1 minute to 1 hour, then alternating light can be expected to cause a depression of photosynthesis because the dark periods may affect the inertia of the stomata opening and closure. Thus, Rabinowitch (1956) explained for this interval, the rate of photosynthesis under intermittent light can only approach, and not exceed, the rate of photosynthesis under continuous light. Sager and Giger (1980) reviewed and analysed the published data using a method to reduce to a common energy (or PPFD) the intermittent and continuous light regimes and concluded that most of the studies (including with algae) supported Rabinowithch's hypothesis. In contrast, McCree and Loomis (1969) reported that the photosynthetic rates of cucumber plants (*Cucumis sativus* L.) under fluctuating light alternated between high (180-360 W m^{-2} PAR) and low (31-63 W m^{-2} PAR) levels at intervals of 0.014 to 3 seconds was 7-9% higher than for steady state continuous light.

Under field conditions, the physiological adaptability of leaves to a fluctuating light environment, related to the net photosynthesis of pastures growing under trees in silvopastoral systems, has received little attention. Studies of photosynthetic response to fluctuating light conditions have been reported for ecological aspects of understorey species in tropical forests characterised by a sunfleck regime (Kirschbaum *et al.*, 1988; Kursar and Coley 1993; Pearcy, 1988; Tinoco-Ojanguren and Pearcy, 1993) and within crop canopies (Pearcy and Seemann, 1990; Sassenrath-Cole and Pearcy, 1994; Pearcy *et al.*, 1996). In a sunfleck regime, sunlight penetrates through small gaps in a canopy, and alters the light or shade status generally on a time frame of seconds to minutes (Pearcy, 1988). In silvopastoral systems the potential range in a time scale is greater (Section 2.2.1). The environmental and physiological controls on leaf photosynthetic rate that operate during fluctuations in light differ from those operating under steady-state conditions (Pearcy *et al.*, 1996).

(i) Leaf photosynthesis from high to low irradiance

When plants experience a change from high to low irradiance, a photosynthesis deactivation process occurs due to a reduction in stomatal conductance (*gs*) (Kirschbaum *et al.*, 1988) and an increase in biochemical limitations (Tinoco-Ojanguren and Pearcy, 1993). A reduction in *gs* under low light in fluctuating light regimes has been reported (Kirschbaum *et al.*, 1988; Pearcy, 1988) and this decline in *gs* would partly explain the decrease in *Pmax*.

Generally, the reduction in *gs* occurs at a slower rate than the *Pmax* reduction under low light (Kirschbaum *et al.*, 1988; Pearcy, 1988; Tinoco-Ojanguren and Pearcy, 1993). This shows that factors other than stomatal closure cause the reduction in *Pmax* during the first five minutes under shade (Pearcy *et al.*, 1996). A description of the non-stomatal limitations that affect photosynthesis was provided by Sassenrath-Cole and Pearcy (1994) who investigated a time course deactivation of RuBisCO and FBPase (fructose-1,6-bisphosphatase) activities at low PPFD (35 μmol m^{-2} s^{-1}) for soybean (*Glycine max* L.) leaves.

(ii) Leaf photosynthesis from low to high irradiance (induction process)

Conversely, for plants going from low to high irradiance there is a lag in the rise of photosynthesis rate to the maximum *Pmax*. This lag time is defined as the 'induction phase' of photosynthesis (Sassenrath-Cole and Pearcy, 1994) and it is dependent on the activity status of photosynthetic enzymes and on *gs* (Pearcy *et al.*, 1996). Under field conditions, the induction state of a leaf is the result of a complex interaction between the dynamics of the light environment and the time courses of stomatal opening and closure, and enzyme activation and deactivation (Tinoco-Ojanguren and Pearcy, 1993). Consequently, differences in the dynamics of non-stomatal and stomatal responses to fluctuating light can determine the capacity of a particular species to utilise the incoming radiation in silvopastoral systems.

The induction phase of photosynthesis has been found to be dependent on three separate processes that operate on different time scales (Pearcy *et al.*, 1996); (i) a fast phase that activates rapidly as PPFD increases, which is associated with limitations in ribulose 1,5-bisphosphate (RuBP) regeneration during the first 1-2 minutes of induction (Sassenrath-Cole and Pearcy, 1992). However, limitations of enzymes in this part of the carbon

reduction cycle by the light activation state are most evident after relatively short low-light periods (<5 minutes) when the other limitations have not yet developed. After long periods (>5 minutes) in low PPFD, this fast phase may be masked by other slower limitations consisting of (ii) the light-activation requirement for RuBisCO and (iii) stomatal opening (Pearcy *et al.*, 1996). The phase of induction dependent on RuBisCO activation requires longer illumination at high PPFD and is largely complete within 7 to 10 minutes after an increase in PPFD. In contrast, stomatal opening may cause a continuing further increase in photosynthesis rate for up to 60 minutes.

Therefore, limitations to enzyme activity generally represent a rapid phase during induction while *gs* contributes to the slower phase of photosynthetic recovery (Kirschbaum and Pearcy, 1988; Sassenrath-Cole and Pearcy, 1992). Sassenrath-Cole and Pearcy (1994) reported that stomatal limitations can occur at any time during induction, but increases in stomatal conductance are the sole cause of increases in assimilation rate after 10 minutes of saturating PPFD when the enzymes are already fully activated. Pearcy and Seemann (1990) reported that for soybean leaves, which had received 180 minutes of shade (< 25 μmol m^{-2} s^{-1}) prior to an increase in PPFD (1200 μmol m^{-2} s^{-1}), photosynthesis increased over the next 20 minutes to a maximum steady-state value while *gs* required nearly 40 minutes. In addition, *Pmax* during induction has been reported to be dependent on the length of the previous low light intensity period experienced by the plant. Tinoco-Ojanguren and Pearcy (1993) found that leaves of *Piper auritum* Kunth. after 1 minute at low light (10-20 μmol m^{-2} s^{-1} PPFD) increased rapidly to full induction values, but after 2 minutes or more in low light the increment of photosynthesis was biphasic.

In summary, the physiological controls (stomatal and non-stomatal factors) on leaf photosynthesis rate that operate during fluctuations in light must be considered to understand the mechanism of *Pmax* deactivation and induction. To date, there is no information in the literature on these processes for understorey pasture species in temperate silvopastoral systems. Thus, leaf photosynthesis functions over time under shade and subsequent induction are necessary for a canopy photosynthesis model for DM prediction in a silvopastoral system.

2.3.1.2 Effect of temperature on *Pmax*

Eagles (1967) and Mitchell and Lucanus (1962) reported that the optimum range for cocksfoot leaf photosynthesis in controlled environments was 20-22 °C. Oizumi *et al.* (1974) found for 'Frode' cocksfoot that the optimum temperature range was 15-22 °C, and this fell slowly to 10 °C but rapidly to a maximum of 35 °C. In contrast, Thornley (1998), using a cubic temperature function for *Pmax*, reported for temperate grasslands in general an optimum temperature of 30 °C for ambient CO_2 conditions.

According to Nie *et al.* (1992), the reduction in *Pmax* at low temperatures cannot be accounted for by stomatal limitations under light-saturating conditions and ambient CO_2 concentrations. Thus, low temperature-induced inhibition probably reflects changes at the chloroplast level rather than limitations to actual leaf gas exchange. At temperatures less than 18 °C the enzyme activities of the Calvin cycle and metabolite transport involved in photosynthesis processes appear to be reduced (Falk *et al.*, 1996).

At high temperatures, it is likely that the photorespiration rate increases with temperature faster than net photosynthesis. Hay and Walker (1989) reported that photorespiration increases with temperature, because higher temperatures reduce the solubility of CO_2 more than O_2, reducing the CO_2/O_2 ratio, and also because high temperature reduces the carboxylase activity of the enzyme which leads to decreased photosynthesis rates.

2.3.1.3 Effect of water on *Pmax*

Water stress has a negative effect on leaf photosynthesis. Jones *et al.* (1980) reported that in water stressed (leaf water potential between –13 and –16 bar) perennial ryegrass swards *Pmax* was reduced by about 45% compared with the irrigated swards at LAI= 2.5. Johns (1978) reported a 50% reduction in gross photosynthesis for water-stressed grasses (relative water content < 60%) compared with irrigated swards. Moderate water-deficit stress reduces photosynthesis primarily by inducing stomatal closure (Chaves, 1991; Slatyer, 1969). However, it is now recognised that the stomata do not respond to changes in leaf water potential until a critical level is reached. Jackson (1974) reported that a field value for leaf water potential of –15.0 bar gave about a 70% decrease in leaf stomatal conductance for cocksfoot plants. More severe levels of water stress can decrease *Pmax* by increasing the mesophyll resistance (Ludlow and Ng, 1976; Kaiser, 1987) and by reducing the RuBP carboxylase activity in water-stressed leaves (O'Toole *et al.*, 1976; Kaiser, 1987;

Antolín and Sánchez-Díaz, 1993).

2.3.1.4 Effect of N on *Pmax*

A positive linear or curvilinear relationship between leaf N% and *Pmax* has been reported for several species (Field, 1983; Hirose and Werger, 1987a; Hilbert *et al.*, 1991). Specifically, Woledge and Pearse (1985) reported that net photosynthesis of perennial ryegrass leaves increased linearly by a slope of 2.38 mg CO_2 dm^{-2} h^{-1} per 1 mg N dm^{-2} at 250 W m^{-2}. The generality of the N-leaf photosynthesis relationship strongly suggests that one or several nitrogenous leaf components directly limit photosynthetic capacity.

The effect of N on *Pmax* per unit leaf area can be explained by the increment of chloroplast content. Increased photosynthetic pigment concentrations such as chlorophyll can be interpreted as giving a greater capacity for light absorption. Decreased chlorophyll formation during nitrogen deficiency is a well-known phenomenon and nitrogen deficiency can also reduce the chloroplasts to about one-half of their normal length (Sundqvist *et al.*, 1980). Leaf photosynthesis is also closely related to leaf nitrogen content because the amount and activity of protein determines the photosynthetic potential of the leaf (Evans, 1996). Prioul *et al.* (1980) found a positive relationship between chlorophyll content and RuBP carboxylase activity along a developing third leaf and a fully expanded leaf of perennial ryegrass seedlings.

2.3.1.5 Effect of leaf age and regrowth duration on *Pmax*

In general as a leaf ages its photosynthetic capacity declines, starting soon after full expansion and well before any visible sign of senescence. Alberda and Sibma (1968), using a photosynthesis crop model, reported that structural changes of pasture were not sufficient to account for the magnitude of the decline phase and this suggested that the photosynthesis capacity of the individual leaves must fall towards the end of a growth period.

(i) Leaf age

For grasses the effect of leaf age on decreasing leaf photosynthesis can occur between different positions on one tiller, and during ageing of leaves in a particular position on the tiller. The vegetative grass sward usually has three green leaves of different ages per tiller (expanding leaf, first and second fully expanded leaves, and senescing leaves). The

youngest expanded leaf (first fully expanded leaf) has been reported to correspond with the maximum photosynthetic capacity in the tiller (Treharne *et al.*, 1968; Woledge, 1972; Woledge and Leafe, 1976; Woledge and Pearse, 1985). Treharne and Eagles (1970) found for two populations of cocksfoot grown in controlled environments that the photosynthetic rate of the growing leaf and the second fully expanded leaf was 20 and 10% lower with respect to the youngest expanded leaf at 25 °C.

Leaf photosynthetic capacity also declines with age from full expansion to senescence. Jewiss and Woledge (1967) indicated that photosynthesis of tall fescue leaves declined from 0.88 μg CO_2 cm^{-2} min^{-1} at full expansion to almost zero at 35 days after complete expansion and this decline was represented by a quadratic function.

A reason for the decline in photosynthesis as leaves age is the decrease in stomatal conductance. Woledge (1972) found that increases in both stomatal and mesophyll diffusion resistances contributed to a 60% fall in photosynthesis when *Lolium temulentum* L. leaves aged from full expansion to 37 days. Also, Woledge (1986) reported that a decrease of stomatal conductance was the main cause of the photosynthesis reduction in white clover leaves with age from full expansion to 35 days.

In addition, the leaf ageing process decreases leaf photosynthesis through its negative effect on enzyme activity and on a decrease of compounds associated with the light reactions (including chlorophyll). Treharne *et al.* (1968) found that cocksfoot photosynthesis per unit of leaf area was maintained at its maximum level for 15-20 days after leaves were fully expanded, but declined rapidly to almost zero photosynthesis after 35 days. This closely paralleled the decline in chlorophyll content which indicated the leaf senescence. Treharne and Eagles (1970) reported a fall of 60% in RuBisCO activity of the youngest fully expanded cocksfoot leaves after 30 days full expansion at 25 °C.

(ii) Regrowth duration

There have been few reports of the effect of regrowth duration on the photosynthetic capacity of leaves that are of the same physiological age, such as the first fully expanded leaf. Parsons *et al.* (1988) proposed, for a photosynthesis model of ryegrass, a function to take into account the decline in the photosynthetic capacity of the youngest fully expanded leaf. In this *Pmax* fell from 1.0 mg CO_2 m^{-2} s^{-1} at LAI< 0.5 to a minimum value of 0.66 mg

CO_2 m^{-2} s^{-1} at LAI= 8. Pearce *et al.* (1965) found that for every LAI unit added to the cocksfoot stand over the range of 3 to 8, leaf photosynthetic efficiency dropped 0.76 mg CO_2 dm^{-2} h^{-1}.

An explanation of the decline in leaf photosynthesis with regrowth duration is that developing leaves from the stem apex, which remains near the soil surface in vegetative swards, are increasingly shaded as the LAI of the sward increases. Consequently, the light level at the base of the plant is low and each tiller in the sward produces a succession of leaves with progressively lower photosynthetic capacities (Woledge and Leafe, 1976; Sheehy, 1977). This is because it is the light conditions experienced by the developing leaf itself that determines its photosynthetic capacity (Robson and Parson, 1978; Prioul *et al.*, 1980). The photosynthetic capacity of successive leaves of perennial ryegrass taken in new expanded leaves from a vegetative sward decreased from 3 g CO_2 m^{-2} h^{-1} at 14 days from cutting (LAI= 1.8) to 0.9 g CO_2 m^{-2} h^{-1} at 53 days (LAI= 6) (Woledge and Leafe, 1976). Woledge (1978) reported similar results for 'S24' perennial ryegrass leaves. Ludlow and Charles-Edwards (1980), on the basis of the work of Acock *et al.* (1978) who measured *Pmax* at three different levels within a tomato canopy, reported a function to predict *Pmax* in grasses based on a linear relationship with the decreasing irradiance as a function of increasing LAI depth in the canopy in which the leaf has grown.

A decrease in leaf photosynthesis in the sward is also expected because the herbage N content decreases over regrowth time. Woledge and Pearse (1985) showed a decrease of 25% in photosynthesis of the youngest expanded leaf of perennial ryegrass after 28 days regrowth. This was mainly due to a decrease in the N content of these leaves (from 4.21% to 3.17%) interacting with shading. Caloin and Yu (1984) and van Keulen *et al.* (1989) reported that even when there is an optimal supply of N, the concentration of N in plants declines with increasing DM accumulation. In older plants, a greater proportion of resources is diverted to structural support and other non-photosynthetic material of low N content. Mobile nutrients, including N, are partially remobilized from senescing leaves and translocated to other parts of the plant, with the result that the concentration of N in leaf material declines during the ageing process (Whitehead, 1995). The effect of decreasing N% on leaf photosynthesis was explored in the previous section.

2.3.1.6 Factors affecting α and θ

The maximum photosynthetic efficiency (α) is determined by the efficiency with which absorbed photons are used for CO_2 assimilation and is related to RuBisCO activity (Kaiser, 1987; Seemann *et al.*, 1987; Lawlor *et al.*, 1989) and photorespiration (Ehleringer and Björkman, 1977; Ehleringer and Pearcy, 1983). The literature shows that factors in addition to *gs* affected *Pmax* (Sections 2.3.1.1, 2.3.1.3, 2.3.1.4 and 2.3.1.5) and therefore it is likely that these factors can also affect α. Marshall and Biscoe (1980a) and Thornley and Johnson (2000) described the parameter θ as the ratio of physical to total resistance to CO_2 transfer. Therefore, depression of α and θ reflects an inability of leaves to operate efficiently under low light and as such, is likely to contribute significantly to reductions in whole canopy photosynthesis and pasture radiation use efficiency. In general, the effect of environmental and management factors on θ has received little attention for pasture species. Thus, unless stated the effect of these factors on α is only described in the present section.

(i) Effect of shade

The effect of low light intensity has shown variable results. Charles-Edwards *et al.* (1974) reported, for six populations of *Lolium* sp., grown in controlled environment conditions a mean decrease in α of about 60% from 250 to 60 W m^{-2}. Long *et al.* (1993) reported a similar α value (mean value of 0.093 ± 0.003 mol CO_2 mol^{-1} photons) for a wide variety of C_3 species from sun or shade environments measured under normal CO_2 pressures (330 µbar) but also under low O_2 pressures (10 mbar) which were used to suppress photorespiration. Similarly, comparisons of the tropical forest understorey species grown in light environments ranging from 1.7 (deep shade) to 24 (55% of full sunlight) mol photons m^{-2} day^{-1} found no differences between species and environments (Sims and Pearcy, 1989). Thus, at low PPFD, the photosynthetic apparatus appears remarkably capable of using the majority of absorbed photons for photochemistry, independent of the light environment in which plants were grown or any genetic adaptation to sun and shade environments.

The contrasting results found in the literature for the response of α to shade, for application to a silvopastoral system, highlights the need to measure potential changes in α under a fluctuating light regime.

(ii) Effect of temperature

A temperature effect on α was reported by Thornley (1998) who found that, for temperate grasslands in general, α decreased by 1.5% per °C as air temperatures increased above 15 °C. Bull (1969) reported that the decrease in α at high temperatures (26 °C) was due to an increase in the photorespiration rate. Similarly, Ehleringer and Björkman (1977) and Ehleringer and Pearcy (1983) reported that photorespiration was the main cause for the reduction in α for C_3 grasses from 0.06 mol CO_2 mol^{-1} at 20 °C to 0.04 mol CO_2 mol^{-1} at 36 °C. In addition, Hay and Walker (1989) suggested that high temperature decreases the carboxylase activity of the enzyme, which can lead to a decrease in α.

(iii) Effect of water stress

Water stress also has been reported to affect α. Thornley (1998) reported that water stress had a theoretical small effect on α with a maximum reduction of 8% at a leaf water potential of –50 bar. Similarly, Jones *et al.* (1980) found only a 6% difference in α between irrigated and water stressed perennial ryegrass swards. A more significant effect of water stress on α was reported for alfalfa (*Medicago sativa* L.) by Antolín and Sánchez-Díaz (1993) who found that it decreased from 0.069 mol CO_2 mol^{-1} in well irrigated plants to 0.017 mol CO_2 mol^{-1} in water stressed plants (leaf water potential of –26 bar).

(iv) Effect of N

Nitrogen content has also been reported to affect α. Hirose and Werger (1987b) reported for *Solidago altissima* L. leaves that α increased linearly with N at a rate of 0.0188 μmol CO_2/μmol PPFD per g N m^{-2}. In contrast, Connor *et al.* (1993) reported no detectable change of α (mean 0.05 mol CO_2 mol^{-1}) in sunflower (*Helianthus annuus* L.) leaves for a range of N contents between 0.63 and 5.0%.

Grindlay (1997) reported that the N compounds whose concentrations are concerned with changing α are likely to be the soluble proteins. These are predominantly the enzymes involved in CO_2 fixation and regeneration of the CO_2 acceptor molecule ribulose 1.5-bisphosphate, and the compounds located in the chloroplast associated with the light reactions.

In addition to effects on α, Hirose and Werger (1987b) reported that increasing tissue N, θ decreased from 0.9 (leaf N of 0.8 g m^{-2}) to 0.6 (leaf N of 2.0 g m^{-2}).

(v) Effect of leaf age

The effect of leaf age or regrowth duration on α has received little attention. Sheehy (1977) found that α of the youngest fully expanded leaf of perennial ryegrass declined from 0.019 to 0.014 mg CO_2 J^{-1} between days 15 and 35 of regrowth.

There is a lack of information in the literature (Section 2.3.1.6) about the influence of the environmental and management factors on α and θ for cocksfoot.

2.3.1.7 Modelling leaf photosynthesis

Tenhunen and Westrin (1979) developed a physiologically based steady-state model of whole leaf photosynthesis (WHOLEPHOT) which described the functional dependence of net photosynthesis in C_3 leaves on [CO_2] and [O_2], incident radiant flux and leaf temperature. Farquhar *et al.* (1980) and Farquhar and von Caemmerer (1982) predicted leaf photosynthesis for C_3 species using a mechanistic model. This model contains equations that represent the rate of ribulose bisphosphate (RuP_2)-saturated carboxylation, the ratio of photorespiration to carboxylation, and the rates of electron transport/photophosphorylation and of 'dark' respiration in the light. Kim and Verma (1991) used Farquhar's model, combined with a stomatal conductance model, to estimate leaf photosynthesis in tallgrass prairie species (*Andropogon gerardii* Vitman, *Sorghastrum nutans* (L.) Nash, *Panicum virgatum* L.). Charles-Edwards (1981) also provided a mechanistic model to predict leaf photosynthesis for changes in leaf temperature, water status and leaf anatomy based on biochemical and biophysical processes. These models are a very important element to understand the biochemical and biophysical processes in leaf photosynthesis. However, these models are complex and the input variables required for leaf photosynthesis prediction (such as maximum velocity of carboxylation and intercellular partial pressure of CO_2) are often difficult to measure in practical situations using field data.

In contrast, if the three parameters of leaf photosynthesis (in particular *Pmax*) are affected by temperature, N, water stress, light and management factors such as cutting regime, then *Pmax*, α and θ are comparatively readily available physiological variables that can be used in the prediction of pasture growth. To be universally applicable they must then be incorporated into a functional pasture growth model. Therefore, the underlying assumption in the relationship presented in Equation 2.1 is that the production of DM is related to

Pmax, α and θ. This relationship has been used to predict growth in pastures (e.g. Sheehy and Cooper, 1973; Sheehy and Peacock, 1975; Thornley, 1998) and crops (e.g. Duncan *et al.*, 1967; Loomis and Williams, 1969; van Keulen and Seligman, 1987) through canopy photosynthesis models. Specifically, the literature shows that *Pmax*, α and θ can be used as physiological variables to assist in the prediction of pasture growth (Equation 2.2).

$$Growth = f(Pmax, \alpha, \theta, R, T, Nu, W, M, C, G) \qquad \textbf{Equation 2.2}$$

Further, this relationship can be modified when *Pmax*, α and θ are restricted by environmental variables, provided the relationships between *Pmax*, α and θ and the individual variables are known (Equation 2.3).

$$Pmax, \alpha, \theta = f(R, T, Nu, W, M, C, G) \qquad \textbf{Equation 2.3}$$

Furthermore, the possibility of interactions between environmental and management factors on pasture growth rates, indicates that factors should be studied in combination rather than isolation. The first step to develop a predictive model of cocksfoot growth requires determination of the individual relationship between *Pmax*, α and θ and the main environmental variables. One approach is to fit a unique generalised model (Equation 2.4) where all factors other than R, T, N, W or M are held constant.

$$Pmax, \alpha max, \theta max = Ppmax, P\alpha max, P\theta max * f(R, T, N, W, M) \qquad \textbf{Equation 2.4}$$

Where *Ppmax*, αmax, θmax represents the potential or maximum *Pmax*, α and θ for individual leaves, and are equivalent to their maximum value in non-limiting conditions.

In its simplest form several authors have suggested that a multiplicative model may be sufficient for predicting *Pmax*, α and θ (e.g. van Keulen and Seligman, 1987; Thornley, 1998). In this process, each of the factors that affect the rate of photosynthesis is fitted to an individual equation when the other four factors are non-limiting and hence their values of $f(x) = 1$. Then the five functions can be joined in a multiplicative model (Equation 2.5).

$$Pmax, \alpha max, \theta max = Ppmax, P\alpha max, P\theta max * [f(R) * f(T) * f(N) * f(W) * f(M)] \qquad \textbf{Equation 2.5}$$

The influence of environmental and management factors on *Pmax*, α and θ have usually been expressed in isolation or with limited explanation of the physiological basis for the responses. In contrast, Thornley (1998) quantifies the important abiotic and biotic factors necessary to develop a comprehensive mechanistic simulation model of grassland ecosystems. However, in his model Thorney did not take into account limitations from regrowth duration and light regimes. Presently, the integrated relationships between shade limitation in fluctuating light regimes and other environmental (temperature, N and water stress) and management (regrowth duration) factors affecting photosynthetic rate of cocksfoot leaves in a temperate silvopastoral system have not been defined. There are currently no known models of pasture growth in a silvopastoral system.

2.3.2 Factors affecting light interception

In addition to leaf photosynthetic factors, canopy photosynthesis also varies according to total canopy LAI and the arrangement of the angular distribution of leaves (i.e. the canopy architecture). Together these determine the interception of solar radiation by a pasture and the distribution of irradiance among individual leaves (de Wit, 1959; Loomis and Williams, 1969; Sheehy and Cooper, 1973).

According to Monteith (1969) diurnal changes in solar radiation dictate the diurnal course of photosynthesis and transpiration, and the vertical gradient of radiant flux in a canopy is a measure of the energy absorbed at different depths. The incident intensity of PPFD on an area of leaf at the level Z in the canopy (Iz) is calculated based on mathematical equations developed by Wilson (1960) where the light from a source (i.e. sun light rays) penetrating a layer of leaves in a canopy is a function of the area of shadow each leaf can cast. This function, which gives the area of light penetrating each foliage layer within the canopy, is in the form of the equation for the Bourguer-Lambert-Beer law and it is equivalent to the equation described by Monsi and Saeki (1953) which used the extinction coefficient (k). The k value is a variable that includes the geometrical aspects of leaf angle, solar elevation angle and LAI. The mathematical equation proposed by Wilson (1960) was corrected by Duncan *et al.* (1967) to estimate the sunlit area of the foliage canopy by considering leaf angle and solar elevation angle. From the responses generated by the simulation model of Duncan *et al.* (1967), Loomis and Connor (1992) reported that with LAI< 2, canopies of horizontal leaves are the most productive. With intermediate LAI of 2-4, leaf angle has

little influence on productivity, but increases in LAI beyond 4, given erect leaves so that the available radiation is spread over more leaf area, lead to progressively greater assimilation.

Maximum pasture production requires complete capture of incident solar radiation and can only be achieved with supporting levels of water and nutrients and non-limiting temperatures. LAI, which depends on the rate of leaf appearance, growth and death of individual tillers and leaves and their morphological changes, has been reported to be dependent on temperature, irradiance, N, water status (Davies, 1988) and light quality (Casal *et al.*, 1987). Also, there is evidence that leaf angle changes with environmental factors (Trenbath and Angus, 1975). Thus, changes in LAI and leaf angle must be known to estimate canopy photosynthesis in full sunlight and shaded conditions.

2.3.2.1 Effect of shade on LAI

Change in light quantity and quality (mainly the decrease of the R:FR ratio) under trees can modify LAI because stem elongation can be promoted and tillering and branching inhibited (Casal *et al.*, 1987; Garnier and Roy, 1988). The changes in R:FR ratio are perceived by understorey plants through the phytocrome system which may change morphogenetic characters in plants (Smith, 1982).

Reduced light intensity and changes in light quality have been reported to reduce tillering and are therefore likely to reduce LAI. Garnier and Roy (1988) reported a 36% reduction of cocksfoot tiller population in France under 33% transmissivity oak tree shade compared with open pasture. Devkota *et al.* (1998) reported for a range of cocksfoot cultivars that the mean tiller number declined 25-30% as the shade environment fell from 77 to 17% PPFD of full sunlight. Mitchell (1955) found that at a temperature of 15 °C, cocksfoot plants in full sunlight had a mean of 10.3 tillers per plant and under shade condition 6.1 tillers per plant. In the Lincoln University silvopastoral experiment, Joshi *et al.* (1999) reported that the number of vegetative and reproductive tillers on cocksfoot plants decreased by 40% at 18% PAR level compared with open pastures. Deregibus *et al.* (1983) showed that after 28 days, the mean number of new tiller per plant of *Lolium* sp. was 16 with a R:FR of 2.2 and decreased to 11 tiller per plant when R:FR declined to 1.1 of similar light intensity. A similar response was reported by Casal *et al.* (1985) and Cassal *et al.* (1987). The physiological basis for the reduction in tillering is that under low irradiance a reduced

supply of current assimilate is preferentially allocated to existing tillers at the expense of axillary buds (Robson *et al.*, 1988). Thus, the effect of low light intensity is not on the rate of site production, but rather on the extent to which sites are filled.

Generally in grasses, high levels of shade will encourage plants to become more etiolated where the taller growth is an effort to gain greater access to available light in competition with neighbouring plants and tillers. Anderson (1978) found that etiolation of cocksfoot was due to cell elongation under shaded environments. According to Kephart and Buxton (1993) etiolation occurs at the expense of root growth, increasing consequently the plant shoot/root ratio under shade. It also appears that shade-intolerant species may show a greater stem elongation response to reduce the R:FR ratio than shade-tolerant species (Smith, 1982). It is likely that leaf area of shaded cocksfoot leaf blades trends to be maintained or increased to maximise light interception at the expense of leaf thickness, resulting in leaves being longer, narrower, and thinner than when grown in full sunlight conditions. This is consistent with Devkota *et al.* (2000) who reported that plants from 10 cocksfoot selections increased the specific leaf area with shade from 15.9 mm^2/mg under 73% of the open PPFD to 21.3 mm^2/mg under 24% of the open PPFD. According to Cooper and Tainton (1968) thinning of leaf blades with shade may result from reduction in cell size.

2.3.2.2 Effect of temperature on LAI

In grasses, temperature has a major effect on LAI through increasing the rate of leaf appearance, leaf expansion and leaf death. The rate of leaf development of a particular pasture species is correlated to the thermal time (or growing degree-days), which is the cumulative temperature above a base that represents the temperature at which growth ceases (Arnold and Monteith, 1974). In general, leaves growing under 'optimum temperatures' extend more rapidly, for a shorter period, to a greater final length; they tend to be longer in relation to their width, achieve a greater specific leaf area and have proportionally more lamina relative to sheath (Mitchell and Lucanus, 1962; Cooper, 1964; Robson, 1974). For example, young plants of 'S170' tall fescue grown at 25 °C produced leaf tissue on the main stem at four times the rate of those grown at 10 °C (Robson, 1974). This was achieved by a doubling of the frequency of leaf appearance (with a matching rise in primordia production) and by leaves extending at four times the rate but for only half the time, to twice the final length. Because both, the time interval between the appearance of

successive leaves and the duration of leaf extension were halved, the number of growing leaves remained fairly constant. The optimum temperature for most aspects of leaf growth tends to be in the region 20-25 °C for most temperate grasses, with the night temperature equal to or slightly lower than that of the day (Evans *et al.*, 1964).

Furthermore, LAI may be indirectly affected by temperature through changes in the tiller population. Optimum temperatures accelerate tiller production in grasses, but mainly through an increased rate of leaf, and hence axillary bud, production (Robson *et al.*, 1988). If tiller number is plotted against leaf number on the main stem instead of against time, effects of temperature very largely disappear (Robson, 1974). Langer (1979) indicated that the optimum temperature for tillering in cocksfoot pastures ranged from 24 to 29 °C.

2.3.2.3 Effect of water on LAI

Irrigation can indirectly increase the radiation interception of a sward by increasing the canopy LAI through a greater leaf expansion and enhancing tillering. Hsiao and Acevedo (1974) reported that the cell expansion is sensitive to water stress, therefore the rate of leaf area expansion of the sward is reduced. For example, Lawlor (1972) showed an 80% reduction in leaf elongation rate of perennial ryegrass when leaf water potential fell from –4 to –10 bars, and elongation ceased at –16 bars.

Irrigation can either increase tiller production or decrease tiller death in the sward consequently affecting the LAI of the pasture. Norris (1982) found for three moisture treatments and a range of grasses that irrigation (maximum potential soil moisture deficit, MSMD, of 41 mm) increased tiller number over control (MSMD of 239 mm) and covered (MSMD of 273 mm) plots. Irrigated plots had higher tillering rates (0.037 tillers tiller^{-1} d^{-1}) than covered plots (0.010 tillers tiller^{-1} d^{-1}), while control plots were intermediate (0.018 tillers tiller^{-1} d^{-1}).

2.3.2.4 Effect of nitrogen on LAI

The influence of N supply on pasture growth has been reported to increase LAI of the sward through an increase in the rate of leaf extension. Wilman and Wright (1983) found that applying 500 kg N/ha/yr compared with none approximately doubled the mean rate of leaf extension of ryegrass.

The fast increment of canopy development (LAI) due to N can also be explained by increases in tiller population and canopy height. Auda *et al.* (1966) showed that the number of tillers of cocksfoot grown in soil/sand mixtures was three times greater when 224 kg N/ha was applied than without an application of N. Wilman and Pearse (1984) reported for perennial ryegrass and tall fescue that N fertiliser increased tiller production from 0.05 tillers $tiller^{-1}$ d^{-1} with 0 kg N/ha to 0.38 tillers $tiller^{-1}$ d^{-1} with 132 kg N/ha, which represented 10 and 50% of new tiller sites, respectively. Nitrogen also promoted fertile tiller numbers in grasses (Langer, 1959, Korte, 1986).

The supply of N also increased the leaf area of the sward by increasing leaf size (Whitehead, 1995). Ryle (1970) reported that for cocksfoot swards in constant-environment conditions, increasing the concentration of nitrate-N in the nutrient solution from 15 to 150 mg N/l increased the average area of individual leaves from 8.5 to 13.5 cm^2, mainly by increasing leaf length.

2.3.2.5 Effect of regrowth duration on LAI

The development of LAI is also dependent on management factors that affect the photosynthetic capacity of the sward. Brougham (1958) showed that ryegrass-clover mixtures increased in growth rate up to 95% light interception and then declined. Pearce *et al.* (1965) reported that on irrigated and fertilised cocksfoot swards reached 95% light interception at LAI of about 5, and that the greatest canopy photosynthesis occurred at LAI between 5 and 6.

Herbage regrowth depends on the rate of appearance, growth and death of individual tillers and leaves and morphological changes over time (Davies, 1988). Duru and Ducrocq (2000) reported that as cocksfoot herbage accumulated up to 80 days of regrowth in N and temperature non-limiting conditions, the leaf appearance rate per tiller decreased and the lamina growth duration, lamina length and life-span increased. The consequence of these interacting factors was that the number of living leaves was fairly constant (3.5 green leaves per tiller).

The total tiller population varied with regrowth time and therefore may modify the LAI of the pasture. As individual tillers become larger, the competition between them increases so that the tiller population decreases. Wilman *et al.* (1976) reported that the number of tillers

produced by four varieties of perennial ryegrass fertilised with 263 Kg N/ha decreased from 5250 tillers/m^2 at 4 weeks to 3410 tillers/m^2 after 10 weeks regrowth. Simon and Lemaire (1987) studied a range of seeding densities of perennial and Italian ryegrass and related the tillering rate with LAI of the sward indicating that as light became limiting at the base of the sward (LAI> 3) tiller buds failed to develop.

Based on the information reviewed in the previous sections (Sections 2.3.2.1 to 2.3.2.5), a predictive relationship between LAI and DM production is needed to take into account the changes in canopy development (canopy height, leaf size, tillers number) due to the environmental and management variables. This relationship then needs to be incorporated into the canopy photosynthesis to determine the foliage development after each day of growth.

2.3.2.6 Factors affecting canopy architecture

One of the main canopy architecture parameters which influences light interception is the extinction coefficient (k).

Shade is an environmental factor in silvopastoral systems that may reduce leaf inclination. Charles-Edwards (1981) demonstrated that there is an optimal canopy k for maximum canopy photosynthesis, which changes with the incident light flux density: the lower the light the more productive pastures with planophile leaves. Thus, horizontal leaves may be able to capture more radiation under shade and hence should maximise the individual leaf photosynthetic input. The pasture leaves under severe shade became more horizontal due to its longer and thinner leaves (Section 2.3.2.1). This is consistent with Deckmyn *et al.* (2000) who reported that cocksfoot leaves drooped from 68.7° to 53.9° as length increased. Adaptation of leaves to shaded environments was reported by McMillen and McClendon (1979) who observed that leaf orientation of 10 woody deciduous dicot species were arranged to nearly vertical in full sun and were more nearly horizontal under 17% of full sunlight. For open 'S345' cocksfoot pastures, Sheehy and Peacock (1975) reported a k daily value of 0.44 and Brown and Blaser (1968) reported a value of 0.50. However, at present there is no information for cocksfoot related to changes in leaf angle or k with light intensity or under fluctuating light regimes. The potential changes in canopy leaf angle of cocksfoot plants grown in fluctuating light regimes is needed for predicting DM in silvopastoral systems using a canopy photosynthesis model.

The pattern of leaf inclination may change as growth proceeds. As a crop lodges, the leaves become more horizontal, the LAI increases greatly, and light penetration into the crop is reduced (Trenbath and Angus, 1975). Pearce *et al.* (1967) reported a decrease in k from 0.38 to 0.25 in *Hordeum vulgare* L. seedlings as LAI increased from 3 to 8. Similarly, de Wit (1959) reported that while the canopy of a young stand of ryegrass was erectophile, it became planophile as the stand aged. Sheehy and Peacock (1977) reported that a decrease in the efficiency of light energy conversion of 24% was observed after a change to a more prostrate form of perennial ryegrass canopy due to lodging.

Although no papers have dealt specifically with the effects of water stress on leaf inclination on grasses, Moran *et al.* (1989) reported that lucerne plants responded to water stress (up to –30 bar plant water potential) by arranging the leaves (cupping response) more vertically than irrigated plants (65.6° vs 48.3° at midday) as an adaptive mechanism to avoid solar radiation.

2.4 Respiration

Utilisation of assimilate for synthesis and maintenance of plant material can be described by two respiratory components, growth and maintenance respiration (McCree and Troughton, 1966; McCree, 1970). Although at the biochemical level the respiratory-chain energetics are probably identical, they have very different practical consequences.

(i) Growth respiration

Growth respiration is a function of daily canopy gross photosynthesis. This represents a loss in material when converting the immediate products of photosynthesis into plant material. The growth respiration coefficient was reported to be one-quarter of the gross photosynthesis (a= 0.25, i.e. the conversion efficiency in biosynthesis is 75%) according to values reported by McCree and Troughton (1966) for white clover and Thornley (1998) for pastures in general. This value is compatible with the range reported by Robson *et al.* (1988) for perennial grasses (a= 0.20-0.35). The conversion efficiency or the coefficient 'a' is unlikely to vary with environmental factors unless the energy coupling in phosphorylation is affected (Penning de Vries, 1972). Therefore, the effect of environmental factors (e.g. shade in silvopastoral systems) may affect growth respiration

through changes in gross photosynthesis.

(ii) Maintenance respiration

Maintenance respiration has been reported to be temperature sensitive and is a fraction of the whole pasture dry weight (McCree and Troughton, 1966). Physiologically, maintenance respiration includes the processes which maintain enzyme pools, cellular structures, gradients of ions and metabolites and also the processes of physiological adaptation that maintain cells as active units in a changing environment (Penning de Vries, 1975).

The maintenance respiration coefficient 'b' has been reported to be a constant value when used in canopy photosynthesis models. Hay and Walker (1989) reported a value of b= 0.012 d^{-1} for barley, Robson *et al.* (1988) reported a constant value of b= 0.014 d^{-1} for ryegrass, Weir *et al.* (1984) used a value b= 0.02 d^{-1} for winter wheat during vegetative growth. However, there is evidence that 'b' changes with environmental factors and with age. For example, it has been reported to change with foliage N content (Johnson *et al.*, 1995) and water stress (Moldau and Rahi, 1983; Thornley, 1998).

The sensitivity of maintenance respiration to temperature proposed by McCree and Troughton (1966) followed a value of Q_{10}= 2.2 over a range of 5 to 30 °C. The theoretical analysis of Penning de Vries (1975) suggests that temperature increase raises the cost of maintenance by a considerable stimulation of protein turnover and of active ion fluxes.

Values of 'b' have been reported to increase with foliage N content. Jones *et al.*, (1978) reported a linear relationship between 'b' and the percentage of protein content for a perennial ryegrass sward adjusted to 15 °C and assuming a Q_{10}= 2. Johnson *et al.* (1995) also proposed a linear relationship between 'b' and N content for grassland in general. Robson and Parsons (1978) reported for perennial ryegrass grown in a controlled environment that 'b' increased from 0.016 d^{-1} in low N (solution containing 3 p.p.m. of N) communities to 0.029 d^{-1} in high N communities (solution containing 300 p.p.m. of N). The relationship between 'b' and N content is supported by a differential maintenance requirement between low-protein and protein-rich materials (Penning de Vries, 1975). Thus, at very low N concentrations, protein turnover is low and has a small maintenance requirement.

The effect of water stress on 'b' for pastures in general was proposed by Thornley (1998) who used a dimensionless correction factor, which decreases exponentially with water stress expressed as leaf water potential. Wilson *et al.* (1980) reported a linear decrease in 'b' of sorghum plants with water stress from 0.055 d^{-1} at leaf water potential of -1 bar to 0.025 d^{-1} at -11 bar. The physiological basis is that with increasing water stress maintenance respiration is reduced due to a decline in the biochemical process related to the enzyme activity in respiration activity of the plant (Penning de Vries, 1975).

A decrease in maintenance respiration, as plant parts age, was reported by Johnson and Thornley (1983) who assumed that the maintenance cost per unit dry weight varied between different leaves ages in a tiller at 20 °C from 0.02 d^{-1} for a growing leaf and the first fully expanded leaf to 0.01 d^{-1} for a senescing leaf. Similarly, Woledge (1986) reported that maintenance respiration per unit dry weight for white clover leaves decreased with age from 5.0 g CO_2 kg^{-1} h^{-1} at full leaf expansion to 3.0 g CO_2 kg^{-1} h^{-1} after 25 days.

On other hand, shading has been reported to have no marked effect on the rate of maintenance respiration (Ryle *et al.*, 1976; Jones *et al.*, 1978).

2.5 Summary

In silvopastoral systems, the productivity of a pasture is dependent on the interaction of environmental (shade, temperature, N and water) and management factors (regrowth duration) (Section 2.2). The influence of each of these factors on cocksfoot has usually been expressed in isolation or by their influence on seasonal production. There is limited explanation of the physiological basis for the responses and there is no predictive capacity for pasture DM production in silvopastoral systems. Therefore, an important research goal is to predict pasture growth rates in silvopastoral systems. One approach to achieve this is to use a physiological mechanism basis to take into account potential interactions between environmental and management factors.

In this review, prediction of canopy photosynthesis was considered the primary process required for prediction of pasture understorey growth. This is in turn influenced by the combination of the photosynthetic capacity of individual leaves (Section 2.3.1),

morphological aspects affecting light interception (Section 2.3.2) and respiration (Section 2.4). Canopy photosynthesis models have been used for different crops and for grasslands under full light regimes. Presently, the integrated relationships between shade limitation in fluctuating light regimes and other environmental and management factors affecting canopy photosynthetic rate of pastures in a silvopastoral system have not been defined, and therefore have not been used to predict pasture growth.

To develop a predictive model of cocksfoot in a silvopastoral system, several steps are proposed:

(i) To create a range of environmental and management situations in the field under different light regimes and to measure cocksfoot DM growth rate and the main canopy characteristics affecting light interception (LAI and canopy leaf angle).

(ii) To derive individual functions for leaf photosynthesis (*Pmax*, α and θ) against temperature, N, water status, regrowth duration and shade. A priority for leaf photosynthesis prediction in silvopastoral systems is to develop mathematical equations to represent the physiological processes (stomatal and non-stomatal limitations) of cocksfoot plants during time under shade and during induction. The individual functions of leaf photosynthesis then need to be integrated into a unique model, which incorporates any interactions among factors.

(iii) To develop a predictive relationship between LAI and DM production to take into account the changes in canopy development due to the environmental and management variables. This relationship then needs to be incorporated into the canopy photosynthesis to determine the foliage development after each day of growth.

(iv) To incorporate the leaf photosynthesis model together with the canopy LAI development function into a canopy photosynthesis model that includes responses to the main environmental and management factors under fluctuating light regimes in silvopastoral systems. The output of this model then needs to be compared with the actual growth rate and DM data of cocksfoot to determine the accuracy of predictions.

CHAPTER 3

Dry matter production and canopy architecture of field grown cocksfoot under different shade, nitrogen and water regimes

3.1 Introduction

In a silvopastoral system, the productivity of a pasture is dependent on the interaction of environmental and management factors under the trees (Section 2.2). These affect the photosynthetic capacity (Section 2.3.1) and architecture of the canopy including LAI and leaf angle (Section 2.3.2). For cocksfoot, under a defined light regime, the main determinants of growth are temperature, water, nitrogen (N) and regrowth duration (Section 2.2). The main aspects of the incoming radiation, which are modified by trees and affect DM production and canopy structure of the understorey, are the light intensity and light quality (Section 2.2.1). The time scale of light/shade fluctuations is dependent on the size of the tree and the development of foliage area of the trees that change with time.

The extent of the effects of the environmental and management factors on DM production depend on seasonal changes and development of trees over time. Therefore, to predict pasture growth rates in the Lincoln University silvopastoral systems it is necessary to quantify the effect of temperature, water, N, regrowth duration and shade on DM production. To do this, a wide range of environmental and DM production conditions are needed. These can then be used to generate and validate a semi-mechanistic mathematical model based on the photosynthetic capacity of leaves and canopy characteristics affecting light interception (Chapters 4-8).

Therefore, the objectives of the research in this chapter were to:

1) describe the main environmental characteristics of the experimental silvopastoral site;

2) create a range of environmental (temperature, N, water) and management (regrowth duration) conditions in the field with different light intensities. The intention was to extend the current light regime in the silvopastoral system and isolate the effect of each of the environmental factors on DM production;

3) quantify any changes in the main understorey canopy characteristics that affect light

interception.

3.2 Material and Methods

This section describes the silvopastoral experimental site and the two experiments within the site, which were used to: (i) extend the light regime of the Lincoln University silvopastoral system by creating four levels of light intensity; (ii) determine the effect of water, herbage N content and regrowth duration on DM production in the silvopastoral system.

3.2.1. Description of the silvopastoral site

3.2.1.1 Establishment

This study was conducted in the Lincoln University silvopastoral experimental area in Canterbury, New Zealand (43° 38'S and 172° 28'E). The original experiment was established in July 1990 to investigate soil/tree/pasture/sheep/climate interactions of five *Pinus radiata* genotypes and six understorey pasture treatments in a split-plot design with three replicates (Mead *et al.*, 1993). The total area planted in trees is about 5.2 ha with 18 main pasture plots of 46.2 x 42.0 m (0.194 ha). After 11 years, the most persistent grass species was cocksfoot, which is the focus of this study.

An adjacent 1 ha site without trees, on the same soil type, also had 18 pasture plots (27.5 x 18 m) sown in September 1990. Of these, three were cocksfoot plots, which were used to provide an open pasture comparison for the silvopastoral experiment.

In all plots both open and under trees, herbage was cut and carried off the site for silage during the first three years of the original experiment but since spring 1993 it has been grazed by sheep.

The 'Grasslands Wana' cocksfoot pastures were originally sown with 'Grasslands Pawera' red clover (*Trifolium pretense* L.), 'Grasslands Huia' white clover and 'Woogenellup' subterranean clover (*T. subterranean* L.).

The pine trees were planted at 1000 stems/ha (7 x 1.4m) and were periodically thinned to the present uniform population of 200 stems/ha with 7 m between rows by 1996. In the

first two years, tree rows were strip sprayed (1 m wide) with herbicide (hexazinone at 2.5 kg a.i/ha) to assist tree establishment. Therefore, plots with trees had only 86% of their area occupied by sown pasture. The silvicultural regime and details of tree characteristics measured during this trial are given in Appendix 1. Crown closure had not occurred at age 10 years.

3.2.1.2 Climate

Long-term average (LTA) meteorological data recorded at Broadfields meteorological station located 3 km north of the silvopastoral site is presented in Table 3.1. The climate is described as sub-humid and temperate with a LTA rainfall of 680 mm, evenly distributed through the year, but evapotranspiration is about double the rainfall which causes frequent soil moisture deficits from October to March. The predominant wind is a cool sea breeze from the north-east, but the site is also exposed to cold-moist south-west gales and warm dry föhn north-west winds.

Table 3.1 Mean monthly long-term (1970-2000) meteorological data for rainfall, solar radiation (SR), maximum (Tmax), minimum (Tmin) and mean daily (Tmean) air temperature, windrun and Penman potential evapotranspiration (Epo.) recorded at Broadfields meteorological station.

Month	Rainfall (mm)	Epo. (mm)	Tmax (°C)	Tmean (°C)	Tmin (°C)	Windrun (km/d)	SR (MJ/m^2)
January	50	153	22.6	18.0	11.4	415	670
February	51	118	21.7	16.4	11.0	397	515
March	59	96	20.1	15.0	9.9	373	422
April	52	63	17.5	12.2	6.7	328	288
May	50	44	13.8	8.7	3.7	305	177
June	63	33	11.2	6.3	1.5	277	126
July	75	37	10.7	6.1	1.4	292	146
August	68	51	12.2	7.6	2.9	340	220
September	40	69	14.2	9.2	4.3	361	339
October	55	105	16.7	11.3	6.0	397	508
November	56	124	18.4	13.1	8.0	398	603
December	61	143	21.3	15.7	10.2	395	673
Annual	679	1036	16.7	11.4	6.4	356	4687

3.2.1.3 Soils

The soil is classified as a Templeton silt loam (Haplusteps) and consists of 1 to 2 m of fine alluvial sediments over gravels. It is medium to free-draining with a moderate capacity to hold moisture (320 mm in the top one meter). The site has only slight changes in topography, but there is variation in depth to the underlying gravels. Neither fertilizer, lime nor irrigation has been applied to the experimental area since its establishment.

Thirty soil cores to 150 mm depth were taken at random within each cocksfoot plot in autumn 1999 and 2000 (Table 3.2). Measurements were made using the Ministry of Agriculture and Fisheries Quicktest (MAF QT) procedures.

Table 3.2 Soil nutrient levels of the experimental sites at the Lincoln University silvopastoral experiment in 1999 and 2000.

Environment	Year	pH	Ca	K	P	Mg	Na	$S(SO_4)$
			m.e./100g	m.e./100g	µg/ml	m.e./100g	m.e./100g	ppm
Open pasture	1999	6.0	5.7	0.36	7	0.92	0.20	3
	2000	6.0	4.4	0.36	6	0.84	0.17	3
Silvopastoral	1999	6.0	4.4	0.41	8	0.76	0.17	3
	2000	5.8	3.8	0.41	8	0.71	0.15	4

Soil tests indicated Olsen-P and $S(SO_4)$ were below optimum for maximum pasture production (Morton *et al.*, 1994), but levels of Ca, K, Mg and Na were adequate. In general, there were no differences between cocksfoot plots in the open and in the silvopastoral site and to be consistent with the long term experimental protocol no basal fertilisers were added to any of the pastures.

3.2.2 Description of the experiments

3.2.2.1 Experiment with four light regimes

This experiment was set-up to measure DM production and the main canopy architecture characteristics of cocksfoot experiencing different levels of a fluctuating light regime.

Within each of the three main cocksfoot plots of the silvopastoral experiment, a study plot of 14.0 x 5.0 m was located in the middle of the 7.0 m wide inter-row under trees and also in the adjacent open pasture plots. Within these study areas, slatted shade structures measuring 3.0 x 2.1 m covered with pine wood slats (150 mm wide) and gaps between slats (150 mm wide) were used to reduce the total incidence of light by approximately 50% (Plate 3.1). This structure provided a bimodal light regime to represent the silvopastoral system (Varella *et al.*, 2001). The shade structures were supported horizontally on a vertically adjustable metal frame, which allowed the shade source to be maintained at 0.3 m above the cocksfoot canopy. For the slatted shade structure, the objective was to create

intervals of sunlight and shade similar to the shade pattern of the radiata pine in the silvopastoral area (Plate 3.1).

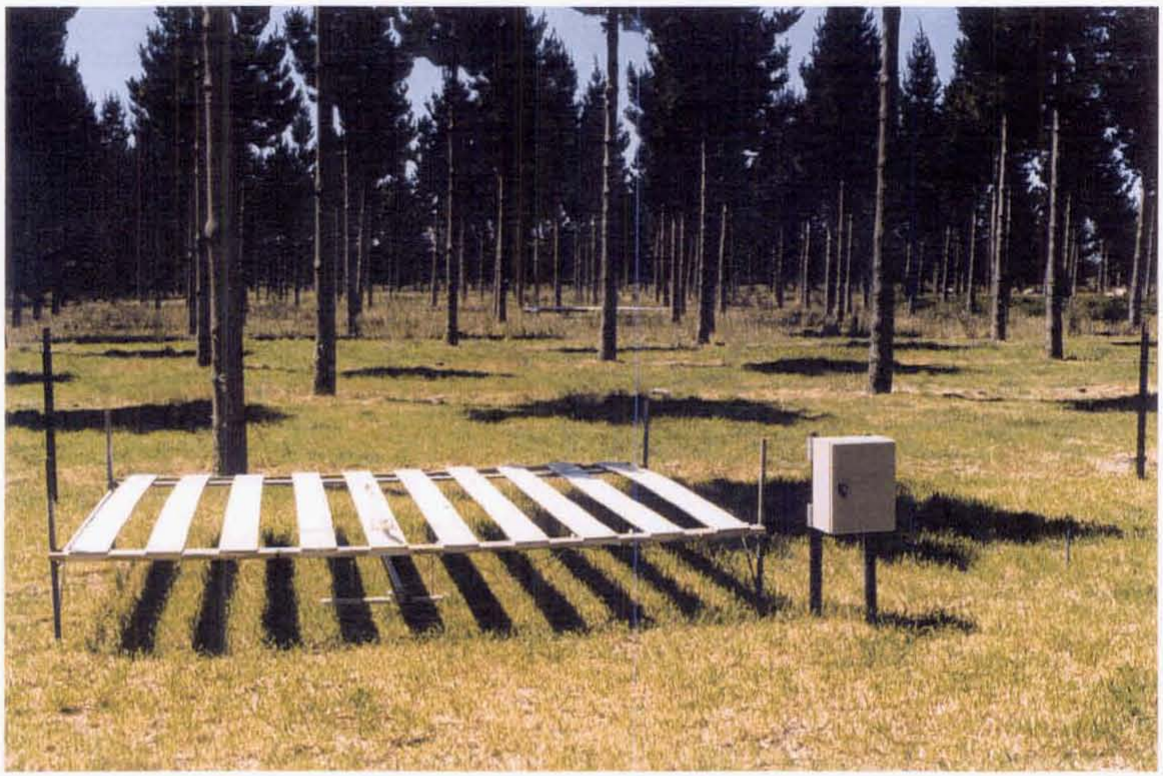

Plate 3.1. Cocksfoot pasture under 10 year-old radiata pine trees (200 stems/ha, pruned up to 6 m height) at Lincoln University silvopastoral experiment which provided a fluctuating light regime of ~58% of open PPFD. In the middle of the 7 m inter-row, slatted shade structures were used to reduce the total incidence of light by approximately 50%. This structure provided a bimodal light regime.

This experiment was arranged with open (100% transmittance) and silvopastoral (~58% transmissivity) plots as main treatments with three replicates. Within each replicate a cocksfoot plot was split into two sub-plots: slatted shade and no slatted shade. This gave four light transmission regime: i) cocksfoot open pasture, ii) cocksfoot pasture under slatted shade, iii) cocksfoot pasture under tree shade, iv) cocksfoot pasture under trees + slatted shade. The trees + slatted shade treatment extended the light regime beyond that experienced under the current silvopastoral situation.

The slatted shade structures were orientated in an East-West direction in the main plots with the slats North-South. They were set up continuously in the plots from September 1999 to May 2001. During periods when main plots were grazed, the shade frames were

removed to avoid damage on plants through sheep using the structures as a camping area. Immediately after each grazing, plots were trimmed with a mower to an even height of 20 mm and slatted frames were replaced to their original positions.

3.2.2.1.1 Grazing management

A flock of shorn Coopworth ewe lambs were rotationally grazed for 7±1 days around the three cocksfoot main plots under trees (28 day rotation with 21±1 days regrowth) (Plate 3.2). A smaller group from the same flock of sheep was grazed in the same rotational pattern around the adjacent open pastures. To avoid overgrazing in the sub-plot areas, sheep were only able to graze for the last 3±1 days of each grazing using an electric fence around the study areas.

All pastures were grazed from 15 September 1999 (initial liveweight of 45±3 kg) to 21 May 2000 and from 21 September 2000 (initial liveweight of 42±5 kg) to 2 April 2001. Because pasture was drought stressed, the grazing was stopped from 16 March to 15 April 2000 and from 26 January to 8 March 2001, to allow pasture to accumulate the minimum pre-grazing mass of 2.0 t/ha.

Stocking rate during grazing periods, over two years, under trees averaged 16 lambs/ha and 25 lambs/ha in the open. Stocking rate was adjusted when necessary after each liveweight measurement (37±5 day intervals) to ensure a similar pasture allowance for both flocks (mean pasture allowance of 3.2 kg DM/hd/d).

3.2.2.1.2 Urine patches

After each grazing rotation, 10 easily identifiable new sheep urine patches per replicate both in the open and under trees (Plate 3.3), were identified in two of the main cocksfoot plots. These were used to separate the main DM growth changes due to light from those of N. At the same time paired control, inter-urine patches, were also selected from within 1 m of each selected urine patch giving a total of 20 sampling points per replicate.

The data were analysed as a split-plot design with light regime (open: 100% transmittance and under tree shade: ~58% transmissivity) as main plots and nitrogen (non-urine patches or urine patches) as the subplot factor with two replicates.

Plate 3.2. Sheep grazing during the November 1999 rotation. A flock of shorn Coopworth ewe lambs was rotationally grazed for 7±1 days around the cocksfoot main plots under trees (28 day rotation with 21±1 days regrowth).

Plate 3.3 New urine patches after 21 days regrowth (November 1999), easily distinguished as dark green patches, were studied to explain the main dry matter growth changes due to light and nitrogen from sheep urine. The mean pasture area covered by urine patches was 30% with a mean diameter of 0.22 m per patch.

3.2.2.2 Exclosure experiment with different shade, water and N levels

In addition to the slatted structures, a second exclosure experiment was set up under trees and in the open (Plate 3.4). This was designed to examine the main yield and canopy architecture changes in cocksfoot due to light, N, water stress and regrowth duration during a season. This experiment also extended the water, herbage N content and regrowth duration conditions in the current silvopastoral system and isolated the effect of temperature, water, N, regrowth duration and shade on DM production.

This experiment was in fenced 6.6 x 6.0 m exclosure plots (Plate 3.4). The experiment was arranged in a 2^3 split-split plot factorial design with two replicates. Cocksfoot open pasture (100% transmissivity) and pasture under tree shade (~58% transmissivity) were the main plot light treatments, irrigation (0 or fully) was the sub-plot factor, and nitrogen (0 or 300 kg N/ha) the sub-sub plot. Sub-sub plots were 2.47 m^2 in area. Irrigation is not a common practice in silvopastoral sites, but this treatment was used to separate shade and water stress effects on pasture production. The sub-plots in the silvopastoral main plot were isolated from tree water extraction by cutting shallow tree roots around boundaries with a sharp spade to a depth of 0.40 m.

The eight treatments were monitored for four 60-day regrowth periods (1 September - 30 October 1999; 1 November - 30 December 1999; 6 January- 6 March 2000; and 8 March – 7 May 2000). A further 110-day regrowth period was measured from 8 May – 16 August 2000. After each period, the next 6.6 x 6.0 m area was fenced in a new position in the grazed pastures of the main plot and each treatment reimposed. Prior to fencing, the new plot areas were trimmed to a uniform stubble height of 20 mm to avoid the effects of any differential grazing on subsequent measurements.

The N was applied as synthetic sheep urine (Fraser *et al.*, 1994) as described in Table 3.3. The synthetic urine-N solution had a concentration of 14.2 g N per litre of de-ionized water. Thus, 5.225 l of solution was applied to the 2.47 m^2 areas to apply an equivalent of 300 kg N/ha (Plate 3.5). This application rate also provided 386 kg K/ha and 30 kg S/ha.

Table 3.3 Chemical composition and mineral concentration per litre of de-ionized water of the synthetic urine-N solution used for the nitrogen treatments in the exclosure experiment.

Compound	**Total** concentration of compound (g/l)	**Nitrogen** (g/l)	**Potassium** (g/l)	**Sulphur** (g/l)
Potassium hydrogen carbonate ($KHCO_3$)	25.7	--	10.0	--
Potassium chloride (KCl)	9.2	--	4.8	--
Potassium sulphate (K_2SO_4)	7.8	--	3.5	1.4
Urea ($CO(NH_2)_2$)	27.6	12.9	--	--
Glycine ($CH_2(NH_2)COOH$)	7.1	1.3	--	--
TOTAL	*77.4*	*14.2*	*18.3*	*1.43*

The full irrigation treatment was timed to prevent the actual soil moisture deficit from exceeding 35 mm or a reduction in volumetric water content (VWC) of 7% in the top 500 mm of soil. Water was applied at an average rate of 15-22 mm per application to ensure a maximum soil moisture content in the top 500 mm of 27%. This was close to the mean field capacity of about 30% (Yunusa *et al.*, 1995). The 3% difference was used to avoid water run-off in the event of rainfall immediately after irrigation. The mean soil moisture content in the top 500 mm was measured every 10 days in spring, autumn and winter, and every 3 days in summer with Time Domain Reflectometry (TDR, Trase Systems, Santa Barbara, USA). Irrigation was applied after the TDR measurements to replace the previous water loss according to a soil moisture deficit water balance (Equation 3.1). During the period of irrigation (I), treatments received an amount of water (A) equal to the difference between potential evapotranspiration (Epo) and rainfall (R) plus I in the previous period,

$$A = \sum Epo - (I+R) \qquad \textbf{Equation 3.1}$$

Actual rainfall and evapotranspiration values for the duration of the experiment were obtained from meteorological data recorded at Broadfields meteorological station 3 km north of the experimental site.

Plate 3.4. A 6.6 x 6.0 m fenced area from the 1 November-30 December 1999 regrowth duration period. This experiment was arranged in a split-split plot factorial design. Cocksfoot pastures in open (100% transmissivity) and under tree shade (~58% transmissivity) were the main plots. Irrigation (0 or fully) was the sub plot factor and nitrogen (0 or 300 kg N/ha) the sub-sub plot.

Plate 3.5. Pasture from an irrigated and N fertilised (300 kg N/ha as synthetic urine) treatment in open conditions after 50 days of regrowth during January-February 2000. Pasture had 5850 kg DM/ha and a LAI of 8. Note the canopy lodging.

The actual amount and timing of water applied for each irrigated treatment and for each regrowth period is shown in Table 3.4. No irrigation was required during the September-October 1999 period.

Table 3.4 Mean amount of water (W) applied (mm) during each regrowth period for the irrigated treatments in open pastures and under trees, with 300 kg N/ha (+N) or without nitrogen.

	Regrowth period				Total
Treatments	Nov-Dec 99	Jan-Feb 00	Mar-Apr 00	May-Aug 00	(mm)
Open W	51	66	62	0	179
Open W+N	75	148	64	0	287
Trees W	57	98	74	14	243
Trees W+N	61	196	79	24	360

The water applied was 23% greater for cocksfoot pasture under trees than in the open, and 35% greater for N compared with no N pastures (Table 3.4).

3.2.3 Physical environmental measurements

3.2.3.1 Air temperature and rainfall

Rainfall measurements were obtained from the Broadfields meteorological station. During the 21 month experimental period, from September 1999 to May 2001, rainfall was 956 mm (Figure 3.1) which was about 197 mm less than the long-term mean for these months. This was mainly because for March-April 2001, rainfall was only 9.2 mm which was approximately 90% less than the long-term mean (Table 3.1).

The air temperature measurements were taken onsite in the open and under trees using a digital temperature sensor (TDC-01A, Monitor Sensors, Queensland, Australia) located 1.5 m above ground, which logged every 6 minutes (resolution ±0.2 °C). The mean daily temperature during June and July 2000 (Figure 3.1) was 1.5 °C warmer than the long-term mean (Table 3.1). The mean daily temperature was similar in the open and under trees (Figure 3.1). In both summers, (December-February 1999/2000 and 2000/2001), the mean temperature under trees was 0.4 °C warmer than in the open, and during winter (June-August 2000) it was 0.2 °C warmer. However, during a sunny day in autumn-winter (maximum temperatures between 10-15 °C) the temperature under trees was up to 3 °C warmer at midday and morning (from 5:00), but the difference was reversed after sun set (Figure 3.2a). In contrast, during sunny hot days in summer (> 28 °C) there was minimal difference in air temperature under trees and open pasture sites (Figure 3.2b).

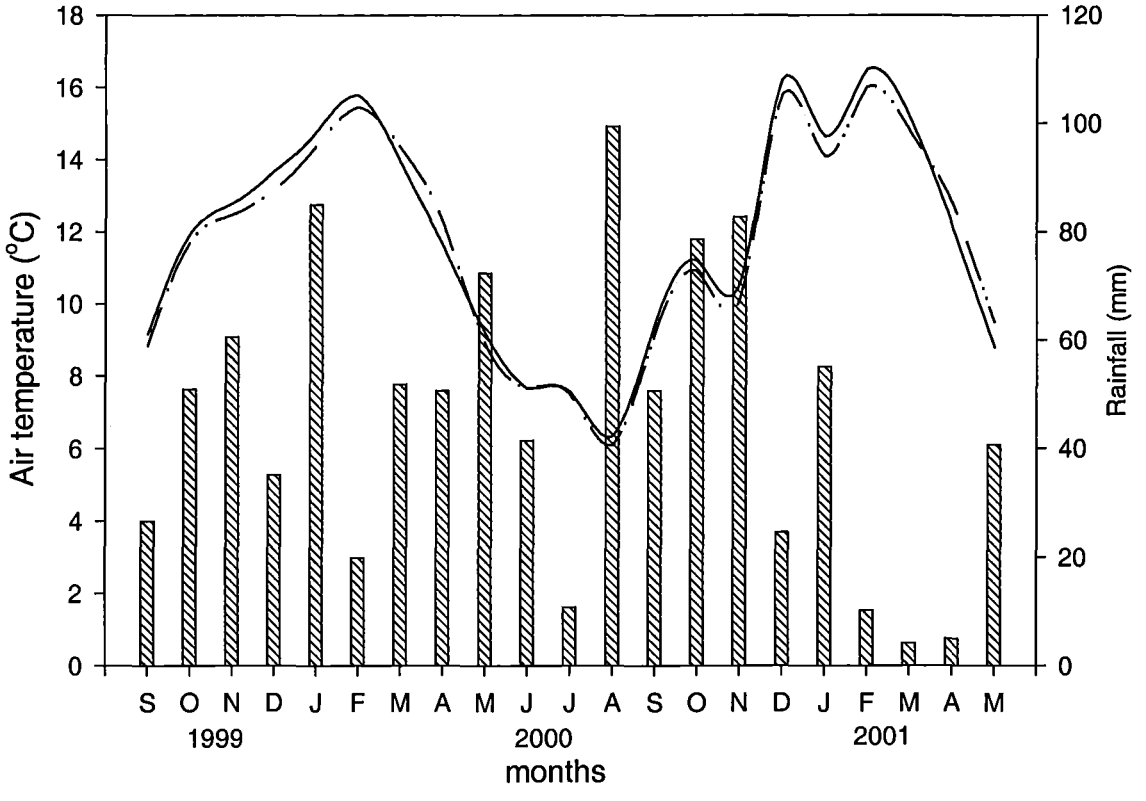

Figure 3.1 Rainfall (▨) and mean monthly air temperature under trees (—) and in the adjacent open (··—) pasture at the Lincoln University silvopastoral experiment from September 1999 to May 2001.

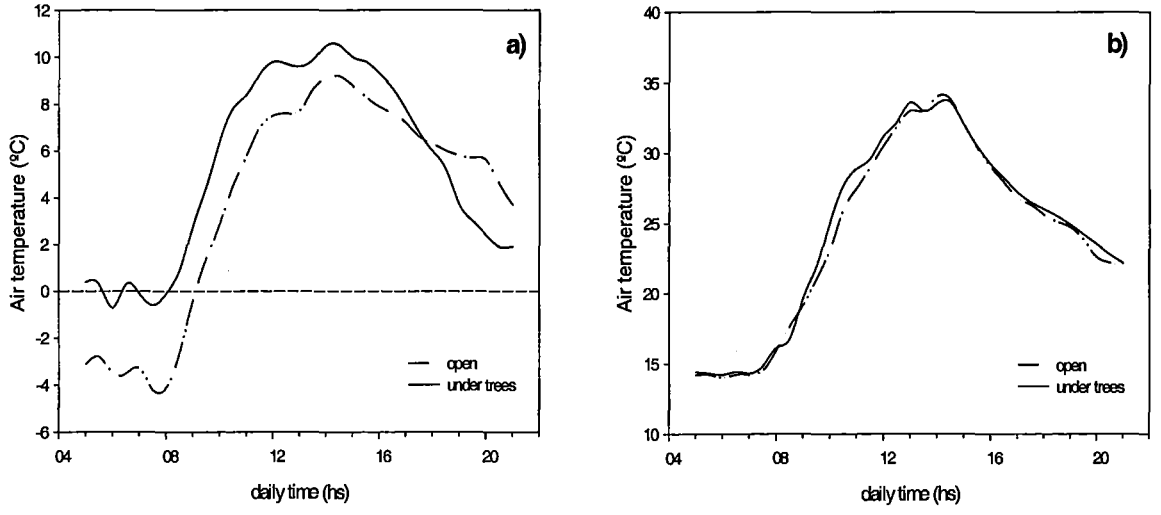

Figure 3.2 Diurnal air temperature under trees and in the adjacent open pasture for sunny days in a) winter (maximum temperature of 10.5 °C, 16 July 2000) and b) summer (maximum temperature of 33.5 °C, 14 February 2001) at the Lincoln University silvopastoral experiment.

3.2.3.2 Soil moisture

3.2.3.2.1 Soil moisture for the experiment with four light regimes

The mean soil VWC in the top 500 mm, was measured every 7 days with TDR (Figure 3.3). In spring and winter, soil moisture was always above 24% and was therefore always greater than half the maximum available water content of the site (mean field capacity= 30%) indicating that the treatments were not moisture stressed during those periods. However, in summer and autumn of both years, pastures were under water stress. On average, pastures under trees had 2.5% less soil VWC than open pastures. The shaded treatment open+slats had a higher soil VWC than open. Similarly, the treatment trees+slats had a higher soil VWC than the pasture under trees. This additional soil VWC under the slatted shade resulted in greater water recharge during winter. For example, in July 2000 the pastures in the open had a soil VWC of 30.5% compared with 32.0% in the open+slats treatment.

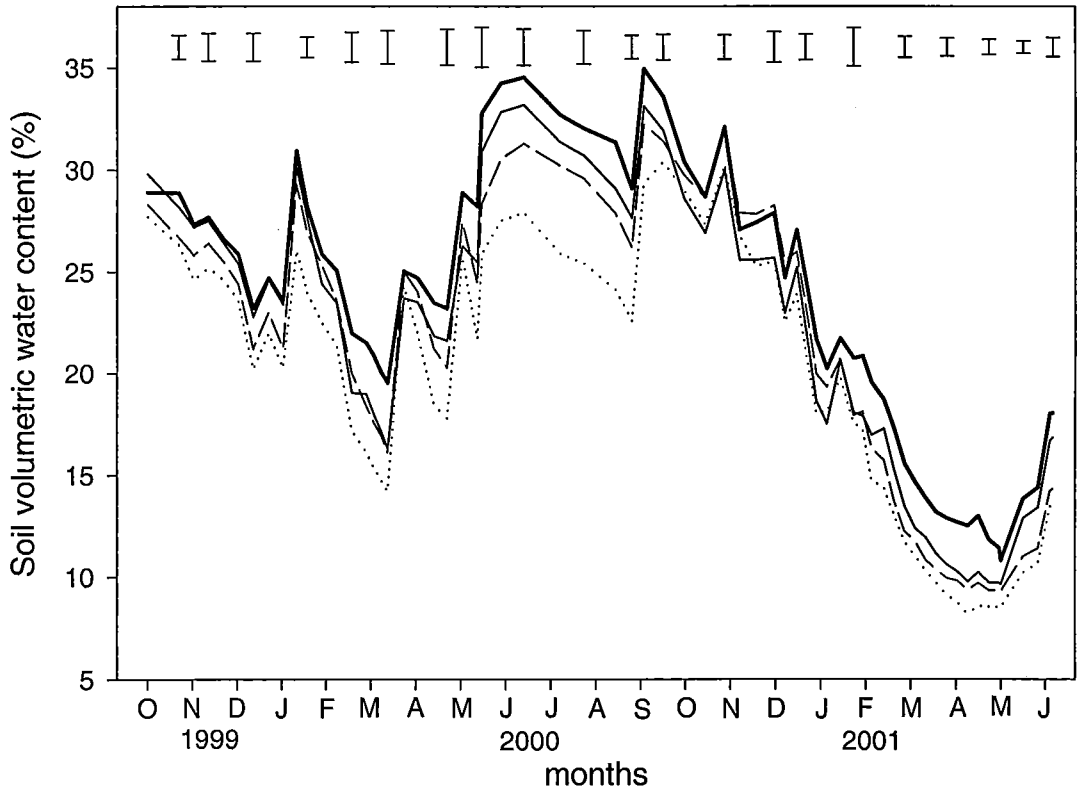

Figure 3.3 Mean soil volumetric water content in the top 500 mm (measured every 7 days) for four shaded treatments: Open (—) (100% transmissivity), open+slats (▬) (~43% transmissivity), under trees (···) (~58% transmissivity) and trees+slats (---) (~24% transmissivity). Bars indicate standard error of the mean (sem). Treatment details are given in Section 3.2.2.1.

3.2.3.2.2 Soil moisture for exclosure plots

The soil VWC for the five growth periods of the exclosure experiment are shown in Figure 3.4. During the first regrowth period in September-October 1999 irrigation was not applied because the soil moisture deficit was less than 35 mm which was a reduction of <7% soil VWC in the top 500 mm. The mean maximum actual soil moisture deficits between treatments and regrowth periods, calculated from the difference for actual soil VWC and field capacity value (VWC= 30%), are summarised in Table 3.5.

Table 3.5 Maximum actual soil moisture deficit (mm) in the top 500 mm for the exclosure experiment with different shade (open and under trees), water (W) and nitrogen (N) levels. Treatment details are given in Section 3.2.2.2.

	Regrowth periods				
Treatment	Sep-Oct 99	Nov-Dec 99	Jan-Feb 00	Mar-Apr 00	May-Aug 00
Open control	17.0	66.8	77.8	70.5	10.0
Open W	17.0[#]	30.0	25.7	15.0	10.0[#]
Open N	25.0	76.5	83.0	78.8	12.5
Open W+N	25.0[#]	35.0	33.5	15.5	12.5[#]
Trees control	20.0	60.5	80.8	97.0	40.5
Trees W	20.0[#]	32.2	30.0	15.0	15.0
Trees N	27.5	72.5	87.5	98.5	42.5
Trees W+N	27.5[#]	35.5	37.0	15.7	15.0
SD	9.25	14.05	27.51	19.50	12.12

[#] Because irrigation was not necessary, values are the same as control treatments.

In most cases, the target for full irrigation treatment was achieved. The maximum soil moisture deficit was in general higher under trees than open pastures. For example, during the March-April regrowth period, the soil moisture deficit for the non-irrigated treatments under trees was 23 mm higher than in the open. Furthermore, the maximum soil moisture deficit averaged 7.5 mm higher in pastures with N than without N.

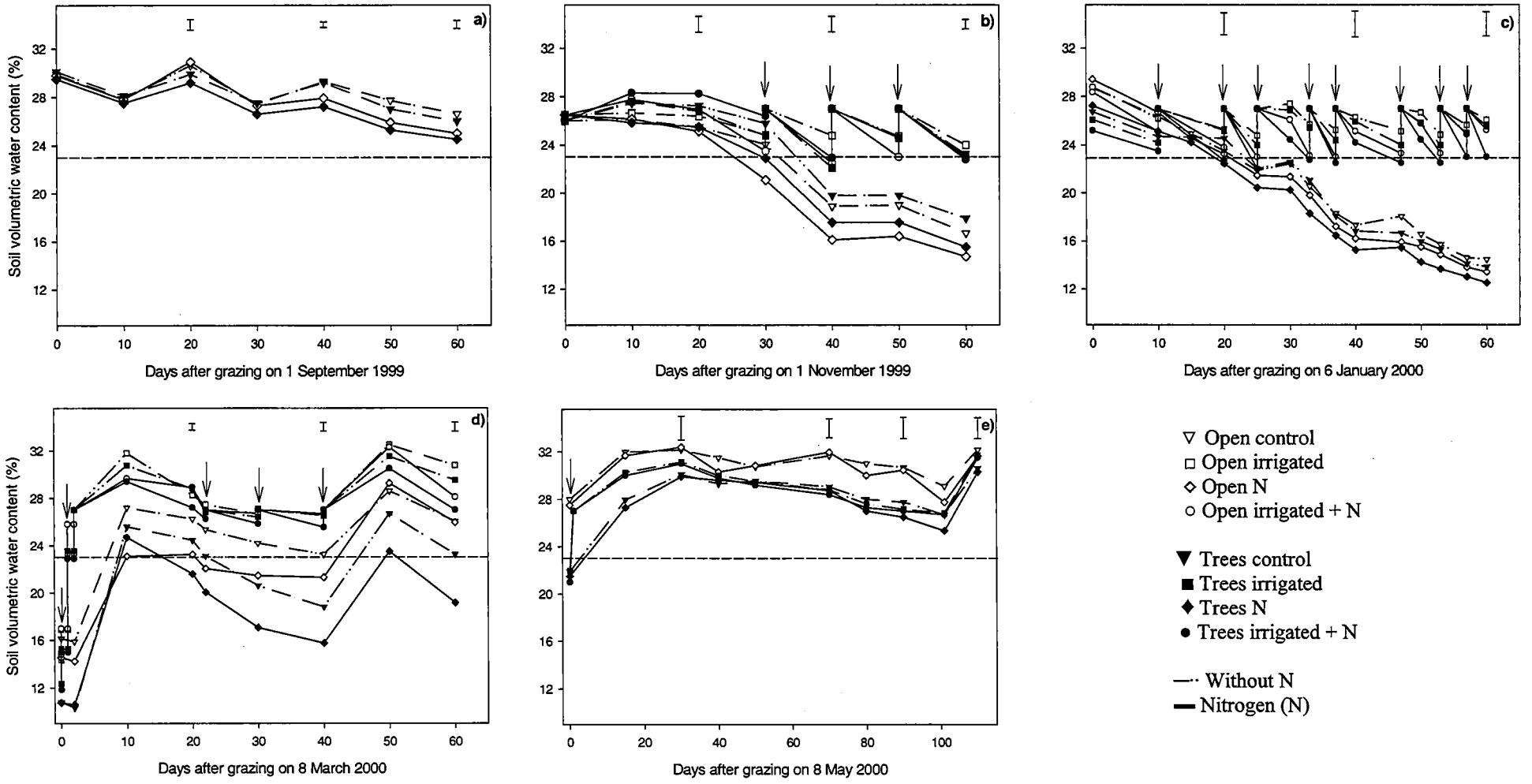

Figure 3.4 Mean soil volumetric water content (VWC) in the top 500 mm over time for two levels of light intensity (open pasture 100% transmittance or pasture under tree shade ~58% transmissivity), two levels of irrigation (0 or fully) and two levels of nitrogen (0 or 300 kg N/ha). Four 60-day regrowth durations (a-d), and a 110-day regrowth duration (e) were used. Dotted lines indicate the lower limit of the irrigation goal (VWC of 23%). Arrows indicate water applications. Bars indicate standard error of the mean (sem).

3.2.3.3 Light quantity

Light intensity was monitored with quantum sensors (Li-cor LI-191SB, Lincoln, Nebraska, USA) installed above and below the slatted shade structures, but above cocksfoot canopy height. This gave a quantitative description of the four levels of shade used in the experiment with four light regimes (open, open+slats, trees and trees+slats) and for the exclosure experiment (open and trees). The quantum sensors measured the photosynthetic photon flux density (PPFD) in the 400-700 nm waveband every 5 minutes by a datalogger with mean PPFD recorded at 30 minute intervals.

The daily PPFD was integrated to calculate the accumulated monthly photosynthetic photons per unit area (Figure 3.5). The maximum photosynthetic photons reaching the cocksfoot pasture was in December (1715-1815 mol/m^2 for open pastures) corresponding to the maximum noon solar angle elevation (69.8° at noon). The minimum (302 mol/m^2 for open pastures) was in June with the lowest noon solar angle elevation of 23°. In December, pastures in the open received 720, 960 and 1220 mol photons/m^2 more than pastures under trees, open+slats and trees+slats, respectively. However, these differences decreased in June.

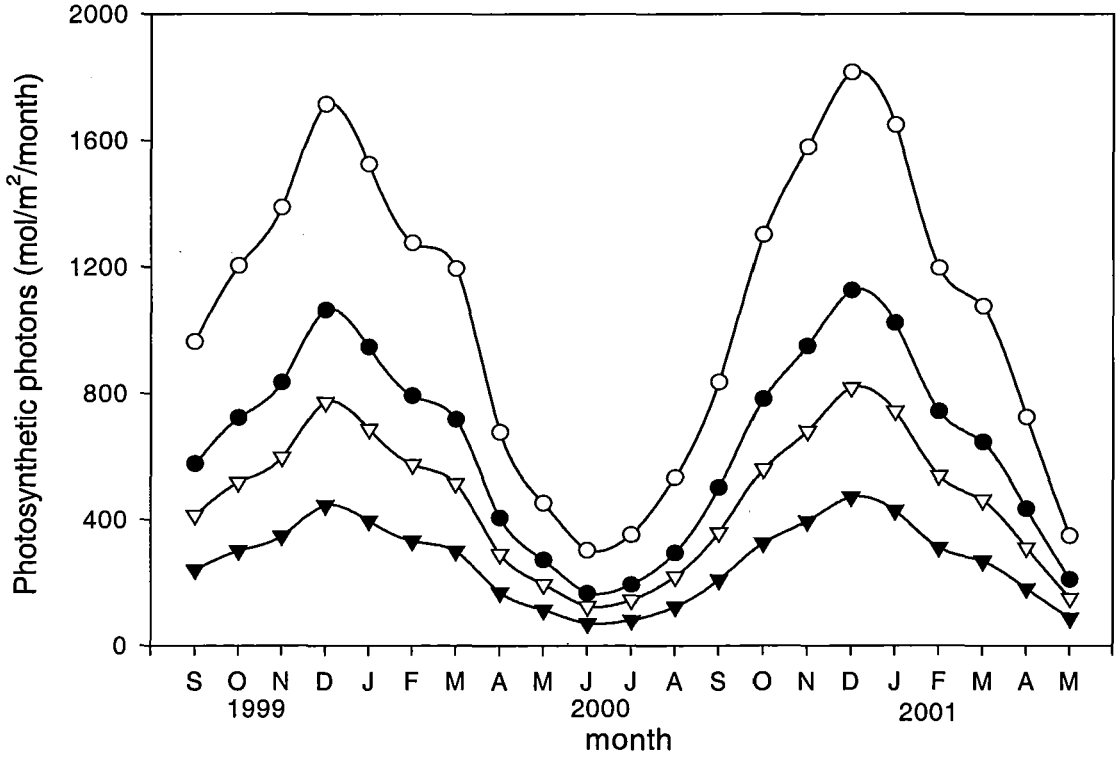

Figure 3.5 Mean monthly photosynthetic photons (400-700 nm waveband) received for cocksfoot pastures from the four shaded treatments: open (○), open+slats (▽), under trees (●) and trees+slats (▼).

The daily PPFD integral in the open for a sunny day in spring or autumn (e.g. 21 September or 21 March at solar angle elevation of 46.5° at noon), summer (21 December at solar angle elevation of 69.8° at noon) and winter (21 June at solar angle elevation of 23.0° at noon), and over a range of cloudy days were used as a reference (100% transmissivity) to calculate the transmissivity of the shade treatments (Table 3.6). This was used to represent the relative reduction of photosynthetic photons in the shaded treatments compared with the open pasture.

Table 3.6 Transmissivity of the shaded treatments as a percentage of the open daily integral photosynthetic photon flux density (PPFD) for sunny days at three different solar angles elevation (seasons) and for a range of cloudy days in Canterbury, New Zealand. Values in parentheses are the total daily integral of PPFD for open expressed as mol photons/m^2/d.

Solar angle at noon	*69.8°*	*46.5°*	*23.0°*	*diffuse light*
Treatments	Summer	Autumn-Spring	Winter	Cloudy days
Open	100% (63.3)	100% (36.0)	100% (10.6)	100% (7-18)
Open+slats	45%	43%	41%	45%
Trees	62%	60%	55%	58%
Trees+slats	26%	25%	23%	20%

The total daily integral photosynthetic photons received in open pasture around the 21 December was 63.3 mol photons/m^2/d which was 6 times higher than in winter (21 June) (Table 3.6). For cloudy days (diffuse light) during summer and spring the total integral daily photosynthetic photons received in open pasture varied between 7 and 18 mol photons/m^2/d depending on the cloud type. The transmissivity under the 10-year-old trees measured in the middle of rows was 62% of the open over a sunny day in summer (at maximum solar elevation), with alternating periods of full sunlight and this decreased to 26% with the addition of the slatted structure. The transmissivity of the shaded treatments decreased with a decrease in solar angle elevation from summer to winter. The transmissivity of the tree shaded treatments during cloudy days (58%) was lower than sunny days in spring and summer (60-62%), but under the slatted shade it remained at 45% between cloudy and sunny days (Table 3.6).

Values of PPFD of individual crown tree shade were measured with a SF-80 Ceptometer (Decagon Device, Cambridge, U.K.) using line transects across the projected shade at

noon. The intensity of the majority (70%) of the individual crown tree shade was 7% of open PPFD. However, there was an area from the edge to about 0.5 m inside the total shaded zone (~6.0 m maximum length x ~5.0 m maximum width) and along the perimeter where the irradiance was gradually reduced from full sun to full shadow (gradient of 23% of open PPFD). Under each slat there was a uniform severe shade of 5% of open PPFD.

3.2.3.4 Light quality

Spectral irradiance from 300 to 1100 nm wavelengths was measured with a Li-Cor LI-1800 spectro-radiometer (Lincoln, Nebraska, USA). Measurements were taken at noon and 17:00 h for a sunny day in spring, which corresponded to solar angle elevations of 46.5° and 17.6°, respectively. Also, measurements were taken at noon for a cloudy day. From the total spectral irradiance data, proportions of red (660 nm) to far-red (730 nm) wavelengths were calculated (Table 3.7).

Table 3.7 Red (660 nm) to far-red (730 nm) ratio at noon and 17:00 h for a sunny and cloudy summer day and for different light conditions.

Light condition	Sunny day at noon (46.5° solar angle)	Afternoon (17:00 h) (17.6° solar angle)	Cloudy day at noon (diffuse light)
Open sun	1.32	1.34	1.29
Open sun under slat	1.28	1.28	-
Open shade under slat	0.74	0.86	1.20
Tree sun	1.24	1.29	-
Tree shade (middle)	0.54	0.83	1.16
Tree shade (edge)	0.90	0.97	-
Tree shade under slat	0.40	0.58	1.16

The R:FR ratio decreased from sun to any of the shaded situations. The minimum value of R:FR was 0.54 at noon in the middle of the tree shade. The R:FR also decreased under the shade of slats. There was a difference in R:FR within the tree shade with higher values along the perimeter (0.5 m inside the shaded zone). There was no difference in R:FR for two different solar angles elevation (noon and afternoon) for full sunlight conditions. However, under the tree shade, the R:FR increased at the lowest solar angle. At noon on the cloudy day, the R:FR was greater under trees and the slatted structure compared with the sunny day, but still less than the R:FR in open.

3.2.4 Biological measurements

Herbage measurements were taken prior to lambs grazing (21±1 days regrowth) for the experiment with four light regimes and the associated urine patches. For the exclosure plots, samples were taken every 10 days.

For all treatments, pasture samples for DM production were obtained from a 0.2 m^2 quadrat cut to 20-25 mm stubble height, except for the paired urine and non-urine patches which were obtained from 0.05 m^2 circular quadrats. The smaller quadrat size (diameter 250 mm) was used to sample completely an individual urine patch (mean diameter ranged from 200 to 300 mm). DM samples were dried in a forced draft oven at 65 °C to constant weight.

The botanical composition of all samples was determined by dissecting an approximately 50 g fresh weight sub-sample from each DM cut before oven drying. Canopy height was measured using a sward stick before herbage harvesting.

The vegetative tiller number was counted as new leaf extension above the grazed leaf sheath height within 3-5 days post-harvest using a circular 0.01 m^2 quadrat. Reproductive tillers were counted at the time of harvest using a 0.2 m^2 quadrat and during November and December 1999 for the exclosure plots.

The area of cocksfoot urine patch covered in main plots was measured both in the open and under trees. This was assessed using six permanent line transects across the plots (27 m long in open and 46 m long under trees) in October (spring), January (summer) and April (autumn) of 1999 and 2000. The mean diameter of individual urine patches and the distances between urine patches were measured using a tape placed on transects.

Urine was collected from sheep grazing the cocksfoot plots to establish the amount of nitrogen applied in urine patches. Urine samples were taken in autumn (18 April 2000) and spring (24 October 2000) from 5 animals grazing cocksfoot under trees and 5 animals grazing cocksfoot in open. Samples were analysed for total nitrogen using the Kjeldahl-N technique.

3.2.4.1 Canopy architecture

The Li-cor LAI-2000 Plant Canopy Analyser (Lincoln, Nebraska, USA) was used to measure leaf area index (LAI), mean canopy leaf angle (mean tilt angle, MTA) and canopy transmittance. The Li-cor LAI-2000 is a hand-held instrument, with optical sensors that includes a fisheye lens and five silicon detectors allowing simultaneous measurement of the radiation coming from the upward hemisphere in five zenithal angles. Canopy transmittance in the five zenithal angles (T_θ) is estimated from measurements successively performed above and below the canopy. From these measurements, inversion of radiative transfer models allows the computation of LAI and MTA (Welles and Norman, 1991). Unlike T_θ, which is directly computed from radiation measurements, LAI and MTA result from model inversion. Accuracy is therefore dependent on the degree to which model assumptions match reality. One of the main assumptions is that foliage elements are randomly distributed.

There are difficulties in measuring total LAI for grasses because the optical sensor head of the instrument is 40 mm high. Therefore, aluminium trenches 40 mm deep x 30 mm wide x 1.2 m long were set up for all treatments so that the top of the sensor was at the soil surface. In this study, measurements were taken from one reading above the canopy followed by five readings beneath, along the trench (transect). As the Li-cor LAI-2000 requires diffuse light to give reliable measurements, the instrument was only used under uniform overcast conditions, or before sunrise and after sunset. To avoid contamination of the measurements by the operator, a 180° view cap was used.

A mean extinction coefficient (k) for the canopy was calculated by considering diffuse radiation interception obtained from measurements of 'gap fraction' measured with the Li-cor LAI-2000 as has been reported for grasses and other plants (Chen *et al.*, 1997; Nouvellon *et al.*, 2000). This is based on the Bourguer-Lambert-Beer' equation described by Monsi and Saeki (1953) (Equation 3.2).

$$I = I_0 e^{-LAI*k} \qquad \textbf{Equation 3.2}$$

Where I is the incident PPFD at a given horizontal level within the canopy (W m^{-2}); I_0 is the incident PPFD above the canopy (W m^{-2}); LAI is the cumulative leaf area index

(dimensionless); k is the extinction coefficient which reflects canopy structure and the position of the sun in the sky.

Derived from Equation 3.1, a plot of $\ln(I/I_o)$ against LAI gives a straight line whose gradient or slope is the extinction coefficient k (Equation 3.3).

$$k = \frac{\ln(I / Io)}{LAI} \qquad \textbf{Equation 3.3}$$

This relationship has been found to give satisfactory descriptions of the penetration of radiation into the canopies of a variety of pasture and crop species (Hay and Walker, 1989). It is important to highlight that these mean values of k calculated from diffuse light (bulked k) are expected to be different from that those calculated for direct sun at different solar angle elevations.

3.2.5 Statistical analysis

Statistical analyses were carried out using the Genstat statistical package (Genstat 5, 1997). Standard error of means (sem) were used to evaluate least significant differences (lsd) at the 0.05 probability level for means separation of the pasture variables. Significant differences for the experiment with four light regimes were determined for each rotation by analysis of variance (ANOVA) according to the split-plot design with three replicates. ANOVA analysis for the urine and non-urine patches was carried out in a split-plot design with two replicates. ANOVA analysis for the exclosure experiment was determined for each harvest according to the split-split plot factorial design with two replicates. Pasture variables were also analysed by considering time as a factor. Thus, this analysis was carried out to detect potential interactions between a pasture variable (such as DM growth rate) and the main environmental factors (such as temperature) which vary with time (seasons).

Based on residual analysis, data obtained from botanical composition were transformed using an arcsine transformation, which is commonly used for analysis of percentage data to remove the skew from distributions (Sokal and Rohlf, 1995). This transformation was carried out before ANOVA.

3.3 Results

3.3.1 Pasture DM production and growth rates

3.3.1.1 Pasture DM production and growth rate for the four light regimes experiment

The mean annual total DM production from February 2000 (when pasture was adjusted to new shaded treatments) to February 2001 (avoiding the atypical dry autumn 2001) was 8.2 t DM/ha/yr in open, 7.3 t DM/ha/yr in open pasture under slat shade, 6.3 t DM/ha/yr under trees shade and 3.8 t DM/ha/yr in the trees+slats treatment.

The differences in pasture DM production were driven by DM growth rates (Figure 3.6). DM growth rate was lower under trees and trees+slats compared with the full sunlight treatment in all seasons. The mean DM production rate of cocksfoot for the grazing seasons (September-April) for the two years was 30 kg DM/ha/d in open, 26 kg DM/ha/d in open+slats, 21 kg DM/ha/d under trees and 14 kg DM/ha/d under trees+slats. For the dry period January-March 2001, pastures in the open under slat shade produced more than the adjacent full sunlight treatment.

There was an interaction ($p< 0.05$) between treatments and time (rotations). This was expressed by seasonal fluctuations in pasture DM growth rates (Figure 3.6). The highest ($p< 0.05$) growth rates occurred during November (mean of 48 kg DM/ha/d in open, 43 kg DM/ha/d in open+slats, 35 kg DM/ha/d under trees and 24 kg DM/ha/d under trees+slats) and there was a rapid decrease in summer (December-February) and winter (June-July). In autumn 2000 (April-May), there was a recovery after summer drought showing a typical bimodal annual growth curve. However, this trend did not occur during autumn 2001. DM production rate was higher in the second year during spring compared with the first year for pastures in the open and under trees, but lower for the shaded treatments; open+slats and trees+slats.

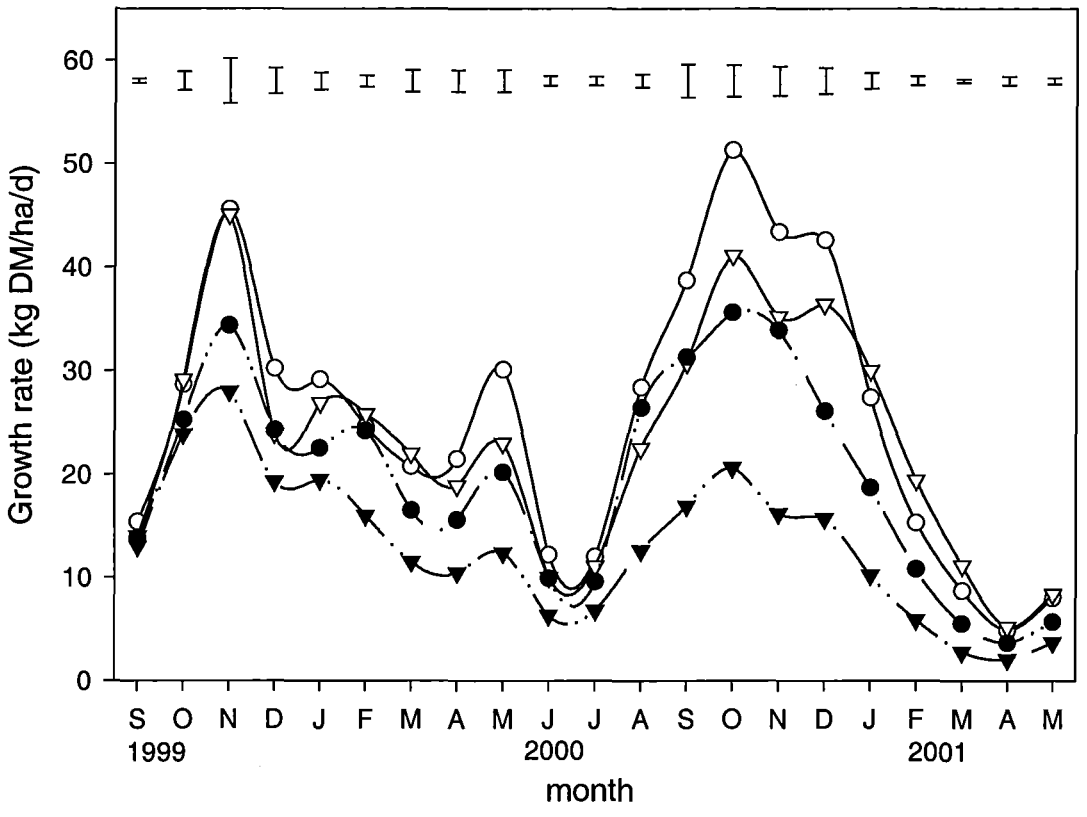

Figure 3.6 Cocksfoot dry matter growth rates (21±1 days regrowth) over time for four shade treatments: open (○) (100% transmissivity), open+slats (▽) (~43% transmissivity), under trees (●) (~58% transmissivity) and trees+slats (▼) (~24% transmissivity). Bars indicate standard error of the mean (sem).

3.3.1.2 Sheep urine N content and pasture production rate from urine patches

The mean area covered by visually obvious urine patches in both open and under trees pastures varied from 25% in October (1999/2000) to 32% in April (1999/2000) with a mean diameter of 0.22 m. Sheep urine had a higher N concentration (g/l) in spring (October) than in autumn (April) in all treatments (Table 3.8), and it was higher for sheep grazing pastures under trees compared with open pastures. Results were used to estimate rate of N applied per hectare based on a mean urination volume by young sheep of 0.15 l (Haynes and Williams, 1993). The rate of N applied per hectare for an individual urine patch varied from 173 to 495 kg N/ha depending on the season and type of pasture grazed (Table 3.8).

Table 3.8 Nitrogen (N) concentration of sheep urine in autumn (April) and spring (October) 2000, and the estimated rate of N applied from sheep urine per hectare to cocksfoot pastures in open and under trees.

Treatment	N (g/l)	Mean urination volume[1] (l)	N applied per urination (g/l)	Mean urination area (m^2)	N in mean urine patch (kg N/ha)
Open autumn	3.46	0.15	0.52	0.03	173
Trees autumn	4.43	0.15	0.66	0.03	221
Open spring	8.97	0.15	1.35	0.03	448
Trees spring	9.90	0.15	1.49	0.03	495

1. Mean urination volume was taken from Haynes and Williams (1993)

The cocksfoot DM production from individual new urine patches compared with non-urine pastures is shown in Figure 3.7. The seasonal fluctuations showed a maximum growth rate during October-November when new urine patches had three times higher ($p< 0.05$) growth rate than the non-urine pastures both in open and under trees. These differences decreased in summer and autumn. For example, in autumn 2001 (soil VWC < 14%) urine patches produced almost the same as paired non-urine areas.

There were no interactions between the shade and N (urine patches). The DM growth rate of new urine patches was lower ($p< 0.05$) under trees than open pastures in all seasons. During September-December, when water was less limiting than in autumn, the growth rate was 96 kg DM/ha/d in open and 72 kg DM/ha/d under trees.

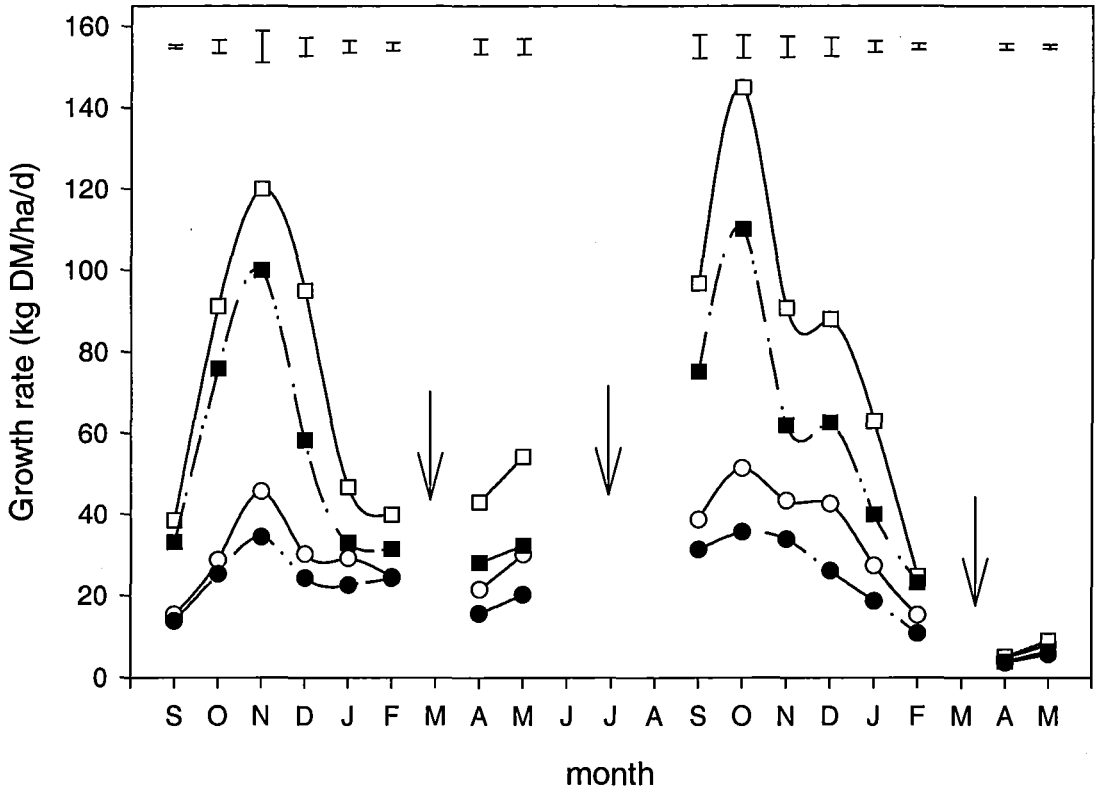

Figure 3.7 Cocksfoot dry matter growth rate (21±1 days regrowth) for urine patches (square symbols) and paired non-urine patches (circle symbols), in open pastures (open symbols) (100% transmissivity) and under trees (solid symbols) (~58% transmissivity). Arrows indicate discontinuity in grazing. Bars indicate standard error of the mean (sem).

3.3.1.3 Pasture DM production and growth rate from the exclosure experiment

On average, the application of N increased ($p < 0.001$) the annual yield by ~14 t DM/ha/yr and irrigation increased ($p < 0.05$) annual yield by ~4.4 t DM/ha/yr (Table 3.9). In contrast, tree shade reduced ($p < 0.05$) total annual yield by ~3.2 t DM/ha/yr.

An interaction occurred between shade and N during the September-October ($p < 0.05$) and during November-December ($p < 0.001$) regrowth periods for DM yield (Table 3.9). This was caused by the higher DM response to increased N in open pastures. The same interaction occurred for DM production rate during all regrowth periods at different times (Figure 3.8).

An interaction also occurred between shade and water during the November-December regrowth period ($p < 0.05$) for DM yield (Table 3.9) and for DM production rate at days 50 and 60. This was caused by the greater response to irrigation in open pastures compared

with under trees. Also, there was an interaction ($p< 0.05$) between treatments with time of regrowth for DM growth rate (Figure 3.8).

In addition, the large differences in DM yield accumulated during the November-December regrowth period were also attributed to the production from reproductive tillers. Shade had a negative effect ($p< 0.001$) on the amount of reproductive DM production. For example, in irrigated plus N fertilised pastures, the reproductive DM accumulated after 60 days regrowth was 1920 kg DM/ha (21% of total) in open and only 650 kg DM/ha (10% of total) under trees.

Table 3.9 Accumulated dry matter yield (kg DM/ha) for different regrowth periods and annual DM production (t DM/ha) for cocksfoot pastures at two light (open and tree shade), two irrigation (0 or fully) and two nitrogen (0 or 300 kg N/ha) levels. Regrowth periods were 60 days for spring, summer and autumn and 110 days for winter (May-August 2000).

	Regrowth period					
Treatment	Sep-Oct 99	Nov-Dec 99	Jan-Feb 00 (kg DM/ha)	Mar-Apr 00	May-Aug 00	Total annual (t DM/ha/yr)
Open control	2650	3260	920	980	1390	9.2
Open W	2650[#]	5340	2230	1440	1390[#]	13.0
Open N	5380	7620	3540	3410	3540	23.5
Open W+N	5380[#]	8970	5980	4690	3540[#]	28.6
Trees control	2580	2340	600	720	1140	7.3
Trees W	2580[#]	3690	2040	1150	1430	10.9
Trees N	4370	5800	3240	2950	2670	19.0
Trees W+N	4370[#]	6830	5780	3940	3210	24.1
sem	110.1	98.7	501.2	120.7	250.9	0.46
Significance						
Shade	*	*	ns	ns	ns	*
W	-	***	*	**	ns	*
N	***	***	***	***	***	***
Interactions						
Shade x W	-	*	ns	ns	ns	ns
Shade x N	*	**	ns	ns	ns	ns
W x N	-	ns	ns	ns	ns	ns
Shade x W x N	-	ns	ns	ns	ns	ns

[#] Because irrigation was not necessary, values are the same as control treatments.
* $p< 0.05$; ** $p< 0.01$; *** $p< 0.001$; ns= no significant differences

The differences in pasture DM production were caused by changes in DM growth rates. The DM growth rate curves showed seasonal differences for all treatments (Figure 3.8). The highest ($p< 0.01$) production rate (154 kg DM/ha/d) occurred in irrigated and N

fertilised open pastures during the November-December 1999 regrowth period when water was non-limiting (mean soil VWC> 23%) and mean air temperature was 13.5 °C. This decreased ($p< 0.001$) to 32 kg DM/ha/d in winter for May-August period (110-day regrowth) when the mean temperature was only 7.0 °C.

The added nitrogen at least doubled ($p<0.001$) DM growth rates in all rotations and in both open and shaded plots. For example, the maximum growth rate in the open W+N during the January-February 2000 regrowth period (mean temperature >15 °C) was 134 kg DM/ha/d compared with 43 kg DM/ha/d in the open W treatment.

Similarly, irrigation increased ($p< 0.05$) DM growth rates. For example, the growth rate was 15 kg DM/ha/d after day 60 of the January-February regrowth period when maximum water stress occurred (soil VWC of 14% in unirrigated plots) compared with 38 kg DM/ha/d for irrigated pastures.

Although there was no significant effect ($p= 0.11$) of shade on DM growth rate, it was consistently lower under trees than in the open. The maximum DM growth rate under trees was 131 kg DM/ha/d in the November-December period for irrigated and N fertilised pastures, but this was 23 kg DM/ha/d lower than for the comparable open pasture.

Regrowth time also affected ($p< 0.001$) DM growth rate. For the irrigated and N fertilised pastures during the November-December and January-February regrowth periods, the DM production increased to a maximum value and then declined. For example, during the January-February regrowth period, the DM production for the open W+N treatment increased from 65 kg DM/ha/d at day 10 to the maximum 137 kg DM/ha/d at day 30, and then declined to 99 kg DM/ha/d after 60 days regrowth.

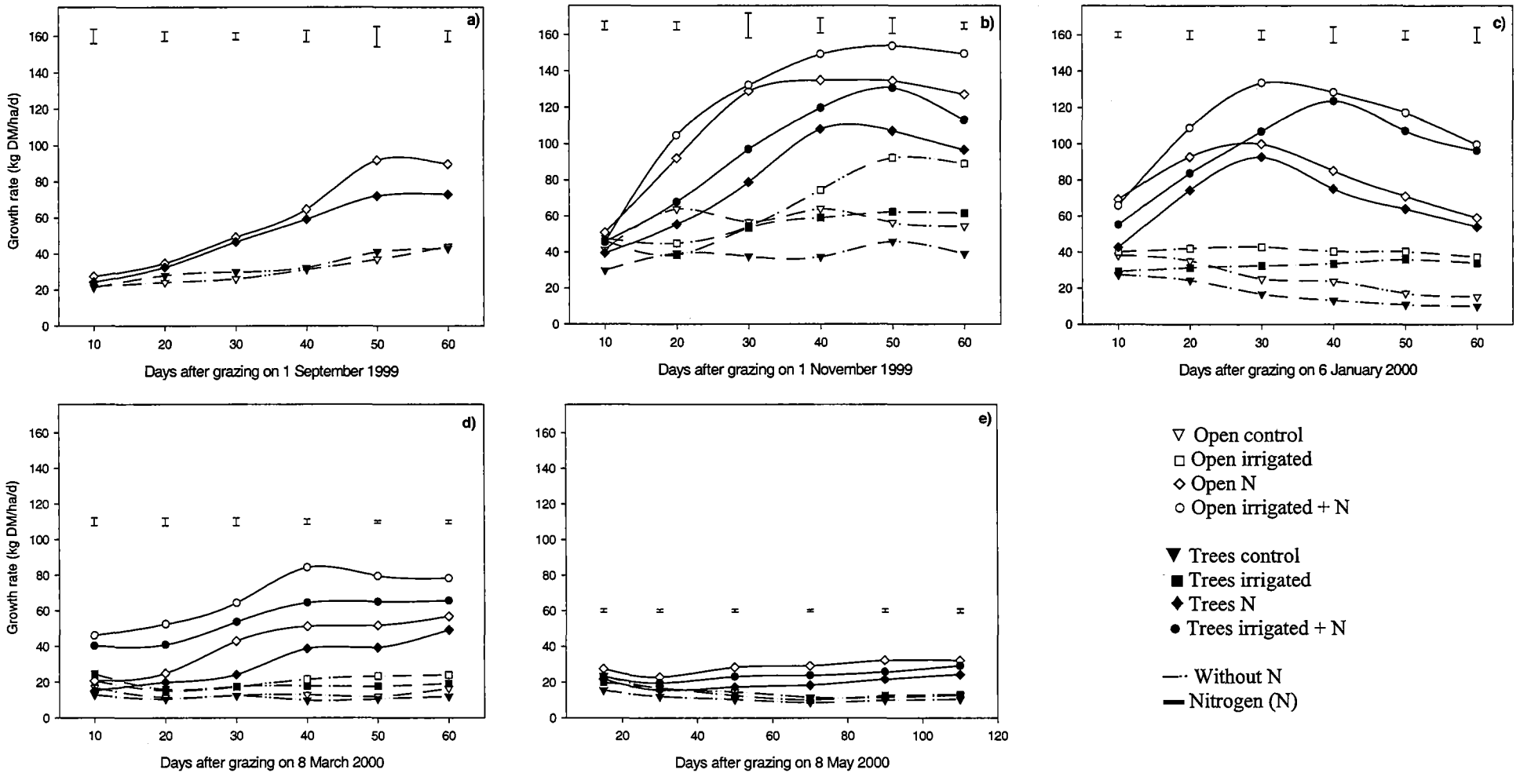

Figure 3.8 Cocksfoot dry matter growth rate (kg DM/ha/d) over time for two levels of light intensity (open pasture: 100% transmittance or pasture under tree shade: ~58% transmissivity), two levels of irrigation (0 or fully) and two levels of nitrogen (0 or 300 kg N/ha). Four 60-day regrowth durations (a-d), and a 110-day regrowth duration (e) were used. Bars indicate standard error of the mean (sem).

3.3.4 Botanical composition

All components of pasture botanical composition (percentage of component contribution to total DM production) of both experiments varied with seasons and for the different shaded, N and irrigated treatments (Appendices 2 and 3). The green cocksfoot component in the pastures ranged from 72 to 96%. The clover and weed components ranged from zero to 15%. Cocksfoot senescent and dead material ranged from 1 to 27%.

3.3.5 Leaf area index (LAI)

3.3.5.1 LAI for the experiment with four light regimes

As for DM production, LAI curves showed seasonal fluctuations (Figure 3.9) which was indicated by the interaction ($p< 0.05$) between treatments and time (rotations). The greatest ($p< 0.05$) LAI occurred in spring during October-November (mean of 4.1 in open, 3.8 in open+slats, 3.0 under trees and 2.2 under trees+slats) and there was a rapid decrease in late summer and winter.

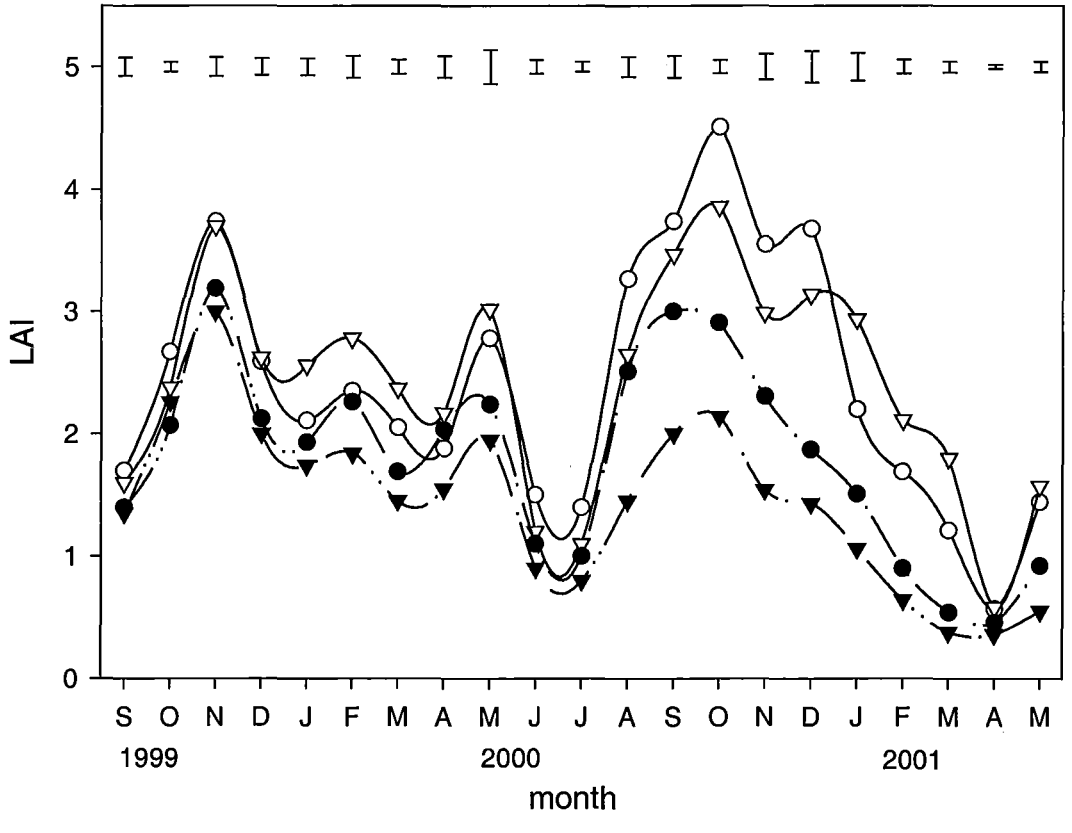

Figure 3.9 Cocksfoot leaf area index (LAI) (21±1 days regrowth) over time for four shade treatments: open (○) (100% transmissivity), open+slats (▽) (~43% transmissivity), under trees (●) (~58% transmissivity) and trees+slats (▼) (~24% transmissivity). Bars indicate standard error of the mean (sem).

The LAI was consistently lower under trees ($p < 0.05$) and trees+slats ($p < 0.01$) compared with the full sunlight treatment in all seasons (Figure 3.9).

3.3.5.2 LAI from the exclosure experiment

Cocksfoot LAI values showed similar responses to N, irrigation and shade over time and the same interactions between factors as for DM production with seasonal fluctuations for all treatments (Figure 3.10). The added nitrogen ($p<0.001$) and irrigation had a positive ($p< 0.05$) effect on LAI in all rotations in open and shaded plots. In contrast, LAI values for pastures under tree shade were lower than in open pastures. As a consequence, after 60 days of regrowth, LAI ranged from 8.2 (in irrigated and N fertilised open pastures during the January-February period) to 2.5 (control pastures under trees during the March-April period).

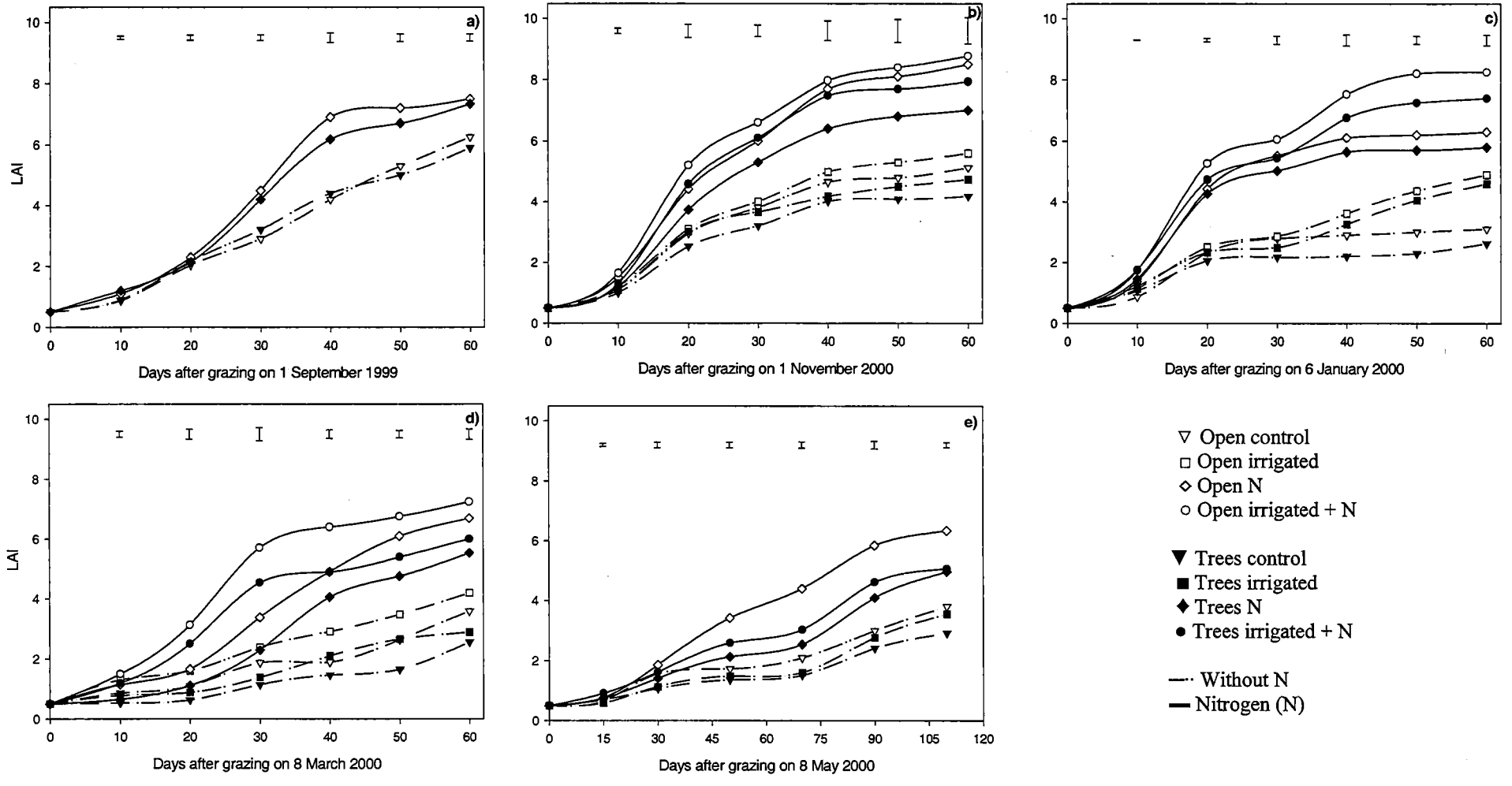

Figure 3.10 Cocksfoot leaf area index (LAI) over time for two levels of light intensity (open pasture: 100% transmittance or pasture under tree shade: ~58% transmissivity), two levels of irrigation (0 or fully) and two levels of nitrogen (0 or 300 kg N/ha). Four 60-day regrowth durations (a-d), and a 110-day regrowth duration (e) were used. Bars indicate standard error of the mean (sem).

3.3.6. Canopy pasture height and tiller population

The changes in cocksfoot LAI were related to variations in morphological aspects of the sward such as canopy pasture height and tiller population.

Details of changes in pasture canopy height and tiller population over time for the experiment with four light regimes are given in Appendix 4. When water was non-limiting (soil VWC > 24%), the cocksfoot canopies under shade were taller ($p< 0.05$) than those grown in full sunlight. During the period of maximum increase in height (October-November), cocksfoot tillers under the shade of trees+slats were etiolated to be 60 mm taller than comparable tillers in full sunlight. In general, cocksfoot tiller population decreased ($p< 0.05$) as shade level increased with a mean vegetative tiller population per m^2 of 5540 in the full sunlight, 5020 in the open+slats treatment, 4720 under trees and 3570 tillers/m^2 in the tree+slats treatment.

Details of the changes in pasture canopy height and tiller population over time from the exclosure experiment are given in Appendix 5. The application of N and irrigation increased ($p<0.001$) canopy height in all rotations and in open and shaded plots. The maximum canopy height in the open W+N at day 40 during the January-February regrowth period was 390 mm compared with 140 mm for the open W treatment. However, there was an interaction between N and regrowth time, whereby canopy height increased to a maximum value and then declined due to lodging. The timing of lodging and the canopy height at which it occurred varied according to treatment and seasons (Appendix 5). In most cases, lodging occurred earlier under shade than full sunlight treatments.

The application of N and irrigation also increased ($p<0.05$) the total tiller population in all rotations and in open and shaded plots to a maximum value and then this declined as indicated by the interaction with time ($p<0.05$) (Appendix 5). For example, total tiller population per m^2 for the open W+N treatment was 8000 at day 20 of the January-February regrowth period and then declined to 6050 at day 60. In comparison, for the same regrowth period, the tiller population in the open W treatment was lower at day 20 (7400 tillers/m^2) than at day 60 (8400 tillers/m^2).

3.3.7. Relationship between DM yield and LAI

DM yield and LAI data from vegetative cocksfoot pastures obtained from each harvest of both experiments (288 data points) were analysed using non-linear regression analysis. The fitted parameters for each treatment of both experiments were compared using an ANOVA. The lack of significant differences in the slope of these relationships meant a single function could be used (Figure 3.11). This relationship was described by an exponential function (Equation 3.4), which resulted in an R^2 of 0.92 and standard error of the estimate (ESE) of DM yield of 404 kg DM/ha.

$$DM = -960 + 916 * e^{(0.25*LAI)} \qquad \textbf{Equation 3.4}$$

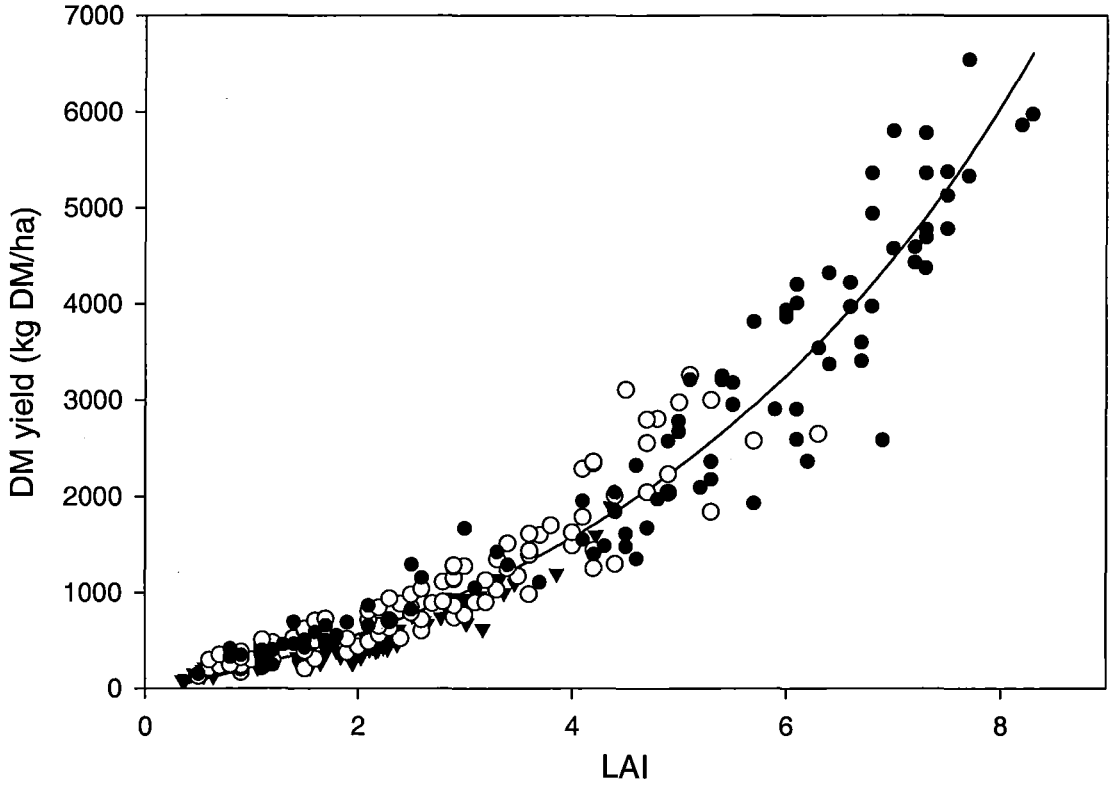

Figure 3.11 Accumulated dry matter (DM) yield (kg DM/ha) against leaf area index (LAI) for vegetative cocksfoot pastures. The line is for the fitted single exponential function (Equation 3.4). Observed data sorted by pasture under shade (▼) (from the four light regimes experiment), and in open pastures with no N fertilised (○) and with 300 kg N/ha (●) (from exclosure experiment).

From 0.5 to 3.0 units of LAI, the relationship was approximately linear and increased at a rate of 370 kg DM/ha per unit of LAI. From this point to LAI= 8 the relationship was curvilinear (Figure 3.11).

3.3.8. Mean canopy leaf angle and extinction coefficient

Under severe shade (trees+slats treatment), the mean canopy leaf angle was 9° lower ($p< 0.01$) than cocksfoot pastures in full sunlight (Table 3.10).

The mean canopy leaf angle for N, irrigation and control pastures at 20 days regrowth was 68±2°. However, in irrigated and N fertilised pastures the mean canopy leaf angle decreased ($p< 0.001$) 28° from day 20 to day 60 of regrowth, being more pronounced after lodging at day 35 (Table 3.10).

Table 3.10 Mean canopy leaf angle for cocksfoot grown under four different light regimes after 21 days regrowth, and during 60 days regrowth during the January-February 2000 period for an irrigated and fertilised (300 kg N/ha) pasture in the open.

Treatment	Mean canopy leaf angle
Open (full sunlight)	68°
Open + slat (~43% transmissivity)	64°
Trees (~58% transmissivity)	65°
Trees + slat (~24% transmissivity)	59°
sem	*0.85*
Regrowth days for open W+N	
day 20	68°
day 30	64°
day 40	55° #
day 50	41°
day 60	40°
sem	*1.21*

Note: # lodging started after 35 days of regrowth.

A mean k value for each canopy was calculated using Equation 3.3 (Section 3.2.4.1) for the four light regimes. Figure 3.12 shows three linear functions where the corresponding slopes represent k. There were differences ($p< 0.05$) between the slopes for open pastures ($k_1= 0.38$) and pastures under trees + slat shade ($k_3= 0.48$). A single value k= 0.42 (k_2) represented the architecture of pastures under the slat shade in open and pastures under trees.

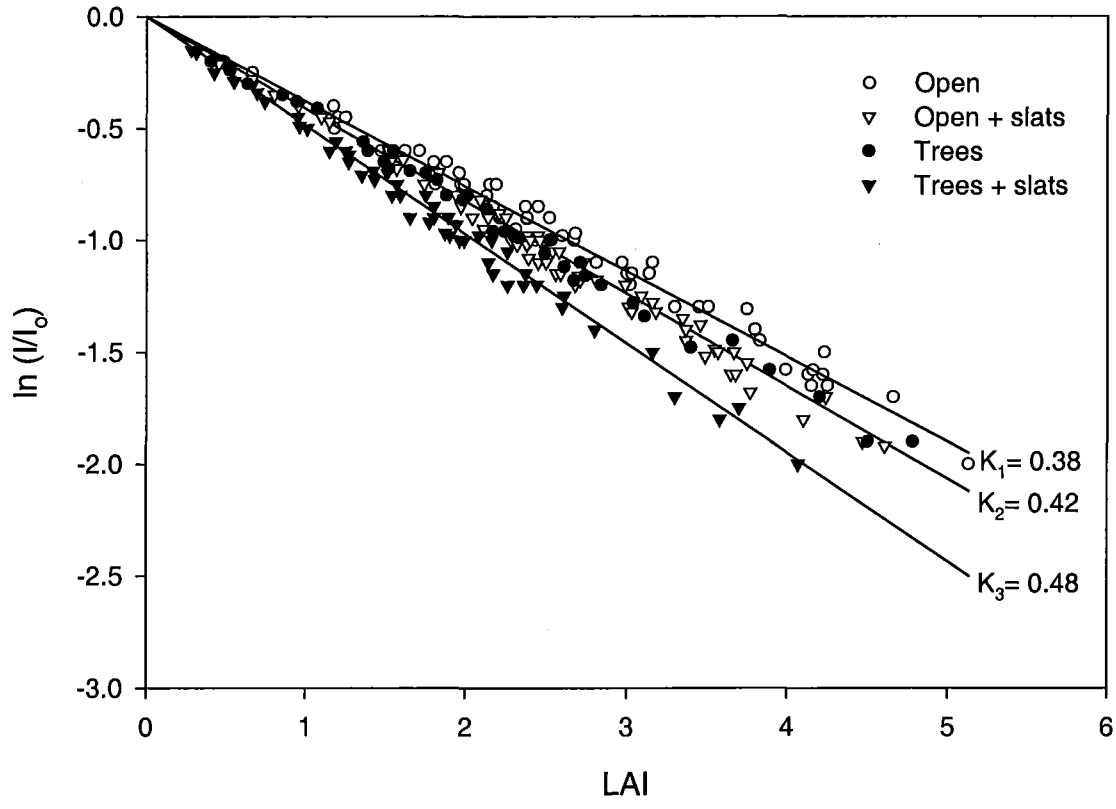

Figure 3.12 Relationship between radiation interception [ln (I/Io)] and leaf area index (LAI). The mean extinction coefficient (k) for diffuse radiation is represented by the slope of linear regression between radiation interception and LAI for the four light intensities: k_1 for open pastures (100% transmissivity), k_2 for pastures under the slat shade in open (~43 % transmissivity) and pastures under tree (~58% transmissivity) and k_3 for pastures under trees+slats (~24% transmissivity). I is the incident PPFD at a given horizontal level within the canopy (W m^{-2}); I_o is the incident PPFD above the canopy (W m^{-2}).

3.4 Discussion

3.4.1 Effect of shade on DM production

The specific component unique to silvopastoral systems is the light regime. In this study, the tree canopy and slatted structures reduced and modified the light available to the understorey cocksfoot pasture. Specifically, the daily PPFD integral for a sunny day in summer (around 21 December at solar angle elevation of 69.8° at noon) was 63.3 mol photons/m^2/d (100% transmissivity) and this was reduced by 38% under trees (62% transmissivity) and 74% under the slatted structures in the silvopastoral system (trees+slats= 26% transmissivity) (Figure 3.5). The reduction in available light quantity for the understorey pasture also changed with cloudy conditions and differences in solar angle elevation throughout the seasons. As a consequence, cocksfoot DM growth rate decreased by 13% under slat shade in the open, 22% under tree shade and 48% under the trees+slats

shade compared with the full sunlight pastures during periods of non-limiting water (soil VWC >25%) and temperature (September- November 1999 and 2000). The reduction in DM growth is in the range reported in the literature (Section 2.2.1).

3.4.2 Effect of N on DM production

The large responses of cocksfoot to N in all seasons indicated a typical state of N stress in these grass dominant pastures. Over two growing seasons (September-April), the mean DM production of individual new urine patches was 60 and 56% higher than non-urine controls in open pastures and under trees, respectively (Figure 3.7). This represented an increase in DM production over the total area of 35% in open and 28% under trees. Between 70-95% of N ingested by animals is returned to the soil in the form of urine and dung (Cameron, 1992) and the N concentration varied with seasons from 173 to 495 kg N/ha (Table 3.8). This indicates that the N excreted in the urine may have varied according to the animal diet.

In irrigated pastures, the application of 300 kg N/ha as synthetic urine increased the total annual yield by 55% in open pastures and 45% under trees (Table 3.9). The potential growth recorded for the Canterbury sub-humid temperate environment in irrigated and N fertilised open pastures (total annual yield of 28.6 t DM/ha/yr), was consistent with potential yields reported in France and Finland (Section 2.2.4). Irrigated and N fertilised pastures under trees (~58% of open PPFD) showed a maximum growth rate of 131 kg DM/ha/d and a total annual yield of 24.1 t DM/ha/yr. The maximum growth rate for irrigated and N fertilised pastures in open during the January-February regrowth period (mean temperature >15 °C) was 134 kg DM/ha/d compared with 43 kg DM/ha/d in the non-fertilised pastures indicating the isolated effect of N on growth rate (Figure 3.8). The variation in DM production found in this study due to N was in the range previously reported in the literature (Section 2.2.4).

3.4.3 Effect of irrigation on DM production

The full irrigation treatments were timed to prevent actual soil moisture deficit of 35 mm in the top 500 mm. The water applied was 23% greater for cocksfoot pasture under trees than in the open, and also 35% greater for pastures fertilised with N than non-fertilised pastures (Table 3.4). The implication is that despite the isolation of subplots by cutting shallow tree roots (0.4 m depth), some of the irrigation water was absorbed by the tree

roots probably from deeper horizons. Also, the increase in DM production and development of cocksfoot pastures with the application of N demanded great amounts of water.

Irrigation had a positive effect on DM production. The maximum growth rate of the irrigated treatment doubled the control at day 60 of the January-February regrowth period when the soil VWC was lowest at 14% control plots (Figure 3.8). In non-fertilised pastures, irrigation increased the total annual yield by 30% in open pastures which was consistent with the results of McBride (1994) for the Canterbury plains.

3.4.4 Interactions between environmental factors and DM production

(i) Interaction with time

Changes in environmental and management factors over time (seasons and regrowth duration) had a strong influence on DM production. For example, the mean daily temperature during this experiment ranged from 6 °C in winter to 16 °C in summer (Figure 3.1) with daily minimum temperatures of 1.4 °C and daily maximum temperatures of 22.6 °C. In addition, as a result of the tree competition, irrigation, regrowth duration and seasonal effect, the soil VWC in the top 500 mm varied from 33 to 8.5% (Figures 3.3 and 3.4). These changes, together with the application of N and regrowth duration, provided a wide range of cocksfoot DM growth rates from 2 to 154 kg DM/ha/d.

The decrease in DM production with shade intensity showed seasonal variation responses (Figure 3.6) with less difference during winter (mean daily air temperatures < 8 °C) and during severe drought (soil VWC < 15%). This indicates that pasture production during winter was limited mainly by low temperatures and by soil water stress in dry conditions. Similarly, Korte *et al.* (1987) reported that low levels of solar radiation do not appear to limit unshaded pasture production in winter. Low temperature is considered to be the major environmental variable limiting pasture production for this season in temperate latitudes. In addition, trees in the silvopastoral plots reduced the soil VWC in all seasons with a mean reduction of 2.5% compared with open pastures due to root competition and the interception of rainfall (Section 2.2.3). These probably also contributed to a reduction in DM growth rate in addition to shade. However, there was some evidence that shade assisted soil moisture conservation during drought periods. For example, from January to

April 2000, the open+slats treatment produced 15% more DM than open pastures as a consequence of 2.2% more soil VWC (Figure 3.6).

There was also seasonal variation in N responses. Irrigated pastures during the January-February regrowth period, when temperature was non-limiting, produced 12.5 kg DM/kg N in open pastures, but the response to 300 kg N/ha declined to 4.4 kg DM/kg N in the non-irrigated treatment due to water stress (soil VWC< 15% in the top 500 mm) (Figure 3.8). This indicated that when water was limiting, N from urine alone resulted in small increases in pasture production in the sub-humid environment of the Canterbury plains. The response to N also decreased in open pastures to 7.2 kg DM/kg N during winter (May-August regrowth period) due to low temperatures (mean daily air temperatures < 7.5 °C). This is consistent with Anslow and Robinson (1986) who reported that the rate of N uptake from perennial ryegrass swards receiving 420 kg N/ha decreased from 3-4 kg N/ha/d in spring to about 0.5 kg N/ha/d in mid-winter (<10 °C).

Regrowth duration also provided variation in DM growth rate over time (Figure 3.8). For the N treatments during the November-December and January-February regrowth periods, the DM production increased to a maximum value and then declined. For example, during the January-February regrowth period (mean air temperature 15 °C), the DM growth rate for the open W+N treatment increased from 65 kg DM/ha/d at day 10 to the maximum 137 kg DM/ha/d at day 30 (LAI= 6.3), and then declined to 99 kg DM/ha/d after 60 days regrowth. The time at which maximum DM production occurred during regrowth periods, which corresponded approximately to a 95% DM accumulation, depended on seasons and on light intensity. Thus, maximum DM production occurred earlier with increments in air temperature and later under tree shade. As LAI increased so did light interception, causing increases in DM up to a critical LAI value of 6.0 (Figures 3.10).

(ii) Interaction between shade and N

Interactions occurred between shade and N caused by the greater responses to increased N levels at high light intensity in open pastures. For example, during the January-February regrowth period, the response was 12.5 kg DM/kg N in open pastures and 10.5 kg DM/kg N under trees. Similarly, in spring (September-October regrowth period) the response was 9.1 kg DM/kg N in open pastures and 6.0 kg DM/kg N under trees. Therefore, cocksfoot response to fertilised N was influenced by variation in light intensity. A similar effect of

light intensity on N response was shown for perennial ryegrass by Deinum (1966). The response to fertiliser N, applied at 25 kg N/ha was 29% greater at high light intensity equivalent to a mid-summer day (2.27 kJ cm^{-2} d^{-1}) compared with a low light intensity situation equivalent to a dull day in mid-winter (0.20 kJ cm^{-2} d^{-1}) and this difference increased to 46% at a rate of 125 kg N/ha.

(iii) Interaction between shade and water

An interaction also occurred between shade and water caused by the greater response to irrigation in open pastures compared with those under trees. For example, after 60 days regrowth in summer (January-February) the full irrigation treatment in open pastures produced 10% more DM than the pasture under trees. A reason for this interaction could be that cocksfoot plants closed their stomata during the severe shade periods and therefore reduced photosynthesis. Thus, growth may be reduced because of stomatal closure in spite of the pasture being irrigated.

In summary, to accurately predict DM production and growth rate of pastures in silvopastoral systems, a canopy photosynthesis model needs to take into account these interactions and also the pasture response to the individual environmental and management factors described.

3.4.5 Relationship between DM yield and LAI

The non-linear relationship between DM yield and LAI (Equation 3.4) for the vegetative cocksfoot sward, indicated that the LAI of the sward increased more slowly in relative terms than the biomass when LAI was greater than 3 units (Figure 3.11). This was consistent with Duru *et al.* (1997) who reported, for cocksfoot, a unique exponential function between LAI and DM for different N levels. One reason for the non-linear relationship, mainly from LAI> 3, would be that the pseudo-stem length and weight increased with LAI (or time of regrowth) and consequently decreased the leaf:pseudo-stem ratio. As a consequence, the proportion of green leaf was reduced. Thus, the increase in the more vertical and heavier pseudo-stem component and the relative decrease in the green leaf component over time gives a greater proportion of DM with a smaller increase in LAI.

The importance of this single relationship between DM yield and LAI is that it includes differences in the cocksfoot canopy due to changes over time in morphological aspects of

the sward, such as tiller population, pasture height (Appendices 3 and 4) and leaf size caused by environmental and management factors. This indicates there is no need to model tiller dynamics and canopy height for predictions of DM growth by a canopy photosynthesis model. The reasons for the morphological changes due to shade, temperature, water, N and regrowth were discussed in Section 2.3.2. This relationship between DM production and LAI can then be used together with a canopy photosynthesis model to determine the foliage (LAI) increment for each day of growth.

3.4.6 Changes in canopy architecture

(i) Effect of shade

Under severe shade (trees+slats treatment), the mean canopy leaf angle was 9° more horizontal than cocksfoot pastures in full sunlight (Table 3.10). The difference in mean canopy leaf angle resulted in differences ($p< 0.05$) in the mean k value for the canopy in diffuse light. Full sunlight pastures had a k= 0.38 compared with k= 0.48 of the pastures under ~24% of the open PPFD (trees + slat shade).

The utilisation of PPFD for growth in temperate grasses has been shown to be influenced by the distribution of light within the grass canopies (Sheehy and Cooper, 1973). This distribution is partly determined by canopy architecture, in particular the angular distribution of leaves. Thus, horizontal leaves may be able to capture more radiation under shade situations and hence should maximise the individual leaf photosynthetic input. This was confirmed by Charles-Edwards (1981) who demonstrated that there may be an optimal canopy k for maximum canopy photosynthesis which changes with the incident light flux density: the lower the light the more productive pasture will have planophile leaves. It seems likely that the cocksfoot leaves under severe shade became more horizontal due to longer and thinner leaves. This is consistent with Deckmyn *et al.* (2000) who reported that cocksfoot leaves drooped from 68.7° to 53.9° as their length increased. Variation in leaf angle from morphological changes such as stem elongation and stem erection has been also reported for other species (Section 2.3.2.6). In this study, shade encouraged plants to become more etiolated where the taller growth may be an effort to gain better access to available light in competition with neighbouring plants and tillers. For example, when water was non-limiting (September-November), shade increased canopy height by approximately 23% for both open+slats and under trees pastures, and by 41% for the trees+slats treatment (Appendix 4). It also appears that the etiolation, and consequently the

more horizontal leaves of shaded cocksfoot plants, responded to a reduction in the R:FR ratio. In the current study, differential absorption of red and far-red light from the tree canopies determined that the R:FR decreased 56% in the middle of the tree shade compared with full sunlight (Table 3.7).

The higher leaf canopy angle (more vertical disposition of leaves) and lower k value for the open cocksfoot pastures compared with shaded pastures meant that PAR penetration was deeper into the canopy, and consequently canopy photosynthesis can be spread over a larger area of leaf. Similarly, Sheehy and Peacock (1975) using solarimeters reported a daily mean value for k of 0.44 for 'S345' cocksfoot. However, Sheehy and Chapas (1976) reported that cocksfoot with 61° mean angle of leaf inclination had k values from 0.11 to 0.16. These values of k reported were lower than the k value presented in this study (k= 0.38) because those k values were calculated from data collected in sunny days around noon when the elevation of the sun was near maximum and also because of the more prostate nature of the field swards. In overcast conditions (as was used to calculate k in this study), diffuse radiation is received from all angles increasing the interception and consequently increasing the value of k. This highlights the need to define the sky condition (sunny or cloudy) and the solar angle elevation for sunny days when k values are reported.

(ii) Effect of regrowth duration

Regrowth duration affected the canopy architecture of cocksfoot pastures when fertilised with 300 kg N/ha and irrigated. The mean canopy leaf angle decreased from 68° at day 20 to 40° at day 60 during the January-February regrowth period (Table 3.10). The decrease in canopy leaf angle may have been caused by the greater tiller and leaf lengths, which promoted lodging starting after 35 days regrowth (LAI >5). Pearce *et al.* (1967) also reported that k of barley plants decreased when LAI was higher than 6 indicating that more horizontal leaves intercepted light at the top of the stand and less was transmitted to lower leaves (Section 2.3.2.6).

Because canopy leaf angles differed, a single value can not be used. Thus, values of k need to be incorporated into the canopy photosynthesis model for DM growth prediction for each shade treatment. Furthermore, the variation in leaf angle with four levels of light intensity and reduction in the R:FR ratio indicates the need for a sub-model that predicts

variation in leaf angle or k for a continuous range of shaded environments. This was not attempted in the present study.

3.5 Conclusions

- A wide range of environmental and management conditions were created through changes in shade intensity, nitrogen, irrigation, regrowth duration and seasonal changes (e.g. temperature, soil moisture) and their interactions. These changes provided a wide range of cocksfoot DM growth rates from 2 to 154 kg DM/ha/d. This indicates the range of values needing to be simulated to predict pasture DM production under the silvopastoral conditions in this study.

- A single relationship between DM production and LAI accounted for most of the variation in cocksfoot canopy development due to changes over time in morphological aspects of the sward (tiller population, pasture height, leaf size) caused by the environmental and management factors. This relationship can be used with a canopy photosynthesis model to determine the LAI increment after each day of growth.

- Severe shade (~24% of the open PPFD) resulted in a more horizontal mean leaf canopy angle. This adaptive feature may have modified the daily integrated PPFD absorbed by a cocksfoot pasture and would be an important input for canopy photosynthesis models working either at an instantaneous or daily time scale.

In the following chapters the prediction of DM production is attempted first by creating an integrated leaf photosynthesis model which predicts the response of net photosynthesis to different environmental and management factors. Secondly, by extending the leaf photosynthesis model to a canopy photosynthesis model to predict pasture growth. The final canopy model also needs to take into account a relationship between daily net carbon gain and LAI which includes the dynamics of tiller morphology and canopy height for the environmental and management variables studied.

CHAPTER 4

Modelling maximum net photosynthetic rate of field grown cocksfoot leaves under different nitrogen, water and temperature regimes

4.1 Introduction

The production of dry matter is related to the maximum leaf photosynthetic rate at light saturation (*Pmax*) and it has been used to predict growth in pastures through canopy photosynthesis models (Section 2.3.1). The first step to develop a predictive model of cocksfoot growth requires determination of the individual relationship between *Pmax* and the main environmental variables. Temperature (T), water (W) and nitrogen (N) have been reported to be the main determinants of cocksfoot growth (Section 2.2).

The research outlined in this chapter aims to determine if a simple multiplicative model of T, W and N can be used to predict *Pmax* for cocksfoot leaves in non-limiting conditions and when one, two, or all three of the factors are limiting. Testing of the model also requires investigation of any interactions among functions (Equation 4.1).

$$Pmax = Ppmax * \left[f_i^T(T) * f_i^W(W) * f_i^N(N) \right] \text{ for range } i \qquad \textbf{Equation 4.1}$$

Where *i*= total physiologically meaningful growth range; *Ppmax* represents the potential or maximum *Pmax* value in non-limiting conditions.

Provided it is biologically based, such a model would provide an initial framework to enable the development of a quantitative prediction of cocksfoot growth in any environment. Thus, the objectives of the research outlined in this chapter are to:

1) derive individual functions for *Pmax* against N, water and temperature for individual cocksfoot leaves;

2) propose biological explanations for each of these functions;

3) test a simple multiplicative model (Equation 2.5, Section 2) for integrating these factors,

and validate this with an independent data set;
4) determine whether prediction was improved by inclusion of any or all of the interactions between these factors (Equation 4.1).

To do this, net photosynthesis for individual cocksfoot leaves was measured in a field experiment that included a wide range of temperature, N and water status conditions. Measurements were taken at a point in the grazing regime, where radiation and canopy architecture were not limiting.

4.2 Materials and methods

4.2.1 Location

For this experiment, only the three open pasture plots (Section 3.2.1) were measured from September 1999 to February 2001. Herbage measurements were taken from two areas. The first was the exclosure areas (Section 3.2.2.2) and the second was the main plot area excluding the exclosure plots, from which urine and non-urine patches were sampled (Section 3.2.2.1).

Measurements were taken immediately prior to sheep grazing after 21 days of pasture regrowth. The caged areas were left for 60 days during this season (Section 3.2.2.2) but only data measured at 21 days after grazing were used in the analyses. After 60 days, cages were placed in new positions and the first 21 days of regrowth were used for further analyses.

4.2.2 Photosynthesis measurements

The photosynthesis rate was measured on a random sample of six of the youngest fully expanded intact leaves from each treatment. All measurements were taken at midday ± 1 hour on cloudless sunny days. Net photosynthesis (μmol CO_2 m^{-2} s^{-1}) and stomatal conductance to water vapour (mol H_2O m^{-2} s^{-1}) were measured in an open infrared gas analysis system (IRGAs) with the instrument "LiCor LI-6400 Portable Photosynthesis System" (Lincoln, Nebraska, USA). This system provides steady light, CO_2, H_2O and temperature conditions for measurement. The sensor head of this apparatus contains a leaf chamber, which is clamped onto a leaf. Net photosynthesis and transpiration are computed by measuring the airflow rate, the incoming and leaf chamber CO_2 and H_2O

concentrations, and leaf area. Light curves with seven light intensities; 0, 100, 250, 500, 750, 1000 and 2000 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), were measured using the "Auto Light Curve Program". The minimum wait time used was 60 seconds for each light intensity, with a 3% coefficient of variation (CV) for each of these intensities. Values of stomatal conductance (*gs*) to water vapour (mol H_2O m^{-2} s^{-1}) were obtained simultaneously with *Pmax* readings.

The measured values of *Pmax* in μmol CO_2 m^{-2} s^{-1} and *gs* in mol H_2O m^{-2} s^{-1} were transformed by dividing the observed values by values obtained in non-limited conditions to give a standardised index value that ranged from 0 to 1. A value of 1 ($Pmax_s$= 1 or gs_s= 1) corresponds to their maximum value found in non-limiting conditions.

Non-limiting temperature values were defined from the highest *Pmax* value found by examining values in the reported optimum temperature range of 20-22 °C (Eagles, 1967; Mitchell and Lucanus, 1962). Non-limiting water status values for *Pmax* were those measured from well-irrigated plants initially in the range of pre-dawn leaf water potential (ψ_{lp}) from –0.2 to –1.2 bar. Non-limiting N values were initially determined from *Pmax* values with a leaf N content >5.0% (Duru *et al.*, 1997; Thornley, 1998). For each factor, the optimum range was expanded to include values that maintained maximal *Pmax* reading. In each case, this expanded optimum range of two factors was used to determine the response of the third under non-optimal conditions.

Overall, 149 photosynthesis measurements were taken in the field. Of these: 19 were used to fit the initial temperature function (N and ψ_{lp} non-limiting), 20 for the N function (temperature and ψ_{lp} non-limiting), and 26 for the ψ_{lp} function (temperature and N non-limiting). A further 62 were used for validation of the simple multiplicative model to predict *Pmax* when two or all three factors were limiting. The remaining 22 observations were used to examine the detected interaction of low N and high temperature.

4.2.3 Herbage and environment measurements

Air temperature, ψ_{lp} and samples for N content were taken on the same day as photosynthesis measurements. Air temperature was measured with a digital temperature sensor (Section 3.2.3.1). Canopy temperature was measured on 20 occasions using an infrared thermometer (Everest Interscience, Model 110, California, USA) to detect any

differences between air and canopy temperatures. Values for ψ_{lp} were obtained from a random sample of five of the youngest fully expanded leaves from each treatment with a pressure chamber (Model 1002, PMS Instrument Co., Corvallis, Oregon, USA). The N content of leaves from a 0.2 m^2 quadrat cut to 25 mm height was determined using the Kjeldahl technique. Samples were dried in a forced draft oven at 65 °C to constant weight and ground in a mill containing a 1mm stainless steel screen.

The leaf chlorophyll content was measured on a random sample of the youngest fully expanded intact leaves at mid position from each treatment to relate to the leaf N content. Chlorophyll was extracted from 2 cm^2 fresh leaf on 60 leaves in 90% acetone after grinding the leaves in a mortar. Absorption was measured at 665 nm (chlorophyll a) and 645 nm (chlorophyll b) using a spectrophotometer (Unicam UV-Visible Spectrometry, Cambridge, UK). The total chlorophyll concentration (g m^{-2}) was calculated from the absorbance measurements using equations from Andrews *et al.* (1984).

4.2.4 Statistical analyses

The data were analysed using linear and non-linear regression to determine the relationship between *Pmax* and each of the environmental factors (T, ψ_{lp} and N). Quadratic, cubic and quartic functions (Thornley, 1998), and Gaussian and Weibull (three and four parameters) functions were fitted to the temperature response. Only a linear function was necessary for the ψ_{lp} response, but different asymptotic functions (Sigmoid, Logistic, Gompertz, Chapman, Hill and Weibull) were fitted for the N response. For modelling simplicity the temperature and nitrogen data were also described using two straight line segments "broken stick" methodology (Draper and Smith, 1998), as has been used previously for simulation of crop canopy photosynthesis (van Keulen and Seligman, 1987).

The coefficient of determination (R^2) and standard error of the estimate (ESE) of $Pmax_s$ were used to select the most appropriate functions. Residuals [(observed measured values – expected model values)] and root mean square deviation (RMSD = $[\Sigma(\text{observed-predicted})^2/n)]^{1/2}$) were calculated to estimate the accuracy of the proposed models.

4.3 Results

The rate of net photosynthesis as a function of PPFD followed the expected non-rectangular hyperbola (Thornley, 1998) in non-limited and limited conditions (Figure 4.1). In this Chapter, analyses focussed on *Pmax* which had a maximum value of 27.4 μmol CO_2 m^{-2} s^{-1} in non-limiting conditions, and the leaf was saturated at 750 μmol m^{-2} s^{-1} PPFD. However, *Pmax* decreased when a single factor (temperature, ψ_{lp} or N) was limiting. For example, *Pmax* was 22.0, 16.7 and 8.5 μmol CO_2 m^{-2} s^{-1} for plants grown at 14 °C (temperature limited), 3.5% N (N limited) or at ψ_{lp} of -10 bar (water stress), respectively. Furthermore, as any factor moved from the optimum, the saturation point changed. For example, as water stress increased, leaf saturation occurred at 500 μmol m^{-2} s^{-1} PPFD. However, the maximum value of *Pmax* was always achieved before 1000 μmol m^{-2} s^{-1} PPFD, a level equivalent to about half of full sunlight.

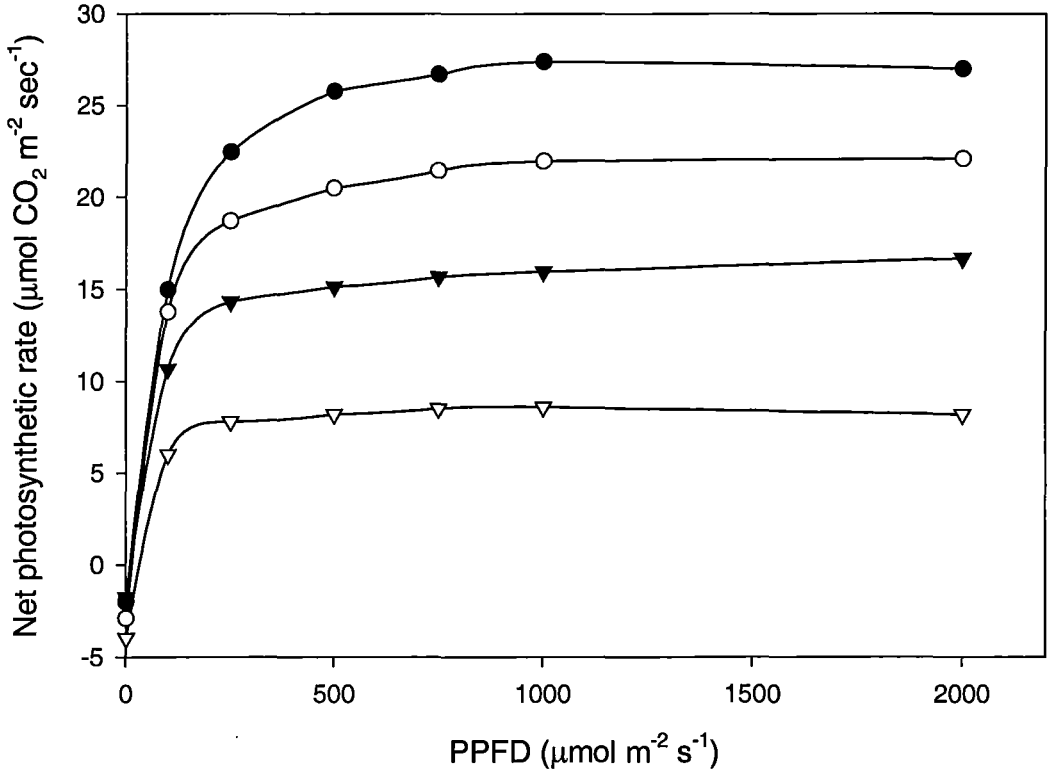

Figure 4.1. Net photosynthetic rate against photosynthetic photon flux density (PPFD) for cocksfoot grown in a field environment with one (temperature 14 °C (▼), nitrogen 3.5% (○) or pre-dawn leaf water potential (ψ_{lp}) of –10 bar (▽)) or no factors limiting (●).

4.3.1 *Pmax* and temperature

The effect of temperature on the rate of net photosynthesis was analysed in irrigated (no water stress) and non-N deficient plants. *Pmax* values were obtained from light curves of cocksfoot grown with air temperatures from 10 °C to 31 °C (Figure 4.2). *Pmax* per unit of leaf increased by 1.6 μmol CO_2 m^{-2} s^{-1} per °C, or 0.058 units of $Pmax_s$ per °C, from 10 to 19 °C and then plateaued at a *Pmax* of 27.4 μmol CO_2 m^{-2} s^{-1} ($Pmax_s$= 1) from 19 to 23 °C. *Pmax* then declined by 2.1 μmol CO_2 m^{-2} s^{-1} per °C, or 0.077 units of $Pmax_s$ per °C, from 23 to 31 °C. This asymmetric response meant a simple quadratic or cubic function (Thornley, 1998) could not be fitted to the data. A Gaussian function was fitted (Equation 4.2) and this gave an R^2 of 0.94 and ESE of $Pmax_s$ of 0.047.

$$Pmax_s = 0.99 * e^{\left[-0.5*\left(\frac{T-20.4}{7.90}\right)^2\right]}$$

Equation 4.2

Where T is the air temperature (°C); *e* is the base of natural logarithms (2.718281).

The data were also described by fitting a "broken stick" model, with inflection points at 19 and 23 °C (Figure 4.2). Extrapolation of the ends of the "broken stick" function predicted $Pmax_s$= 0 ≡ *Pmax*= 0 at 2 and 37 °C, compared with 3 and 40 °C from the Gaussian function.

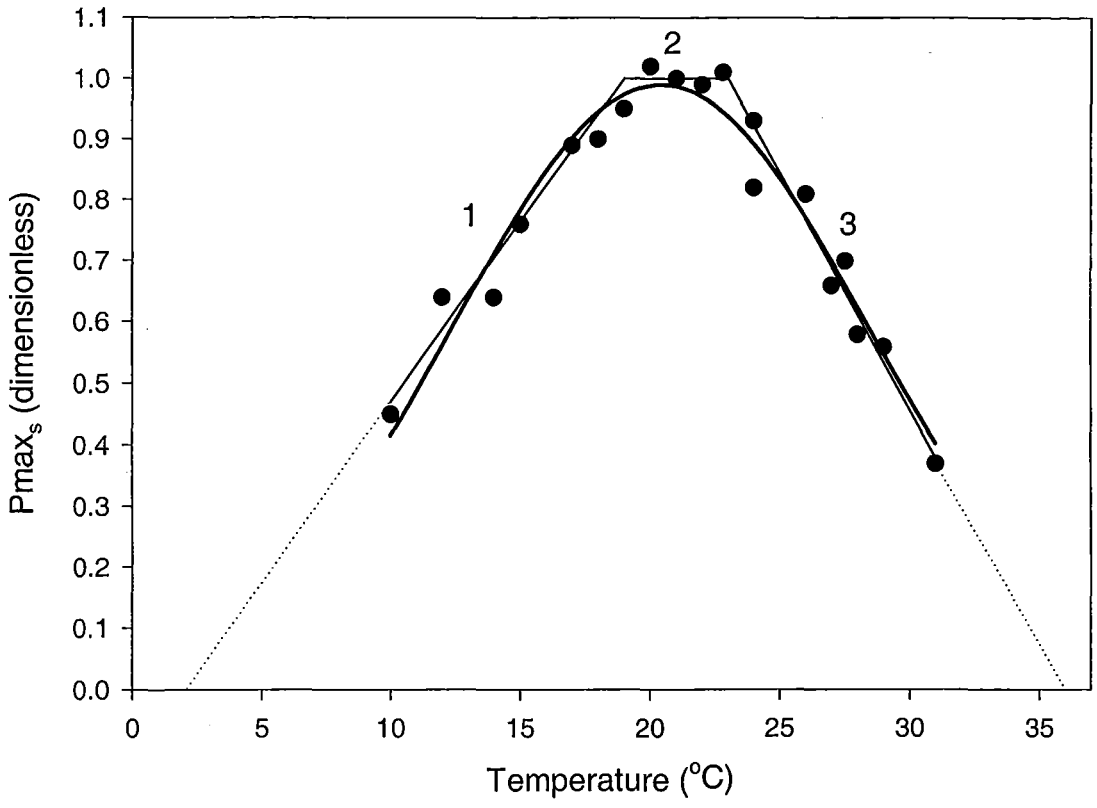

Figure 4.2. Standardised rate of net photosynthesis (*Pmax$_s$*) against temperature for cocksfoot grown under field conditions where nitrogen and water were non-limiting. *Pmax$_s$*= 1 ≡ *Pmax* = 27 μmol CO_2 m^{-2} s^{-1}. The fitted Gaussian function model (—) and a three part (1, 2 and 3) "broken stick" model (—) are indicated, with extrapolation to *Pmax$_s$*= 0 (····) for the 'broken stick' model.

4.3.2 *Pmax* and nitrogen content (N%)

The effect of leaf N% on the rate of net photosynthesis was analysed in irrigated plants (no water stress) within the optimum temperature range (19-23 °C). The leaf N content ranged from 1.5 to 5.9%. The same maximum value of 27.4 μmol CO_2 m^{-2} s^{-1} (*Pmax$_s$*= 1) was measured from 5.2 to 5.9% N (Figure 4.3). From this point *Pmax* decreased at a rate of 3.0 μmol CO_2 m^{-2} s^{-1}, or 0.115 units of *Pmax$_s$*, per 1% N down to 2.6% leaf N content. This was followed by a further decline of 11.3 μmol CO_2 m^{-2} s^{-1}, or 0.409 units of *Pmax$_s$*, per 1% N down to the lowest recorded value of 1.5% N. This relationship was described by an asymtotic Weibull function (Equation 4.3), which resulted in an R^2 of 0.98 and ESE of *Pmax$_s$* of 0.03.

$$Pmax_{s} = 1.08 * \left[1 - e^{-\left(\frac{N - 2.22 + 1.57 * \ln 2^{1/0.84}}{1.57} \right)^{0.84}} \right]$$

Equation 4.3

Where N is the nitrogen content (%).

The data were also fitted using a "broken stick" model with points of inflection at 2.6 and 5.2% N (Figure 4.3).

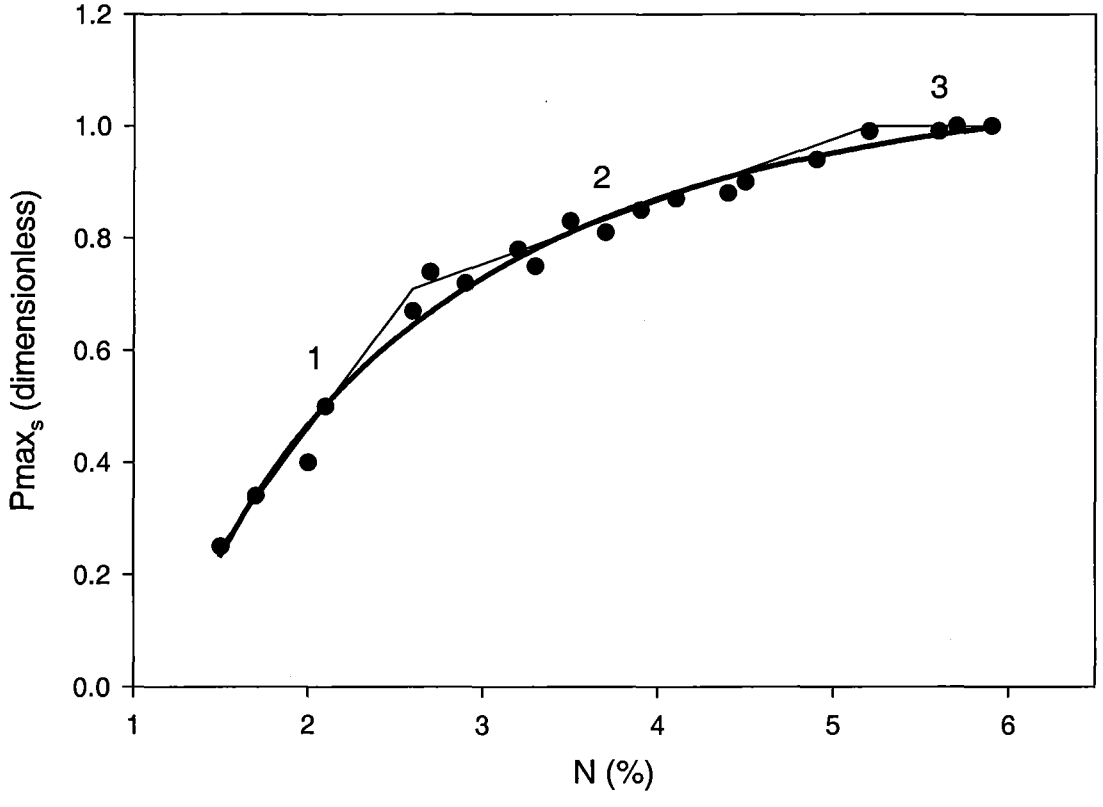

Figure 4.3. Standardised rate of net photosynthesis ($Pmax_s$) against nitrogen percentage for cocksfoot grown under field conditions where temperature and water were non-limiting. $Pmax_s$= 1 ≡ $Pmax$ = 27 μmol CO_2 m^{-2} s^{-1}. Fitted Weibull function model (—) and a three part (1, 2 and 3) "broken stick" model (—) are indicated.

4.3.3 Relationship between nitrogen content and total chlorophyll concentration.

Total chlorophyll concentration per unit area (CHL) ranged from 0.05 to 0.96 g m^{-2} as N% increased from 1.5 to 5.9% N (Figure 4.4). The maximum value of CHL was measured from 5.5 to 5.9% N. From this point CHL decreased at a rate of 0.026 g m^{-2} per 1% N to

2.6% leaf N content. This was followed by a decline of 0.009 g m^{-2} per 1% N to the lowest recorded value of 1.5% N. This relationship was described by an asymptotic sigmoid function (Equation 4.4), which resulted in an R^2 of 0.97 and ESE of CHL of 0.05.

$$CHL = 0.05 + \frac{0.93}{1 + e^{-\left(\frac{N-3.76}{0.62}\right)}}$$

Equation 4.4

Where *CHL* is the total chlorophyll concentration (g m^{-2}); N is nitrogen %.

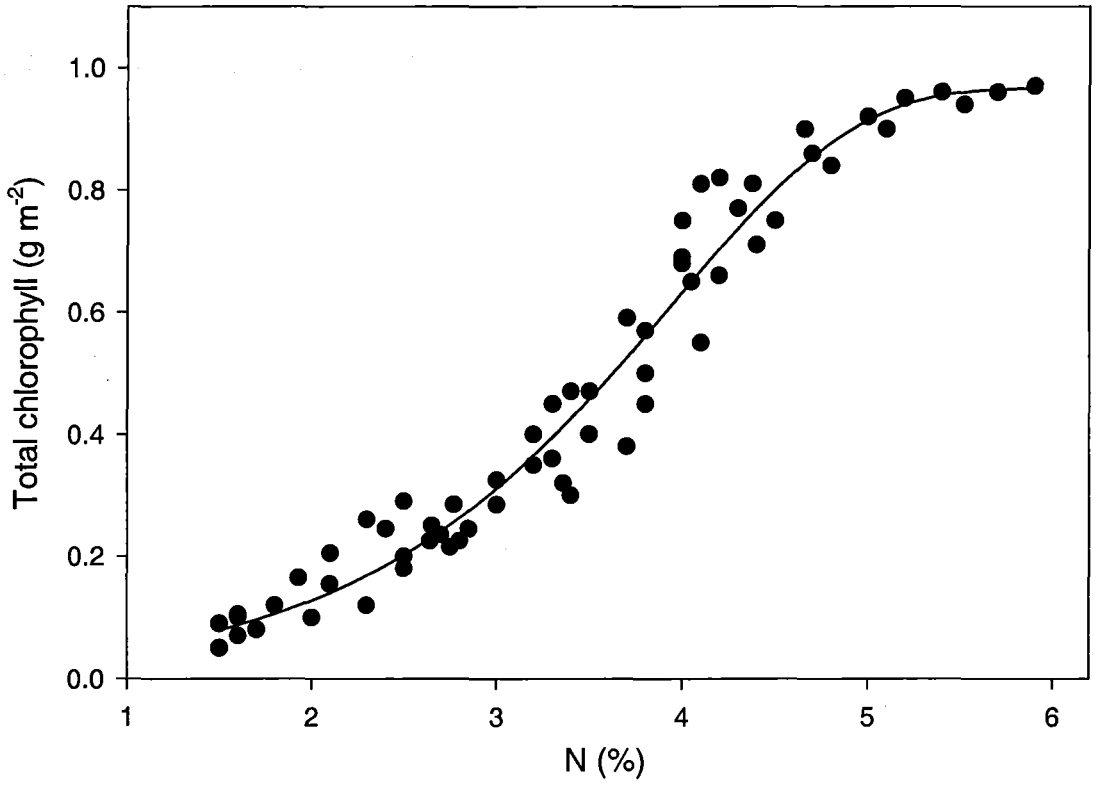

Figure 4.4. Total chlorophyll concentration per unit of area (g m^{-2}) against nitrogen percentage for cocksfoot grown under field conditions. Fitted asymptotic sigmoid function (—) is indicated.

4.3.4 *Pmax* and water stress

The effect of water stress, expressed as ψ_{lp}, on *Pmax* was analysed in the optimum temperature range with leaf N $\geq$ 5.2%. The range of ψ_{lp} in the present work was from –0.1 bar to –16.0 bar which corresponded to soil volumetric water contents (VWC) in the top 500 mm of 32 and 11%, respectively. There was a strong negative relationship between *Pmax* and ψ_{lp} (Figure 4.5). The maximum value of *Pmax* of 27.4 μmol CO_2 m^{-2} s^{-1} ($Pmax_s$= 1) was measured from –0.1 to –1.2 bar (from 30 to 27% soil VWC). From this point, *Pmax* decreased linearly (Equation 4.5) (R^2= 0.98; ESE= 0.042) at the rate of 2.1 μmol CO_2 m^{-2} s^{-1}, or 0.078 units of $Pmax_s$, per bar of ψ_{lp} as water stress increased to –14.0 bar. Beyond this, *Pmax* reached a constant negative value (from -0.1 to -0.5 μmol CO_2 m^{-2} s^{-1}) which indicated that total respiration was higher than photosynthesis under severe water stress.

$$Pmax_s = 1.0716 - 0.0765\ \psi_{lp}\ [\text{Range } -1.2 \text{ to } -14.0 \text{ bars}] \qquad \textbf{Equation 4.5}$$

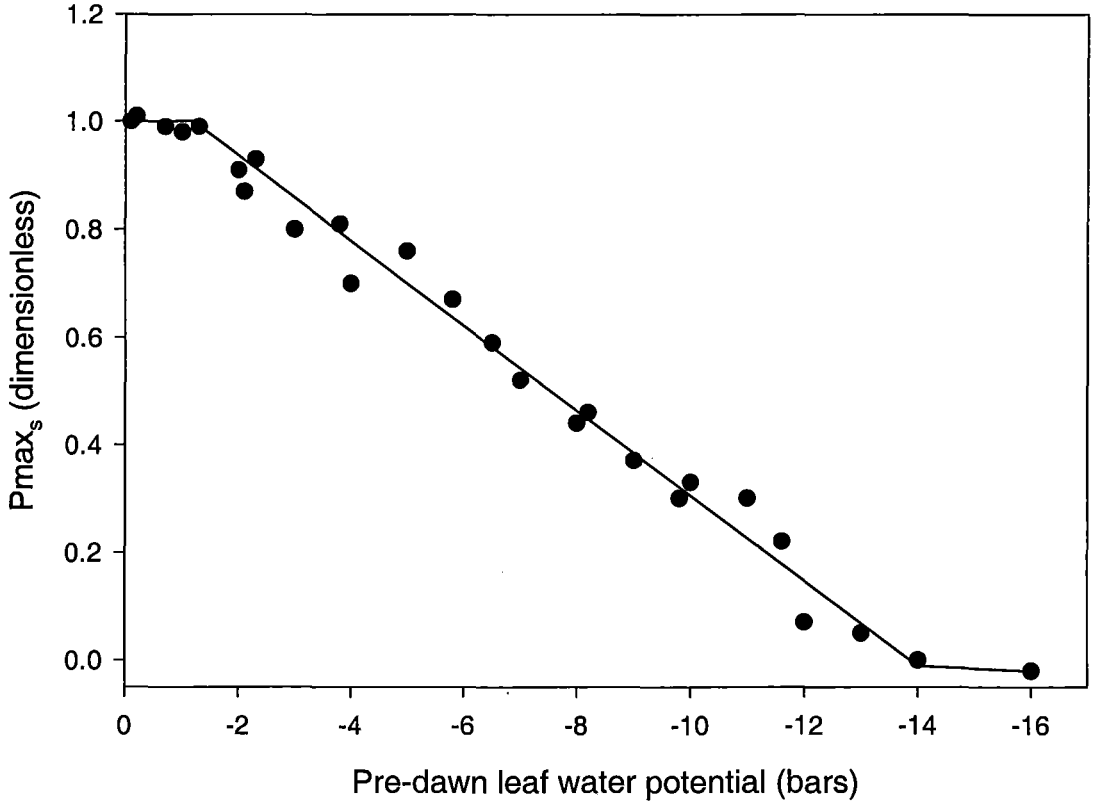

Figure 4.5. Standardised rate of net photosynthesis ($Pmax_s$) against water stress for cocksfoot grown under field conditions where temperature and nitrogen were non-limiting. $Pmax_s$= 1 ≡ *Pmax* = 27 μmol CO_2 m^{-2} s^{-1}.

4.3.5 Relationship between ψ_{lp} and soil VWC

There was a strong quadratic relationship (R^2= 0.98 and ESE of ψ_{lp}= 0.47) between ψ_{lp} and soil VWC (Figure 4.6) when all data in the present experiment were included. From saturation point (34%) to 27% soil VWC, ψ_{lp} decreased from –0.2 to –1.2 bar (Equation 4.6). Then, ψ_{lp} decreased at an average rate of 0.7 bar per 1% of soil VWC. Values of ψ_{lp} reached a constant of –16.7 bar when soil VWC was $\leq$ 11%.

$$\psi_{lp}= -37.32 + 2.23\ SM - 0.033\ SM^2 \qquad \textbf{Equation 4.6}$$

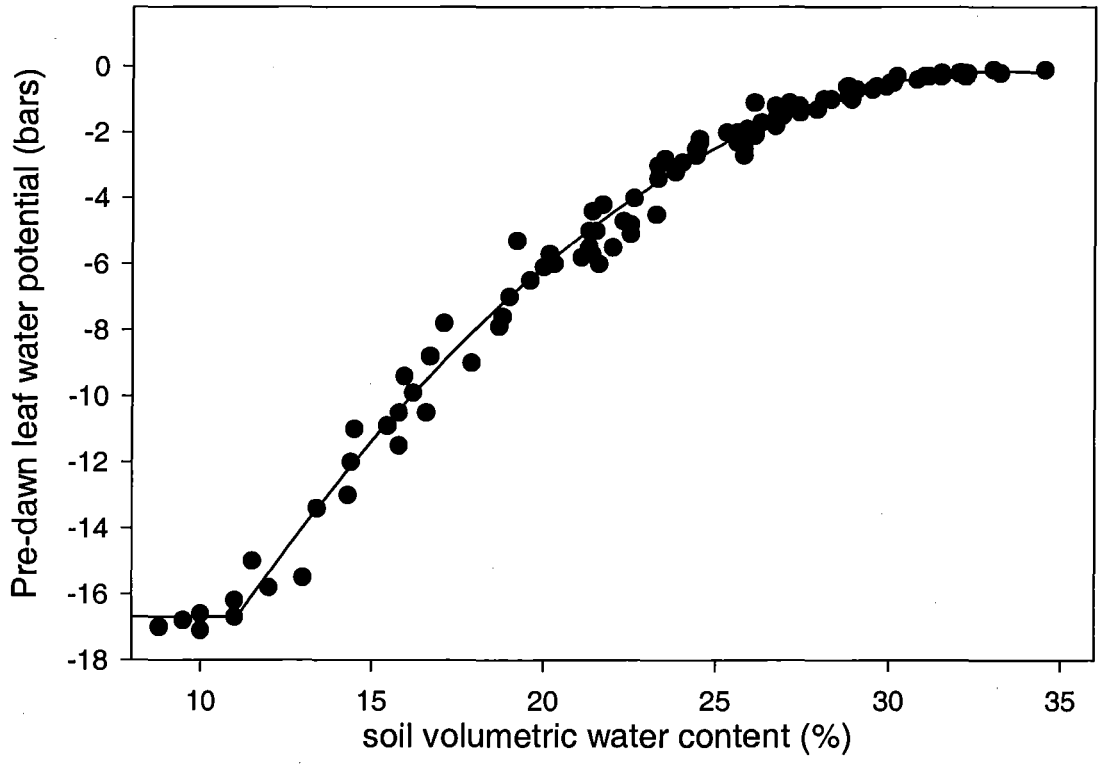

Figure 4.6. Pre-dawn leaf water potential against soil volumetric water content up to 500 mm depth.

4.3.6 Stomatal conductance

The maximum rate of *gs* was 0.45 mol H_2O m^{-2} s^{-1} in non-limiting conditions and the minimum value recorded was 0.0001 mol H_2O m^{-2} s^{-1}. Least squares regression analysis showed a positive linear relationship between gs_s and $Pmax_s$ for changes in ψ_{lp} with a coefficient for the slope of 1.0 (Figure 4.7a). The negative ordinate axis intercept value showed that values of gs_s close to zero were related to negative values of $Pmax_s$. In

contrast, there was no significant relationship between gs_s and $Pmax_s$ for the range of foliage N concentrations and temperatures measured (Figure 4.7b and Figure 4.7c). Specifically, for temperature and N, $Pmax_s$ varied from 0.2 to 1.0, but gs_s values ranged between 0.87 and 1.0. The exceptions were three outlying temperature values with gs_s of 0.79 at 28 °C, 0.73 at 29 °C and 0.65 at 31 °C (Figure 4.7b).

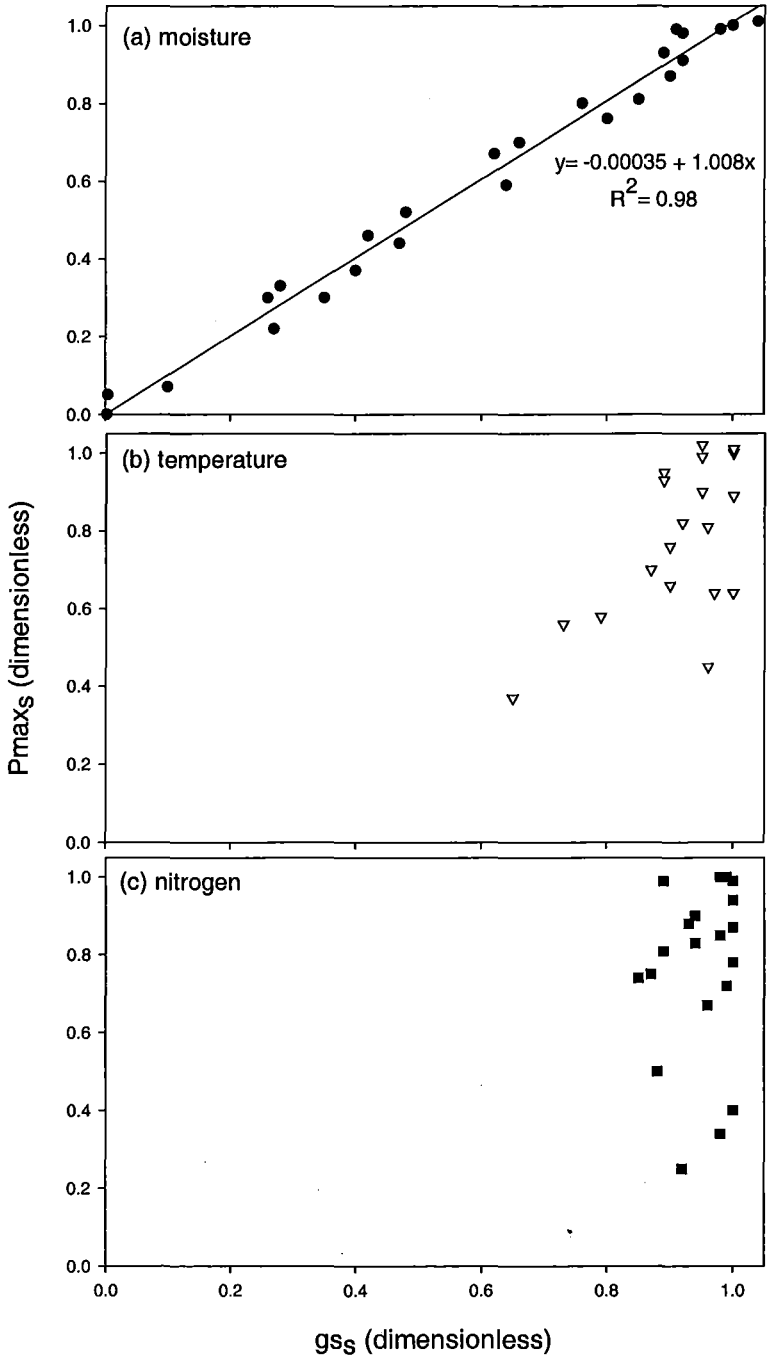

Figure 4.7. Standardised rate of net photosynthesis ($Pmax_s$) against standardised rate of stomatal conductance (gs_s) for cocksfoot growing in field conditions; (a) at different leaf water potentials where temperature and nitrogen were non-limiting (•); (b) at different temperatures where nitrogen and water were non-limiting (▽); (c) at different foliage nitrogen contents where temperature and water were non-limiting (■).

4.3.7 Empirical model for *Pmax* in cocksfoot

Using the three individual empirical "broken stick" functions of the main factors affecting *Pmax* (Equations 4.2, 4.4 and 4.5) enabled a simple multiplicative model (Equation 2.5) to be tested for the prediction of *Pmax* when two or three factors were constrained (Equation 4.7). For each function $Pmax_s$= *Ppmax*= 1.0 ≡ 27.4 μmol CO_2 m^{-2} s^{-1} and this indicates the factor was non-limiting. At $Pmax_s$= 0 no photosynthesis was occurring (*Pmax*= 0). The rate of constraint of each of the factors was defined by the following matrices;

$$Pmax = Ppmax * \begin{Bmatrix} 10 & 0.47 \\ 19 & 1.00 \\ 23 & 1.00 \\ 31 & 0.38 \end{Bmatrix} * \begin{Bmatrix} 1.5 & 0.25 \\ 2.6 & 0.70 \\ 5.2 & 1.00 \\ 5.9 & 1.00 \end{Bmatrix} * \begin{Bmatrix} -0.1 & 1.00 \\ -1.2 & 1.00 \\ -14.0 & 0.00 \\ -16.0 & -0.05 \end{Bmatrix}$$

f (T) x (°C) y (*Pmaxs*) | f (N) x (%N) y (*Pmaxs*) | f (ψ_p) x (ψ_p) y (*Pmaxs*)

Equation 4.7

Simulated results for the multiplicative photosynthesis model were compared with 62 data points (Figure 4.8) collected during the trial period where two or three factors were outside their determined optimum range. The average value of the RMSD (0.12) was about 21.5% of the mean observed *Pmax* values. The model adequately simulated *Pmax* when any two factors (water, N or temperature) were limiting but there was less accuracy when all factors were limiting. In particular, *Pmax* was underestimated by the model for a group of points in the observed range of 0.2 - 0.5 *Pmax* (Figure 4.8).

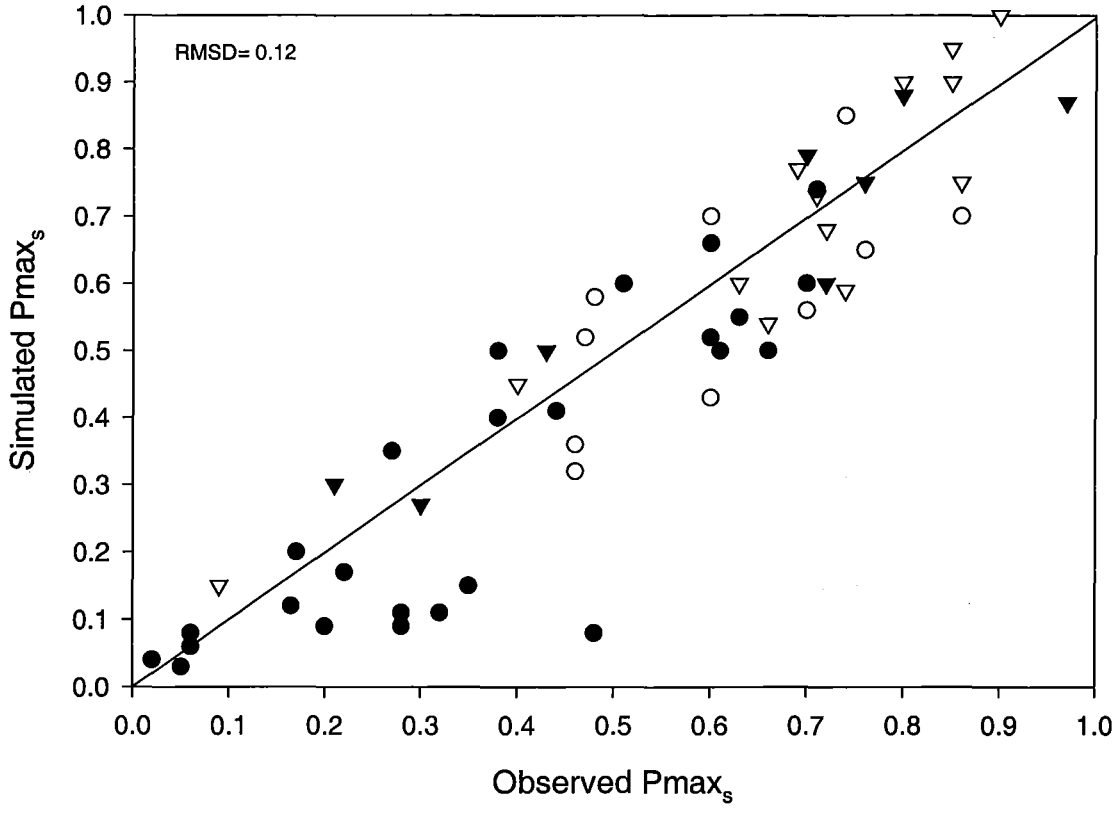

Figure 4.8. Simulated versus observed values for standardised rate of net photosynthesis (*Pmax$_s$*) sorted by four groups (water non-limiting (▽), nitrogen non-limiting (▼), temperature non-limiting (○) and all factors limiting (●)) for cocksfoot leaves grown in a field experiment. Simulated data were based on the multiplicative model proposed in Equation 4.7.

To identify the reasons for the low simulated *Pmax*, when all factors were limiting, residual analysis was used (Figure 4.9). For this, predicted values under limiting conditions of N and ψ_{lp} were sorted across the three temperature groups (sub-optimum, optimum and supra-optimum). Linear regression analyses of residuals for each factor combination were used to detect interactions between factors. Thus, if the slope (β) of the regression differed significantly from zero, an interaction was indicated.

There was no significant interaction ($\beta = 0$) for temperature and ψ_{lp} (Figure 4.9a). Most of the residuals (62%) were less than ±0.10 units from the predicted *Pmax* and evenly distributed across the ψ_{lp} range. This indicated acceptable accuracy for these situations. In contrast, for temperature groups across N% there was an even distribution of the residuals when temperature was ≤ 23 °C, but there was an interaction ($\beta \neq 0$) between low N content (≤ 2%) and temperatures above 23 °C (Figure 4.9b).

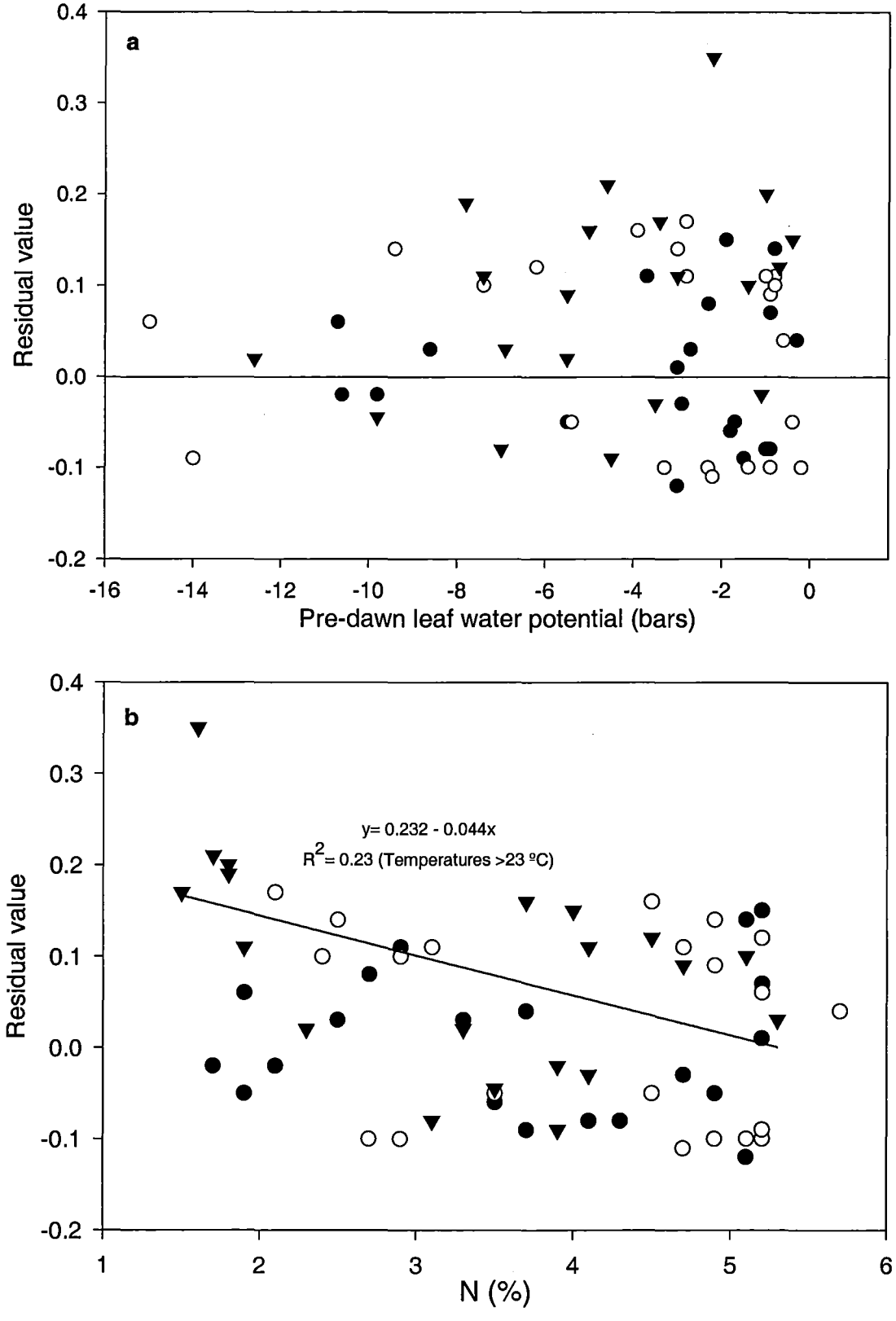

Figure 4.9. Residual [(observed – simulated values)] of standardised rate of net photosynthesis ($Pmax_s$) against (a) the leaf water potential range and (b) against nitrogen (N%) analysed for three temperatures groups (< 19 ºC (•), 19-23 ºC (○) and > 23 ºC (▼)).

4.3.8 Low nitrogen and high temperature effect on *Pmax*

Twenty-two data points collected during the trial period were used in a non-linear regression analysis to determine the effect of temperature on *Pmax* for irrigated plants (ψ_{lp}>-2.0 bar), when N content was ≤ 2%. Using the "broken stick" approach, the rate of constraint for the interaction was defined by the following matrix;

$$\text{Pmax}= \begin{array}{c} \begin{matrix} \text{x (°C)} & \text{y} \end{matrix} \\ \left\{ \begin{matrix} 10 & 0.44 \\ 19 & 1.00 \\ 25 & 1.00 \\ 31 & 0.50 \end{matrix} \right\} \end{array}$$

Equation 4.8

Compared with the original temperature function (Equation 4.7), the rate of increase from 10 to 19 °C was similar; the range of optimum temperatures for *Pmax* ($Pmax_s$= 1) increased by 2 °C to 25 °C; but from 25 to 31 °C the decrease in $Pmax_s$ was faster (Equation 4.8) (0.083 $Pmax_s$ units per °C) than for the non-N deficient temperature function. Despite this, at 31°C $Pmax_s$ was 0.5 compared with 0.38 units in the non-N deficient situation (Figure 4.10).

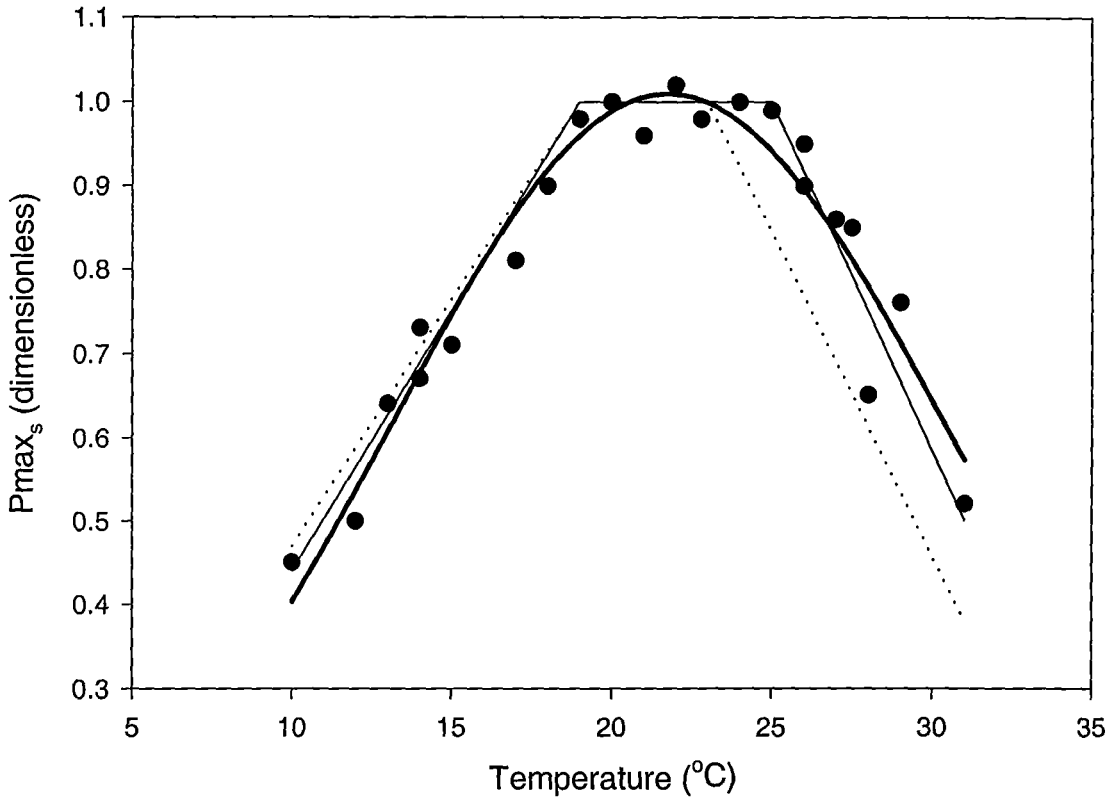

Figure 4.10. Standardised rate of net photosynthesis (*Pmax$_s$*) for cocksfoot grown in low nitrogen (≤ 2%) field conditions. The fitted Gaussian function model (—) and the "broken stick" model for interaction Equation 4.8 (—) and the "broken stick" model without interaction Equation 4.7 (····) are indicated.

The relationship between temperature and *Pmax$_s$* for low N% (Figure 4.10) was also described by a Gaussian function, which resulted in an R^2 of 0.92 and ESE for *Pmax$_s$* of 0.048 (Equation 4.9).

$$Pmax_s = 1.01 * e^{\left[-0.5*\left(\frac{T-21.7}{8.68}\right)^2\right]}$$

Equation 4.9

Where T is the air temperature (°C).

When the air temperature was > 28 °C, the canopy temperature of irrigated plants in the non-N deficient cocksfoot pasture was up to 2 °C colder than N deficient (≤ 2%) plants.

4.3.9 Modelling *Pmax* in cocksfoot- including the interaction

Detection of the interaction between low N and high temperature meant that the initial multiplicative model was modified (Figure 4.11).

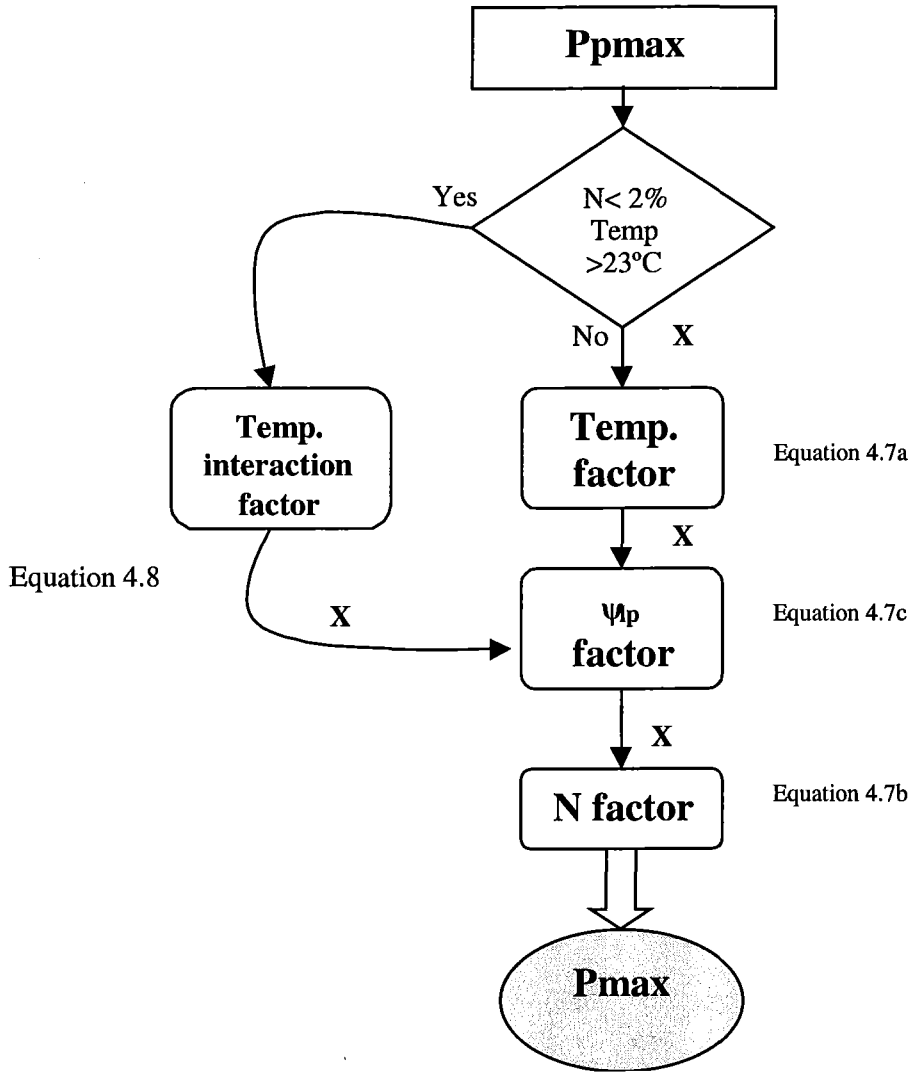

Figure 4.11. Diagram of the modified multiplicative model for prediction of *Pmax* for cocksfoot leaves under different temperature (from 10 to 31 °C), nitrogen (from 1.5 to 5.9% N) and soil moisture (from–0.1 to –16.0 bar pre-dawn water potential) environments. Individual equations are indicated. *Ppmax* represents the potential or maximum *Pmax* value in non-limiting conditions (*Ppmax*= 1.0 ≡ 27.4 μmol CO_2 m^{-2} s^{-1}).

Simulated results for the modified multiplicative photosynthesis model were then compared with the original validation set (Figure 4.12) and the RMSD decreased from 21.5% to 17.8% of the mean observed $Pmax_s$ values.

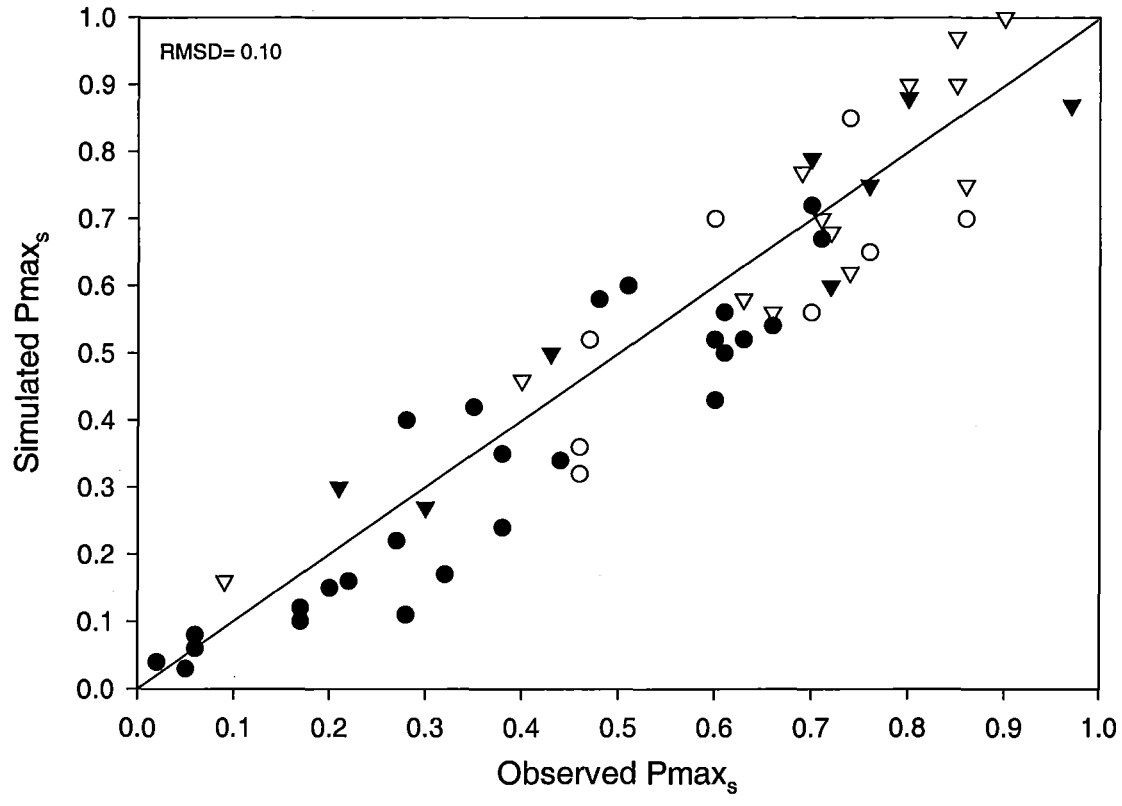

Figure 4.12. Simulated versus observed values for standardised rate of net photosynthesis (*Pmax*$_s$) sorted by four groups (water non-limiting (▽), nitrogen non-limiting (▼), temperature non-limiting (○) and all factors limiting (●)) for cocksfoot leaves grown in a field experiment. Simulated data was based on the modified multiplicative model proposed in Figure 4.11.

4.4 Discussion

4.4.1 Model accuracy

The use of the modified multiplicative model (Equation 4.1) resulted in the development of an empirical model (Figure 4.11) which predicted *Pmax* for a wide range of temperature, N and soil moisture environments. Validation of the model indicated approximately 78% of the variation in *Pmax* could be accounted for using these three factors as single functions. This was increased to 82% by the addition of a N x temperature interaction function (Equation 4.8). However, the addition of the interaction function for situations of low N% and high air temperatures requires validation.

To expand this single-leaf photosynthesis model to predict net canopy photosynthesis requires consideration of canopy architecture (LAI and leaf angles) and solar elevations, but the individual factor responses also provide a basis for varying the radiation use

efficiency (RUE) response across a range of environmental conditions. Factors that decrease *Pmax* also lower RUE (Sinclair and Muchow, 1999). Therefore the proposed model could be used for calibrating models which utilise RUE.

The individual functions for temperature, N% and water status were empirically derived and summarised into easily transferable coefficients using "broken stick" regressions. The success of this approach for predicting *Pmax* is reliant on these relationships holding in environments outside those from which they were derived. To confer repeatability, they must have a biologically meaningful basis and should be consistent with previous reports based on single factor analysis for cocksfoot.

4.4.2 Temperature function

The three stages of the "broken stick" function for the temperature response of *Pmax* (Figure 4.2) showed an optimum temperature range for *Pmax* of 19 to 23 °C. This was consistent with the 20-22 °C optimum range reported for controlled environment conditions (Eagles, 1967; Mitchell and Lucanus, 1962). Similarly, Oizumi *et al.* (1974) found for 'Frode' cocksfoot that the optimum temperature range was 15-22 °C, and this fell slowly to 10 °C but rapidly to a maximum of 35 °C. In contrast, Thornley (1998), using a cubic temperature function for *Pmax*, reported an optimum temperature of 30 °C for ambient CO_2 conditions.

The poor relationship between $Pmax_s$ and gs_s (Figure 4.7b) suggests that changes in stomatal conductance were not responsible for the reduction in *Pmax*. The reduction in *Pmax* at low temperatures cannot be accounted by stomatal limitations under light-saturating conditions and ambient CO_2 concentrations (Nie *et al.*, 1992). Thus, low temperature-induced inhibition probably reflects changes at the chloroplast level rather than limitations on actual leaf gas exchange. For example, at temperatures less than 18 °C the enzyme activities and metabolite transport involved in photosynthesis processes appear to be reduced for cocksfoot. Falk *et al.* (1996) reported that sub-lethal low temperatures could exert a reversible limitation on photosynthetic rate due to thermodynamic constraints on enzyme catalysed reactions of the Calvin cycle.

In addition, *Pmax* declined with temperatures above 23 °C. In contrast, for grasslands in general Thornley (1998) reported that *Pmax* started to decline from its maximum value at

30 °C to zero at 45 °C. Knievel and Smith (1973) showed that temperatures above 28 °C greatly reduce cocksfoot growth. It seems likely that the photorespiration rate of non-N limited and irrigated leaves of cocksfoot increased with temperature faster than net photosynthesis. Hay and Walker (1989) reported that photorespiration increases with temperature, because higher temperatures reduce the solubility of CO_2 more than O_2, reducing the CO_2/O_2 ratio, and also because high temperature affects the carboxylase activity of the enzyme which leads to decreased photosynthesis rates. It is also possible that the maintenance respiration increased with temperature due to enhanced enzyme activities (positive Q_{10} values) as has been shown for other species (McCree, 1974; Woledge and Dennis, 1982).

The three values of gs_s which indicated a closure in stomata (Figure 4.7b) at temperatures above 28 °C, when relative humidity (RH) was 48% and wind run was 15 km/h could be a consequence of a high transpiration rate from the leaves of irrigated plants exceeding the rate of absorption from the roots. Kramer (1969) indicated that the rate of water absorption and transpiration were controlled by different sets of factors, and in some circumstances are not perfectly synchronised.

4.4.3 Nitrogen function

There was a strong positive relationship between N% and *Pmax*, defined empirically by an asymptotic Weibull function and simplified to a three stage "broken stick" (Figure 4.3). For cocksfoot, this response showed that 2.6% N content was a critical value below which *Pmax* was severely restricted. Again, the lack of relationship between $Pmax_s$ and gs_s (Figure 4.7c), indicated factors other than stomatal conductance caused the reduction in *Pmax*. In this study leaf N was estimated from leaves of bulked dry matter samples. In a pastoral context it is the N content of the bulked sample that is usually analysed for feed quality. Thus, in practical terms any relationship between leaf *Pmax* and bulked N after 21 days reduces the need for two measurements. However, it would have been more accurate to have used the N% of the leaf where the photosynthesis measurement was taken.

The effect of N on *Pmax* per unit leaf area can be explained by the increment of chloroplast content in cocksfoot leaves. Increased photosynthetic pigment concentrations such as chlorophyll can be interpreted as giving a greater capacity for light absorption. The application of 300 kg N/ha and urine patches increased the total chlorophyll content in the

cocksfoot leaves from 0.06 g/m^2 in plants with N deficiency to 0.96 g/m^2 in non-N deficient plants (Figure 4.4). Decreased chlorophyll formation during nitrogen deficiency is a well-known phenomenon and nitrogen deficiency can also reduce the chloroplasts to about one-half of their normal length (Sundqvist *et al.*, 1980).

Furthermore, photosynthesis is closely related to leaf nitrogen content because the amount and activity of protein determines the photosynthetic potential of the leaf (Evans, 1996). For example, RuBisCO activity, obtained from gas exchange measurements, was reported to vary positively in proportion to leaf nitrogen content of a tropical forest understory herb (*Alocasia macrorrhiza* (L.) G. Don.) (Sims and Pearcy, 1989). Similarly, Prioul *et al.* (1980) found a positive relationship between chlorophyll content and RuBisCO activity along a developing third leaf and a fully expanded leaf of ryegrass seedlings.

The effect of N on *Pmax* could be modified by their influence on leaf anatomy. Lawlor *et al.* (1989) using flag leaves of winter wheat described the effect of N-deficiency on the reduction of cell number and cell volume, and also on the size and distribution of the chloroplasts within a cell. Changes of both the chloroplast and cell volumes were also described by Evans (1988). Ultimately, any reduction in *Pmax* will limit canopy expansion and therefore light interception leading to differences in pasture growth (Donohue *et al.*, 1981; Moloney *et al.*,1993).

4.4.4 Water function

There was a negative linear relationship between *Pmax* and the water status (Figure 4.5) of the plants expressed as pre-dawn leaf water potential (ψ_{lp}). Effects of water stress on net photosynthesis can be caused by stomatal and non-stomatal factors. In this study, the linear reduction in stomatal conductance to water vapour was the main factor that reduced *Pmax*, from 0.45 mol H_2O m^{-2} s^{-1} in irrigated plants (ψ_{lp}= -0.2 bar) to 0.0001 mol H_2O m^{-2} s^{-1} in plants under severe water stress (ψ_{lp}= -16 bar) (Figure 4.7a). Moderate water-deficit stress reduces photosynthesis primarily by inducing stomatal closure (Chaves, 1991; Slatyer, 1969). However, it is now recognised that the stomata do not respond to changes in leaf water potential until a critical threshold level is reached. Jackson (1974) reported that a field value for diurnal leaf water potential of –15.0 bar gave about a 70% decrease in leaf stomatal diffusion rate for cocksfoot plants. In the present study, it is likely that pre-dawn ψ_{lp} of –14 bar (*Pmax*= 0) fell progressively during the day reaching a maximum negative

value at noon when the radiation and temperature were highest and consequently the leaf stomata conductance decreased. More severe levels of water stress can decrease the rate of net photosynthesis per unit leaf area by increasing the mesophyll resistance (Ludlow and Ng, 1976; Kaiser, 1987) and by reducing the RuBP carboxylase activity (O'Toole *et al.*, 1976; Kaiser, 1987; Antolín and Sánchez-Díaz, 1993) in water-stressed leaves.

In this study, the effect of water stress on individual leaf net photosynthesis has been examined. However, the main consequence of severe water stress on cocksfoot production probably results from a reduction of leaf area as has been reported for ryegrass (Leafe *et al.*, 1977).

There was a strong relationship between ψ_{lp} and soil VWC (0-500 mm depth) (Figure 4.6). Water moves along a gradient of decreasing water potential from the soil, through the plant, to the atmosphere. The progressive changes in soil (ψ_s) and plant water potential as the soil dries out are characterised by marked diurnal fluctuations in leaf water potential (dependent on environment factors) with ψ_s setting the limit of recovery possible by the plant during the night (Turner and Begg, 1977). Thus, plant water potential returns to a value equal to soil water potential at pre-dawn time ($\psi_{lp}= \psi_s$). However, as soil VWC decreases to permanent wilting point, the ψ_{lp} falls to be equal to the osmotic potential and ψ_{lp} does not fully recover. The constant value of ψ_{lp} when soil VWC was 8 to 10% in this study suggests that there was mainly evaporation from soil surface because at this level of ψ_{lp} transpiration would be very low due to low stomatal conductance at $Pmax= 0$. Because ψ_{lp} is difficult to measure, this relationship provides an alternative method to predict $Pmax_s$ (Equation 4.5) from the soil VWC in this experiment.

The physiological reasons for the effects of the three factors on *Pmax* are summarised in Table 4.1.

Table 4.1. Summary of the effect of nitrogen (N), water (W) and temperature (T) on the rate of leaf net photosynthesis (*Pmax*).

Factor	Function	Maximum *Pmax* range	Minimum *Pmax* values	Extrapolated *Pmax*= 0 points	Biological impact
Air T (°C)	Inverted parabola (Gaussian)	19 to 23 °C	10 °C 31 °C	3 °C 40 °C	Low T reduces enzyme activity High T increases photorespiration and maintenance respiration
Leaf N (%)	Two stage linear	≥5% N	< 1.6% N	0.9% N	N increases chlorophyll, then increases RuBisCO activity
Leaf ψ_{lp} (bar)	Asymtotic (Weibull)	-0.1 to –1.2 bar	-14 to -16 bar	-14 bar	Water stress decreases stomatal conductance, increases mesophyll resistance, and decreases RuBisCo activity

4.4.5 Factor interaction

Only one interaction was detected between factors and this was only for the limited condition of low N (≤ 2%) and high temperatures (> 23 °C). In plants with low N content, the range of optimum temperatures for *Pmax* increased (19-25 °C) and the lower limit at 31 °C was higher (*Pmax*= 0.5). One reason for these differences could be that the rate of photorespiration in non-N deficiency leaves was higher than leaves with low N content when the temperature increased over the optimum range. The photorespiration cycle is initiated in the chloroplasts by the oxygenation of RuBP, which is the consequence of the active-site chemistry of RuBisCO (Hay and Walker, 1989). The higher N% was related to increased total chlorophyll content and this may increase the RuBisCO activity. Therefore, an increment in photorespiration may be expected mainly in high temperature environments with adequate RuBisCo. Photorespiration was reported to be over 50% of net photosynthesis at 35 °C, but only 10% at temperatures below 15 °C (Hay and Walker, 1989).

Contrary to this, at high temperatures (>28 °C), there was a difference between the canopy and air temperatures, which had the opposite effect of the interaction function (Equation 4.8). Canopy temperature of plants with >2% N was up to 2 °C cooler than N deficient plants (≤ 2%). This suggests that stomata were wider open and evaporative cooling and photosynthetic activity were greater in the high N plants than the low N plants. This was supported by measurements of stomatal conductance for irrigated plants at 29 °C, which

measured 0.30 mol H_2O m^{-2} s^{-1} when N was available but only 0.24 mol H_2O m^{-2} s^{-1} when $N \leq 2\%$. Thus, differences in canopy temperature cannot explain the interaction which implies differences were caused by an increment in photorespiration for non-N deficiency plants. The difference between air and leaf temperature, particularly when photosynthesis was restricted indicates that, where possible, canopy temperature should be used directly to fit photosynthesis models. Nevertheless, in this study air temperature, which is commonly available, was accurate in the prediction of leaf net photosynthesis in cocksfoot in most situations.

4.5 Conclusions

- Temperature, leaf N% and leaf water status of cocksfoot plants modified the utilisation of solar energy for the photosynthetic activity in leaves.

- The modified version of the multiplicative model explained about 80% of the variation in the maximum rate of net photosynthesis for individual leaves of cocksfoot. Thus, net photosynthesis as a potential input variable to predict growth in pastures was satisfactorily predicted using three main variables (temperature, nitrogen content and water status of the plants).

- Further *Pmax* functions, which include leaf age and different light intensities, coupled with canopy architecture (LAI and leaf angles) and solar elevations are needed to extend the models applicability for dry matter prediction in field studies.

In Chapter 5, the effect of regrowth duration on *Pmax* is evaluated and incorporated into the multiplicative model as a fourth factor.

CHAPTER 5

Maximum net photosynthetic rate of field grown cocksfoot leaves under different regrowth duration

5.1 Introduction

The use of the modified multiplicative model (Equation 4.1) resulted in an empirical model (Figure 4.11) which predicted the maximum leaf photosynthetic rate (*Pmax*) for a wide range of temperature, N and soil moisture environment conditions (Section 4.3). However, the productivity of a pasture is also dependent on management factors, such as the frequency and severity of defoliation. These affect the age of leaves and consequently their photosynthetic capacity (Section 2.3.1.5).

For grasses the effect of leaf age on leaf photosynthesis (i) in different positions on one tiller and (ii) during ageing of leaves in a particular position on a tiller has been quantified (Section 2.3.1.5). However, the effect of regrowth duration on the photosynthetic capacity of leaves that are of the same physiological age, such as the first fully expanded leaf, has received less attention (Section 2.3.1.5). Furthermore, the interaction of leaf age with other environmental factors has not been defined for cocksfoot. In this chapter the focus is on examining how regrowth duration affects *Pmax* of the youngest expanded leaf and its integrated relationship with temperature, N and water stress.

Therefore, the objectives of the experiment reported in this chapter were to:

1) derive an individual function for *Pmax* against regrowth duration for individual youngest fully expanded cocksfoot leaves;

2) propose biological explanations for the function derived;

3) integrate this regrowth duration function into the modified multiplicative model proposed in Figure 4.11 for environmental factors (temperature, N and water status);

4) validate this expanded model with independent data and identify any interactions among all four factors.

5.2 Materials and methods

For the current experiment only the three exclosure areas in open plots were measured from September 1999 to September 2000 (Section 3.2.2.2). To derive the individual function for *Pmax* against regrowth duration measurements were taken from the exclosure plots where nitrogen (300 kg N/ha) and irrigation were applied, and during the period of non-limiting temperature (November-December 1999 and January-February 2000).

To integrate the regrowth duration function into the modified multiplicative model, when other factors (temperature, N and water status) were limiting, measurements were taken from all treatments in exclosure plots during spring (September-October 1999), autumn (March-April 2000) and winter (May-August 2000), and from the treatments without irrigation and without applied N in the November-December and January-February regrowth periods (Section 3.2.2.2).

5.2.1 Photosynthesis measurements

The net photosynthesis rate (μmol CO_2 m^{-2} s^{-1}) was measured on a random sample of six of the youngest fully expanded intact leaves from vegetative tillers after 20, 25, 35, 40, 45, 55 and 60 days of regrowth. This meant that a new sample of 6 leaves was taken for each measurement date. All measurements were taken at midday ± 1 hour on cloudless sunny days when N, temperature and ψ_{lp} were non-limiting as defined in Chapter 4 (Equation 4.7, Section 4.3.7). Light photosynthesis curves, values of stomatal conductance (gs) to water vapour (mol H_2O m^{-2} s^{-1}) and their standardised index values ($Pmax_s$ or gs_s) were obtained as described in Section 4.2.2.

Overall, 119 photosynthesis measurements were taken in the field. Of these: 30 were used to fit the initial regrowth duration function in non-limiting conditions, and 67 were used for validation of the modified multiplicative model (Figure 4.11) when two, three or all four factors were limiting (temperature, N, ψ_{lp} and regrowth duration). A further 22 observations were used to examine the only interaction detected between regrowth duration and low ψ_{lp}. Data used to fit and validate the interaction function were taken from 40 and 60 days regrowth and from caged urine patches (N non-limiting) in the main plots sampled in January-February 2001 (Section 3.2.2.1.2) when temperature was non-limiting but soil moisture was low (Section 3.2.3.2.1).

5.2.2 Herbage measurements

From the January-February 2000 60-day regrowth period (Section 3.2.2.2), where nitrogen (300 kg N/ha) and irrigation were applied (corresponding with the period of non-limiting temperature) leaf chlorophyll content, N content (total, leaf and pseudo-stem) and tiller morphology were measured every 10 days. The leaf chlorophyll content per unit of area (g m^{-2}) was measured on a random sample of the youngest fully expanded intact leaves at mid-position following the methods described in Section 4.2.3. Samples for N content were taken on the same day as photosynthesis measurements. The N content of leaves and pseudo-stems from a 0.2 m^2 quadrat cut to 25 mm height was determined using the Kjeldahl technique. The total herbage N content was calculated from the weighted mean of the dry matter leaf:pseudo-stem ratio.

Morphological measurements were taken on a random selection from 20 dominant tillers per plot on each measurement date. Dominant tillers were defined as those positioned at the top of the canopy. From these the length of the youngest expanded leaf (with visible ligule), pseudo-stem height (height of the sheath from the above-ground soil level up to the ligule of the youngest expanded leaf), lamina width at mid position and number of green leaves per tiller were measured. The leaf:pseudo-stem ratio was calculated from dry weight of each component in dominant tillers.

5.2.3 Analyses

The data were analysed using non-linear regression analysis to determine the relationship between *Pmax* and regrowth duration. A linear function was used for the interaction detected between regrowth duration and ψ_{lp} and for modelling simplicity the data were also described using a two straight line segments "broken stick" methodology (Section 4.2.4). Values of R^2 and ESE $Pmax_s$ were used to select the most appropriate functions. Residuals and RMSD were calculated to estimate the accuracy of the models proposed (Section 4.2.4).

5.3 Results

The rate of net photosynthesis as a function of PPFD had a similar asymptotic shaped response function for different regrowth durations (Figure 5.1). All functions followed the expected non-rectangular hyperbola (Thornley, 1998) and *Pmax* was 27.4 μmol CO_2 m^{-2} s^{-1} in non-limiting conditions (20 days regrowth) with leaf saturation at 1000 μmol m^{-2} s^{-1} PPFD. However, *Pmax* decreased as regrowth duration increased. For example, *Pmax* was 23.7 and 14.2 μmol CO_2 m^{-2} s^{-1} for the youngest fully expanded leaf of plants grown for 40 and 60 days, respectively. Furthermore, as the regrowth duration increased beyond 20 days, the saturation point decreased and was 500 μmol m^{-2} s^{-1} PPFD at day 60.

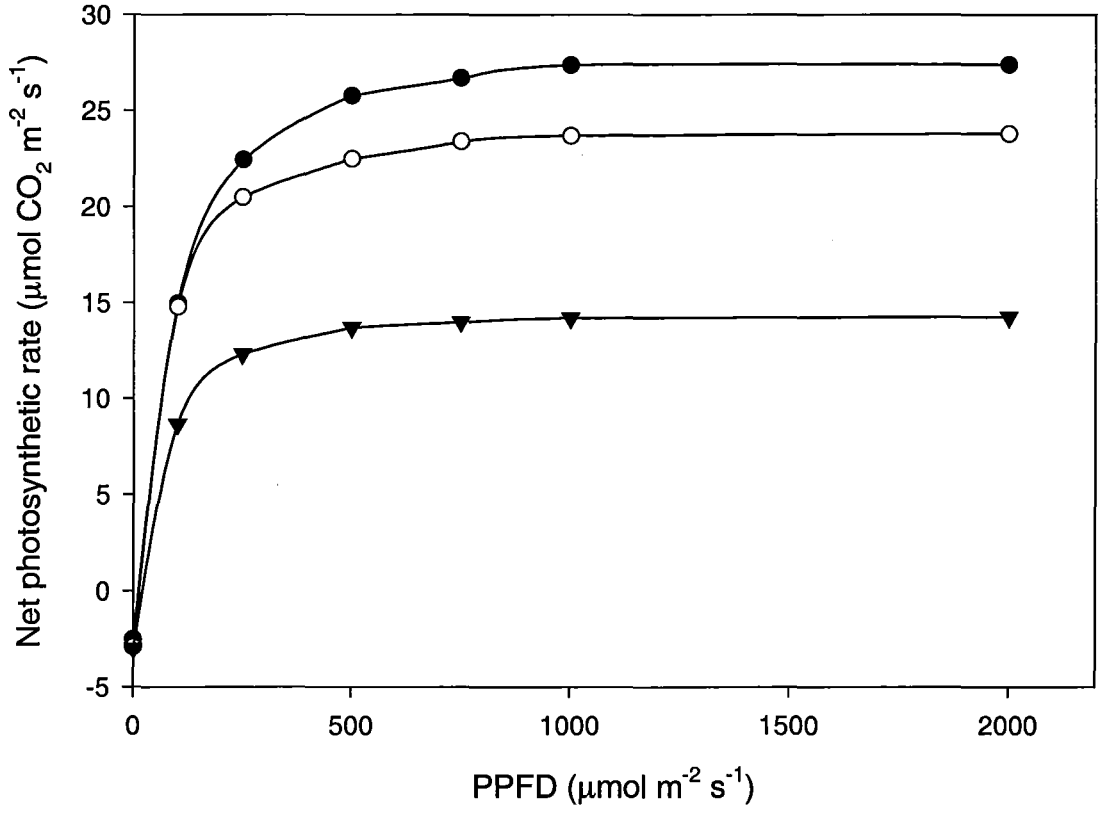

Figure 5.1 Net photosynthetic rate against photosynthetic photon flux density (PPFD) for youngest expanded leaf of cocksfoot grown in a field environment with regrowth duration non-limiting at 20 days (●) and with regrowth after 40 (○) and 60 (▼) days.

5.3.1 *Pmax* and regrowth duration

The *Pmax* of successive newly expanded leaves was progressively reduced with regrowth duration and this reduction was more pronounced after lodging occurred at 35 days regrowth. From 20 to 25 days regrowth, *Pmax* per unit of leaf was constant at its maximum value ($Pmax_s$= 1) but it then decreased by 0.42 μmol CO_2 m^{-2} s^{-1} per day of regrowth, or

0.0154 units of $Pmax_s$ per day (Figure 5.2). A quadratic function was fitted (Equation 5.1) to the measured data and this gave an R^2 of 0.95 and ESE of $Pmax_s$ of 0.034.

$$Pmax_s = 0.872 + 0.0104\ Tr - 0.0003\ Tr^2 \qquad \textbf{Equation 5.1}$$

Where Tr is time of regrowth in days.

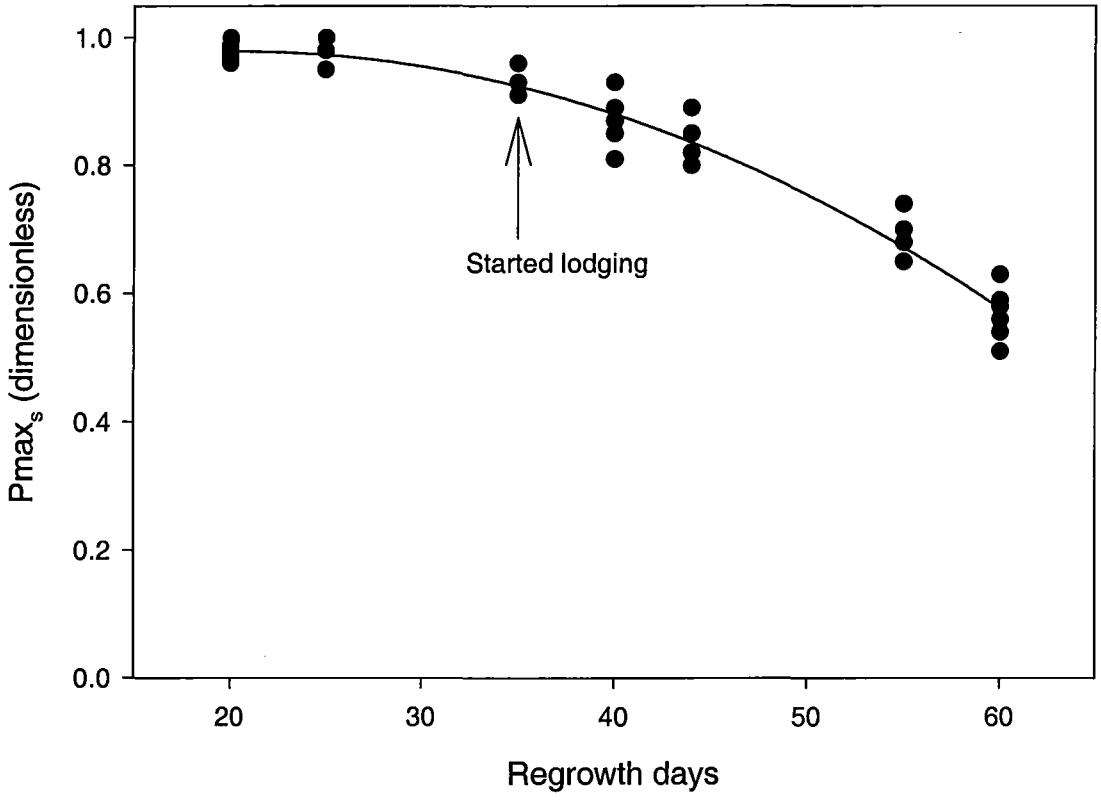

Figure 5.2. Standardised rate of net photosynthesis ($Pmax_s$) against regrowth duration for cocksfoot grown under field conditions where temperature, N and water were non-limiting. $Pmax_s = 1 \equiv Pmax = 27.4$ μmol CO_2 m^{-2} s^{-1}. The fitted quadratic function (Equation 5.1) is indicated (—).

5.3.2 Morphology

Tiller morphology was also affected by the duration of regrowth. As the number of days increased, successive youngest fully expanded leaves increased in length from 110 mm at day 10 to 510 mm after 60 days (Figure 5.3). Similarly, the pseudo-stem height increased from 29 to 200 mm from 10 to 60 days regrowth, respectively. The width at mid position of the youngest expanded leaf remained almost constant over time with a mean value of

5.5 ±0.22 mm. The number of green leaves per tiller reached a maximum value of 3.6 leaves at 20 days regrowth and then decreased slightly to 3.4 leaves per tiller.

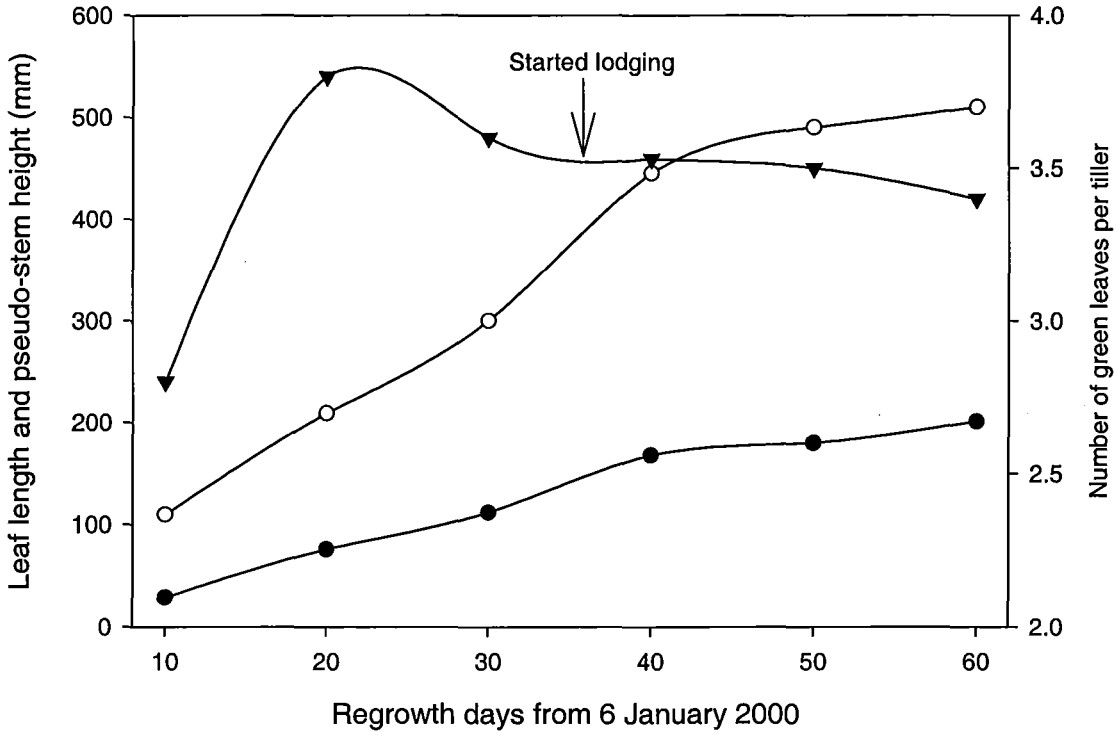

Figure 5.3. Length of the youngest expanded leaf (○), pseudo-stem height (●) and number of green leaves per tiller (▼) of dominant tillers measured over a 60 day regrowth period in non-limiting (temperature, N% and water) conditions from January-February 2000.

5.3.3 Nitrogen content

In all plant parts the herbage N content declined with regrowth duration and was lowest at the end of the rotation (Figure 5.4). The leaf:pseudo-stem ratio increased from 2.1 at day 10 to 2.5 at day 20 and then declined to 1.2. This change in the leaf:pseudo-stem ratio resulted in a decrease in total herbage N content from 5.4 (day 10) to 2.6% N (day 60) (Figure 5.4). The maximum N content of leaves was measured at the first reading after 10 days regrowth (5.8% N) and then it declined at about 0.04% d^{-1}. Similarly, for the pseudo-stem, N% decreased from 4.4 to 1.2% from day 10 to day 60.

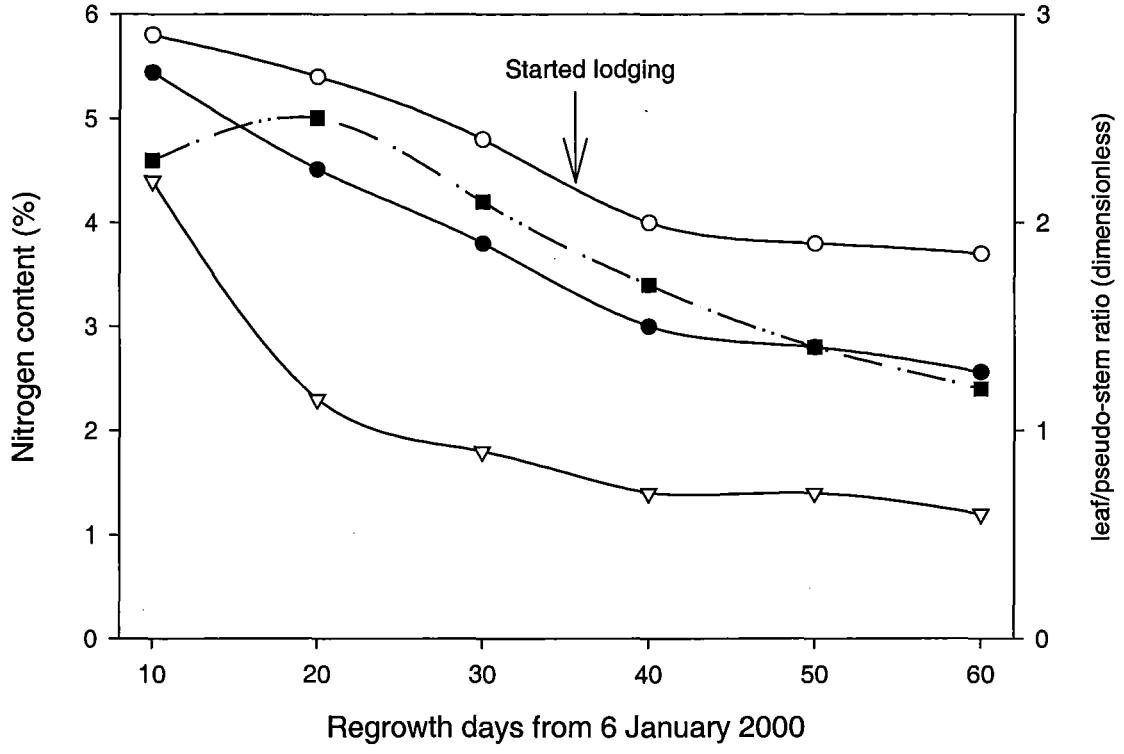

Figure 5.4. Variation of total herbage nitrogen content (●), leaf N content (○), pseudo-stem N content (▽) and leaf:pseudo-stem ratio (■) over a 60 day regrowth period in non-limiting (temperature, N% and water) conditions from January-February 2000.

5.3.4 Chlorophyll content

The chlorophyll content per unit of leaf area (CHL) of consecutive youngest expanded leaves was almost constant up to 30 days regrowth with a maximum mean value of 0.96 g m^{-2} (Figure 5.5). From this point, CHL reduced at a rate of 0.011 g m^{-2} d^{-1} reaching a minimum value of 0.60 g m^{-2} at day 60.

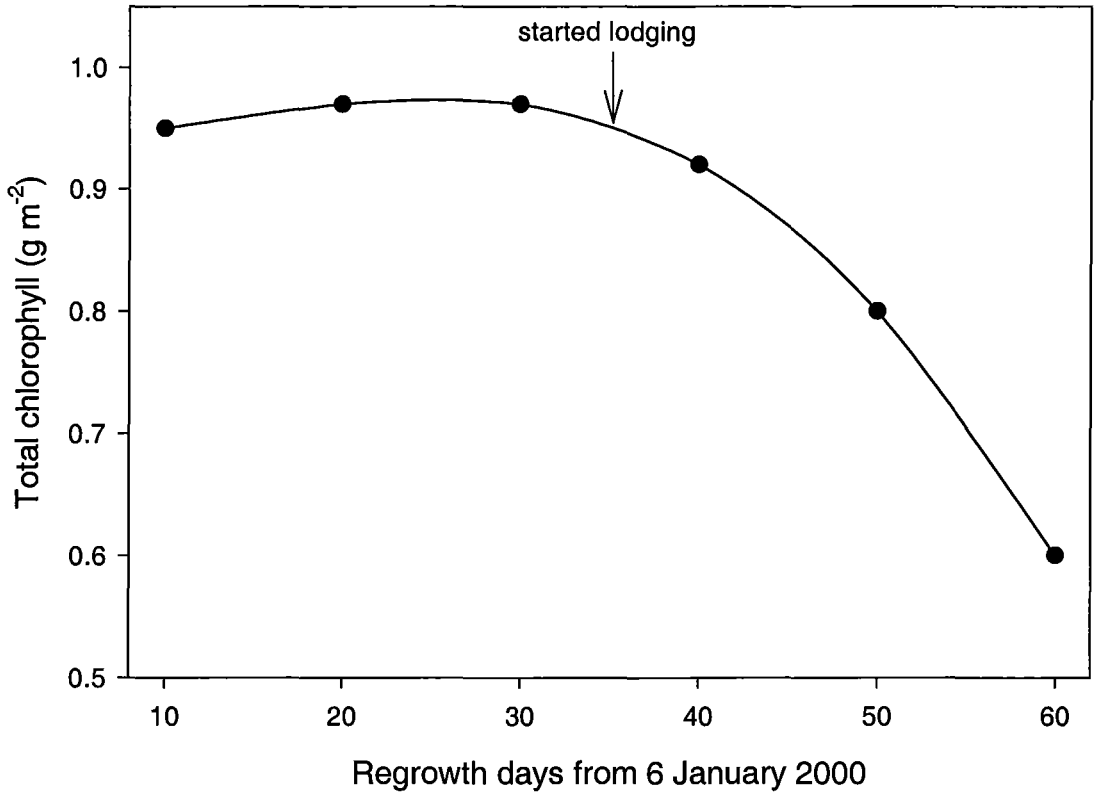

Figure 5.5. Mean total leaf chlorophyll content per unit of area (g m^{-2}) over a 60-day regrowth period in non-limiting (temperature, N% and water) conditions from January-February 2000.

5.3.5 Stomatal conductance

The maximum rate of *gs* was 0.45 mol H_2O m^{-2} s^{-1} (gs_s= 1) in non-limiting conditions (day 20) and the minimum value recorded was 0.31 mol H_2O m^{-2} s^{-1} at day 60. Least squares regression analysis showed a positive linear relationship between gs_s and $Pmax_s$ for changes in regrowth duration with a coefficient for the slope of 1.33 (Figure 5.6).

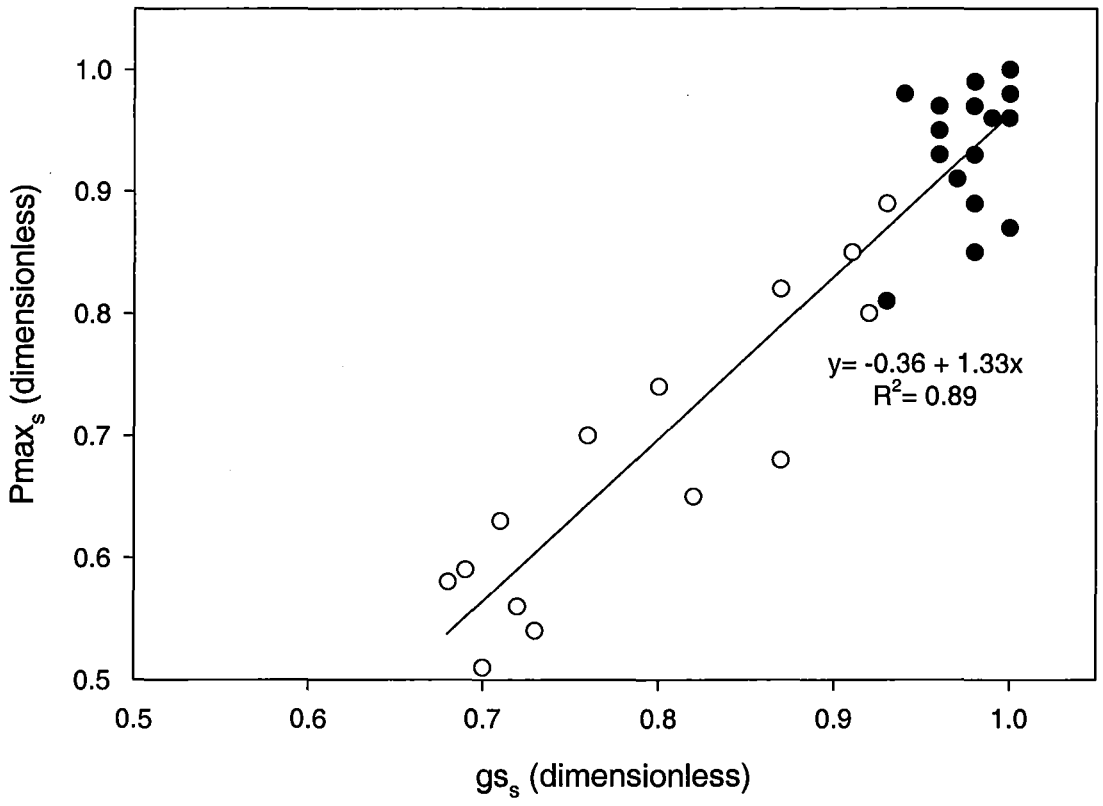

Figure 5.6. Standardised rate of net photosynthesis (*Pmax$_s$*) against standardised rate of stomatal conductance (*gs$_s$*) for cocksfoot growing in field conditions where temperature, N and water were non-limiting. Data sorted by two regrowth period groups: 20-40 (•) and 40-60 (○) days.

5.3.6 Modelling *Pmax* in cocksfoot- a modified version incorporating the regrowth duration function

The function obtained for regrowth duration (Equation 5.1) was an additional factor used to expand the multiplicative model presented in Figure 4.11 (Section 4.3.9). For each function *Pmax$_s$*= *Ppmax*= 1.0 ≡ 27.4 μmol CO_2 m^{-2} s^{-1} and this indicates the factor was non-limiting. At *Pmax$_s$*= 0 no photosynthesis was occurring (*Pmax*= 0).

Simulated results for the multiplicative photosynthesis model were compared with 67 data points (30 data points from 40 days regrowth and 37 data points from 60 days regrowth) collected during the trial period where regrowth duration and one, two or three other factors were outside their defined optimum range (Figure 5.7).

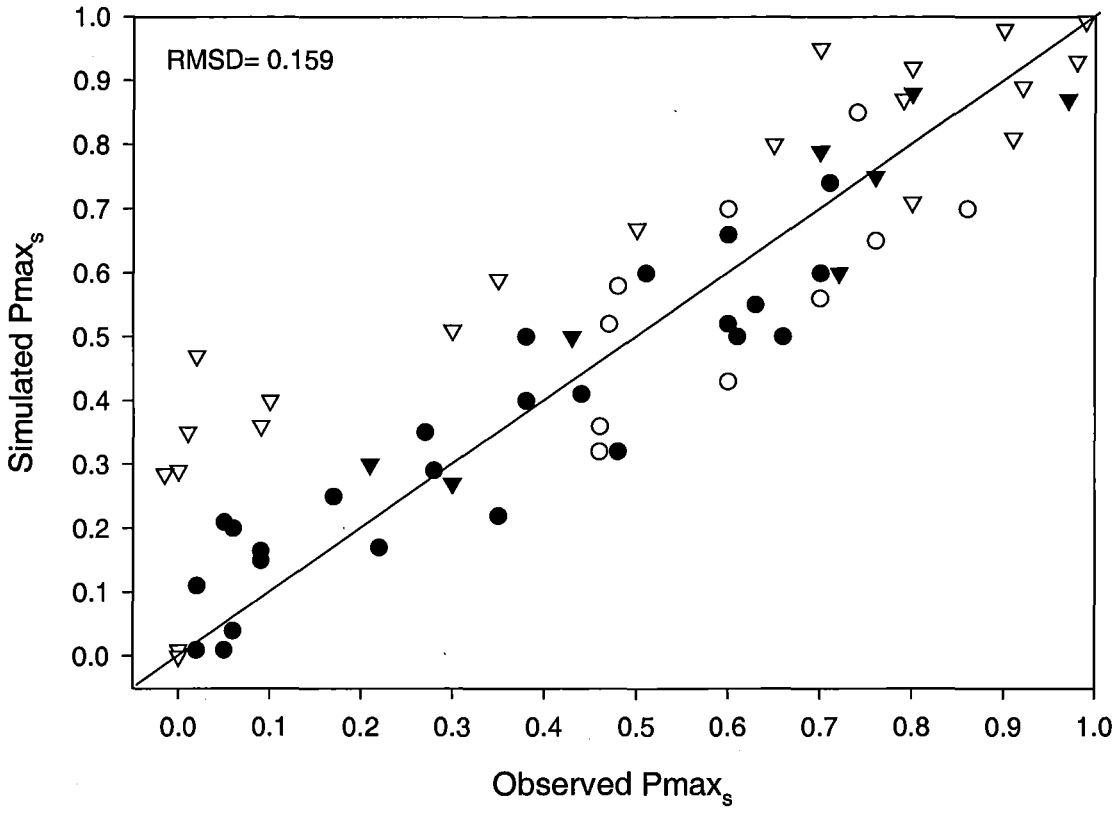

Figure 5.7. Simulated versus observed values of standardised rate of net photosynthesis (*Pmax$_s$*) sorted by four groups (water limiting (▽), nitrogen limiting (▼), temperature limiting (○) and all factors limiting (●)) for cocksfoot leaves grown in a field experiment. Simulated data were based on the multiplicative model proposed in Figure 4.11 (Section 4.3.9) incorporating regrowth duration as other factor (Equation 5.1).

The average value of the RMSD (0.16) was about 31% of the mean observed *Pmax* values. The model adequately simulated *Pmax$_s$* when regrowth duration and N or temperature or all factors were limiting. However, the prediction of *Pmax$_s$* was less accurate when regrowth duration and water were both limiting. Thus, *Pmax$_s$* was overestimated by the model for a group of 40-60 days regrowth measurements with an observed range of 0.01 – 0.2 *Pmax$_s$* (Figure 5.7) and ψ_{lp} values between –5.0 and –14.0 bar.

Regression analyses of these residuals for each factor combination were used to identify interactions between factors (as described in Section 4.3.7). Analyses indicated no significant interaction ($\beta = 0$) between the regrowth duration factor and temperature, N or all factors limiting (Figure 5.8). Most of the residuals (85%) were less than ±0.20 units from the predicted *Pmax$_s$* and evenly distributed. This indicated acceptable accuracy for these situations. In contrast, there was an interaction ($\beta \neq 0$) between regrowth duration and

water limited leaves for the predicted $Pmax_s$ values (Figure 5.8).

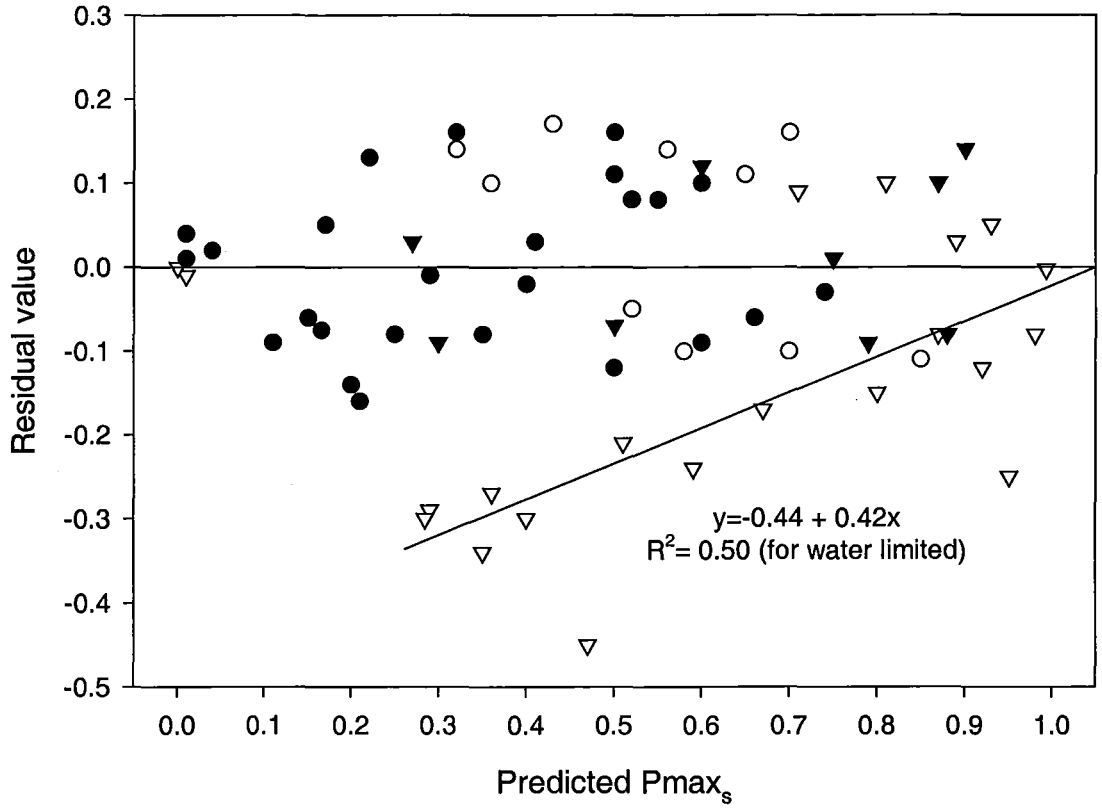

Figure 5.8. Residual [(observed – simulated values)] of standardised rate of net photosynthesis ($Pmax_s$) against the predicted values sorted by four groups (water limiting (▽), nitrogen limiting (▼), temperature limiting (○) and all factors limiting (●)) for cocksfoot leaves grown in a field experiment.

5.3.7 Interaction between regrowth duration and leaf water potential (ψ_{lp}) on *Pmax*

Twenty-two additional observations, collected during the trial period, were used to explore the interaction between 40-60 days of regrowth and ψ_{lp}. The range of ψ_{lp} was from –0.1 bar to –16.0 bar. There was a strong negative relationship between *Pmax* and ψ_{lp} (R^2= 0.97, ESE= 0.042) (Figure 5.9). The maximum value of *Pmax* ($Pmax_s$= 1) was measured from –0.1 to –1.2 bar (from 30 to 27% soil VWC). Compared with the original ψ_{lp} function where regrowth duration was non-limiting (Equation 4.5 in Section 4.3.4), the range of optimum ψ_{lp} was similar; but from –1.2 to –10.0 bar the decrease of $Pmax_s$ was faster (0.114 units per bar) (Equation 5.2) than when the regrowth duration was non-limiting (0.078 units per bar). Furthermore, $Pmax_s$ reached a zero value at –10.0 bar compared with –14.0 in Equation 4.5.

$$Pmax_s = 1.067 - 0.102\ \psi_{lp}\ [\text{Range } -1.2. \text{ to } -10.0 \text{ bar}] \qquad \textbf{Equation 5.2}$$

Using the "broken stick" approach, the rate of constraint for the interaction was defined by the following matrix;

$$\text{Pmax} = \begin{array}{c} \begin{matrix} x\ (\psi_{lp}) & y\ (Pmax_s) \end{matrix} \\ \left\{ \begin{matrix} -0.1 & 1.00 \\ -1.2 & 1.00 \\ -10.0 & 0.00 \\ -16.0 & -0.05 \end{matrix} \right\} \end{array}$$

Equation 5.3

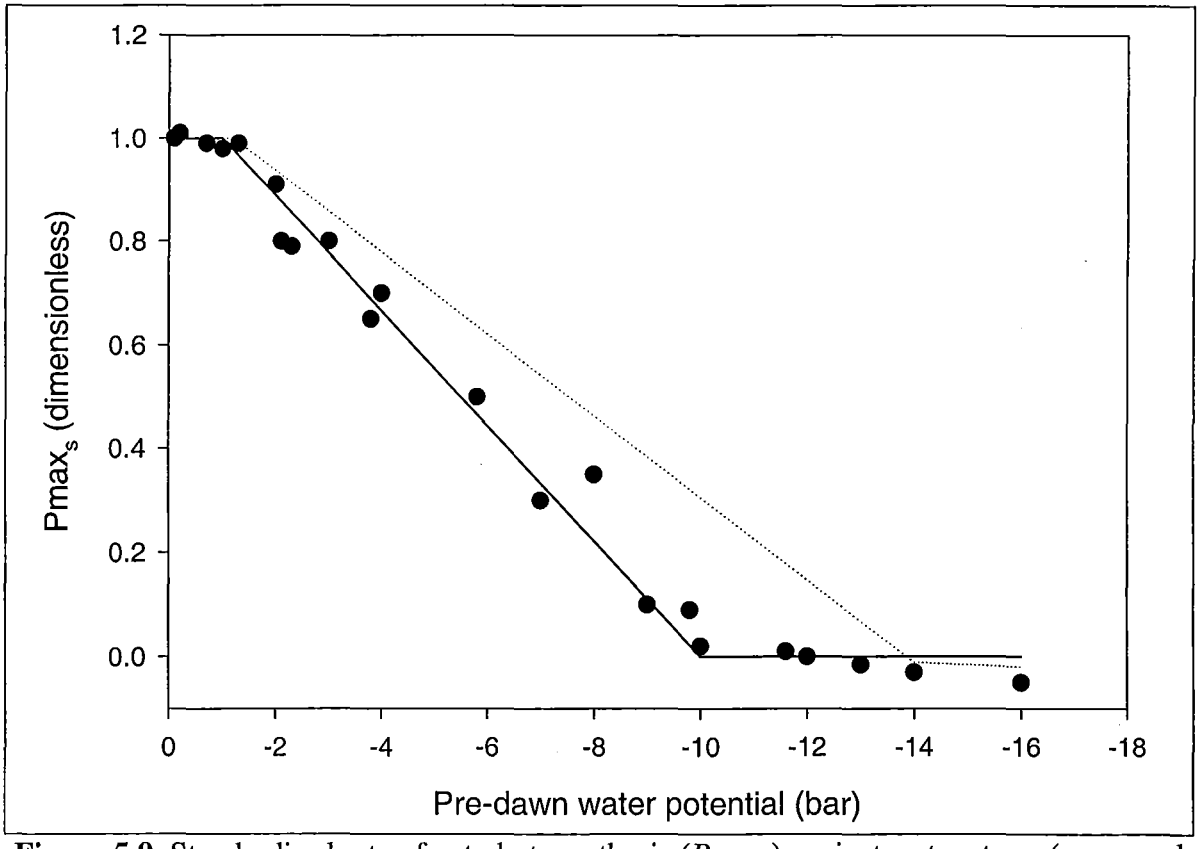

Figure 5.9. Standardised rate of net photosynthesis (*Pmax$_s$*) against water stress (expressed as pre-dawn water potential, ψ_{lp}) for cocksfoot grown under field conditions where regrowth duration was limiting (40-60 day regrowth). The "broken stick" model for interaction Equation 5.3 (—) and the "broken stick" model without interaction for 21 days regrowth Equation 4.5 (····) are indicated.

5.3.8 Stomatal conductance of the regrowth duration and leaf water potential (ψ_{lp}) interaction

The maximum rate of gs_s for regrowth duration limiting conditions (40-60 days) was 0.45 mol H_2O m^{-2} s^{-1} for well irrigated plants (ψ_{lp} = -0.1 to –0.7 bar) and the minimum value recorded was 0.0001 mol H_2O m^{-2} s^{-1}. Least squares regression analysis showed a positive linear relationship between gs_s and $Pmax_s$ for changes in ψ_{lp} with a coefficient for the slope of 1.05 (Figure 5.10a). The negative ordinate axis intercept value showed that values of gs_s close to zero were related to negative values of $Pmax_s$.

A comparison of the effect of ψ_{lp} on gs_s was made between regrowth non-limiting (21 days, data from Section 4.3.6) and regrowth limited conditions (40-60 days). The range of optimum gs_s (gs_s= 1) was similar (ψ_{lp} = -0.1 to –0.7 bar) (Figure 5.10b). However, from this point the linear decrease of gs_s was faster for regrowth limited plants (0.087 units of gs_s per bar) than for the regrowth non-limiting situation (0.080 units gs_s per bar). Then, gs_s reached the minimum value at –10.0 bar for regrowth limiting compared with –13.0 bar for the regrowth non-limiting situation (Figure 5.10b).

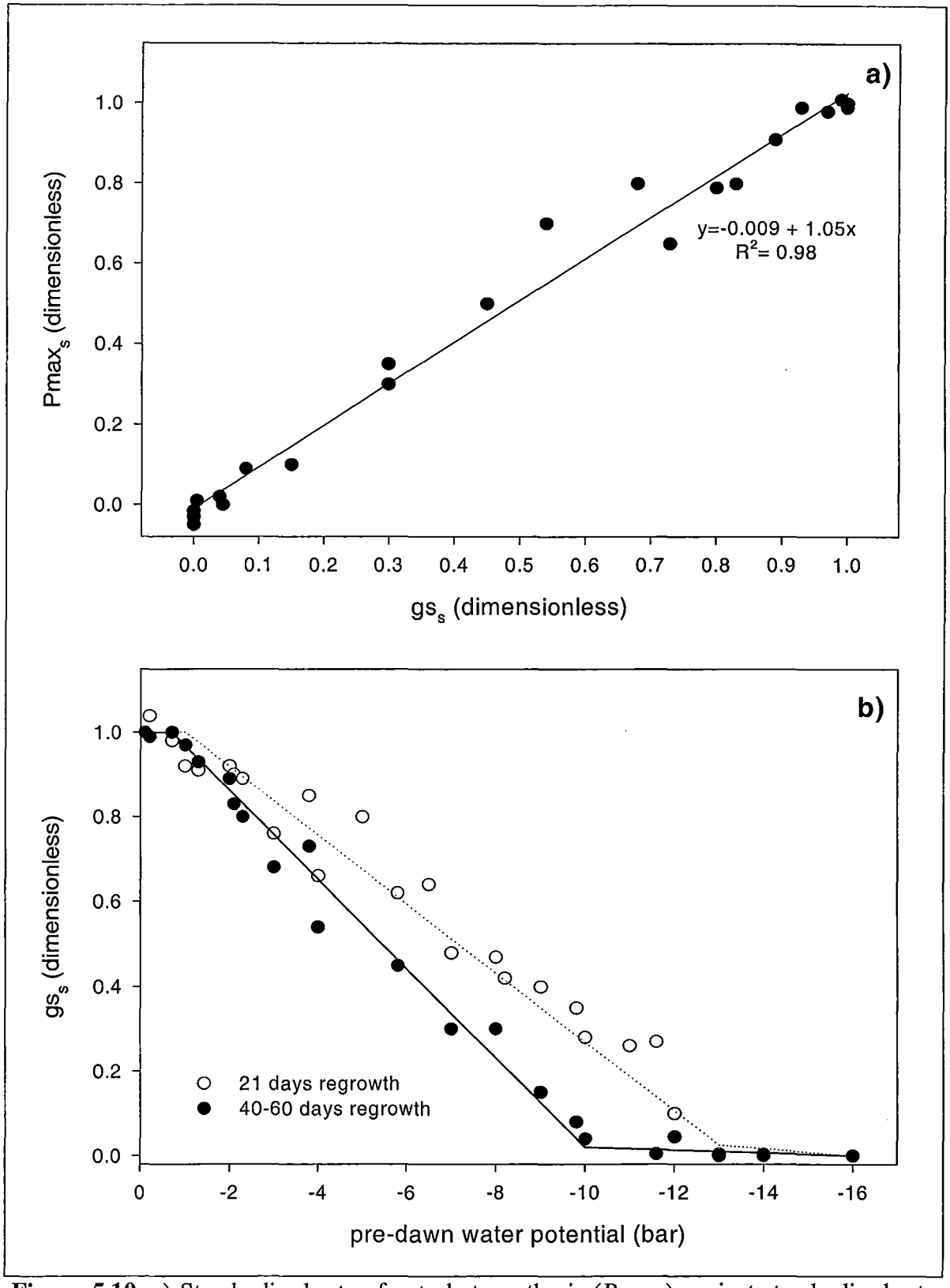

Figure 5.10. a) Standardised rate of net photosynthesis ($Pmax_s$) against standardised rate of stomatal conductance (gs_s) for interaction Equation 5.3 for cocksfoot grown in field conditions; b) Standardised rate of stomatal conductance (gs_s) against pre-dawn water potential for interaction Equation 5.3 (•) and without interaction for 21 days regrowth Equation 4.5 (○) for cocksfoot grown in field conditions where temperature and N were non-limiting.

5.3.9 Modelling *Pmax* in cocksfoot- including regrowth duration and the ψ_{lp} interaction.

Detection of the interaction between regrowth duration (40-60 days) and ψ_{lp} meant that the multiplicative model required modification (Figure 5.11).

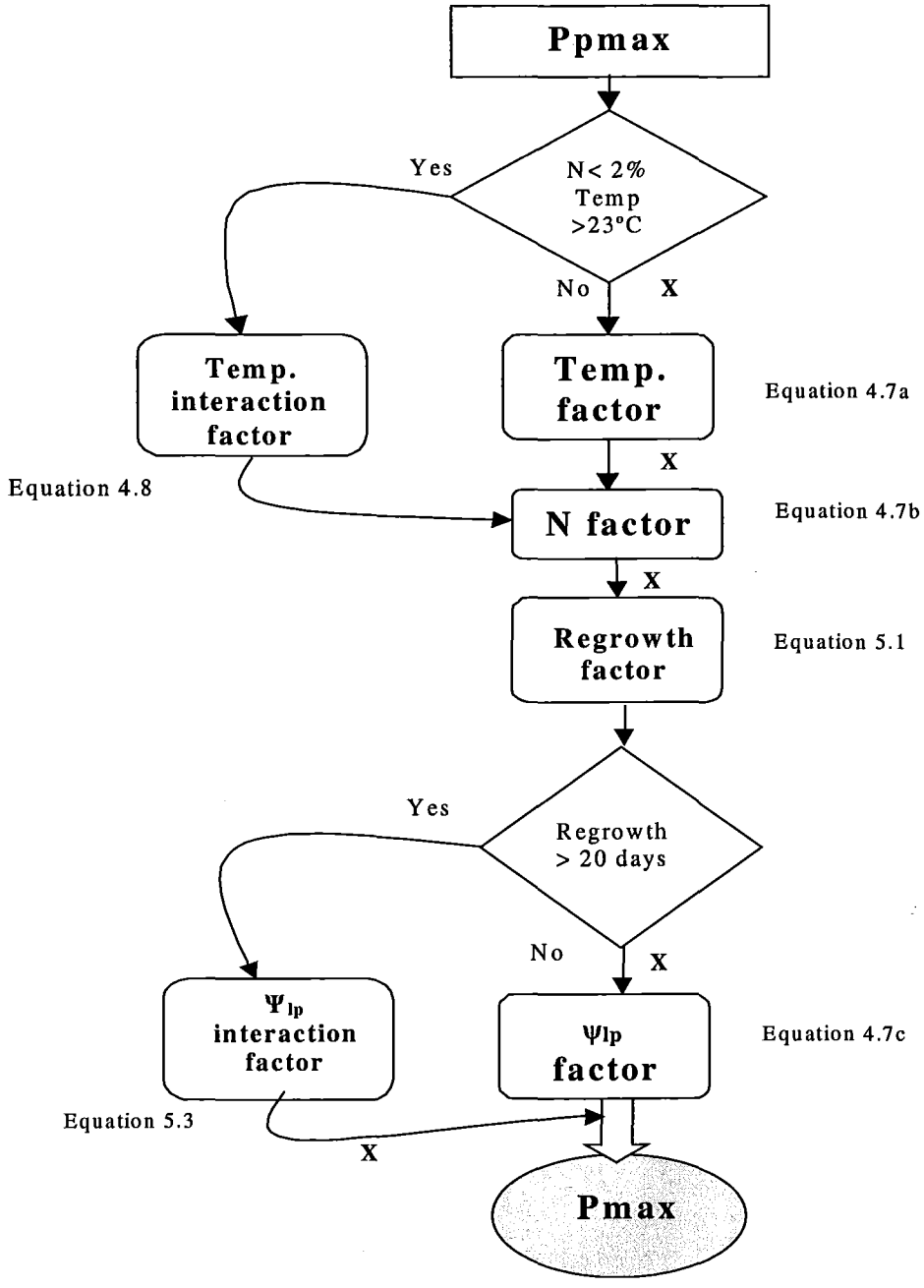

Figure 5.11. Diagram of the modified multiplicative model for prediction of *Pmax* for cocksfoot leaves under a wide range of temperature, nitrogen, soil moisture environments and for different regrowth periods. Individual equations are indicated. *Ppmax* represents the potential or maximum *Pmax* value in non-limiting conditions (*Ppmax*= 1.0 ≡ 27.4 μmol $CO_2 m^{-2} s^{-1}$).

Simulated results for the modified multiplicative photosynthesis model (Figure 5.11) were then compared with the original validation set and showed the RMSD decreased from 31% to 20% of the mean observed $Pmax_s$ values (Figure 5.12) compared with the 18% for the three factor model excluding the regrowth duration limitation (Section 4.3.9).

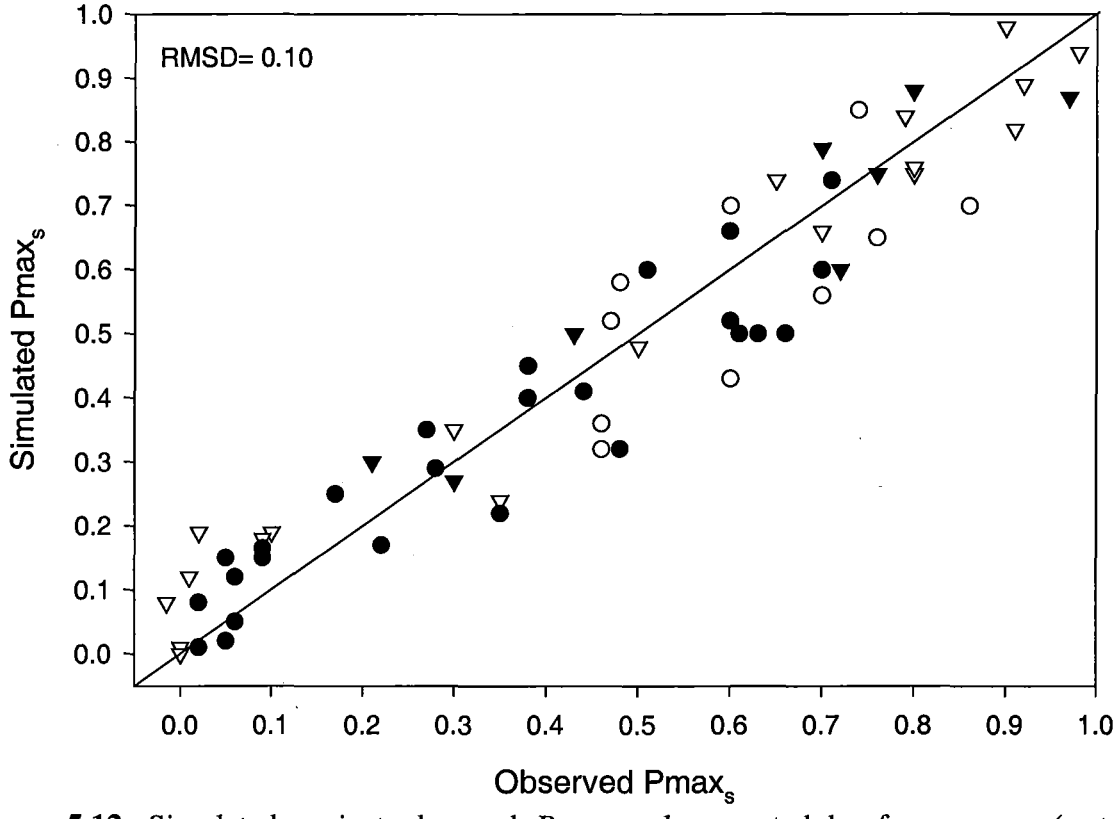

Figure 5.12. Simulated against observed $Pmax_s$ values sorted by four groups (water limiting (▽), nitrogen limiting (▼), temperature limiting (○) and all factors limiting (●)) for cocksfoot leaves grown in a field experiment. Simulated data was based on the multiplicative model proposed in Figure 5.11.

5.4 Discussion

5.4.1 Model accuracy

The use of the modified multiplicative model proposed in Figure 4.11 (Section 4.3.9) resulted in the development of a predictive model (Figure 5.11) for *Pmax* over a range of temperature, N and soil moisture environments and for the management factor of regrowth duration. Validation of this model indicated 80% of the variation in *Pmax* was accounted for using these four factors and the addition of a regrowth duration by water status interaction function (Equation 5.3). However, this interaction function for situations of 40 or more days regrowth and water stress remains to be validated. The individual function for regrowth duration was empirically derived and demonstrated the flexibility of the multiplicative model to incorporate additional factors. The observed response of *Pmax* has been attributed to regrowth duration but it was confounded by lodging from day 35.

5.4.2 Regrowth duration function

There was a negative quadratic relationship between *Pmax* and regrowth duration (Figure 5.2). In this field study, the youngest expanded leaves at the beginning of a growth period had the highest photosynthetic capacity ($Pmax_s$= 1) and this decreased up to 48% after 60 days of regrowth. The decline in the photosynthetic capacity of successive leaves of perennial ryegrass has been also reported (Wodledge and Leafe, 1976; Woledge, 1978) (Section 2.3.1.5). Thus, Equation 5.1 which takes into account the decline in *Pmax* of the youngest expanded leaf due to regrowth duration, is required to enable the prediction of pasture growth by a canopy photosynthesis model that uses leaf photosynthesis as a driving factor. Similarly, Parsons *et al.* (1988) proposed, for a photosynthesis model used for ryegrass, a function to take into account the decline in the photosynthetic capacity of the fully youngest leaf. In their results, *Pmax* fell from 22.7 μmol CO_2 m^{-2} s^{-1} at LAI= 0 to a minimum value of 15.0 μmol CO_2 m^{-2} s^{-1} at LAI= 8.

5.4.2.1 Direct factors affecting *Pmax* with regrowth duration

The effect of regrowth duration on net photosynthesis of leaves can be caused directly by changes in tiller morphology which are related to an ageing effect, and by shading within the canopy.

(i) Morphology changes related to ageing effect on leaf photosynthesis

The first consideration is that successive youngest expanded leaves may be older as regrowth days progress. This is consistent with the tiller morphology measured in this study over 60-day regrowth where the lamina length and pseudo-stem height increased (Figure 5.3) suggesting that the youngest expanded leaves at day 60 were actually older than those at day 10. Duru and Ducrocq (2000) reported that as cocksfoot plants grew up to 80 days of regrowth (N and temperature non-limiting conditions), the leaf appearance rate per tiller decreased and the lamina growth duration, lamina length (from 14 to 44 cm) and life-span (from 362 to 580 degree-days) increased with its insertion level (sheath length). Similarly, Robson *et al.* (1988) indicated that as a grass tiller ages, the proportion of old to young leaf increases because if the pseudo-stem in grasses is left intact, the emerging leaves will be relatively long and appear more slowly. Wilman *et al.* (1977) reported that the longevity of Italian ryegrass leaves increased from 6.8 to 40.1 days after 8 weeks regrowth. Furthermore, because leaf appearance rate decreased and life-span increases during regrowth, the number of living leaves is fairly constant (Duru and Ducrocq, 2000). This was also shown in this experiment with an almost constant mean value of 3.5 green leaves per tiller confirming greater longevity of leaves at the end of a rotation. Thus, a dominant tiller at 60 days of age may be proceeding through a senescence process even though it has the youngest fully expanded leaf for the plant on it. Wilman *et al.* (1977) reported that Italian ryegrass tiller longevity was less than 70 days.

These changes in tiller morphology over time may influence photosynthetic efficiency through an ageing process. For grasses, the negative effect of leaf age on leaf photosynthesis in different positions on one tiller, and during ageing of leaves in a particular position on the tiller has previously been reported (Jewiss and Woledge, 1967; Treharne *et al.*, 1968; Treharne and Eagles, 1970; Woledge, 1972; Robson and Parson, 1978) (Section 2.3.1.5).

(ii) Shading within the canopy

Nitrogen and irrigation increased leaf photosynthesis early in the growth period (Section 4.3.2) but, by stimulating leaf expansion (Figure 5.3) and mutual shading, may indirectly depress it later. Therefore, a further explanation of the decline in *Pmax* with regrowth duration may be that developing leaves from the stem apex, which remains near the soil surface in vegetative swards, are increasingly shaded as the LAI of the sward increases.

Consequently, the light level at the base of the plant decreases over time. For the January-February 60-days period, lodging occurred at 35 days of regrowth (Appendix 5) with LAI= 6.5 (Section 3.3.5.2). As a result, each tiller in the sward produced a succession of leaves with progressively lower photosynthetic capacities. As a consequence, it is likely that when they emerged into full sunlight they were unable to make full use of the available energy (Woledge and Leafe, 1976; Sheehy, 1977). This is because it is the light conditions experienced by the developing leaf itself, that determines its photosynthetic capacity (Prioul *et al.*, 1980). Similar to the present study, Sheehy (1977) reported a 50% reduction in *Pmax* of the youngest expanded leaf after 37 days regrowth when lodging occurred. Also, Robson and Parson (1978) reported that the photosynthetic capacity of leaves, when the canopy was fully light-intercepting, is repressed by shading during development. However, Woledge and Pearse (1985) concluded that it was not possible to separate the roles of shading during growth and reduced N content in leaves in causing the reduction in photosynthetic capacity of the leaves of the N fertilised sward in the later stages of regrowth.

5.4.2.2 Impact of ageing effect and low light intensity at the apex on *Pmax* with regrowth duration

Changes in tiller morphology (ageing effect) and shading within the canopy with regrowth duration may have a consequent effect on *Pmax* through changes in stomatal conductance, leaf N and chlorophyll content and maintenance respiration rate.

(i) Nitrogen content

A likely cause of the decrease in *Pmax* in the sward is that the herbage and leaf N content decreased over regrowth time (Figure 5.4). Even when there is an optimal supply of N, the concentration of N in plants declines with increasing age or DM accumulation. This is because, in older plants, a greater proportion of resources is diverted to structural support and other non-photosynthetic material of low N content (Caloin and Yu, 1984; van Keulen *et al.*, 1989). In this study, this effect is shown by the increase in the relative amount of pseudo-stem compared with leaf DM (Figure 5.4). Mobile nutrients, including N, are partially remobilized from senescing leaves and translocated in the phloem to other parts of the plant, with the result that the concentration of N in leaf material declines during the ageing process (Whitehead, 1995).

Using Equation 4.3 (Section 4.3.2), the decline in leaf N content from 5.8 to 4.0% (Figure 5.4) indicates that 10% of the decline in *Pmax* at day 60 would be accounted by the impact of N% (Figure 5.2). In contrast, Woledge and Pearse (1985) reported that a decrease from 4.21% to 3.17% in the N content of perennial ryegrass leaves after 28 days of regrowth accounted for 25% reduction in photosynthesis of the youngest expanded leaf.

The reasons for a strong relationship between *Pmax* and leaf N content were explored in Chapter 4 (Section 4.4.3). These were mainly the increase in soluble proteins (predominantly enzymes activities) and the increase of compounds associated with the light reactions (including chlorophyll). In this experiment, the decrease in leaf N content with regrowth was consistent with the decrease in chlorophyll content of the youngest expanded leaf (Figure 5.5). Treharne *et al.* (1968) also found for cocksfoot a decrease in leaf chlorophyll content with age. Treharne and Eagles (1970) reported a fall of 60% in RuBisCO activity of the youngest fully expanded cocksfoot leaves after 30 days full expansion at 25 °C.

(ii) Stomatal conductance (*gs*)

Another reason for the decline in *Pmax* as leaves age, or as regrowth duration progresses, is the decrease in *gs* (Figure 5.6). In this study, *gs* decreased linearly by 30% from 20 to 60 days regrowth. Similarly, Woledge (1972) found that increases in both stomatal and mesophyll diffusion resistances contributed to a 60% fall in photosynthesis when *Lolium temulentum* L. leaves aged from full expansion to 37 days. Also, Woledge (1986) reported that a decrease of stomatal conductance (from 22 to 6 mm s^{-1}) was the main cause of the photosynthesis reduction (from 25.2 to 8.2 μmol CO_2 m^{-2} s^{-1}) in white clover leaves with age (from full expansion to 35 days). These changes in *gs* may confirm that the youngest expanded leaf at day 60 was older than those at day 10.

(iii) Maintenance respiration rate

An increase in maintenance respiration may also decrease *Pmax* over regrowth time. Maintenance respiration is proportional to plant dry weight (McCree, 1974), so the heavier weight of the youngest expanded leaf at day 60 of regrowth is expected to increase its maintenance respiration and therefore decrease the net photosynthesis rate. An indication of this reduction was obtained from light curves at zero PPFD (Figure 5.1) where maintenance respiration increased from –2.5 μmol CO_2 m^{-2} s^{-1} at 20 days regrowth to –3.0

μmol CO_2 m^{-2} s^{-1} at day 60. The small magnitude of this change suggests it would be only a minor contributor to the decrease in *Pmax*. However, according to Penning de Vries (1975) the constant of proportionality falls as the plant parts age. Johnson and Thornley (1983) assumed that the maintenance cost per unit dry weight varied between cohorts of leaves of different ages. For a grass growth model, the authors applied maintenance respiration coefficients decreasing with leaf age at 20 °C (0.02 d^{-1} for a growing leaf and the first fully expanded leaf, 0.015 d^{-1} for the second fully expanded leaf and 0.01 d^{-1} for senescing leaf). Similarly, Woledge (1986) reported that maintenance respiration per unit dry weight for white clover leaves decreased with age from 5.0 at fully leaf expansion to 3.0 g CO_2 kg^{-1} h^{-1} at 25 days. Also, a 30-35% decrease of maintenance respiration per unit leaf area from full expansion to 37 days was reported by Woledge (1972) for *Lolium temulentum* leaves.

The physiological reasons for the effects of regrowth duration on *Pmax* are summarised in Table 5.1.

Table 5.1 Summary of the effect of regrowth duration on *Pmax*.

Function	**Maximum *Pmax***	**Minimum *Pmax***	**Direct factors**	**Consequential biological impact**
quadratic	20-25 days	60 days	Ageing process Low light at apex	Lower N and chlorophyll content Decrease in stomatal conductance Increase in maintenance respiration

Regardless of the mechanisms related to the reduction in *Pmax*, the function derived empirically (Equation 5.1) provided a useful framework to predict *Pmax* of the youngest fully expanded leaf from 10 to 60 days of regrowth and to deal with the interaction between regrowth duration and water stress conditions.

5.4.3 Interaction factor between regrowth duration and ψ_{lp}

Only one interaction was detected between factors and this was for a limited condition of water stress (ψ_{lp}> -1.2 bar) and 40-60 days regrowth duration. This was mainly the consequence of more complete stomatal closure (Figure 5.10b). For example, at ψ_{lp} of –10 bar, *gs* was 0.009 and 0.25 mol H_2O m^{-2} s^{-1} for limiting and non-limiting regrowth

conditions, respectively. One reason for these differences could be that at the same pre-dawn water potential the leaf water potential at midday (when *Pmax* measurements were taken) of 40-60 day regrowth plants fell progressively more rapidly reaching more negative values than leaves after only 21 days regrowth. Because the rate of transpiration is dependent, in part, on leaf area (de Wit, 1978; Jensen *et al.*, 1990), 40-60 day regrowth plants with greater LAI than 21 days regrowth (Section 3.3.5.2) may have closed their stomata more to reduce the water loss.

5.4.4 Use of the regrowth duration function into a canopy photosynthesis model and limitations

To incorporate these results from leaf photosynthesis measurements into a canopy photosynthesis model several factors need to be considered. Firstly, the vegetative grass sward usually has three green leaves of different ages per tiller (expanding leaf, first and second fully expanded leaves, and senescing leaves). In this study, the effect of regrowth duration on *Pmax* was only carried out on the youngest expanded leaf (first fully expanded leaf). The *Pmax* of this leaf corresponds with the maximum *Pmax* in the tiller (Jewiss and Woledge, 1967; Treharne *et al.*, 1968; Treharne and Eagles, 1970; Woledge, 1972; Woledge and Leafe, 1976; Woledge and Pearse, 1985). Therefore, the model presented in this study may represent the maximum potential decline in *Pmax* with regrowth.

Secondly, because lodging occurred at 35 days regrowth, with a LAI= 6.5 when temperature, N and water were non-limiting, the foliage became progressively more prostate and the mean leaf angle decreased which would affect light interception (Section 3.3.8).

Thirdly, the principal morphological change that occurred with regrowth was a greater proportion of the DM pseudo-stem component over time (Figure 5.4). This reduced the proportion of green leaf as the main photosynthetic component. Davidson and Milthorpe (1966) calculated that the rate of photosynthesis per unit area of leaf sheaths was about one-third of that of leaf laminae in cocksfoot. Consequently, this model may underestimate the effect of leaf age on canopy photosynthesis even though accurately measuring the potential leaf photosynthesis of the youngest expanded leaves. Additional measurements of leaves of different ages would be required to create a comprehensive model.

5.5 Conclusions

• For field grown vegetative cocksfoot swards, the youngest expanded leaf at 21 days regrowth had a *Pmax* equivalent to the non-limiting conditions defined in Chapter 4. Over the next 39 days, *Pmax* of successive newly-expanded leaves declined by 48%.

• The decline in *Pmax* was attributed to (i) differences in leaf age which were shown by changes in tiller morphology over time, and (ii) shading within the canopy during leaf expansion. These factors consequently affected *Pmax* by decreasing the leaf N% and chlorophyll content, and by decreasing stomatal conductance.

• The modified version of the multiplicative model explained about 80% of the variation in *Pmax* for individual leaves of cocksfoot using temperature, N% and ψ_{lp} and the interaction with regrowth duration.

• The incorporation of the individual function for regrowth duration demonstrated the flexibility of the multiplicative model to incorporate new factors. Further expansion would be possible for any other factor which may aid explanation of some of the remaining 20% variation.

In the next chapter, the response of *Pmax* to different sunlight regimes, similar to those likely to be experienced in an silvopastoral system, will be analysed. Then, the fitted mathematical equations will be incorporated into the modified multiplicative model proposed in Figure 5.11 (Section 5.3.9) for integrating the shade factor with temperature, N%, ψ_{lp} and regrowth duration.

CHAPTER 6

Maximum net photosynthetic rate of cocksfoot leaves under different field shade and environment conditions

6.1 Introduction

In field environments plants can experience frequent fluctuations in irradiance from full sun to shade. The extent of shading can alter the efficiency of conversion of energy to dry matter by affecting light interception and the photosynthetic activity of individual leaves (Section 2.3.1.1). In this chapter, the focus is on silvopastoral systems where understorey plants experience frequent and rapid fluctuations in irradiance from full sun to shade caused by tree canopy shading. In these systems the duration of full sunlight/shade periods is dependent on the size of the tree, crown shape, tree planting density, silvicultural practices and the development of the foliage area of the trees (Section 2.2.1).

The effects of different uniform light energy levels on photosynthesis have been reported for cocksfoot (Section 2.3.1.1). However, the physiological adaptability of leaves to a fluctuating light environment, related to the net photosynthesis of pastures growing under trees in silvopastoral systems, has received little attention.

Of interest in this chapter are the environmental and physiological controls on photosynthesis rate that operate during fluctuations in light in silvopastoral systems. These differ from those operating under steady-state conditions (Section 2.3.1.1). When plants experience a change from high to low irradiance, a photosynthesis deactivation process occurs due to a reduction in stomatal conductance (*gs*) and an increase in biochemical limitations (Section 2.3.1.1). For plants going from low to high irradiance there is a lag in the rise of photosynthesis rate to the maximum *Pmax*. This lag time is defined as the 'induction phase' of photosynthesis and it is dependent on the activity status of photosynthetic enzymes and on *gs* (Section 2.3.1.1).

Furthermore, in silvopastoral systems the productivity of a pasture is dependent on the interaction of environmental and management factors that affect the photosynthetic capacity of the sward as described in Equation 2.5 (Section 2.3.1.7). According to Ong *et al.* (1991), when the availability of a single factor in an agroforestry system (soil VWC, nutrient level and the amount of light) falls below the plants' combined demands, competition begins. The integrated relationship between shade limitation and other environmental (temperature, N and water stress) and management (regrowth duration) factors affecting photosynthetic rate of cocksfoot leaves in a silvopastoral system has not been defined.

This chapter reports two components of competition in a silvopastoral system. The first part is related to light availability, and examines the response of *Pmax* of cocksfoot leaves to light and dark fluctuations, as likely to be experienced in a silvopastoral system, when other factors are non-limiting. In the second part, the measured response of *Pmax* to sunlight fluctuations is integrated with the main environmental and management factors used previously in this study (Chapters 4 and 5) of the silvopastoral system. The aim of the research was to provide a framework to develop quantitative predictions of cocksfoot growth in a silvopastoral environment where all these factors operate to influence understorey productivity.

There are six objectives of this chapter.

1) To determine the light regime (periods of sun/shade and light intensity) in the silvopastoral system described in Chapter 3 and then, create artificial structures to simulate this light regime and extend the time scale of the light regime (described in Chapter 3) to longer periods of shade. This is analogous to an extended light regime affected by an increase in the tree canopy.

2) To evaluate the effect of time under severe shade (5% of open photosynthetic photon flux density, PPFD), which represents the light intensity in the middle of pine crown shade, and moderate shade (50% of open PPFD) on *Pmax* and *gs* of individual leaves of cocksfoot when temperature, N, water and regrowth duration were non-limiting.

3) To determine the induction state of cocksfoot leaves on *Pmax* and *gs* at different physiologically relevant times after severe shade (based on the potential tree crown size).

4) To develop mathematical equations to represent the physiological processes measured in objectives 3 and 4 for a silvopastoral system.

5) To determine the relative importance of stomatal and non-stomatal limitations on photosynthetic rate of plants exposed to severe and moderate shade, as an explanation of the observed response in *Pmax*.

6) To modify and test the multiplicative model (Figure 5.11) after integrating the shade factor with temperature, N, water status and regrowth duration factors and to validate this model with an independent data set that also examines whether there were significant interactions between these factors.

6.2 Materials and methods

6.2.1 Experiment to evaluate the effect of time under shade on *Pmax* and *gs*

The first part of this investigation involved creating artificial structures to simulate the light regime in the silvopastoral system described in Chapter 3 and then to extend the time scale of this light regime to longer periods of shade.

The three main cocksfoot plots under trees were used in this experiment (Section 3.2.2.1) giving three replicates of each treatment. The experiment ran from September 1999 to February 2001 during which time plots were rotationally grazed by sheep (Section 3.2.2.1.1).

6.2.1.1 Shade treatments and light regime measurements

Light intensity was monitored with quantum sensors as described (Section 3.2.3.3).

(i) *Tree shade*

The daily PPFD integral under the 10-year-old trees, measured in the middle of rows, was 62% of the open PPFD over a sunny day in summer (at maximum solar elevation), with alternating periods of full sunlight (1700-1900 μmol m^{-2} s^{-1} PPFD at midday) and severe shade (129-130 μmol m^{-2} s^{-1} PPFD) (Figure 6.1a). The duration of light and shade changed from an interval of full sunlight and shade of 45-60 minutes from 8:00 to 11:00 and 17:00 to 20:00, but 90–120 minutes around midday. The light regimes from the radiata pine trees and other structures used for the present experiment are summarised in Table 6.1.

Within the silvopastoral experiment, two artificial structures were used in addition to the tree shade to evaluate the effect of time under shade and light intensity on *Pmax* and *gs*. These structures were placed to avoid overlap with the tree shade during measurement periods.

(ii) *Severe shade*

A wide wooden shade structure was sited in the middle of the 7 m inter-row. This structure was made with a solid 2.4 x 1.8m timber top area, and was supported on an adjustable height frame to allow the shade source to be maintained at 0.3 m above the cocksfoot canopy. This structure provided 0-180 minutes of uniform severe shade of 5% of open PPFD transmissivity (85-95 μmol m^{-2} s^{-1} PPFD) with a bimodal light regime analogous to the silvopastoral system (Table 6.1). This structure is representative of the light regime of a pine tree silvopastoral system with an extended time of shade periods, and was used to represent increased shade from more developed pine stands.

(iii) *Moderate shade*

A second structure covered with black shade cloth over a 2.3 x 1.8m area was sited in the middle of the inter-row, and was supported horizontally on a vertically adjustable metal frame to provide constant 50% of open PPFD (850-950 μmol m^{-2} s^{-1} PPFD at midday) (Figure 6.1c) as continuous shading for 0-180 minutes (Table 6.1). This structure may represent the shade of a less dense tree crown shade than radiata pine (i.e. when some light penetration occurs). However, it was mainly used to examine if leaf photosynthesis response to a 50% of open PPFD was affected by the temporal light pattern experienced by plants. In agroforestry research, artificial shading with continuous partial shade has been used widely to simulate light reduction (Varella *et al.*, 2001). Thus, this treatment was used to determine if the understorey pasture response was similar to that in fluctuating light conditions.

Table 6.1. Shade sources used to generate different light regimes and a description of how the data was used for development of a model for a silvopastoral system.

Shade source	Light regime	Use
Trees	Fluctuating *tree shade*[1]/full sunlight[2] at intervals of 90-120 min	To define structures and for validation of Equation 6.2
Wide wooden structure	180 min *severe shade*[3] and 45 min full sunlight[1]	To fit functions for Equations 6.2 and 6.4 of time under shade and recovery from shade for *Pmax* and *gs*, and for validation of these equations.
Cloth structure	Continuous *moderate shade*[4]	To fit functions for Equations 6.3 and 6.5 of time under shade for *Pmax* and *gs*, and for validation.
Slated structure	Fluctuating *severe shade*[3]/full sunlight[2] at intervals of 110-120 min	For validation of Equation 6.2
Windbreak	300 min under *tree shade*[3]	For validation of Equation 6.2

[1] 7% of open PPFD; [2] 1700-1900 μmol m^{-2} s^{-1} PPFD at midday; [3] 5% of open PPFD; [4] 50% of open PPFD.

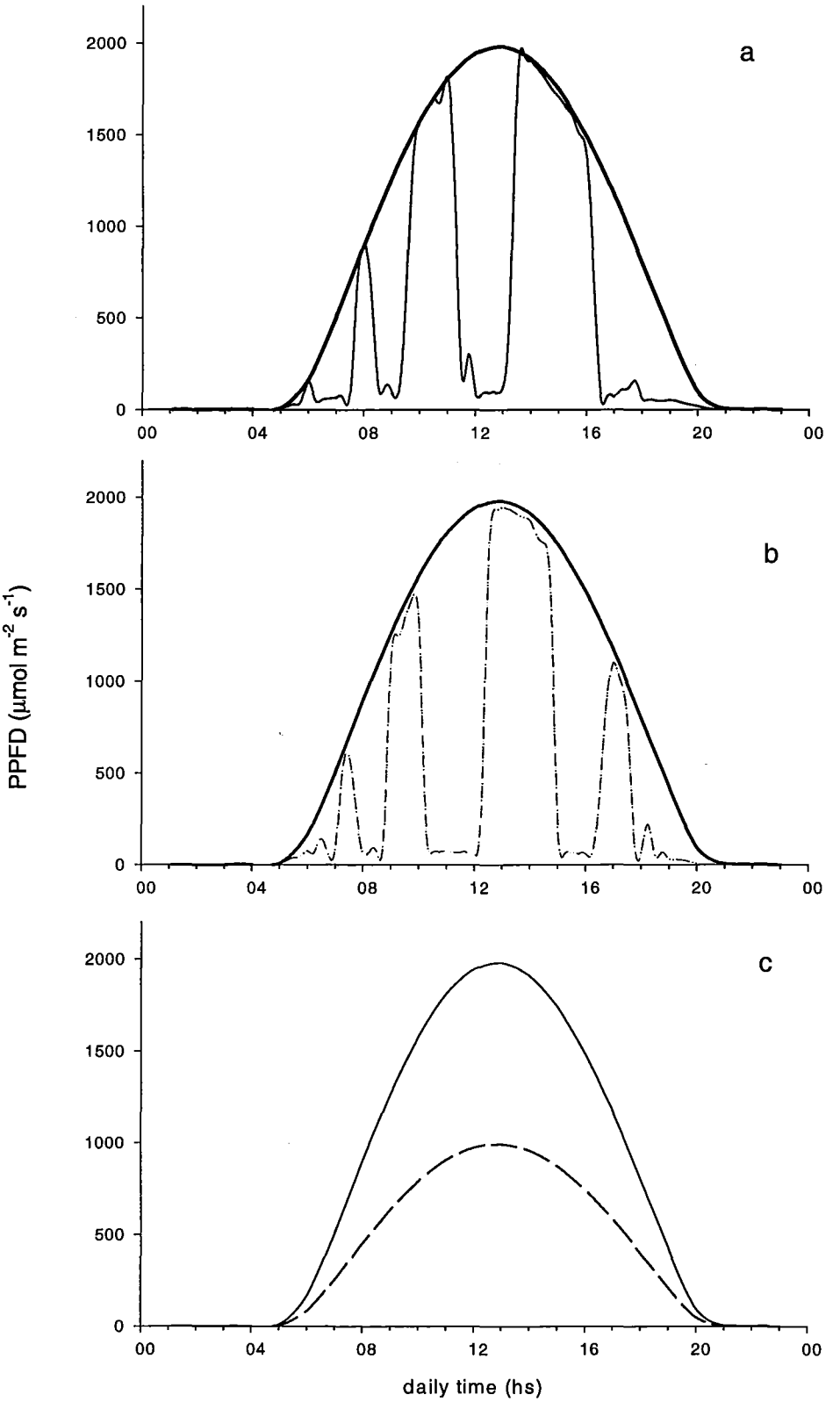

Figure 6.1. Photosynthetic photon flux density (PPFD) received on a typical sunny summer day (23 December 1999) in Canterbury, New Zealand for cocksfoot in the open (▬) and (a) under trees (—), (b) under a slat structure (··—··) and (c) under a cloth structure (--). Note: these light regimes were used to validate the effect of time in shade on net maximum photosynthesis rate (*Pmax*) presented in Equation 6.2.

6.2.1.2 Photosynthesis measurements

The photosynthesis rate and *gs* were measured on a sample of the youngest fully expanded intact leaves on six different tillers from each treatment. Tillers were randomly selected from readily identifiable dark green urine patches (Section 3.2.2.1.2) to ensure N was non-limiting (Section 4.3.2). Measurements were taken after tillers have been exposed to at least 90 minutes of full sunlight. Also, measurements were only taken when temperature (Section 4.3.1), water (Section 4.3.5) and regrowth duration (Section 5.3.1) were non-limiting. All measurements were taken at midday ± 1 hour on cloudless sunny days, 21 days after grazing.

The effect of time under severe (wide wooden) and moderate (cloth) shade on the rate of net photosynthesis and *gs* was measured. During induction, photosynthesis and *gs* were also measured for plants exposed to 30, 60 and 180 minutes under severe shade until full induction was reached. Induction measurement from plants exposed 60 minutes under severe shade was chosen because this represented the average situation in the Lincoln University silvopastoral system. Induction measurements after 30 and 180 minutes of severe shade were selected to evaluate the recovery in *Pmax* and *gs* which may represent silvopastoral systems with narrower and wider tree crowns. Because, the cloth structure provided a continuous shade of 50% of open PPFD over a day, induction measurements were not taken for this treatment.

Light photosynthesis curves, values of *gs* and their standardised index values ($Pmax_s$ or gs_s) were measured as defined in Section 4.2.2. Overall, 102 photosynthesis and *gs* measurements were taken in the field. Of these: 36 were used to fit the severe shade function, 16 for the moderate cloth shade function, and 50 for the recovery functions during induction.

The induction state of the leaf (IS) at any time (IS_t) was calculated (Equation 6.1) from independent observations collected 1, 2 and 10 minutes after the return to full sunlight for plants previously exposed to 30, 60 and 180 minutes of severe shade. Data were analysed as a one-way ANOVA;

$$IS_t = (A_t/A_{ss}) \times 100 = Pmax_s \times 100 \qquad \textbf{Equation 6.1}$$

Where A_t is the assimilation rate at time t, measured in minutes from the light increase, and A_{ss} is the steady-state, light saturated assimilation rate after induction is complete ($Pmax_s$= 1 in the present work). Thus, *($Pmax_s$ x 100)* can be used to calculate the induction state, which is equivalent to IS_t calculated in Equation 6.1. IS_1, IS_2, IS_{10} serve as indicators of the stomatal and RuBisCO limitations imposed by the induction requirement (Pearcy *et al.*, 1996) at t= 1, 2 and 10 minutes, respectively.

6.2.1.3 Validation of shade and induction functions

(i) *Severe shade*

A further 84 observations in the silvopastoral site from four environments with different shade conditions were used to validate the model for the time course of severe shade and induction (Table 6.1). These were from plants grown;

i) during 180 minutes under severe shade of a wide wooden structure (5% of open PPFD) (14 measurements). During this period, 32 additional measurements were taken to validate the three recovery functions proposed for the net photosynthesis induction.

ii) in the middle of shade from trees (7% of open PPFD) after 40-45 minutes of severe shade (14 measurements);

iii) under shade of a slat structure (Section 3.2.2.1) with gaps between slats (150 mm wide) which created an environment with a transition time between shade (5% of open PPFD) and sun of 110–120 minutes from 10:30 to 15:00 hours (Figure 6.1b). For the slatted shade structure, the objective was to create intervals of sunlight and severe shade (Figure 6.1b) similar to a nearby experimental radiata pine silvopastoral area. These, 14 measurements were taken at the middle of the slatted shade, which corresponded to plants exposed to 55-60 minutes of severe shade;

iv) under severe shade of radiata pine trees in an adjacent windbreak with shade of 7% of open PPFD (10 measurements). These measurements were taken to test if the model proposed for *Pmax* under severe shade reached a steady-state after 300 minutes.

(ii) *Moderate shade*

A further 14 independent data points from plants grown under cloth shade (50% of open PPFD) in the silvopastoral site were used to validate the corresponding time course model proposed for that environment.

6.2.2 Experiment to evaluate the effect of shade on *Pmax* interacting with temperature, water stress, N and regrowth duration.

The second part of this process was to evaluate the effect of shade on *Pmax* when temperature, water, N and regrowth duration were limiting using the modified multiplicative model proposed in Figure 5.11 (Section 5.3.9). Measurements were taken throughout the year to provide a range of temperature and water conditions. Measurements were from two areas within the silvopastoral experiment where trees provided fluctuating shade. The first was exclosure areas, which were left for 60 days (Section 3.2.2.2). After 60 days, cages were placed in new positions.

The second area was in the main plot excluding the exclosure plots. Samples were taken from urine and non-urine patches, either under the shade of a slat structure or not (Section 3.2.2.1). In this area, measurements were taken immediately prior to sheep grazing after 21 days of pasture regrowth.

6.2.2.1 Measurements

The photosynthesis rate and *gs* were measured on a random sample of six of the youngest fully expanded intact leaves from each treatment as was described in Section 6.2.1. Limiting temperature, water status and N values were defined from data outside the optimum range for *Pmax* (Chapter 4). All measurements were taken at midday ± 1 hour on cloudless sunny days. Limiting regrowth duration measurements were taken from leaves of vegetative tillers of 40 and 60 days regrowth from within the exclosure areas. Measurements under shade were taken at the middle of the slatted shade which corresponded to plants exposed to 55-60 minutes of severe shade (5% PPFD) and in the middle of shade from trees (7% of open PPFD) after 45-60 minutes of shade.

Air temperature, pre-dawn leaf water potential (ψ_{lp}) and samples for N content were taken on the same day as photosynthesis measurements as described in Section 4.2.3. Canopy temperature was measured on 50 occasions (on the same days as photosynthesis measurements) using an infrared thermometer (Section 4.2.3) to detect any differences between air and canopy temperature in irrigated plants exposed to full sunlight and under shade. A problem for predicting leaf photosynthesis using air temperature is that

understorey canopy temperature may be reduced by tree shade due to a reduction in radiation (Section 2.2.2).

Overall, 81 photosynthesis measurements were taken in the field for validation of the multiplicative model for *Pmax* (Figure 5.11, Section 5.3.9) when two, three, four or all five factors were limiting (temperature, N, ψ_{lp}, regrowth duration and shade). A further 44 observations were used to examine the interaction detected between time under severe shade (5% PPFD) and water stress (sorted by two water stress groups: ψ_{lp}= -4 to –8 bar and –8 to –13 bar).

6.2.3 Analyses

The data were analysed using linear and non-linear regression to determine the relationships between *Pmax* and *gs* with time under slat or cloth shade, and with recovery time during induction. Different asymptotic (Sigmoid, Logistic, Gompertz, Chapman, Hill and Weibull) and exponential decay functions were fitted for the time under shade variable. Linear functions were only used for the relationship between *Pmax* and time during induction. R^2 and ESE of $Pmax_s$ and gs_s were used to select the most appropriate functions (Section 4.2.4). Residuals and RMSD were calculated to estimate the accuracy of the proposed models (Section 4.2.4). The maximum standard error of coefficients (Max. SE), which correspond to the highest SE value, for the linear functions during induction are presented. For situations where only one time under shade (i.e. in the middle of the slatted shade or in the middle of the tree shade) (Section 6.2.1.3) was validated, the unique simulated value was compared with the mean of the observed values and its standard deviation (StD) was indicated.

6.3 Results

6.3.1 Experiment to evaluate the effect of time under shade on *Pmax* and *gs*

The maximum *Pmax* value was 26.5 μmol CO_2 m^{-2} s^{-1} saturated at 1000 μmol m^{-2} s^{-1} PPFD for plants grown in full sunlight. As the time under severe shade (5% of open PPFD) increased, both *Pmax* and the saturation point changed (Figure 6.2). After 2 minutes shade, *Pmax* was 21.8 μmol CO_2 m^{-2} s^{-1} saturated at 1000 μmol m^{-2} s^{-1} PPFD and after 180 minutes *Pmax* was 10.7 μmol CO_2 m^{-2} s^{-1} saturated at 250 μmol m^{-2} s^{-1} PPFD. From these results, a standardised value of $Pmax_s$= 1 was calculated and this corresponded to *Pmax*= 26.5 ±0.3 μmol CO_2 m^{-2} s^{-1}, or *Pmax* in non-limiting conditions.

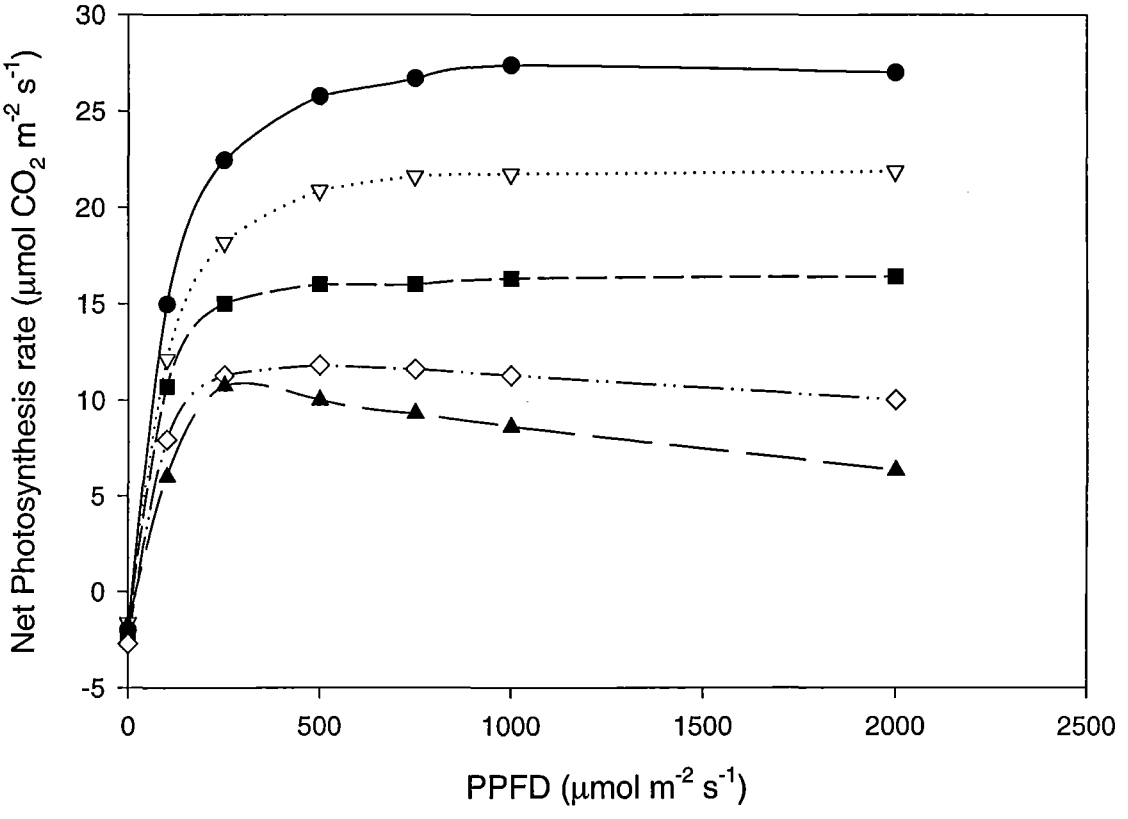

Figure 6.2. Net photosynthesis rate against light intensity (photosynthetic photon flux density, PPFD) for cocksfoot grown in a field environment at full sun (●), and as a function of different times under severe shade: 2 minutes (▽), 20 minutes (■), 60 minutes (◇) and 180 minutes (▲). The level of light intensity in the open situation was 1900 μmol m^{-2} s^{-1} PPFD and in severe shade was 85-95 μmol m^{-2} s^{-1} PPFD.

6.3.1.1 Response of *Pmax* to shade

(i) *Severe shade (5% of the open PPFD)*

$Pmax_s$ values were calculated from light curves of cocksfoot from 1 to 180 minutes under severe shade (Figure 6.3). From full sun to 1 minute under severe shade, $Pmax_s$ decreased from 1 to 0.83. From 1 minute under shade the decrease in $Pmax_s$ was non-linear against time. From 1 to 30 minutes under shade $Pmax_s$ decreased by 0.40 μmol CO_2 m^{-2} s^{-1} per minute or 0.015 units of $Pmax_s$ per minute of severe shade. From 30 to 60 minutes $Pmax_s$ decreased by 0.079 μmol CO_2 m^{-2} s^{-1} per minute of severe shade or 0.003 units of $Pmax_s$ per minute, and by 0.026 μmol CO_2 m^{-2} s^{-1} per minute of severe shade or 0.001 units of $Pmax_s$ per minute from 60 to 180 minutes. However, from 140 minutes under severe shade, *Pmax* reached a steady-state asymptote of 0.375 units of $Pmax_s$.

These results enabled an exponential decay function to be fitted (Equation 6.2) which gave an R^2 of 0.89 and ESE of $Pmax_s$ of 0.049.

$$Pmax_s = 0.30 * e^{\left(\frac{47.3}{ts+44.1}\right)}$$

Equation 6.2

Where t_s is the time under severe shade (5% of the open PPFD) in minutes.

(ii) *Moderate shade (50% of the open PPFD)*

Under cloth shade (50% of the open PPFD) a different exponential function (Equation 6.3) was required due to a slower decline compared with severe shade, and the higher steady-state value of 0.76 units of $Pmax_s$ after approximately 120 minutes (Figure 6.3).

This relationship had an R^2 of 0.86 and ESE of $Pmax_s$ of 0.037.

$$Pmax_s = 0.43 * e^{\left(\frac{274}{tm+315}\right)}$$

Equation 6.3

Where t_m is the time under moderate shade (50% of the open PPFD) in minutes.

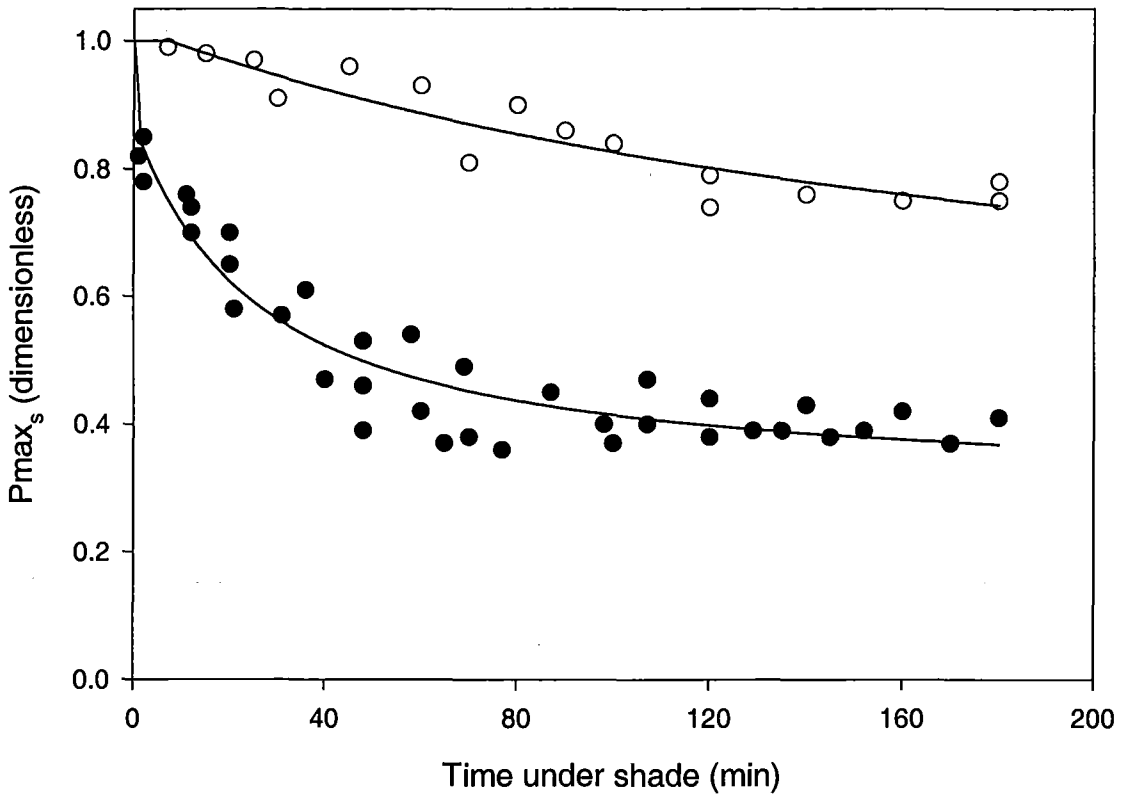

Figure 6.3. Time course of standardised rate of net photosynthesis (*Pmax$_s$*) for cocksfoot grown under field conditions in response to two light intensities: slat shade (•) at 85-95 μmol m^{-2} s^{-1} PPFD and cloth shade (○) at 850-950 μmol m^{-2} s^{-1} PPFD. *Pmax$_s$*= 1 ≡ *Pmax*= 26.5 μmol CO_2 m^{-2} s^{-1}. The fitted exponential decay functions are from Equations 6.2 and 6.3 as indicated in the text.

6.3.1.2 Effect of time under shade on stomatal conductance (*gs*)

(i) *Severe shade (5% of the open PPFD)*

From full sun to 1 minute under severe shade, *gs$_s$* decreased from 1 to 0.98 (Figure 6.4a). From this point, and similarly to *Pmax*, *gs* decreased as a non-linear function of time under severe shade. From 1 to 20 minutes, *gs* decreased by 0.0012 mol H_2O m^{-2} s^{-1} per minute of severe shade or 0.003 units of *gs$_s$* per minute, by 0.0025 mol H_2O m^{-2} s^{-1} per minute or 0.006 units of *gs$_s$* from 20 to 100 minutes, and by 0.0004 mol H_2O m^{-2} s^{-1} per minute or 0.001 units of *gs$_s$* from 100 to 180 minutes. After 100 minutes, *gs* reached an asymptotic value of 0.38 units of *gs$_s$*.

A sigmoidal function (Equation 6.4) was fitted to the measured data and this gave an R^2 of 0.97 and ESE of *gs$_s$* of 0.034.

$$gs_s = 0.38 + \frac{0.62}{1 + e^{-\left(\frac{t_S - 56.5}{-17.1}\right)}}$$

Equation 6.4

Where t_s is the time under severe shade (minutes).

(ii) *Moderate shade (50% of the open PPFD)*

Values of gs_s for cocksfoot under shade cloth also declined at a non-linear rate over time (Figure 6.4b). However, the rate of decline was less and the minimum value (0.76 units of gs_s) obtained after 175 minutes was higher than those obtained from the severe shade treatment.

This relationship was also described by a sigmoidal function (Equation 6.5), which resulted in an R^2 of 0.98 and ESE of gs_s of 0.010.

$$gs_s = 0.74 + \frac{0.26}{1 + e^{-\left(\frac{t_m - 127}{-22.2}\right)}}$$

Equation 6.5

Where t_m is the time under moderate shade in minutes.

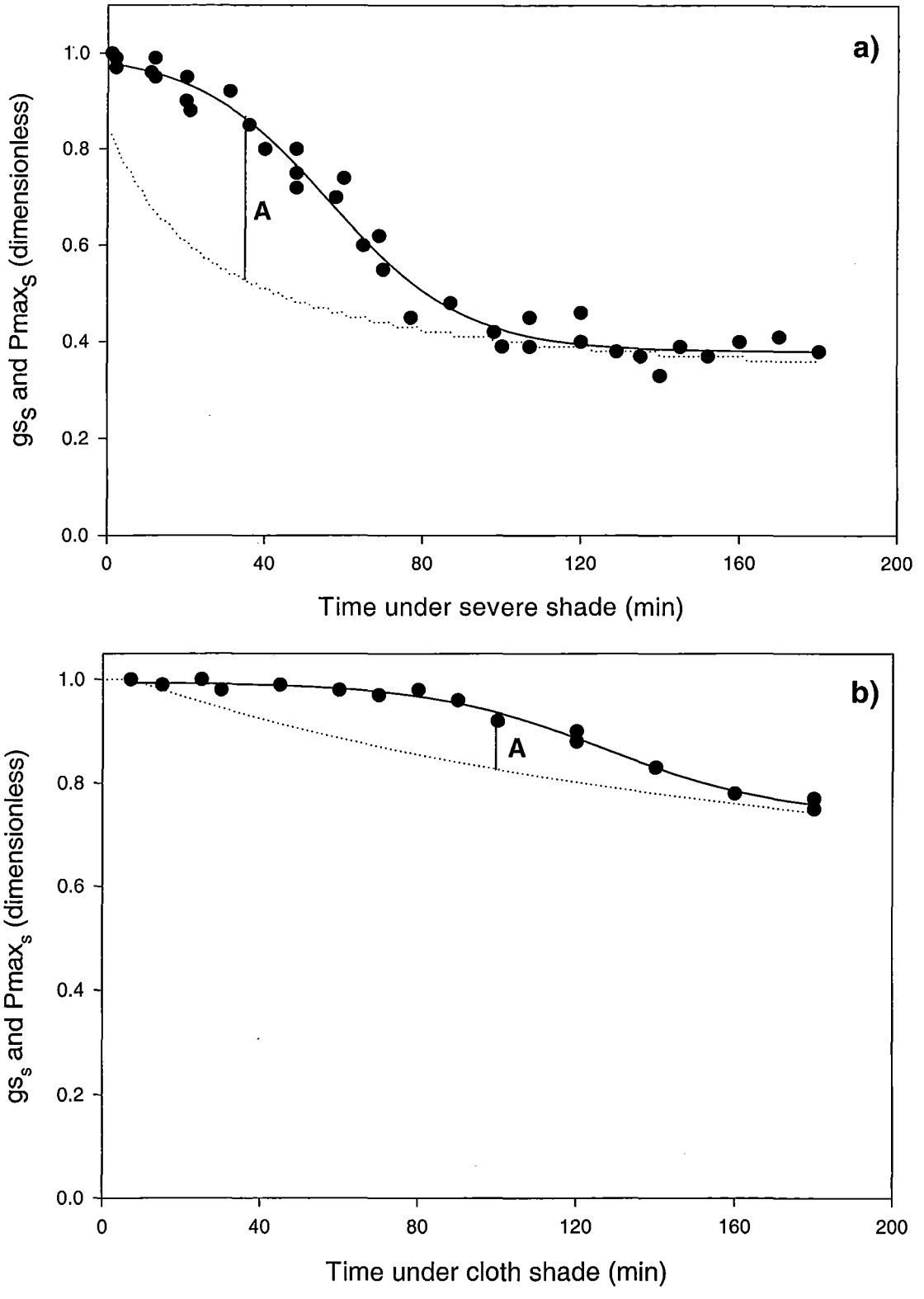

Figure 6.4. Time course of standardised rate of stomatal conductance (gs_s) for cocksfoot grown under field conditions in response to (a) severe shade (•) at 85-95 μmol m^{-2} s^{-1} PPFD and (b) to moderate shade (○) at 850-950 μmol m^{-2} s^{-1} PPFD. gs_s= 1 ≡ gs= 0.41 mol H_2O m^{-2} s^{-1}. The fitted sigmoidal functions for gs_s (—) and the corresponding $Pmax_s$ functions (····) from Figure 6.3 are indicated. The segments **A** indicate the time and magnitude of maximum difference between gs_s and $Pmax_s$.

6.3.1.3 Induction of *Pmax* after different times under severe shade

There were two distinct aspects to the induction process of net photosynthesis after severe shade (Figure 6.5a). Firstly, there was a biphasic induction process of $Pmax_s$ which was represented by two linear equations with an inflection point fitted using a 'broken stick' analysis (Draper and Smith, 1981) (Table 6.2). The mean slope of $Pmax_s$ induction for *phase I* was 6.2 μmol CO_2 m^{-2} s^{-1} per minute of exposure to full sunlight compared with only 0.3 μmol CO_2 m^{-2} s^{-1} per minute of full sunlight for *phase II*.

Secondly, the time required to reach full induction ($Pmax_s$= 1) was dependent on the previous time spent under severe shade which influenced the start point for *phase I* and subsequently the duration of *phase II*. The time required for full induction of *Pmax* was 15, 20 and 37 minutes after the increase of PPFD (full sun) for plants which had been 30, 60 and 180 minutes under severe shade, respectively.

Table 6.2. Linear functions for the two phases of the relationship between standardised rate of net photosynthesis ($Pmax_s$) and time (minutes) in full sunlight after different shade intervals (Ts) for cocksfoot grown under field conditions.

Time under shade (minutes)	Phase I	Phase II
30	$Pmax_s$= 0.55 + 0.290*Ts (R^2= 0.96)	$Pmax_s$= 0.83 + 0.011*Ts (R^2= 0.88)
60	$Pmax_s$ = 0.45 + 0.226*Ts (R^2= 0.97)	$Pmax_s$= 0.77 + 0.012*Ts (R^2= 0.83)
180	$Pmax_s$ = 0.37 + 0.183*Ts (R^2= 0.86)	$Pmax_s$= 0.65 + 0.010*Ts (R^2= 0.88)
Max. SE of coefficients	*(0.045) (0.052)*	*(0.026) (0.0011)*

Notes: (i) At time 0 the light increased from 85-95 μmol m^{-2} s^{-1} PPFD to 1700-1900 μmol m^{-2} s^{-1} PPFD. (ii) $Pmax_s$= 1 corresponds to *Pmax*= 26.5 μmol CO_2 m^{-2} s^{-1}.

The slope of *phase I* was dependent on the duration under shade prior to returning to full sun. Thus, plants exposed to 30 minutes of shade increased $Pmax_s$ at a rate 37% faster than those exposed to 180 minutes of shade. In contrast, the slope of *phase II* was similar for plants shaded from 30, 60 or 180 minutes (Table 6.2). The start point of $Pmax_s$ prior to induction also affected the time necessary to reach full induction ($Pmax_s$= 1). For plants shaded for 30 minutes the start point of *phase I* was higher ($Pmax_s$= 0.55) than for 60 ($Pmax_s$= 0.45) or 180 ($Pmax_s$= 0.37) minutes (Figure 6.5a). These values affected the

induction state (IS) or duration of recovery of $Pmax_s$ from shade to full sun depending on the duration of the previous shade exposure (Table 6.3). The IS_1 for plants after 30 minutes under severe shade was 20 and 34% higher than for plants which had been 60 and 180 minutes under severe shade, respectively. However, the relative difference for IS between plants exposed to 30 and 180 minutes under severe shade decreased at IS_{10}.

Table 6.3. Induction state (IS) (%) after 1 (IS_1), 2 (IS_2) and 10 (IS_{10}) minutes in full sunlight (1700-1900 μmol m^{-2} s^{-1} PPFD) for plants previously exposed to 30, 60 and 180 minutes of severe shade at 85-95 μmol m^{-2} s^{-1} PPFD.

Time under shade (minutes)	IS_1	IS_2	IS_{10}
30	84	86	94
60	67	79	89
180	55	67	75
sem	*1.5*	*1.6*	*1.3*
significance	***	***	***

*** indicates a significant difference at $p < 0.001$.

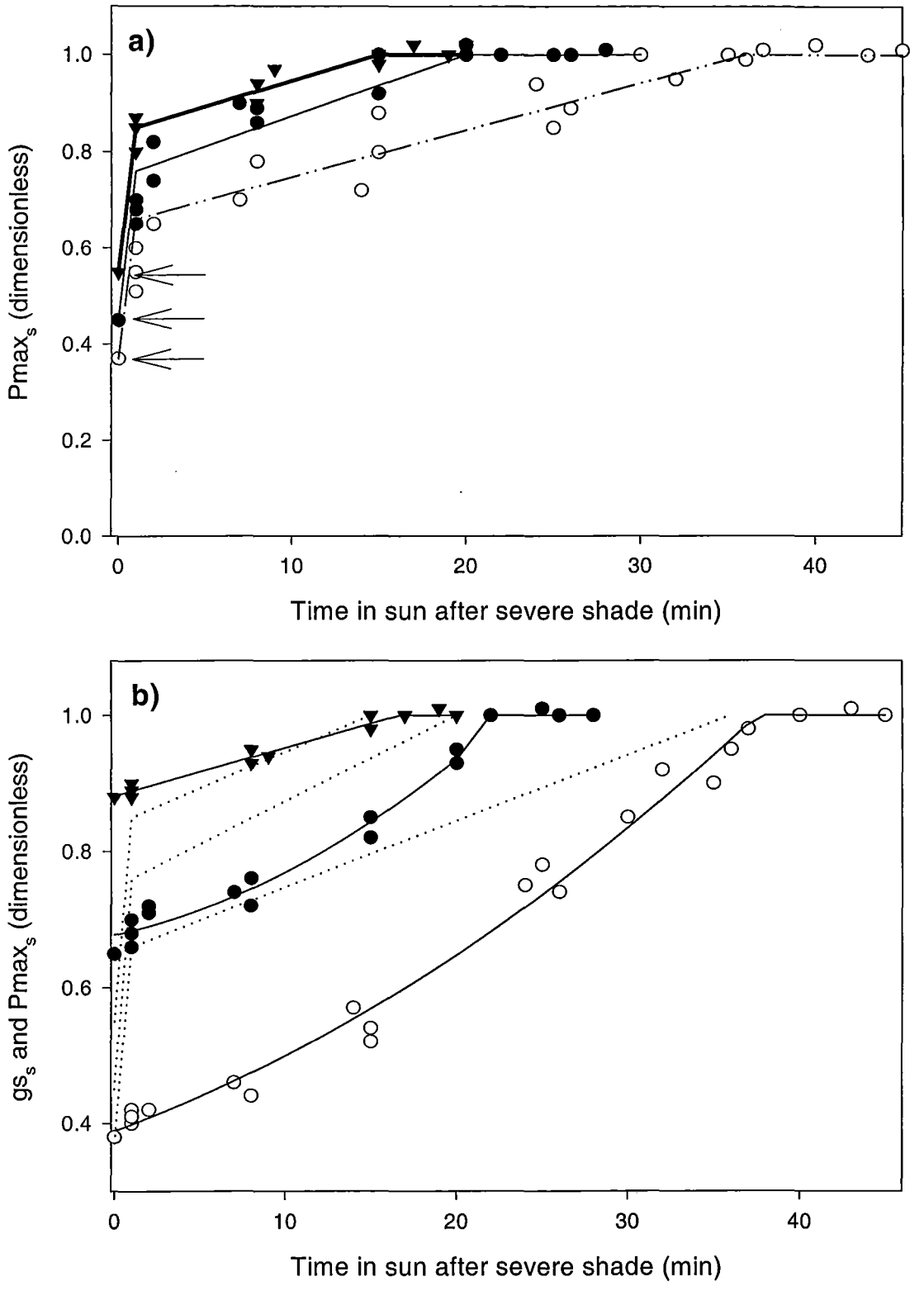

Figure 6.5. Time courses of the increase in (a) standardised rate of net photosynthesis (*Pmax*$_s$) and (b) standardised rate of stomatal conductance (*gs*$_s$) (—) and *Pmax*$_s$ (····) for cocksfoot grown under field conditions during induction. Reactivation of photosynthesis was determined after 30 minutes (▼), 60 minutes (●) and 180 minutes (○) of severe shade. At time 0 the light increased from 85-95 μmol m^{-2} s^{-1} PPFD to 1700-1900 μmol m^{-2} s^{-1} PPFD. *Pmax*$_s$= 1 ≡ *Pmax*= 26.5 μmol CO_2 m^{-2} s^{-1}. *gs*$_s$= 1 ≡ *gs*= 0.41 mol H_2O m^{-2} s^{-1}. Arrows indicate the start point for *Pmax*$_s$ at time 0.

6.3.1.4 Recovery of stomatal conductance (*gs*) after different times under severe shade

When PPFD was increased from 5% to full sun, the initial values of gs_s differed (Figure 6.5b) being 0.88, 0.68 and 0.39 units for plants exposed to 30, 60 and 180 minutes of severe shade. There was almost no change in gs_s values from the previous situation under shade and after the first minute of sun exposure. After 30 minutes of severe shade, gs_s increased linearly at a rate of 0.0028 mol H_2O m^{-2} s^{-1} per minute of sun exposure from 1 to 15 minutes when it reached unity. For the other two situations, the increment of gs_s was represented by a quadratic function (Table 6.4) and the time to reach a value of 1.0 unit was 25 and 40 minutes for plants exposed to 60 and 180 minutes of severe shade, respectively.

Table 6.4. Functions describing recovery to the maximum standardised stomatal conductance (gs_s= 1) rate over time for cocksfoot grown under field conditions during induction after different intervals of severe shade. At time 0 the light increased from 85-95 µmol m^{-2} s^{-1} PPFD to 1700-1900 µmol m^{-2} s^{-1} PPFD. gs_s= 1 corresponds gs= 0.41 mol H_2O m^{-2} s^{-1}.

Time under severe shade (minutes)	**Equation**	
30	$gs_s = 0.88 + 0.007*Ts$	R^2= 0.96; SE= 0.009
60	$gs_s = 0.68 + 0.005*Ts + 0.0004*Ts^2$	R^2= 0.95; SE= 0.021
180	$gs_s = 0.39 + 0.009*Ts + 0.0002*Ts^2$	R^2= 0.98; SE= 0.029
Max. SE of coefficients	*(0.013) (0.0021) (0.0001)*	

Ts= is the time (minutes) in sun after being under severe shade.

6.3.1.5 Calculation of stomatal and non-stomatal limitations for *Pmax* under severe shade

The decrease in *gs* occurred at a slower rate than the reduction in *Pmax* for plants exposed to increasing periods of shade (Figure 6.4). The maximum difference between gs_s and $Pmax_s$ was 0.34 units after 35 minutes under severe shade (Segment A, Figure 6.4a) and 0.12 units after 90 minutes of moderate shade (Segment A, Figure 6.4b). Similarly, during induction, the difference between gs_s and $Pmax_s$ was 0.12 units one minute after plants were returned to full sun for plants previously exposed to 60 minutes of severe shade, and 0.26 units 2 minutes after the return to full sun for plants previously exposed to 180

minutes of severe shade (Figure 6.5b). The difference between $Pmax_s$ and gs_s over time indicates that both reduction and recovery in $Pmax_s$ were due to stomatal (s_s) and non-stomatal (ns_s) limitations. Therefore, the total standardised limitation (T_s) on $Pmax_s$ can be expressed as:

$$T_s = s_s + ns_s$$
$$\Rightarrow T_s = 1 - Pmax_s \qquad \textbf{Equation 6.6}$$

Assuming a rapid recovery of the non-stomatal limitation during induction (Ins_s), corresponding to the rapid *phase I* (Figure 6.5a), then the stomatal limitation (Is_s) influenced the recovery of $Pmax_s$ during the slower *phase II*. Thus, in this method we assumed that after 5 minutes of recovery, all the limitation in $Pmax_s$ was due to the rate of stomatal opening. This assumption is supported by previous studies which indicate biochemical factors are the most important limitation on photosynthesis at the beginning of the induction process (Kirschbaum and Pearcy, 1988; Pearcy and Seemann, 1990; Sassenrath-Cole and Pearcy, 1994). Calculation of the stomatal limitation on photosynthetic rate during induction was carried out by a regression analysis between gs_s and the stomatal limitation. This was derived from $Pmax_s - gs_s$ for plants exposed for 30, 60 and 180 minutes of severe shade during *phase II* of induction (Figure 6.5b). The relationship was described by a quadratic function (Figure 6.6), which resulted in an R^2 of 0.90 and ESE of Is_s of 0.024 (Equation 6.7).

$$Is_s = Pmax_s - gs_s$$
$$\Rightarrow Is_s = 0.39 - 0.22\, gs_s - 0.16\, gs_s^2 \qquad \textbf{Equation 6.7}$$

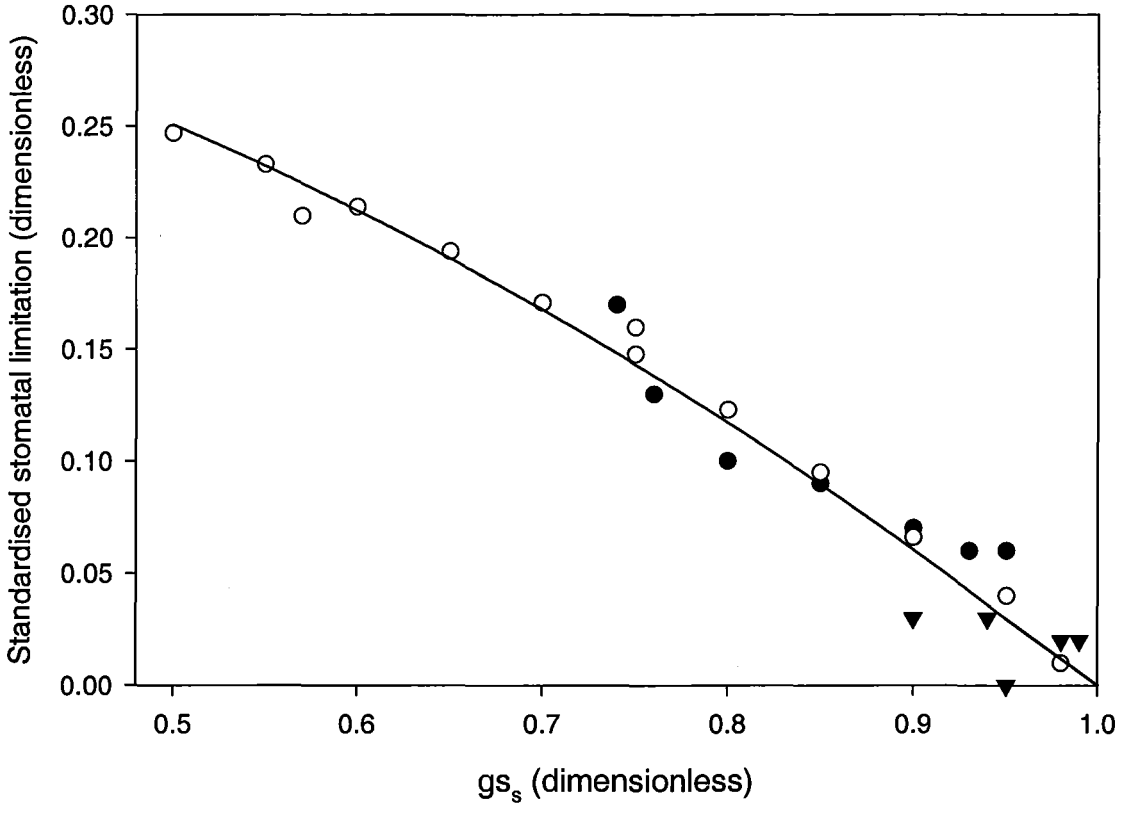

Figure 6.6. Relationship between standardised stomatal limitation and standardised stomatal conductance (gs_s) during induction for cocksfoot grown under severe shade (85-95 μmol m^{-2} s^{-1} PPFD) in field conditions. gs_s measurements during induction corresponded to the *phase II* (after 5 minutes of induction) in Figure 6.5a for plants exposed to 30 (▼), 60 (●) and 180 (○) minutes of shade before exposure to full sun (1700-1900 μmol m^{-2} s^{-1} PPFD).

The values of the standardised non-stomatal limitation were then calculated by the difference between total limitation and stomatal limitation during induction.

$$Ins_s = It_s - Is_s$$

$$\Rightarrow Ins_s = (1 - Pmax_s) - Is_s \qquad \textbf{Equation 6.8}$$

The relative importance of stomatal and non-stomatal limitations on photosynthetic rate of leaves during the time under shade was calculated using Equation 6.7 for the limitation due to stomatal closure presented in Figures 6.4a and 6.4b, and using Equation 6.6 for the total limitation. The limitations for non-stomatal effects were derived from Equation 6.8. Figure 6.7 shows the interpolated response of stomatal (s_s) and non-stomatal (ns_s) limitations for

severe and moderate shade. The rate of total, stomatal and non-stomatal limitations on $Pmax_s$ over time had similar shaped response functions for severe and moderate shade.

The increase in total limitation (T_s) was non-linear against time under shade. From 1 to 60 minutes under severe shade, T_s increased by 0.0062 units per minute and from 60 to 180 minutes t_s increased by 0.0008 units per minute of severe shade (Figure 6.7a). In contrast, for cloth shade, T_s increased at a rate of 0.0014 units from 1 to 180 minutes under moderate shade (Figure 6.7b). The stomatal limitation on photosynthetic rate showed a sigmoidal response whereby the magnitude and timing of limitation was dependent on the shade intensity. For plants under severe shade the maximum stomatal limitation of 0.28 units was at 100 minutes (Figure 6.7a), and for plants under moderate shade the highest limitation of 0.14 units was found after 180 minutes of shade (Figure 6.7b).

Similarly, the magnitude and timing of non-stomatal limitation on photosynthetic rate was dependent on shade intensity. Under severe shade it increased by 0.0067 units from 1 to 40 minutes and then reached a maximum limitation value of 0.36-0.38 units (Figure 6.7a). In contrast, under moderate shade, non-stomatal limitation reached a maximum value of 0.13-0.14 units after 100 minutes under shade.

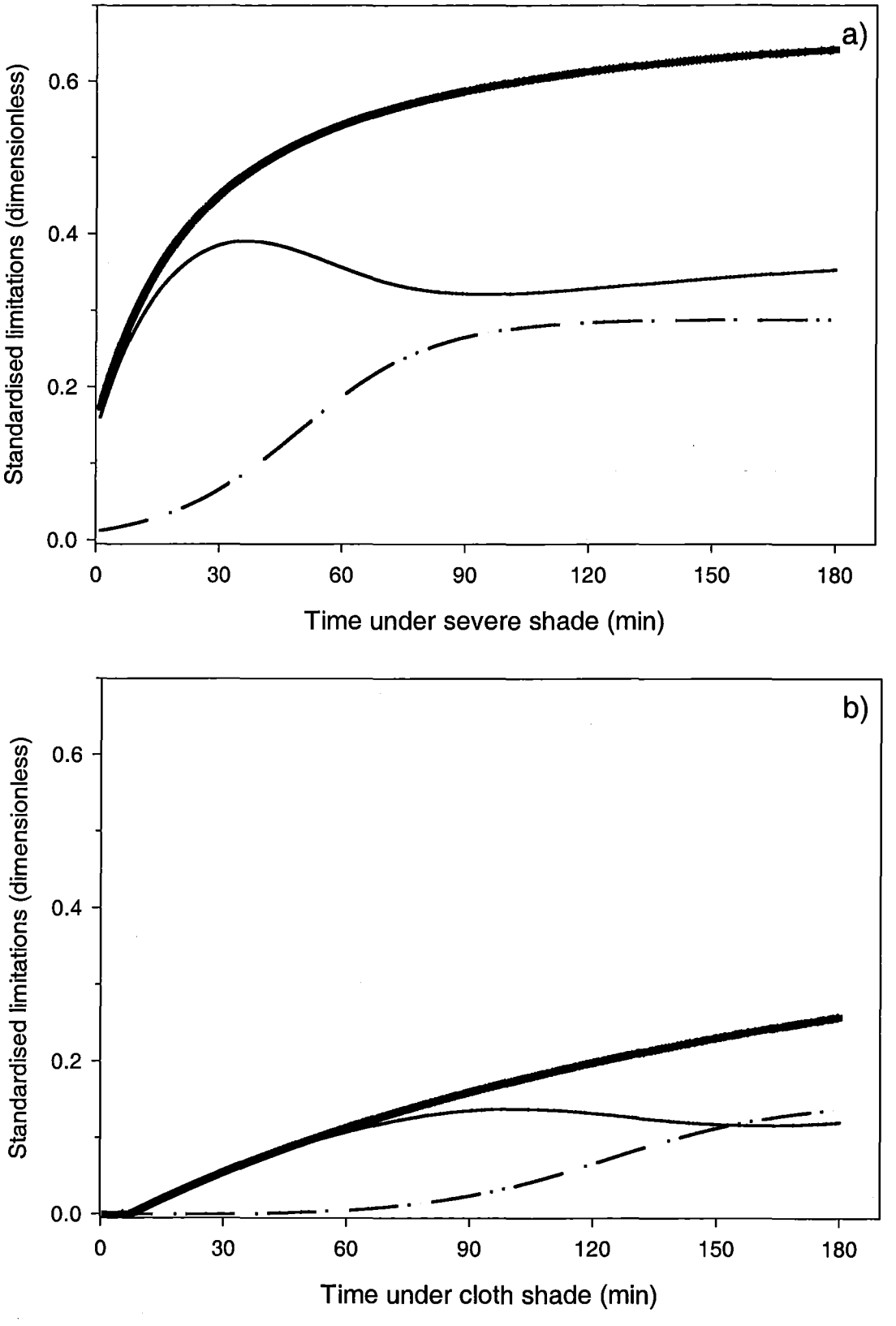

Figure 6.7. Time courses of total (—), stomatal (··—··) and non-stomatal (—) standardised limitations on photosynthetic rate for cocksfoot grown under field conditions in response to two light intensities: (a) severe shade at 85-95 μmol m^{-2} s^{-1} PPFD and (b) moderate shade at 850-950 μmol m^{-2} s^{-1} PPFD.

6.3.1.6 Validation of *Pmax* equations under different shade conditions

The predicted results from Equation 6.2 were compared with independent data from plants grown under shade in a range of conditions (Table 6.1). The model adequately simulated $Pmax_s$ when time under shade was limiting (Figure 6.8). The average values of RMSD were about 12%, 19% and 18% of the mean observed $Pmax_s$ values for 180 minutes under the wide wooden shade, 55-60 minutes after slatted shade and 300 minutes under the windbreak shade, respectively. However, the model underestimated $Pmax_s$ at 0.59 units of observed $Pmax_s$, which corresponded to the middle tree shade (average value of RMSD= 23%).

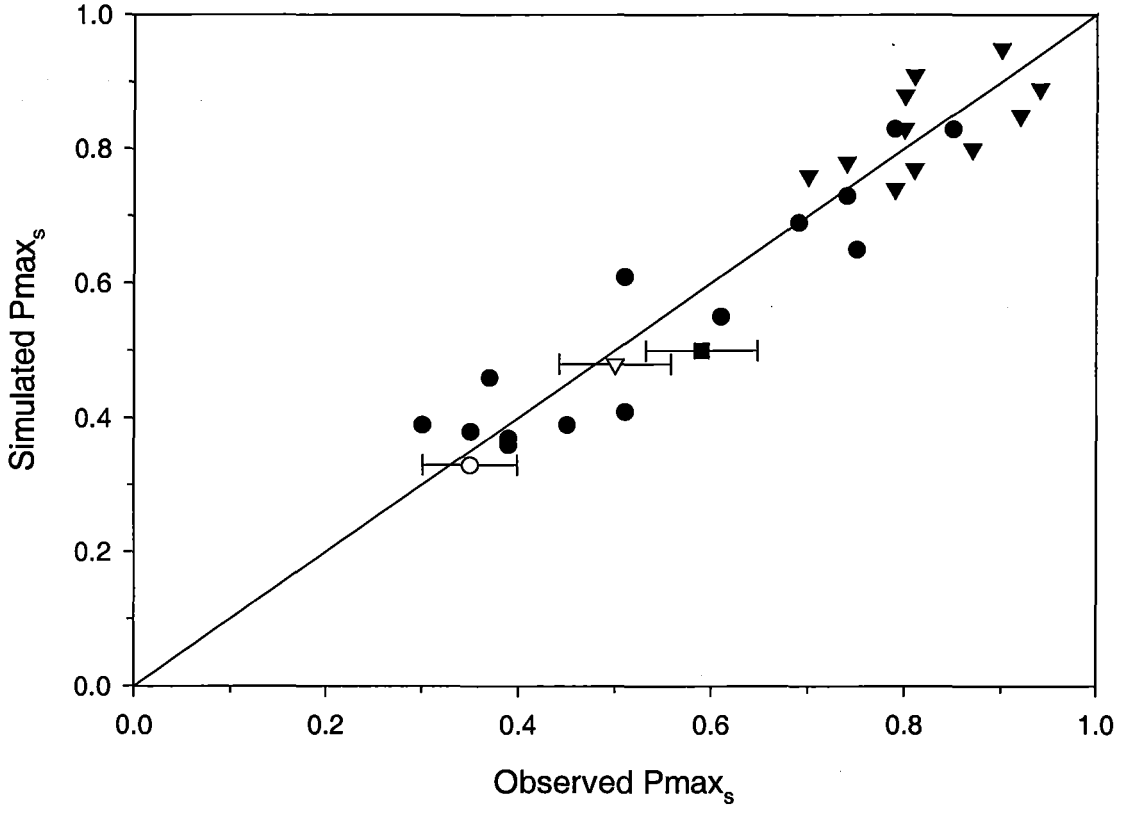

Figure 6.8. Simulated against observed $Pmax_s$ for time course of low-light deactivation, sorted by five groups: middle tree shade at 40-45 minutes (■) (root mean square deviation, RMSD= 0.130), slat shade at 55-60 minutes (▽) (RMSD= 0.109), time course up to 180 minutes under severe shade (●) (RMSD= 0.065), time course up to 180 minutes under cloth shade (▼)(RMSD= 0.060) and windbreak shade at 300 minutes (○) (RMSD= 0.097). Simulated data were based on Equations 6.2 and 6.3. Bars indicate standard deviation of mean.

In addition, simulated results from Equation 6.3 were compared with the data points collected for validation. The prediction of $Pmax_s$ was accurate over 180 minutes under cloth shade. The average value of RMSD was 7% of the mean observed $Pmax_s$ values.

Figure 6.9 shows the distribution of predicted $Pmax_s$ for the six linear recovery functions versus observed $Pmax_s$ values. This showed that the model for these six situations was predicted accurately. The average RMSD values were about 9%, 10% and 12% of the mean observed $Pmax_s$ values for plants which had been shaded for 30, 60 and 180 minutes, respectively.

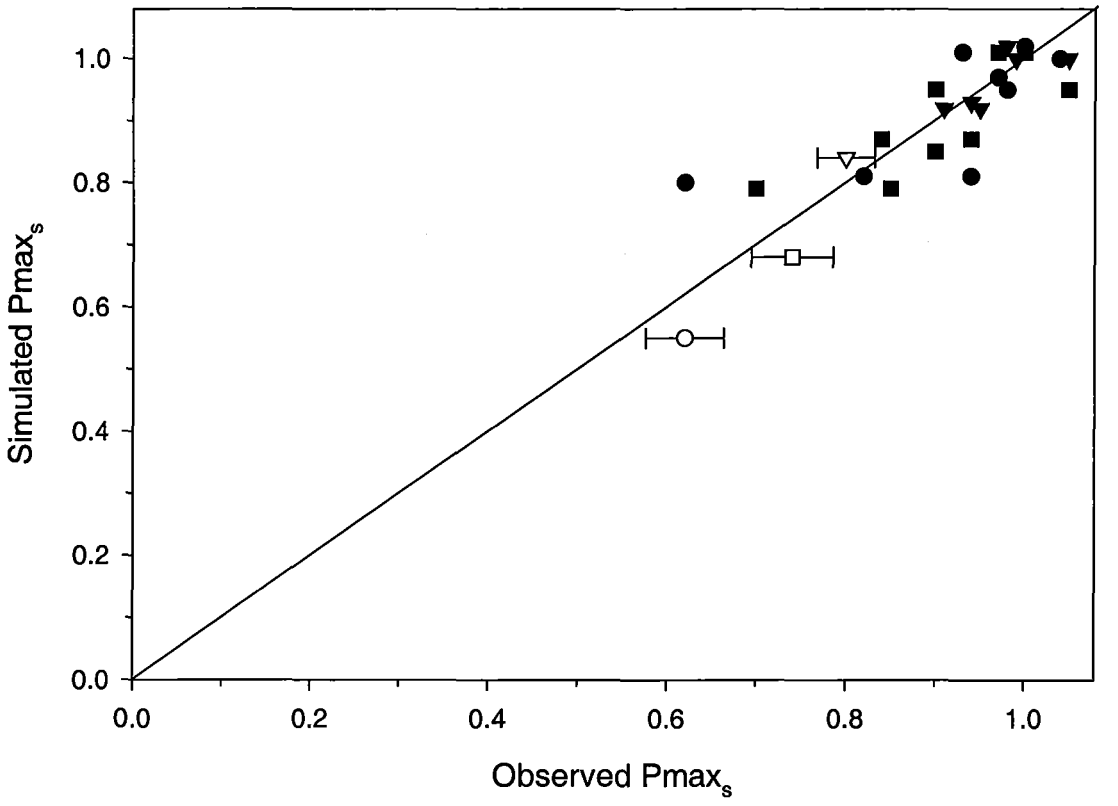

Figure 6.9. Simulated against observed $Pmax_s$ for time course of induction, sorted by six groups: phases *I* (∇) and *II* (▼) for plants exposed to 30 minutes of severe shade (85-95 μmol m^{-2} s^{-1} PPFD) (root mean square deviation, RMSD= 0.050), phases *I* (□) and *II* (■) for plants exposed to 60 minutes of severe shade (RMSD= 0.077), and phases *I* (○) and *II* (●) for plants exposed to 180 minutes of severe shade (RMSD= 0.093). The light increased to 1700-1900 μmol m^{-2} s^{-1} PPFD. Phases *I* and *II* correspond to the fast and slow phases as described in Figure 6.5a. Simulated data were based on Equations 6.4, 6.5 and 6.6. Bars indicate standard deviation of mean.

6.3.2 Effect of shade on *Pmax* interacting with temperature, water stress, N and regrowth duration.

Having established functions to describe the response to shade and subsequent induction process, it is necessary to incorporate these into the multiplicative model (Figure 5.11; Section 5.3.9) and assess any interactions between factors.

6.3.2.1 Canopy and air temperature

To determine which temperature to use for prediction of *Pmax* in shade conditions, air and canopy temperatures were measured. Air temperature was higher than canopy temperature both in sun and under tree shade (after 45-60 minutes under 7% PPFD) situations at midday (when photosynthesis measurements were taken) and this difference increased with increasing air temperatures. However, the magnitude of the difference between air and canopy temperature (T_{a-c}) varied between sun and shade situations according to exponential functions (Figure 6.10). From 10 to 20 °C air temperature, the mean T_{a-c} was 0.3 °C in sun plants compared with 2.6 °C under the shade. At an air temperature of 31 °C, the mean maximum value of T_{a-c} was 2.0 °C for plants in sun and 7.4 °C for plants under shade. For this reason, canopy temperature was used to validate the multiplicative model of *Pmax* for plants under shade when air temperature was limiting (temperatures > 24 °C). The need for this modification has already been suggested theoretically in Section 4.4 for the low N (≤ 2%) and high temperature (>28 °C) situation though dismissed on that occasion because its practical significance was minimal for pasture grown in the open.

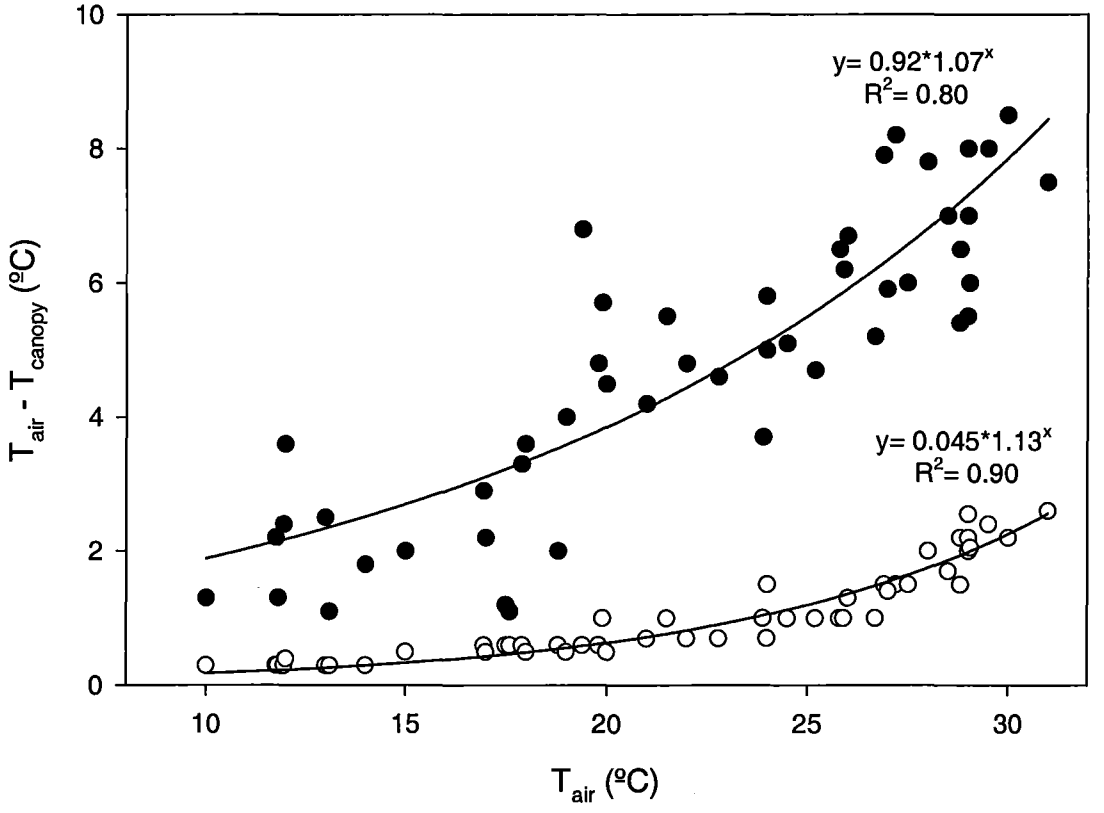

Figure 6.10 Difference between air (T_{air}) and canopy (T_{canopy}) temperature against T_{air} for field irrigated cocksfoot plants in sun (○) and under tree shade (●) situations (7% of open PPFD) within the agroforestry site. Data corresponds to midday measurements in sunny days.

6.3.2.2 Multiplicative model

The function obtained for time under severe shade (Equation 6.2) was incorporated as a fifth factor into the modified multiplicative model (Section 5.3.9). For each function $Pmax_s$= $Ppmax$= 1.0 ≡ 27.4 μmol CO_2 m^{-2} s^{-1} when the factor was non-limiting. At $Pmax_s$= 0 no photosynthesis was occurring ($Pmax$= 0).

Simulated results for the multiplicative photosynthesis model were compared with 81 data points collected during the trial period where shade and one, two or all other factors were outside their defined optimum range (Figure 6.11). The average value of the RMSD (0.14) was about 32% of the mean observed $Pmax_s$ values. The model adequately simulated $Pmax$ when shade, temperature, N, regrowth duration or all factors were limiting. However, the prediction of $Pmax_s$ was less accurate when shade (after 40-60 minutes of severe shade) and water were limiting. Thus, $Pmax_s$ was underestimated by the model for a group of points in the observed range of 0.40 – 0.55 $Pmax$ (Figure 6.11) which corresponded to

values of ψ_{lp} between –4.0 and –13.0 bar. However, the model adequately predicted *Pmax* from ψ_{lp} –14.0 to –16.0 bar under shade. In this situation *Pmax* reached zero or a constant negative value (from -0.1 to -0.5 μmol CO_2 m^{-2} s^{-1}) which was consistent with those values found without shade (Equation 4.5, Section 4.3.4). This indicated that total respiration was higher than gross photosynthesis under severe water stress with or without shade.

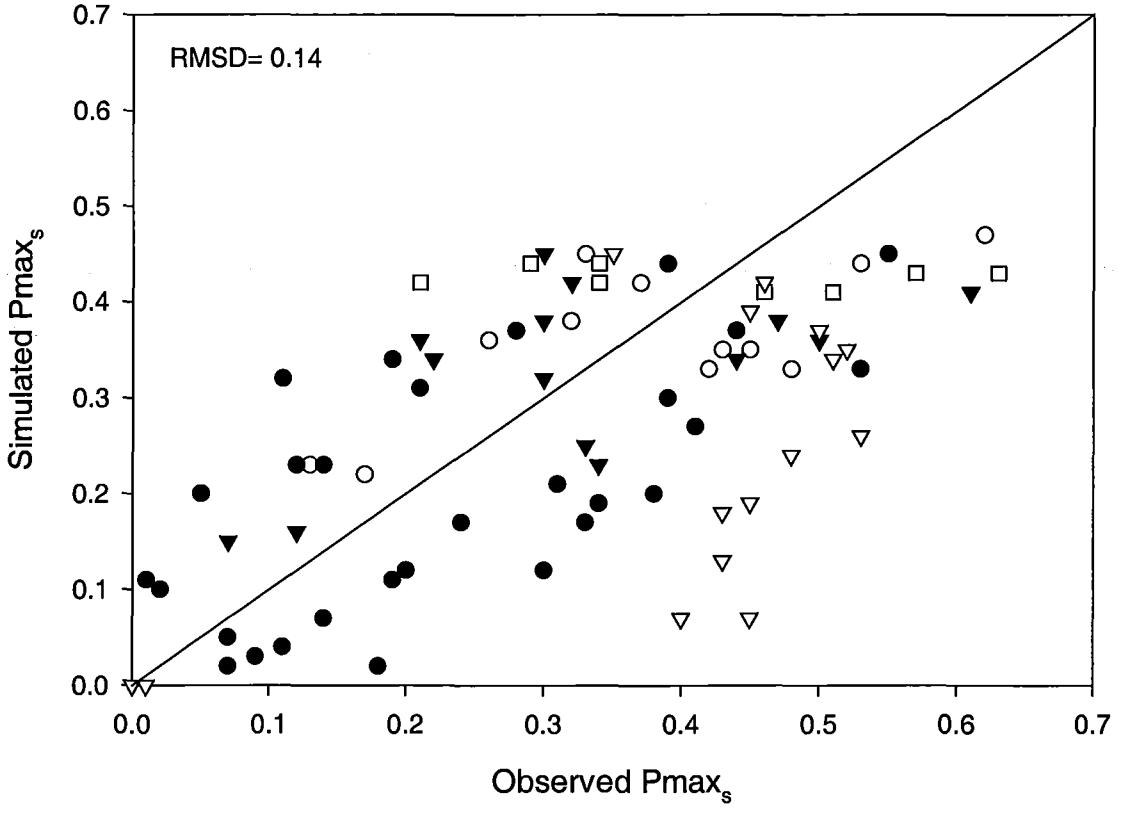

Figure 6.11 Simulated against observed *Pmax*$_s$ values sorted by five groups (water limited (▽), nitrogen limited (▼), temperature limited (○), regrowth duration limited (□) and all factors limited (●)) for cocksfoot leaves grown under severe shade (85-95 μmol m^{-2} s^{-1} PPFD) in a field silvopastoral experiment. Simulated data were based on the multiplicative model proposed in Figure 5.11 (Section 5.3.9) incorporating the shade function as an additional factor (Equation 6.2).

Regression analyses of these residuals for each factor combination were used to identify interactions between factors (as described in Section 4.3.7). There was no significant interaction ($\beta = 0$) between the shade limiting factor and temperature, N or when all factors were limiting (Figure 6.12). Most of the residuals (74%) were < ±0.15 units from the predicted *Pmax* and evenly distributed. This indicated acceptable accuracy for these situations. In contrast, for the water limited, there was an interaction ($\beta \neq 0$) with shade

(Figure 6.12). This indicated that the reduction in *Pmax* was not accurately represented by a multiplicative reduction between shade and water stress factors.

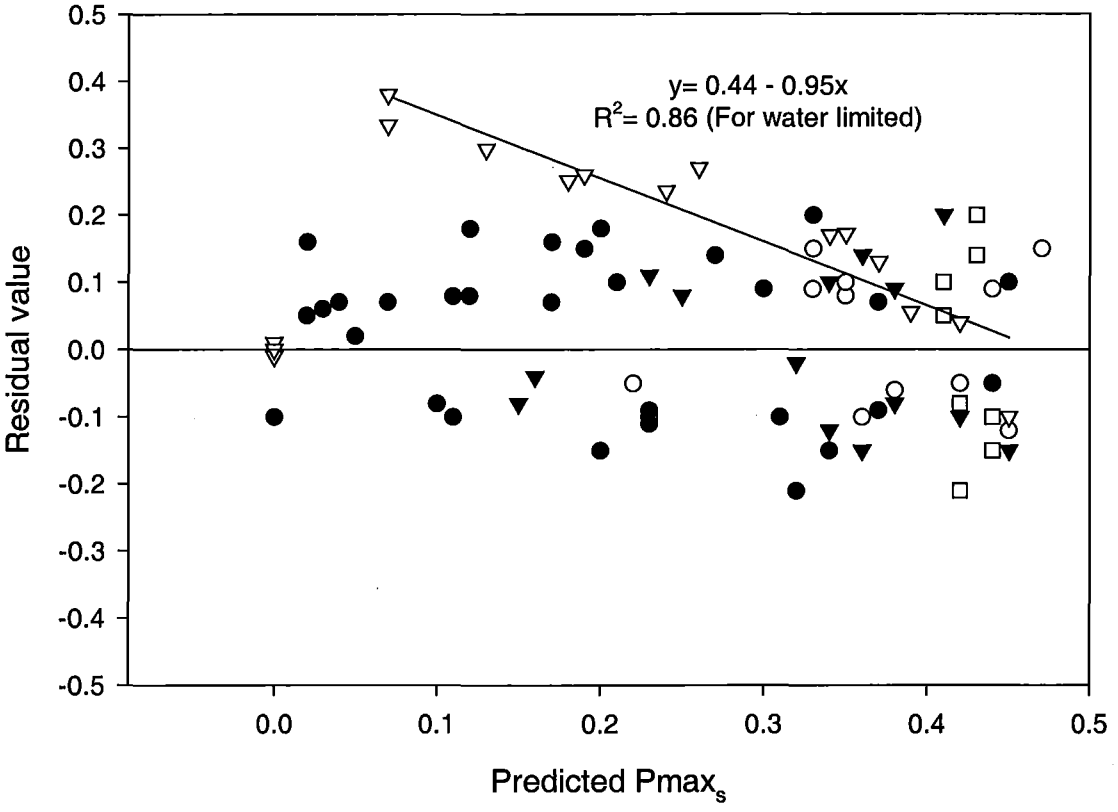

Figure 6.12 Residual [(observed – simulated values)] of $Pmax_s$ against the predicted values sorted by five groups (water limited (▽), nitrogen limited (▼), temperature limited (○), regrowth duration limited (□) and all factors limiting (●)) for cocksfoot leaves grown in a field experiment.

6.3.2.3 Severe shade and leaf water potential (ψ_{lp}) interaction effect on *Pmax*

The interaction between severe shade and ψ_{lp} was explored using 42 data points collected during the trial period when temperature, N and regrowth duration were non-limiting. $Pmax_s$ values were calculated from light curves of cocksfoot grown from 1 to 180 minutes under severe shade and water stress from ψ_{lp}= -4.0 to –13.0 bar. $Pmax_s$ under severe shade showed two different responses according to the level of water stress (Figure 6.13a). From full sun ($Pmax_s$= 1) to 1 minute under shade, the decrease in $Pmax_s$ ($Pmax_s$= 0.52 for ψ_{lp}= -4.0 to –8.0 bar and $Pmax_s$=0.40 ψ_{lp}= -8.0 to –13.0 bar) was considered linear. These values of $Pmax_s$ were lower than those found for the original shade function without water stress ($Pmax_s$= 0.83) (Section 6.3.1.1).

From 1 minute under shade the decrease in rate of $Pmax_s$ was non-linear against time and depended on the water stress level. From 1 to 30 minutes under shade $Pmax_s$ decreased similarly for both groups of water stressed plants by 0.17 μmol CO_2 m^{-2} s^{-1} per minute of severe shade or 0.0065 units of $Pmax_s$ per minute of severe shade. From 30 to 60 minutes $Pmax_s$ decreased by 0.012 μmol CO_2 m^{-2} s^{-1} or 0.00045 units of $Pmax_s$ per minute of severe shade. However, $Pmax_s$ reached a steady-state asymptote of 0.28 units of $Pmax_s$ at 46 minutes for ψ_{lp}= -4.0 to –8.0 bar plants and 0.19 units of $Pmax_s$ at 35 minutes for ψ_{lp}= -8.0 to –13.0 bar plants. These asymptote values were lower and were reached earlier compared with the original shade function (Section 6.3.1.1).

Exponential decay functions were fitted for ψ_{lp}= -4.0 to –8.0 bar plants (Equation 6.9) (R^2= 0.95; ESE= 0.022) and ψ_{lp}= -8.0 to –13.0 bar plants (Equation 6.10) (R^2= 0.94; ESE= 0.021).

$$Pmax_s = 0.28 + 0.25 * e^{(-0.086 * t_s)}$$

Equation 6.9

$$Pmax_s = 0.18 + 0.21 * e^{(-0.090 * t_s)}$$

Equation 6.10

Where t_s is the time under severe shade in minutes.

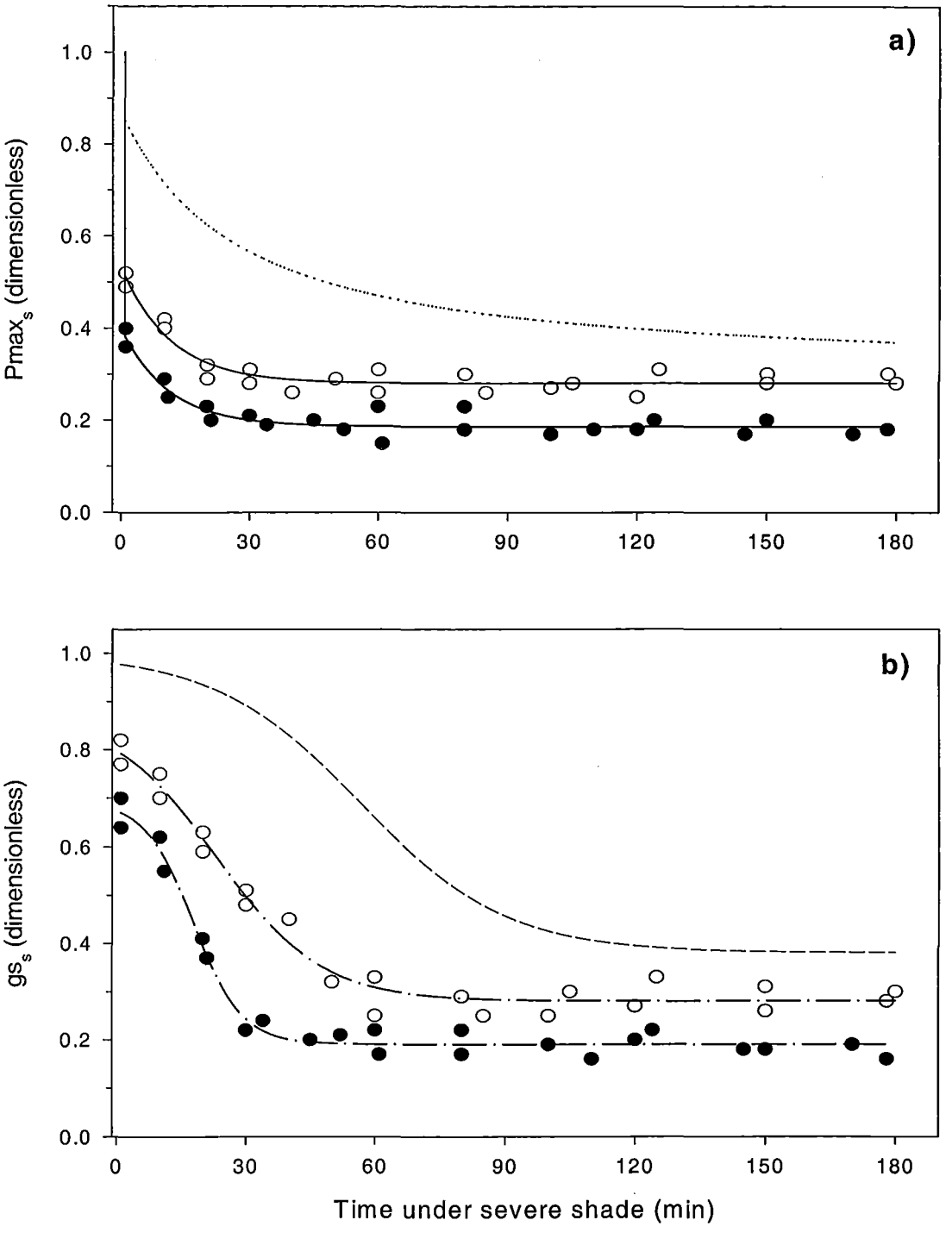

Figure 6.13 Time course of (a) standardised rate of net photosynthesis ($Pmax_s$) and (b) standardised rate of stomatal conductance (gs_s) for cocksfoot grown under severe shade (85-95 μmol m^{-2} s^{-1} PPFD) field conditions in response to water stress status: ψ_{lp}= -4 to –8 bar (○) and ψ_{lp}= -8 to –13 bar (●). $Pmax_s$= 1 corresponds to $Pmax$= 26.5 μmol CO_2 m^{-2} s^{-1}. gs_s= 1 corresponds gs= 0.41 mol H_2O m^{-2} s^{-1}. The fitted exponential decay $Pmax_s$ functions for both groups of water stress limiting (—) from Equations 6.9 and 6.10, and the fitted decay function for water non-limiting (····) (Equation 6.2) are indicated. The fitted sigmoidal functions for gs_s for both groups of water stress limiting (—·) from Equations 6.11 and 6.12 and the sigmoidal function for water non-limiting (----) (Equation 6.4) are indicated.

6.3.2.4 Severe shade and leaf water potential (ψ_{lp}) interaction effect on *gs*

From full sun (gs_s= 1) to 1 minute under severe shade, the decrease in gs_s (gs_s= 0.82 for ψ_{lp}= -4.0 to –8.0 bar and gs_s=0.70 ψ_{lp}= -8.0 to –13.0 bar) was considered to be linear. Again, these values were lower than the original shade function without water stress (gs_s= 0.98) (Section 6.3.1.3). From this point, and similarly to *Pmax*, *gs* per unit of leaf decreased as a non-linear function of time under severe shade (Figure 6.13b). After 83 minutes under shade, *gs* reached an asymptotic value of 0.28 units of gs_s for plants with ψ_{lp}= -4.0 to –8.0 bar. For plants with ψ_{lp}= -8.0 to –13.0 bar, *gs* reached an asymptotic value of 0.19 units after 45 minutes of severe shade. These asymptote values were lower and reached earlier than for the original shade function (Section 6.3.1.3).

Sigmoidal functions were fitted for ψ_{lp}= -4.0 to –8.0 bar plants (Equation 6.11) (R^2= 0.97; ESE= 0.031) and ψ_{lp}= -8.0 to –13.0 bar plants. (Equation 6.12) (R^2= 0.98; ESE= 0.024).

$$gs_s = 0.28 + \frac{0.59}{1 + e^{-\left(\frac{t_s - 23.2}{-12.2}\right)}}$$

Equation 6.11

$$gs_s = 0.19 + \frac{0.50}{1 + e^{-\left(\frac{t_s - 17.9}{-5.69}\right)}}$$

Equation 6.12

Where t_s is the time under severe shade in minutes.

6.3.2.5 Modelling *Pmax* in cocksfoot- including shade and ψ_{lp} interaction.

Detection of the interaction between severe shade and ψ_{lp} meant that the multiplicative model was modified to enable this situation to be included (Figure 6.14).

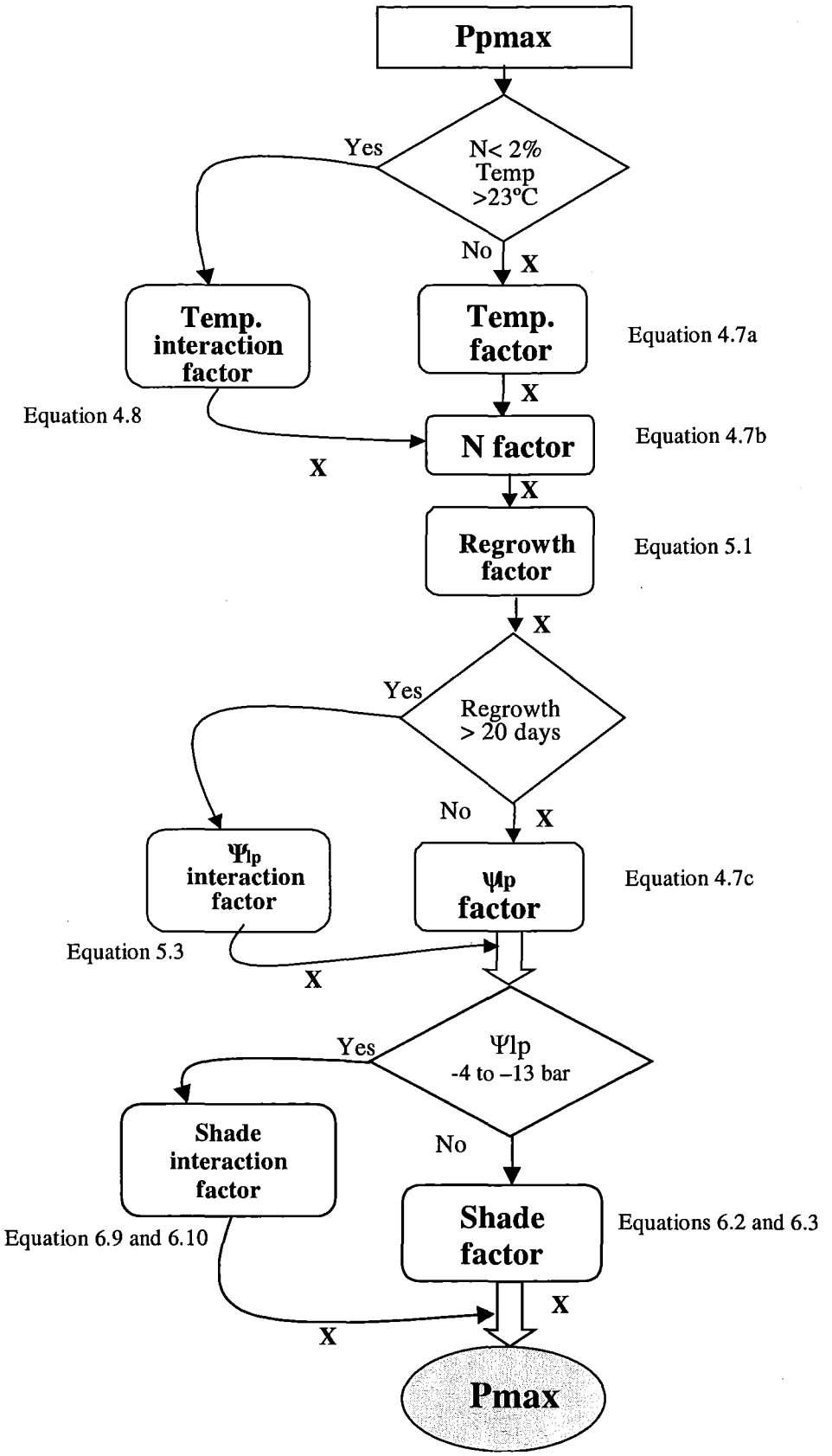

Figure 6.14 Diagram of the final multiplicative model for prediction of *Pmax* for cocksfoot leaves under a wide range of temperature, nitrogen, soil moisture environments, different regrowth periods and time under shade. Individual equations are indicated. *Ppmax* represents the potential or maximum *Pmax* value in non-limiting conditions (*Ppmax*= 27.4 μmol CO_2 m^{-2} s^{-1}).

Simulated results for the modified multiplicative photosynthesis model (Figure 6.14) were then compared with the original validation set and showed the RMSD decreased from 32% to 22% of the mean observed $Pmax_s$ values (Figure 6.15).

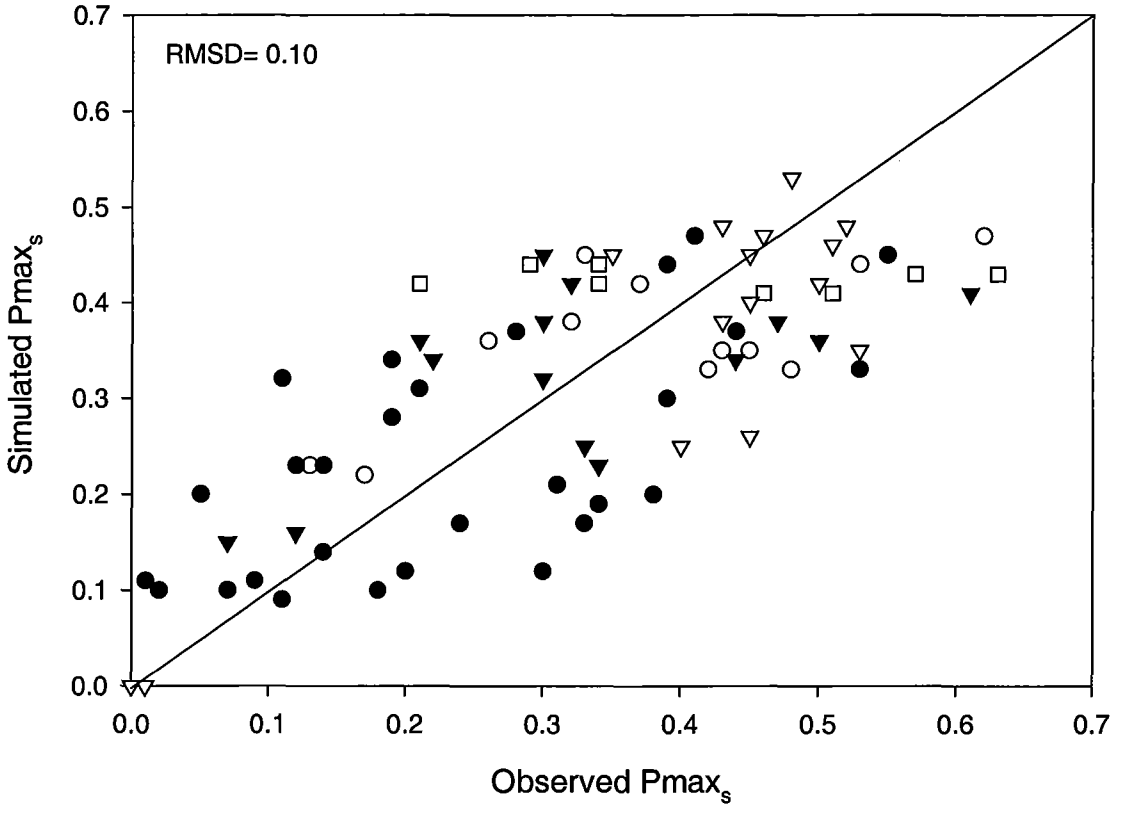

Figure 6.15 Comparison between simulated and observed $Pmax_s$ sorted by five groups (water limiting (▽), nitrogen limiting (▼), temperature limiting (○), regrowth duration limiting (□) and all factors limiting (●)) for cocksfoot leaves grown under severe shade (85-95 μmol m^{-2} s^{-1} PPFD) in a field agroforestry experiment. Simulated data were based on the final modified multiplicative model proposed in Figure 6.14.

6.4 Discussion

6.4.1 Practical implications of the fitted functions for shade duration and light intensity

The daily light regime under the 10-year-old trees in the middle of rows with alternating periods of full sunlight and shade (7% of the open PPFD) ranging from 45-60 minutes (morning and afternoon) to 90–120 minutes (around midday) was accurately simulated using a slat structure (Figure 6.1). However, in silvopastoral systems the period of full sunlight and shade may change over time according to the development of tree crowns and silvicultural practices applied during the rotation length. The use of the artificial structures (Section 6.2.1.1) widened the available range of time (0 to 180 minutes) of severe or moderate shade. Therefore, by calculating the time course of shade for a particular tree canopy (from different tree planting density, age, pruning and thinning intensities, etc.) in a silvopastoral system, it is possible to use the fitted *Pmax* functions (Equations 6.2 and 6.3) for situations of up to 180 minutes of shade. In contrast, the induction process (from low to high light levels) was only evaluated for plants which had been exposed to three periods under severe shade (30, 60 and 180 minutes) (Figure 6.5a). This may limit the use of the model for different situations of tree crown size in silvopastoral systems. However, for different durations under shade it is likely that *Pmax* will follow the parallel biphasic responses observed during the induction process in this experiment (Table 6.2). Values for additional time periods could be interpolated from the current results as an estimate of the expected response.

The functions fitted also provided predictions of *Pmax* at two light intensities. The artificial severe shade treatment (5% of the open PPFD) was used to generate a function (Equation 6.2) to represent the light regime from adjacent pine trees, which projected periods of shade of 7% of the open PPFD and periods of full sunlight where induction processes occurred. In contrast, the moderate shade (50% of the open PPFD) function (Equation 6.3) represented a continuous reduced light regime without induction processes. Because there are no induction processes for leaves exposed to cloth shade, it is likely that the daily canopy photosynthesis would be overestimated under this light regime compared with the same mean daily light intensity but under a fluctuating regime of full

sunlight/shade periods. For this reason, the use of artificial cloth shade may not accurately simulate the photosynthesis response of the understorey in silvopastoral systems.

Results from the present study show that the fluctuating light regimes influenced the net leaf photosynthesis rate of cocksfoot plants depending on the time and intensity of the full sunlight/shade periods (Figure 6.4). As the time under severe shade (5% of the open PPFD) was longer, the level of *Pmax* decreased and the subsequent duration to reach full induction increased. Thus, trees with a larger crown could be expected to reach lower levels of *Pmax* and take longer to return to $Pmax_s$= 1. This response to the temporal pattern of shade would result in an overall change in canopy carbon gain over a day for cocksfoot plants. Therefore, there is a need to incorporate the time course of shade affecting leaves into any canopy photosynthesis model of silvopastoral systems.

6.4.2 Accuracy and limitations of fitted functions for shade

The success of the approach used for predicting *Pmax* is that it can be used in environments outside those in which the equations were derived. The individual photosynthesis and stomatal functions over time for the two levels of shade and subsequent induction were empirically derived and summarised into easily transferable coefficients. Validation indicated at least 80% of the variation in $Pmax_s$ was accounted for by these functions, except for data from the middle of tree shade (Figure 6.8). In this situation, Equation 6.2 accounted for 77% of the variation. The difference between observed and predicted values for the middle of the tree shade (0.09 units of $Pmax_s$) situation was equivalent to 21 minutes of shade at 5% PPFD compared with that predicted at 40 minutes (19 minutes earlier). One reason for the underestimation of $Pmax_s$ could be the irregular shade intensity in the tree perimeter. In this experiment, there was 7% PPFD in the majority of the individual crown tree shade (70%), but there was also an area from the edge to 0.5 m inside the shaded zone and along the perimeter where the irradiance was gradually reduced from full sun to full shadow (gradient of 23% PPFD or a decrease rate of 24 $\mu mol\ m^{-2}\ s^{-1}$ PPFD per minute) (Section 3.2.3.3). This represented 16 minutes under shade above the 7% of open PPFD used in the derivation. To improve this prediction, it would be necessary to use Equation 6.3 during this time followed by a switch to Equation 6.2 as the higher shade level occurred. This anomaly highlights the importance of accurately describing the light environment under tree plantations before the models

proposed in this study could be expected to predict photosynthesis in a silvopastoral systems. Validation, using the windbreak data (300 minutes at 7% of open PPFD), suggested that the time course function for severe shade would reach a steady-state after 180 minutes.

6.4.3 Effect of time in shade on *Pmax*

The photosynthetic rate of individual cocksfoot leaves exposed from high to low light intensity decreased as a function of the magnitude and duration of the PPFD level previously experienced (Figure 6.3). The minimum value of $Pmax_s$ was for plants grown at 5% of open PPFD and this was 51% lower than for those grown at 50% of open PPFD (Figure 6.3). This result was consistent with data from a controlled environment study by Frank and Barker (1976). They reported a decrease in the rate of net photosynthesis of about 80% from 1160 to 200 μmol m^{-2} s^{-1} PAR for a whole cocksfoot plant. Similarly, Eagles and Treharne (1969) reported that the photosynthetic rate, on a chlorophyll basis, was 60% lower as light intensity decreased from 144 to 48 W m^{-2} for a natural Norwegian population of cocksfoot. In contrast, Singh *et al.* (1974) found that photosynthesis per unit leaf area (13.2 μmol CO_2 m^{-2} s^{-1}) of cocksfoot did not respond to different light intensities from 30 to 100% of full sunlight, but no explanation for this anomaly was reported.

(i) Stomatal conductance limitation under shade

The decrease in $Pmax_s$ in the first 30 minutes after entering shade at 5% of open PPFD was 92% faster than those grown at 50% of open PPFD (Figure 6.3). A reduction in *gs* occurred under low light (Kirschbaum *et al.*, 1988; Pearcy, 1988; Tinoco-Ojanguren and Pearcy, 1993) and this would explain the differences in the rate of decrease in *Pmax*. For example, values of gs_s indicated stomata closed 83% faster, during the first 100 minutes, for plants grown at 5% of open PPFD (Figure 6.4a) than those at 50% of open PPFD (Figure 6.4b). This was consistent with the observed rate of stomatal closure reported previously for *Lolium temulentum* L. leaves (Woledge, 1972). In contrast, according to Frank and Barker (1976) stomata diffusion resistance for water vapour of cocksfoot growing in a controlled environment did not respond to different light levels indicating that leaf photosynthesis was limited by the mesophyll resistance (Section 2.3.1.1.1).

(ii) Non-stomatal limitation under shade

The rate of *gs* limitation occurred slower than the rate of reduction in *Pmax* reduction rate under shade (Figure 6.4). This indicates that factors other than stomatal closure caused the reduction in *Pmax* during the initial period under shade. In this study, the non-stomatal limitation was 92% greater than the stomatal limitation after 10 minutes under severe shade (Figure 6.7a). Similarly, for moderate shade there was almost no stomatal limitation after 30 minutes under shade, but the non-stomatal limitation on $Pmax_s$ was 0.054 units (Figure 6.7b). The magnitude of the maximum non-stomatal limitation, and the time required to reach this maximum value under severe shade were both 2.5 times greater than under moderate shade. These data were consistent with a two compartment system driving the reduction in $Pmax_s$, where one compartment acts as a buffer to reduction in the non-stomatal limitation process. A description of the non-stomatal limitations that affected photosynthesis was provided by Sassenrath-Cole and Pearcy (1994) who investigated a time course deactivation of RuBisCO and FBPase (fructose-1,6-bisphosphatase) activities at low PPFD (35 μmol m^{-2} s^{-1}) for soybean leaves. In this work, the authors found that after 5 minutes at low PPFD, the FBPase activity was insufficient to support the maximal light-saturated rate of photosynthesis and that RuBisCO activity declined slower, retaining half-maximal activity after 20 minutes at low PPFD. A similar mechanism may explain the current results for cocksfoot.

The probable physiological reasons for the effects of shade on *Pmax* are summarised in Table 6.5.

Table 6.5 Summary of the effect and biological explanation of shade on *Pmax*

Factor	Function	Maximum *Pmax* values	Minimum *Pmax* values	Biological impact
Severe shade (5% of open PPFD)	Exponential decay	0 min in shade	> 140 min	Slow decrease in stomatal conductance Fast deactivation of non-stomatal factors (RuBisCO and FBPase activities)
Moderate shade (50% of open PPFD)	Exponential decay	0 to 5 min in shade	> 120 min	Same reasons above but reduced magnitude.

6.4.4 Induction of photosynthesis

Pmax at any given time during induction was dependent on the duration of the previous low light (5% PPFD) period (Table 6.3). For example, IS_2 was 22% lower in plants exposed to 5% PPFD for 180 minutes than those exposed for 30 minutes. This influence of shade duration on the recovery of *Pmax* during induction has been reported for other species (Sassenrath-Cole and Pearcy, 1994; Chazdon and Pearcy, 1986; Tinoco-Ojanguren and Pearcy, 1993) (Section 2.3.1.1.2).

(i) Stomatal conductance limitation during induction

The increase in *gs* occurred slower than the *Pmax* increment during the first 10 minutes of induction and was almost constant in the first minute (Figure 6.5b). Changes in stomatal conductance contributed mainly to the second slower phase of photosynthetic induction (*phase II*). Similarly, Sassenrath-Cole and Pearcy (1994) reported that stomatal limitations can occur at any time during induction, but increases in stomatal conductance are the sole cause of increases in assimilation rate after 10 minutes of saturating PPFD when the enzymes are already fully activated. Pearcy and Seemann (1990) reported that for soybean leaves, which had received 180 minutes of shade (2% of the full sunlight PPFD) prior to an increase in PPFD (1200 $\mu mol\ m^{-2}\ s^{-1}$), photosynthesis increased over the next 20 minutes to a maximum steady-state value while *gs* required nearly 40 minutes to recover the maximum value.

(ii) Non-stomatal conductance limitation during induction

The implications is that factors other than an increase in *gs* caused the increment in *Pmax* during the first minutes of induction (*phase I*). In the present work, the maximum non-stomatal limitation during induction was 0.12 units at 1 minute after full sun for plants exposed to 60 minutes under shade and 0.26 units at 2 minutes after full sun for plants exposed to 180 minutes under shade (Figure 6.5b). Sassenrath-Cole and Pearcy (1992) reported that during the first 1-2 minutes of induction a fast phase was associated with limitations in ribulose 1,5-bisphosphate (RuBP) regeneration. After long periods in low PPFD, this fast phase may be masked by other slower limitations consisting of the light-activation requirement for RuBisCO which is largely complete within 7 to 10 minutes after an increase in PPFD (Pearcy *et al.*, 1996).

6.4.5 Hysteric response

Pmax, *gs*, and stomatal and non-stomatal limitations exhibited a hysteric response. The increase in photosynthesis and stomata opening during induction (Figure 6.5) were faster than the decrease and closing of stomata when leaves were exposed to severe shade (Figures 6.3 and 6.4). The rate of decrease in $Pmax_s$ during 60 minutes of severe shade was 67% slower than the rate of increase of *Pmax* to reach the maximum saturated value ($Pmax_s$= 1) after 20 minutes of full sunlight. Similarly, the rate of decrease in *gs* during 60 minutes of severe shade was 59% slower than the subsequent rate of increase to gs_s= 1. This was consistent with Kirschbaum *et al.* (1988) who found a faster opening (20 minutes to reach the maximum value after a single sunfleck) than closing of stomata (60 minutes to return to the steady state level at a low light of 10 μmol quanta m^{-2} s^{-1}), particularly in response to 5 minutes of sunflecks in leaves of the tropical forest understory plant *Alocasia macrorrhiza*.

The asymmetric response in *Pmax* rate may be due to a faster opening after the return of sunlight or to a slower deactivation of enzymatic activities compared with their activities during induction. This hysteric response by the non-stomatal limitation is also consistent with a two component system with a buffered reduction and unbuffered recovery for the non-stomatal limitation. For example, the decrease in rate for non-stomatal limitations during 60 minutes under severe shade was 94% slower than the increase rate of non-stomatal limitations during induction until *Pmax* reached the maximum saturated value ($Pmax_s$= 1). Sassenrath-Cole and Pearcy (1994) reported for soybean leaves a slower low-light deactivation of enzymatic activity (RuBisCO and Ru5P kinase) than under high-light activation (1500 μmol m^{-2} s^{-1}) for a minimum of 20 minutes.

The consequence of these hysteric responses is a likely reduction in the efficiency of utilisation of sunlight in fluctuating light regimes with longer shade periods. This is because the incoming irradiance (sun gaps versus shade area) generally decreases with time in silvopastoral systems, due to tree crown development, and it is therefore likely that over time cocksfoot leaves will rarely be fully induced. For example, cocksfoot full induction after 180 minutes under shade occurred after almost 40 minutes. If in this situation less than 40 minutes of high PPFD occurred, then the induction state would be a function of the immediate past light environment. Further study to quantify the dynamics

of the induction response in fluctuating light environments are required to assess the quantitative role in photosynthetic activity.

6.4.6 Difference between air and canopy temperatures

As expected, air temperature at midday was higher than canopy temperature in irrigated plants in the agroforesty site (Figure 6.10). For plants in full sunlight the canopy temperature was up to 2 °C (at air temperature 30-31 °C) cooler than air temperature suggesting that stomata were wide open and transpirational cooling was occurring. The effect of this difference on *Pmax* was discussed in Section 4.4.5.

For plants under shade, the canopy temperature was up to 7.4 °C cooler than air temperature and up to 5.4 °C cooler than canopy temperature of plants in full sunlight at the same time (Figure 6.10). This difference was probably caused by the energy balance of leaves through a reduction in the incoming radiation (Section 2.2.2). For example, at midday on a sunny summer day, the total radiation was 1000 W m^{-2} in the sun and only 50-70 W m^{-2} under the middle of the tree shade.

The difference between air and canopy temperature, particularly when photosynthesis was restricted for a combination of shade and high air temperatures (mainly air temperatures > 24 °C), indicates canopy temperature needs to be used directly to fit or to use photosynthesis models. Alternatively, if only air temperatures are available, a predictive model to predict canopy temperatures from air temperatures needs to be created as a function of different shade intensities and temperature levels. In this study, the equations to predict canopy temperature under pine tree shade (Figure 6.10) will be used in Chapter 8 for predicting canopy photosynthesis in a silvopastoral system.

6.4.7 Interaction factor between shade and ψ_{lp}

Only one interaction was detected between environmental and management factors coupled with the light regime, and this was for the limited condition of time under severe shade (5% PPFD) and water stress (ψ_{lp}= -4 to –13 bar). There were three distinct aspects to the reduction process of *Pmax* after severe shade occurred in water stressed plants.

Firstly, $Pmax_s$ did not decrease in a multiplicative way when these two factors were limiting. For example, $Pmax_s$ reached a steady-state asymptote of 0.28 units of $Pmax_s$ for

plants grown at ψ_{lp}= -4.0 to –8.0 bar and 0.19 units of $Pmax_s$ for plants grown at ψ_{lp}= -8.0 to –13.0 bar (Figure 6.13a). However, from the multiplicative model (Figure 6.11) it was expected that $Pmax_s$ should have reached a steady-state asymptote of 0.16 and 0.02 units of $Pmax_s$ for these two groups, respectively. In silvopastoral systems, the effects of shade and water stress on cocksfoot and grasses in general have been reported on a seasonal dry matter basis (Braziotis and Papanastasis, 1995; Devkota *et al.*, 1997, 1998; Joshi *et al.*, 1999), but there is no information about the physiological reasons for this interaction. The magnitude of the effect of each factor (shade or water stress) in the interaction reducing *Pmax* also depends on species adaptations. Zhang *et al.* (1995) reported for a sub-shrub (*Encelia farinosa* A. Gray) that the water stress factor (ψ_{lp}= –20 bar) was more important than shade (40% of full sunlight) in the reduction of *Pmax*. Thus, while *Pmax* was reduced only by 6% due to shade, the effect of water stress in shaded plants decreased *Pmax* by 85%. In contrast, Alvino *et al.* (1994) reported for pepper (*Capsicum annuum* L.) leaves that experienced both low irradiance (30% of full sunlight) and water stress (-28 bar) reduced photosynthesis, although the decrease was greater due to reduced irradiance.

Stomatal closure has been reported to be an important cause of the reduction in *Pmax* at low light (Kirschbaum *et al.*, 1988; Pearcy, 1988; Tinoco-Ojanguren and Pearcy, 1993) and under water stress environments (Chaves, 1991; Slatyer, 1969). In this experiment, stomatal conductance at the steady-state (asymptotic value) was lower when shade and ψ_{lp} were both limiting (0.28 units of gs_s for plants with ψ_{lp}= -4.0 to –8.0 bar and 0.19 units of gs_s for plants with ψ_{lp}= -8.0 to –13.0 bar) than when either of these factors was limiting alone (e.g. gs_s= 0.36 for shade only limiting). However, in water stressed plants (ψ_{lp}< -4.0 bar), stomata also did not close in a multiplicative way under severe shade and this could be one reason for the non-multiplicative reduction in *Pmax* when both factors were limiting. Similarly to *gs*, the non-stomatal factors may not follow a multiplicative function when severe shade and water stress are present.

Secondly, the decrease in $Pmax_s$ during the initial period under severe shade was faster for plants grown with water stress than those grown without water stress. For example, after 1 minute under shade the decrease in $Pmax_s$ was 2.8-fold and 3.5-fold faster for plants grown with ψ_{lp}= -4.0 to –8.0 bar and ψ_{lp}= -8.0 to –13.0 bar, respectively than those grown without water stress (Figure 6.13a, Table 6.6). This response could be mainly due a combination of a faster decrease in *gs* and non-stomatal limitations (Table 6.6).

Table 6.6 Effects of standardised stomatal conductance limitation and non-stomatal limitation on the standardised rate of net photosynthesis $Pmax_s$ after 1 minute under severe shade (5% of open PPFD) for two groups of water stressed plants. Values represents the decrease in $Pmax_s$, gs_s and non-stomatal limitation expressed in standardised units from previous shade to 1 minute under shade obtained from Figures 6.13a and 6.13b.

Water status	*Stomatal limitation* ($1 - gs_s$)	*Non-stomatal limitation* ($gs_s - Pmax_s$)	*Total limitation* ($1 - Pmax_s$)
Water non-limiting	0.01	0.16	0.17
ψ_{lp}= -4.0 to –8.0 bar	0.18	0.30	0.48
ψ_{lp}= -8.0 to –13.0 bar	0.30	0.30	0.60

Similarly, the decrease in $Pmax_s$ after 30 minutes entering shade was faster for plants grown with ψ_{lp}= -4.0 to –8.0 bar ($Pmax_s$= 0.30) and ψ_{lp}= -8.0 to –13.0 bar ($Pmax_s$= 0.20), respectively than for those grown without water stress ($Pmax_s$= 0.55). This response could be mainly due to a lower start point of $Pmax_s$ after 1 minute under shade (Figure 6.13a) and a faster closure of stomata occurring during the first 30 minutes under low light for water stressed plants compared with irrigated plants. For example, values of gs_s indicated a closure in stomata 59% and 72% faster than irrigated plants during this period for plants grown with ψ_{lp}= -4.0 to –8.0 bar and ψ_{lp}= -8.0 to –13.0 bar, respectively. Knapp and Smith (1988) also reported a more rapid decrease in *gs* in response to fluctuations in irradiance (5 minutes shade periods alternating with 8 minutes of full sunlight) in water stressed versus a non-water stressed subalpine herb. Similarly, Knapp and Smith (1990) reported a 30% faster stomatal closure for a subalpine herb under water stress (ψ= -31 bar) during the dark period (< 600 μmol m^{-2} s^{-1} PPFD) of a fluctuating light regime than irrigated plants. The faster closure of stomata for water stressed plants may determine that $Pmax_s$ reached the minimum steady-state (asymptote value) 94 and 105 minutes earlier than those without water stress. Furthermore, a benefit of the rapid stomatal response of cocksfoot leaves (to changes in light intensity under water stress) would be reduced water loss and increase water use efficiency during shade. This is because transpiration is reduced via stomatal closure at the same time photosynthesis is reduced by low light and water stress interaction.

Thirdly, as occurred with irrigated plants, the reduction of *gs* in water stressed plants occurred slower than the reduction in *Pmax* under severe shade. For example, the maximum difference between gs_s and $Pmax_s$ was 0.34 units at 10 minutes under shade for plants grown at ψ_{lp}= -4.0 to –8.0 bar and 0.33 units at 5 minutes under shade for plants grown at ψ_{lp}= -8.0 to –13.0 bar. The magnitude of this difference between gs_s and $Pmax_s$ was similar to the value found for irrigated plants (0.34 units), but it occurred later after 35 minutes (Figure 6.4a). Therefore, cocksfoot plants that experience water stress during alternating light/shade intervals appear to have a more sensitive response pattern in *gs* than plants grown in full sunlight, which means more closely tracking responses in *Pmax*. However, as occurred with irrigated plants, factors other than stomatal closure may cause the reduction in *Pmax* during the first minutes under shade of water stressed plants.

6.4.8 Accuracy of the final multiplicative model with five factors

The second part of this chapter aimed to integrate the light regime response of *Pmax* with temperature, N, soil moisture and regrowth duration into the multiplicative model proposed in Figure 5.11 (Section 5.3.9). This resulted in the extension of the empirical model (Figure 6.14). Validation of this model indicated 78% of the variation in *Pmax* could be accounted for using these five factors by the addition of the shade x water status interaction function (Equations 6.9 and 6.10). These interaction functions for situations of water stress (ψ_{lp}= -4 to –13 bar) and time under severe shade (1 to 180 minutes) still need to be independently validated.

The success of using the five factors and the interaction factor for predicting *Pmax* shows that predictions were transferable from open to shade conditions and suggests a similar approach could be used in other silvopastoral environments (outside those in which these equations were derived).

The sequential evaluation of the final multiplicative model for the five factors is given in Table 6.7.

Table 6.7 Sequential evaluation of the final multiplicative model for the five factors through the root mean square deviation (RMSD) analysis expressed as a percentage of the mean observed $Pmax_s$ values.

Main effects					Interactions				
T	ψ_{lp}	N%	RD	Shade	T x N	RD x ψ_{lp}	Shade x ψ_{lp}	RMSD	Results Section
✓	✓	✓						22%	4.3.7
✓	✓	✓			✓			18%	4.3.9
✓	✓	✓	✓		✓			31%	5.3.7
✓	✓	✓	✓		✓	✓		20%	5.3.9
✓	✓	✓	✓	✓	✓	✓		31%	6.3.2.2
✓	✓	✓	✓	✓	✓	✓	✓	22%	6.3.2.5

T= air temperature; ψ_{lp}= water status; N= herbage nitrogen content; RD= regrowth duration.

6.5 Conclusions

• The light regime of the silvopastoral system used in this study was characterised by periods of full sunlight/shade and the light intensity, which was accurately simulated using slat structures.

• The photosynthesic rate (*Pmax*) of individual cocksfoot leaves decreased (exposed from high to low light intensity) and increased (from low to high light intensity) as a function of the magnitude and duration of the PPFD level previously experienced. The individual photosynthesis functions over time for the two levels of shade and subsequent induction were empirically derived and summarised into easily transferable coefficients.

• Stomatal and non-stomatal factors were jointly responsible for the reduction and induction of *Pmax*, with their relative importance depending on the duration and intensity of shade.

- Water stress was an important factor that influenced the *Pmax* reduction under shade through more sensitive stomatal and non-stomatal responses.

- The difference between air and canopy temperature when photosynthesis was restricted for a combination of shade and high air temperatures (mainly air temperatures > 24 °C) determined that canopy temperature needs to be used directly for photosynthesis modelling.

- Defining the *Pmax* functions (temperature, N, water status, regrowth duration and shade) in the multiplicative model for individual leaves of cocksfoot is the first step to developing a pasture production model in silvopastoral systems through its incorporation in a canopy photosynthesis model.

The multiplicative model proposed for *Pmax* (Figure 6.14) can now be incorporated into a canopy photosynthesis model as a variable to predict cocksfoot growth in a silvopastoral system. In the next chapter, the response of α and θ (the other two parameters which represent the rate of leaf photosynthetic capacity) to temperature, N, water status, regrowth duration and shade will be evaluated by fitting individual functions and integrating these functions into a comprehensive model.

CHAPTER 7

Modelling photosynthetic efficiency and convexity of the light-response curve for field grown cocksfoot leaves under different environmental and regrowth duration conditions

7.1 Introduction

The rate of net photosynthesis as a function of PPFD generally follows a non-rectangular hyperbola with three parameters: θ (a dimensionless parameter indicating the degree of curvature or convexity), α (the initial slope of the light response curve or photosynthetic efficiency also referred as the quantum yield or photochemical efficiency in the liteature) and *Pmax* (the asymptote) (Section 2.3.1). The effects of environmental factors and regrowth duration on *Pmax* have been presented in Chapters 4, 5 and 6. In this chapter, the analysis is focussed on how these factors affect α and θ.

Values of α are determined by the efficiency with which absorbed photons are used for CO_2 assimilation and are related to the RuBP carboxylase enzyme (RuBisCO) activity and photorespiration (Section 2.3.1.6). In this study, there were indications that factors other than stomatal conductance (*gs*) also affected photosynthesis (e.g. N and shade). Therefore it is likely that these factors also affect α.

Marshall and Biscoe (1980a) and Thornley and Johnson (2000) describe θ as the ratio of physical to total resistance to CO_2 transfer within a leaf. Therefore, depression of α and θ both result in a reduced capability of leaves to operate efficiently under low light. As a consequence, there is likely to be a reduction in whole canopy photosynthesis and RUE.

Both α and θ have been used in crop and pasture canopy photosynthesis models to predict growth. However, in most of the canopy photosynthesis model, α and θ are assumed constant values (Section 2.3.1.7). In contrast, Thornley (1998) reported functions for α depending upon temperature and leaf water status but assumed no effect of N. Hirose and Werger (1987a) varied α and θ in a sub-model of the canopy photosynthesis model using

two linear regressions correlated with leaf N concentration. However, the relationship between environmental (temperature, N, water stress and shade) and management (regrowth duration) factors on α and θ has usually been expressed in isolation or with limited explanation of the physiological basis for the responses. The influence of these factors on α and θ of cocksfoot leaves in a silvopastoral system has not been defined.

The objectives of the research outlined in this chapter were to:

1) derive individual functions for α and θ against temperature, N, water status, regrowth duration and shade (intensity and time course) for individual cocksfoot leaves;

2) propose biological explanations for each function derived;

3) develop a mathematical model to integrate these functions into the simple multiplicative model proposed in Equation 4.1, and validate this model with an independent data set.

7.2 Materials and Methods

7.2.1 Photosynthesis measurements

The net photosynthesis rate measured on youngest fully expanded intact leaves from light curves obtained in Chapters 4, 5 and 6 were used to fit non-rectangular hyperbola (Marshall and Biscoe, 1980a; Thornley, 1998). The mathematical form of this equation is:

$$0 = \theta P_n^2 - [P_{max} + \alpha I_l]) P_n + \alpha I_l P_{max}$$

and is;

$$Pn = \frac{1}{2\theta}\left\{\alpha I_1 + Pmax - \left[(\alpha I_l + Pmax)^2 - 4\theta\alpha I_l Pmax\right]^{1/2}\right\}$$

Equation 7.1

Where P_n is the rate of single leaf net photosynthesis (μmol CO_2 m^{-2} s^{-1}), I_l is the irradiance incident on a leaf (μmol m^{-2} s^{-1} PPFD), α is the initial slope of the light response curve or photosynthetic efficiency (μmol CO_2/μmol PPFD or mg CO_2 J^{-1}), θ is the degree of curvature (dimensionless), and *Pmax* is the maximum rate of net photosynthesis (μmol CO_2 m^{-2} s^{-1}).

Overall, 163 photosynthesis light curves were fitted to analyse the effect of each individual factor on α and θ. Of these:

(i) 19 were used to fit light curves when only temperature was limiting (Section 4.3.1),

(ii) 20 when only N was limiting (Section 4.3.2),

(iii) 26 when only pre-dawn leaf water potential (ψ_{lp}) was limiting (Section 4.3.4),

(iv) 30 when only regrowth duration only was limiting (Section 5.3.1),

(v) 36 during 180 minutes under severe shade of a wide wooden structure (5% of the open PPFD) (Section 6.3.1.1) and 16 for its respective recovery during induction (Section 6.3.1.3),

(vi) 16 for the moderate shade (50% of open PPFD) of a cloth structure (Section 6.3.1.1).

A further 46 were used for model validation to predict α when two, three, four or all five factors were limiting.

The calculated values of α in μmol CO_2/μmol PPFD and θ (dimensionless) were transformed by dividing the observed values by values obtained in non-limited conditions, as defined for *Pmax* (Section 4.2.2), to give a standardised index value that ranged from 0 to 1. A value of 1 (α_s= 1 or θ_s= 1) corresponds to the maximum value found for α or θ in non-limiting conditions.

7.2.2 Analyses

The data were analysed using linear regression and non-linear regression analysis to determine the relationships between α and θ and each of the environment and management variables. For modelling simplicity these variables were also described using a two straight line segments "broken stick" methodology (Section 4.2.4). Values of R^2 and ESE of α_s were used to select the most appropriate functions.

In an integrated analysis, a simple multiplicative model (Equation 4.1) and a model using a 'law of the minimum factor' as has been used for simulation of crop growth (Jones *et al.*, 1986) and for nutrient supply effects on crop yields (Black, 1993) were used to test the prediction of α for cocksfoot leaves when one, two, three, four or all of the factors were limiting. The 'law of the minimum factor' model proposed the axiom: 'when a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factor' (Black, 1993). Residuals and RMSD were

calculated to estimate the accuracy of the proposed models (Section 4.2.4).

7.3 Results

The non-rectangular hyperbola fitted to the light response data of leaf net photosynthesis explained over 99% of the total variance.

7.3.1 Photosynthetic efficiency (α) and temperature

The α values were obtained from light curves of cocksfoot grown with air temperatures from 10 °C to 31 °C (Figure 7.1). The maximum value of 0.036 μmol CO_2/μmol PPFD or 0.0069 mg CO_2 J^{-1} (α_s= 1) was measured from 10 to 24 °C. From this point α decreased linearly at a rate of 0.001 μmol CO_2/μmol PPFD, or 0.028 units of α_s per °C up to 31 °C. The data were described by fitting a "broken stick" model (Equation 7.2), with inflection point at 24 °C (R^2= 0.91; ESE= 0.022).

x (°C)	y (α_s)
10	1.00
24	1.00
31	0.80

Equation 7.2

The range of optimum temperatures for α_s (α_s= 1) was greater than for $Pmax_s$ (Equation 4.7 in Section 4.3.7), and the magnitude of reduction at 31 °C was lower (Figure 7.1).

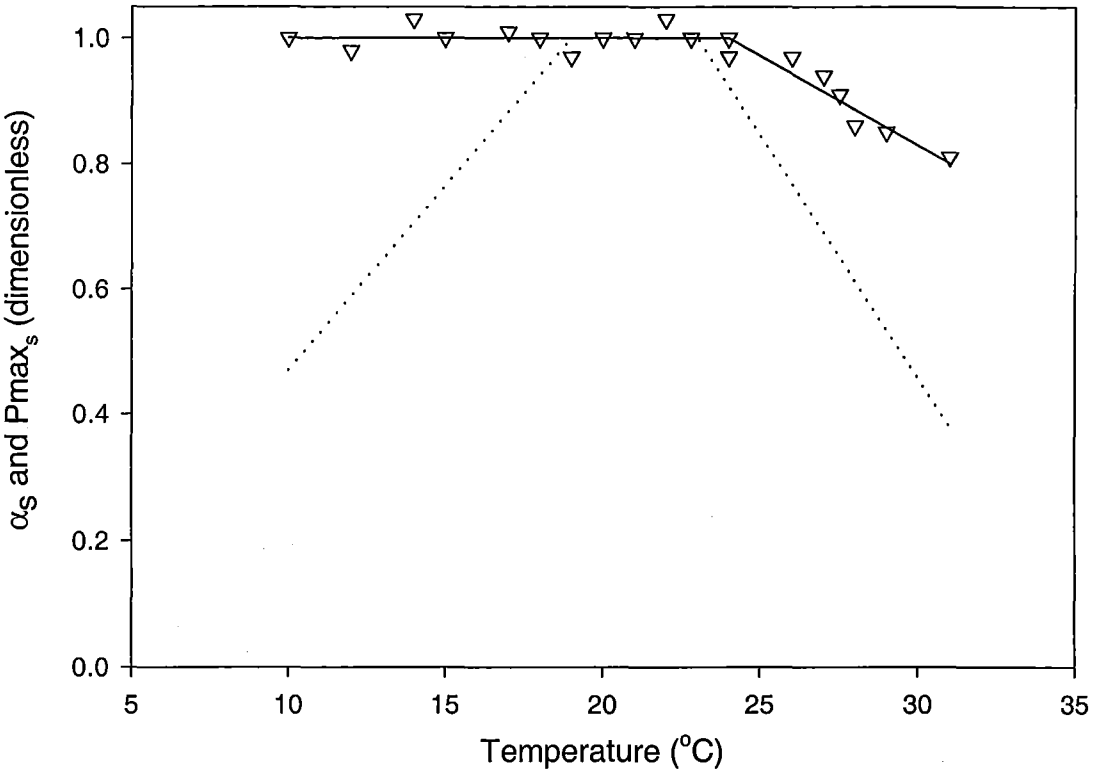

Figure 7.1 Standardised rate of photosynthetic efficiency (α_s) against temperature for cocksfoot grown under field conditions where other factors were non-limiting. α_s= 1 ≡ α= 0.036 μmol CO_2/μmol PPFD. The fitted "broken stick" model for α_s (—) and the "broken stick" model for $Pmax_s$ (····) are indicated.

7.3.2 Photosynthetic efficiency (α) and nitrogen content (N%)

The leaf N content ranged from 1.5 to 5.9%. The same maximum value of 0.036 μmol CO_2/μmol PPFD or 0.0069 mg CO_2 J^{-1} (α_s= 1) was measured from 4.0 to 5.9% N (Figure 7.2). From this point α decreased linearly at a rate of 0.0061 μmol CO_2/μmol PPFD, or 0.17 units of α_s per 1% N reduction to 1.5% leaf N content. The data were described by fitting a "broken stick" model (Equation 7.3), with inflection points at 4% N (R^2= 0.95; ESE= 0.031).

x (%N)	y (α_S)
1.5	0.57
4.0	1.00
5.9	1.00

Equation 7.3

The optimum range for α_s (α_s= 1) was greater and the minimum value was higher (Figure 7.2) than these found for $Pmax_s$ (Equation 4.7 in Section 4.3.7).

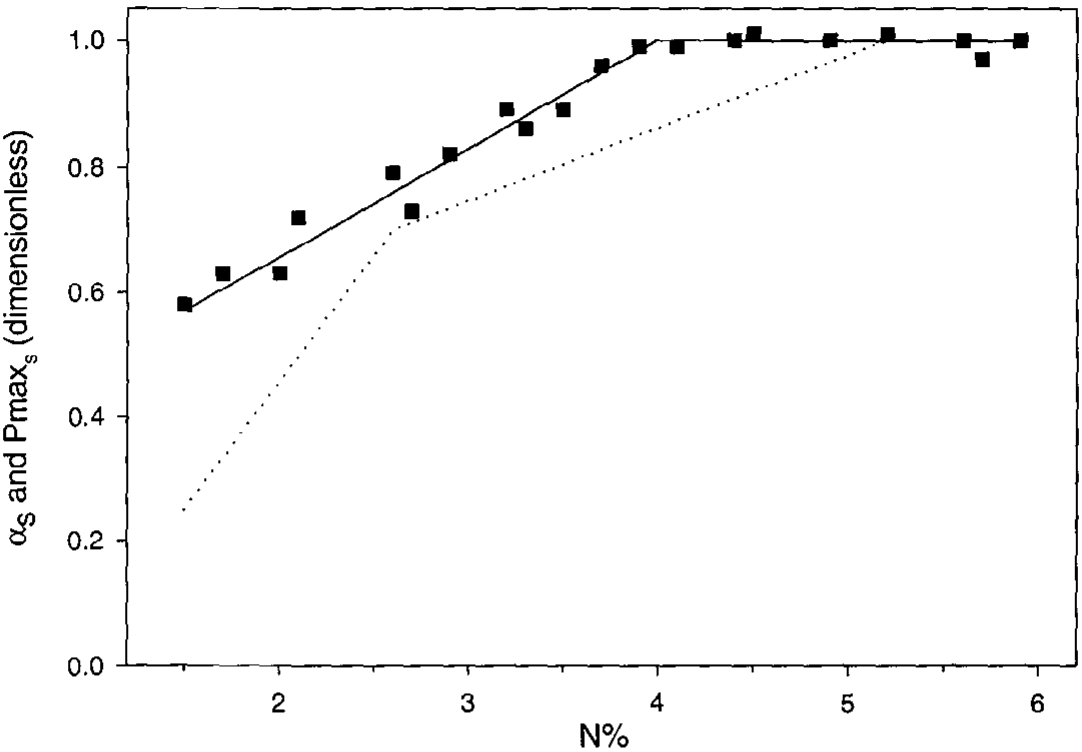

Figure 7.2 Standardised rate of photosynthetic efficiency (α_s) against nitrogen percentage for cocksfoot grown under field conditions where other factors were non-limiting. α_s= 1 ≡ α= 0.036 μmol CO_2/μmol PPFD. The fitted "broken stick" model for α_s (—) and the "broken stick" model for $Pmax_s$ (···) are indicated.

7.3.3 Photosynthetic efficiency (α) and water stress

The range of ψ_{lp} was from –0.1 bar to –16.0 bar which corresponded to a soil VWC in the top 500 mm of 32% and 11%, respectively. The maximum value of α of 0.036 μmol CO_2/μmol PPFD (α_s= 1) was measured from –0.1 to –10.0 bar (from 30 to 16% soil VWC). From this point, α decreased linearly at the rate of 0.0017 μmol CO_2/μmol PPFD, or 0.048 units of α_s, per bar of ψ_{lp} as water stress increased to –16.0 bar (Figure 7.3). The data were described by fitting a "broken stick" model (Equation 7.4), with an inflection point at –10.0 bar (R^2= 0.93; ESE= 0.028).

$$\begin{array}{cc} x\ (\psi_{lp}) & y\ (\alpha_s) \\ \left\{\begin{array}{cc} -0.1 & 1.00 \\ -10.0 & 1.00 \\ -16.0 & 0.71 \end{array}\right\} & \end{array}$$

Equation 7.4

The range of optimum ψ_{lp} for α_s (α_s= 1) was greater and the minimum value was higher at −16.0 bar (Figure 7.3) than for $Pmax_s$ (Equation 4.7 in Section 4.3.7).

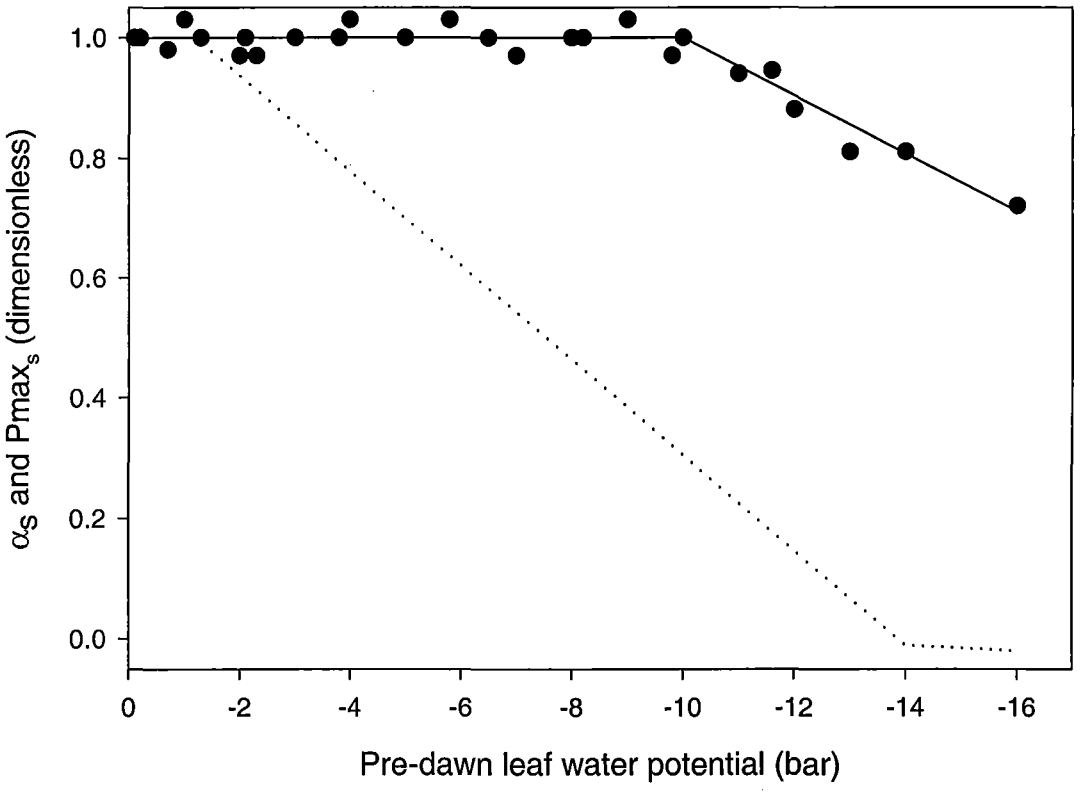

Figure 7.3 Standardised rate of photosynthetic efficiency (α_s) against water stress for cocksfoot grown under field conditions where other factors were non-limiting. α_s= 1 ≡ α= 0.036 μmol CO_2/μmol PPFD. The fitted "broken stick" model for α_s (—) and the "broken stick" model for $Pmax_s$ (····) are indicated.

7.3.4 Photosynthetic efficiency (α) and regrowth duration

The α values were obtained from light curves on a random sample of six of the youngest fully expanded intact leaves from vegetative tillers after 20, 25, 35, 40, 45, 55 and 60 days of regrowth. The α_s values of successive newly expanded leaves were progressively reduced with regrowth time (Figure 7.4). From 20 to 40 days regrowth, α was almost constant at a maximum value of 0.036 μmol CO_2/μmol PPFD (α_s=1). From this point, α decreased at a rate of 0.0002 μmol CO_2/μmol PPFD per day of regrowth, or 0.006 units of α_s per day. A quadratic function was fitted (Equation 7.5) to the measured data and this gave an R^2 of 0.84 and ESE of α_s of 0.021.

$$\alpha_s = 0.968 + 0.0036\ Tr - 0.0001\ Tr^2 \qquad \textbf{Equation 7.5}$$

Where Tr is time of regrowth in days.

The minimum value of α_s at day 60 was α_s = 0.83 compared with 0.55 for the $Pmax_s$ function (Equation 5.1 in Section 5.3.1) (Figure 7.4).

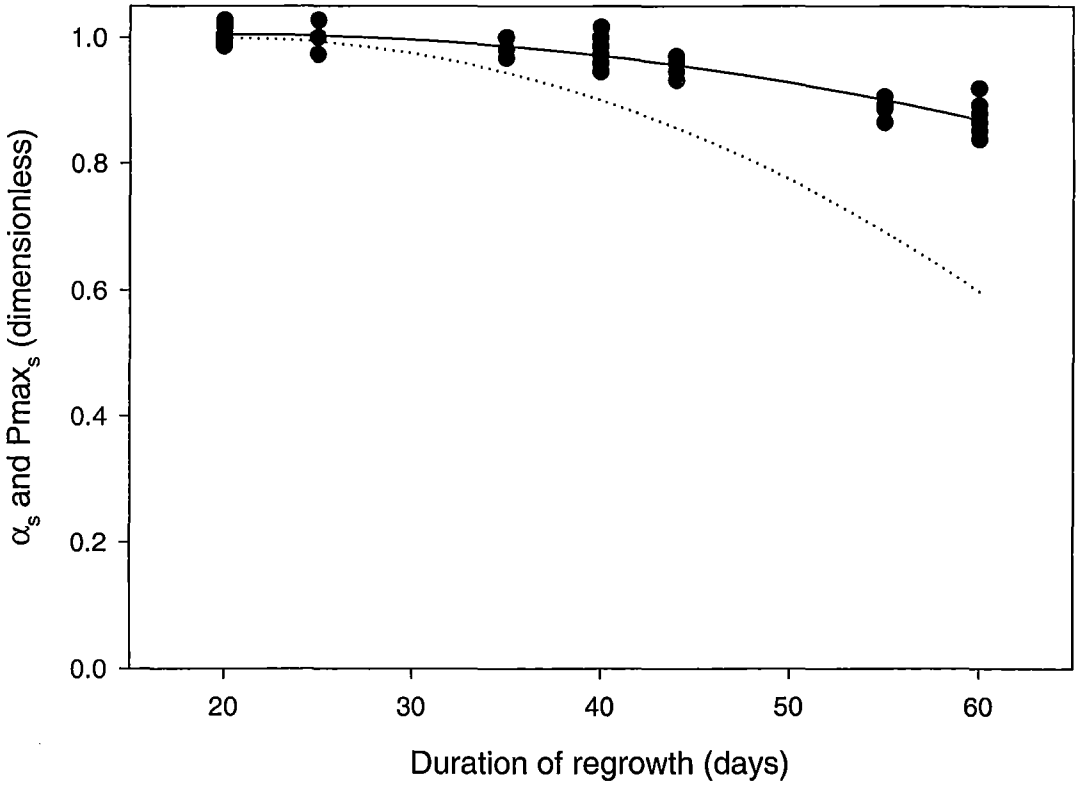

Figure 7.4 Standardised rate of photosynthetic efficiency (α_s) against regrowth duration for cocksfoot grown under field conditions where other factors were non-limiting. α_s= 1 ≡ α= 0.036 μmol CO_2/μmol PPFD. The fitted quadratic equation for α_s (—) and the model for $Pmax_s$ (····) are indicated.

7.3.5 Effect of time in shade on photosynthetic efficiency (α)

(i) Severe shade (5% of the open PPFD)

The α_s values were calculated from light curves of cocksfoot grown from 1 to 180 minutes under severe shade (Figure 7.5a). From full sun (α_s= 1 ≡ α= 0.036 µmol CO_2/µmol PPFD = 0.0069 mg CO_2 J^{-1}) to shade, the decrease in α_s was non-linear against time. From 1 to 20 minutes under shade α_s decreased by 0.0004 µmol CO_2/µmol PPFD per minute of severe shade or 0.012 units of α_s per minute. From 20 to 180 minutes, α_s decreased by 0.000016 µmol CO_2/µmol PPFD per minute of severe shade or 0.00044 units of α_s per minute. However, from 60 minutes under shade, α_s reached a steady-state asymptote of 0.74 units which was higher than for *Pmax* ($Pmax_s$= 0.37) (Figure 7.5a).

The exponential decay function (Equation 7.6) fitted to the measured data gave an R^2 of 0.78 and ESE of α_s of 0.045.

$$\alpha_s = 0.72 * e^{\left(\frac{2.76}{t_S + 6.99}\right)}$$

Equation 7.6

Where t_s is the time under severe shade in minutes.

(ii) Moderate shade (50% of the open PPFD)

Under cloth shade a different exponential function (Equation 7.7) was required due to the slower decline of α_s compared with severe shade. From full sun (α_s= 1) to shade, the decrease in α_s was non-linear against time (Figure 7.5b). From 1 to 20 minutes under shade α_s decreased by 0.00024 µmol CO_2/µmol PPFD per minute of moderate shade or 0.0067 units of α_s per minute. From 20 to 180 minutes, α_s decreased by 0.000014 µmol CO_2/µmol PPFD per minute of moderate shade or 0.0004 units of α_s per minute. However, from 40 minutes under moderate shade, α_s reached a steady-state asymptote of 0.92 units which was higher than for *Pmax* ($Pmax_s$= 0.76) (Figure 7.5b).

The exponential decay function (Equation 7.7) fitted to the measured data gave an R^2 of 0.81 and ESE of α_s of 0.026.

$$\alpha_s = 0.88 * e^{\left(\frac{2.41}{tm+11.2}\right)}$$

Equation 7.7

Where t_m is the time under moderate shade in minutes.

(iii) Induction of α after severe shade

There was a biphasic induction process of α_s represented by two linear equations with an inflection point fitted using the 'broken stick' analysis (Equation 7.8) (R^2= 0.81; ESE= 0.032). The mean slope of α_s induction for *phase I* was 0.073 units of α_s per minute of full sunlight or 0.0026 μmol CO_2/μmol PPFD per minute compared with only 0.0011 units of α_s per minute of full sunlight or 0.00004 μmol CO_2/μmol PPFD per minute for *phase II* (Figure 7.5a). The time required for full induction after 180 minutes under severe shade for α_s was similar to that for $Pmax_s$ (Figure 7.5a). However, at the end of the *phase I*, α_s had almost reached the full induction value after 3 minutes of full sunlight (α_s= 0.96), but $Pmax_s$ had only reached a value of 0.65.

t (min)	y (α_s)
0	0.74
3	0.96
37	1.00

Equation 7.8

Where t is the time in full sunlight after being under severe shade (minutes)

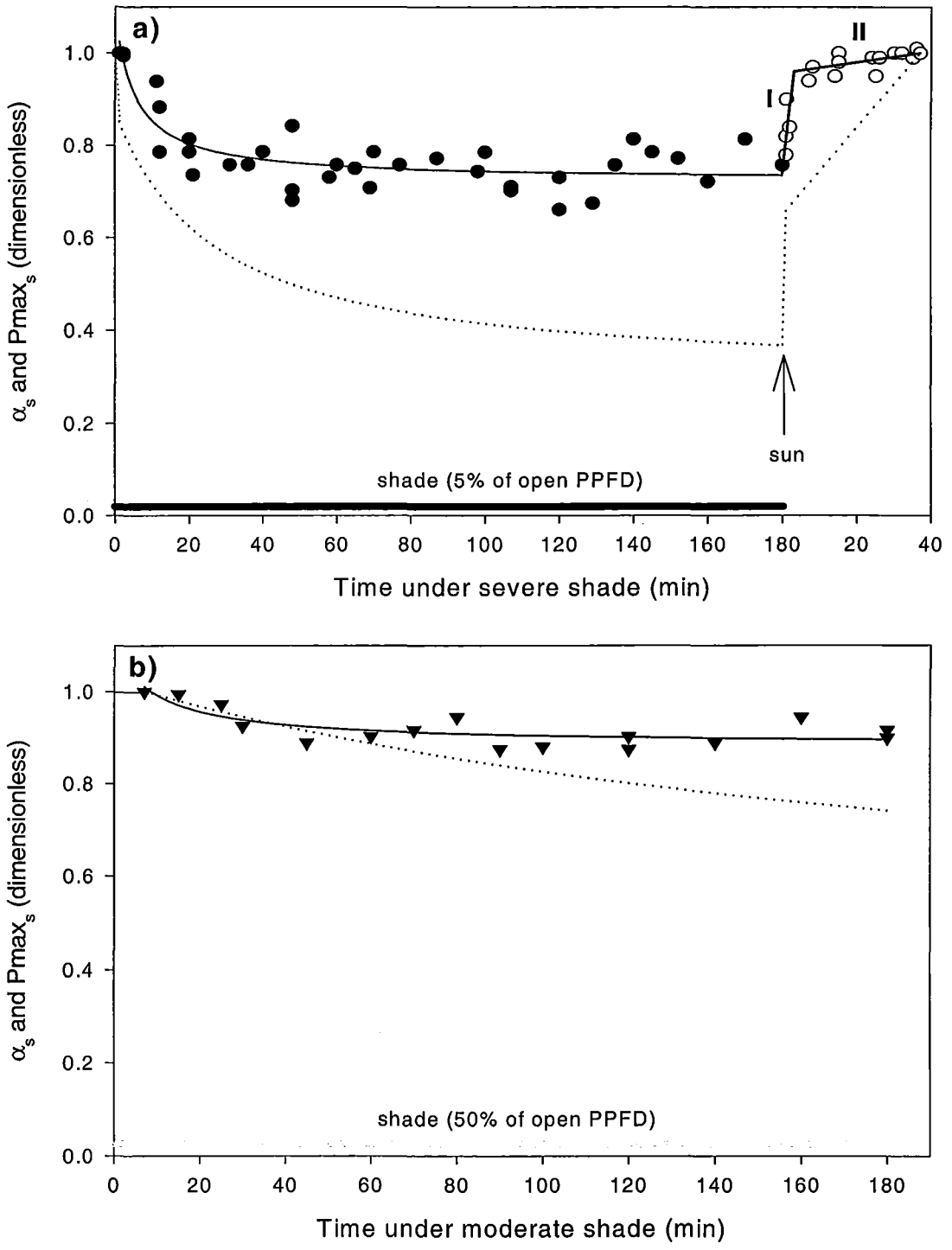

Figure 7.5 Time course of standardised rate of photosynthetic efficiency (α_s) for cocksfoot grown under field conditions in response to:
(a) severe shade (•) at 85-95 μmol m^{-2} s^{-1} PPFD and time courses of the increase in α_s during induction (○) in full sunlight (1700-1900 μmol m^{-2} s^{-1} PPFD). The fitted exponential decay function from Equations 7.6, the two part (*phases I and II*) fitted "broken stick" (Equation 7.8) model for α_s induction and the function for $Pmax_s$ (‴) are indicated.
(b) moderate shade (▼) at 850-950 μmol m^{-2} s^{-1} PPFD. The fitted exponential decay function from Equations 7.7 and the function for $Pmax_s$ (‴) are indicated.
Note: α_s= 1 corresponds with α= 0.036 μmol CO_2/μmol PPFD.

7.3.6 Degree of curvature (θ) and temperature, N, water stress, regrowth duration and shade

There was no relationship between θ and any of the environmental and regrowth duration variables (Figures 7.6a-f) with a mean value of 0.96 ±0.02.

7.3.7 Empirical model for photosynthetic efficiency (α) in cocksfoot leaves

The five individual empirical "broken stick" and non-linear functions of the main factors affecting α_s (Equations 7.2, 7.3, 7.4, 7.5 and 7.6) were tested by a simple multiplicative model (Equation 7.9) when more than one factor was constrained. For each function $\alpha_s = \alpha_{sp} = 1.0 \equiv 0.036$ CO_2/µmol PPFD and this indicates the factor was non-limiting. At $\alpha_s = 0$ photosynthetic efficiency was zero ($\alpha = 0$) and thus no photosynthesis was occurring.

$$\alpha_s = \alpha_{sp} * [f(T) * f(N) * f(W) * f(R) * f(Shade)] \qquad \textbf{Equation 7.9}$$

Where α_{sp} represents the potential or maximum α_s units for individual leaves, and is equivalent to α in non-limiting conditions.

Simulated results for the multiplicative model of α_s were compared with 46 data points (Figure 7.7) collected during the trial period when four or all five factors were outside their determined optimum range. The average value of the RMSD (0.19) was about 30% of the mean observed α values and α was underestimated for all points in the observed range. This was confirmed by residuals analysis giving a mean positive value of 0.17. The results of this validation indicated that the reduction in α when more than one factor was limiting did not follow a multiplicative form (Figure 7.7).

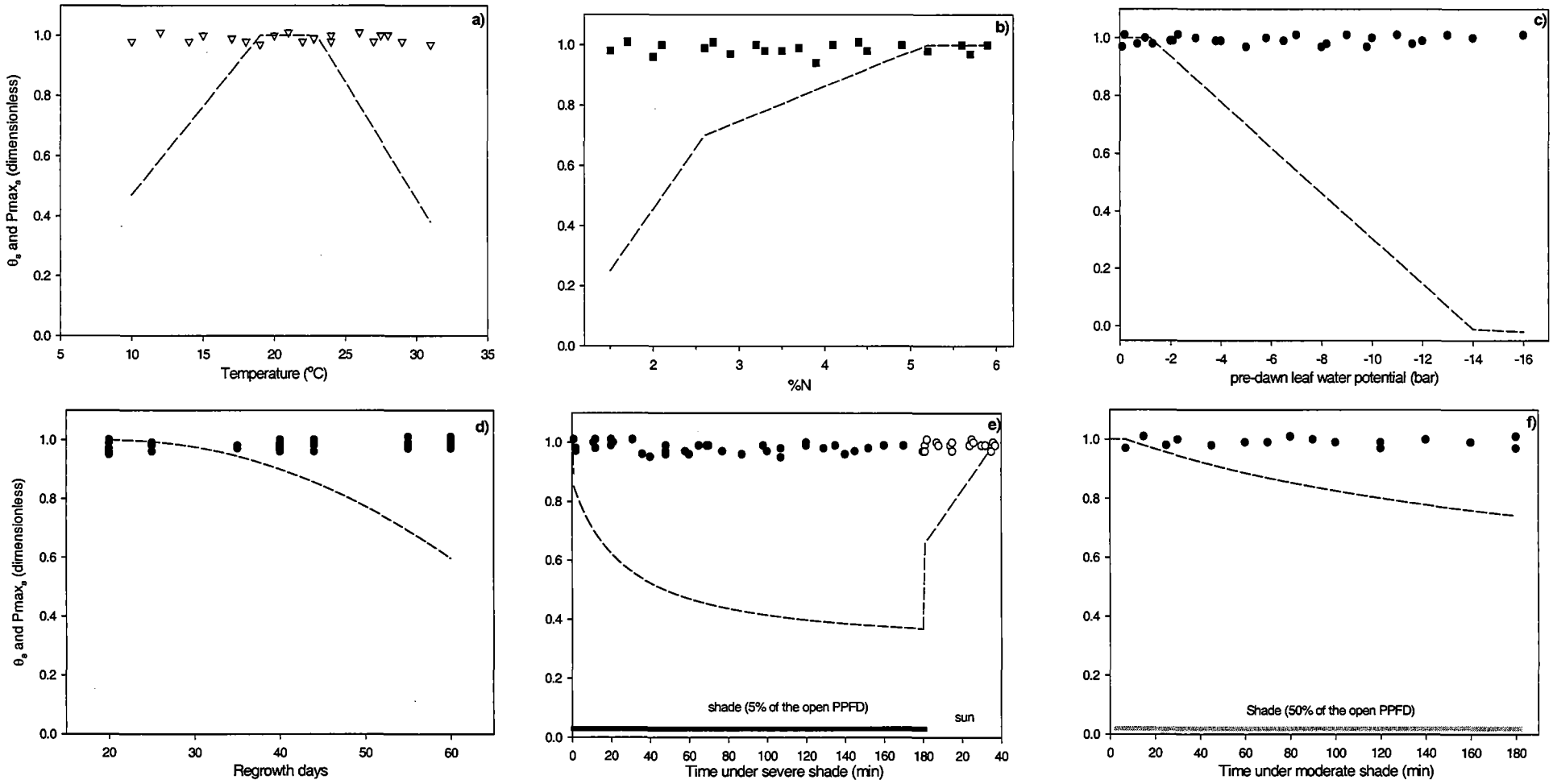

Figure 7.6 Standardised degree of curvature (θ_s) of the leaf light curve response against a) temperature, b) herbage N content, c) water status, d) regrowth duration, e) time course under severe shade (85-95 μmol m^{-2} s^{-1} PPFD) and its induction process, and f) time course under moderate shade (850-950 μmol m^{-2} s^{-1} PPFD). The maximum θ value was 0.98 (θ_s= 1).

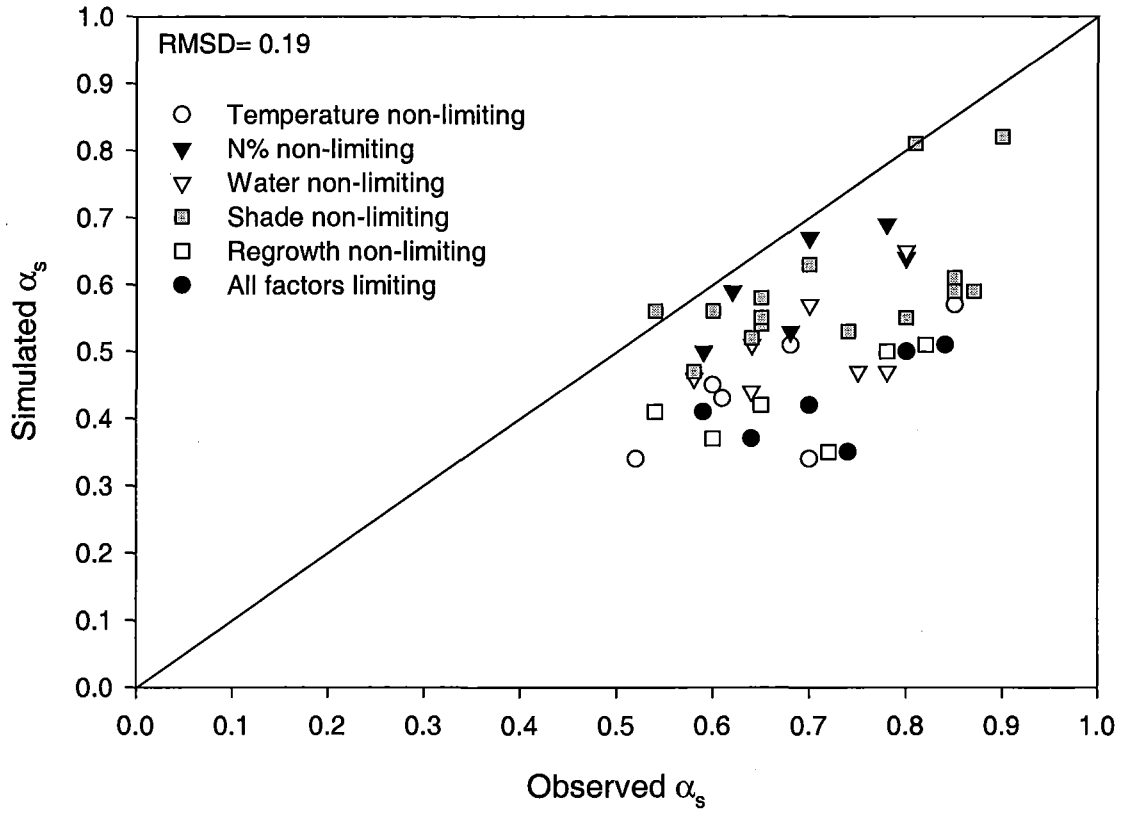

Figure 7.7 Simulated versus observed standardised rate of photosynthetic efficiency (α_s) sorted by five groups: temperature non-limiting, herbage nitrogen content non-limiting, water non-limiting, regrowth duration non-limiting, shade non-limiting and all factors limiting for cocksfoot leaves grown in field conditions. Simulated data was based on the multiplicative model proposed in Equation 7.9.

In contrast, when the five individual functions of the main factors affecting α_s, were tested in a 'law of the minimum factor' model (Equation 7.10), α_s was adequately simulated.

$$\alpha_s = \alpha_{sp} * [f(T) \text{ or } f(N) \text{ or } f(W) \text{ or } f(R) \text{ or } f(Shade)]_{min} \qquad \textbf{Equation 7.10}$$

Simulated results for this model were then compared with the original validation set (Figure 7.8a) and showed that the value of the RMSD (0.08) decreased from 30 to 12% of the mean observed α_s values. Regression analyses of residuals for each factor combination were used to detect the possibility of any interactions between factors (as described in Section 4.3.7). There was no significant interaction ($\beta = 0$) for temperature, N, water, shade and all factors (Figure 7.8b). Most of the residuals (82%) were less than ±0.10 units from

the predicted α_s, evenly distributed across the predicted range, and with a mean value close to zero (-0.005). This indicated acceptable accuracy for these situations.

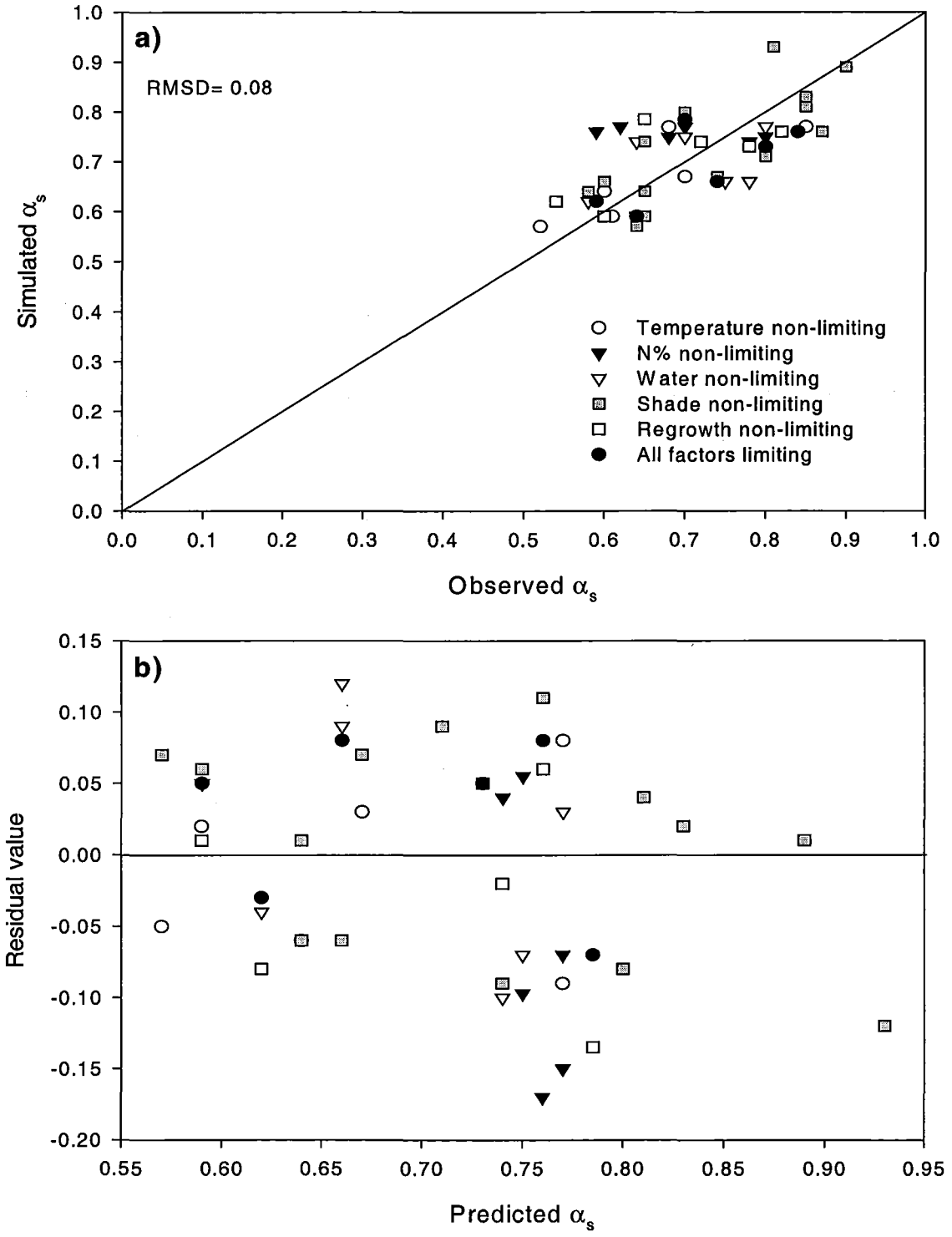

Figure 7.8 a) Simulated versus observed standardised rate of photosynthetic efficiency (α_s) and b) residuals of α_s against predicted values. Data sorted by five groups: temperature non-limiting, herbage nitrogen content non-limiting, water non-limiting, regrowth duration non-limiting, shade non-limiting and all factors limiting for cocksfoot leaves grown in field conditions. Simulated data was based on the 'law of the minimum factor' model proposed in Equation 7.10.

7.4 Discussion

7.4.1 Model accuracy

The variation in α measured for the range of environmental and management factors reported in this study indicates that a constant value of α was inappropriate and should not be used in a canopy photosynthesis model for predicting pasture growth. The 'law of the minimum factor' model (Equation 7.10) resulted in the development of an empirical model, which accurately predicted α for a wide range of temperature, N, water status, regrowth duration and shade conditions. Validation of the model indicated approximately 88% of the variation in α was accounted for using these five factors as single functions without recourse to interactions. This confirms that the rate of α was controlled by the most limiting factor when temperature, N, water status, regrowth duration and/or shade were limiting.

The individual factor responses also provide a basis for varying the RUE response across a range of environmental conditions. Factors that decrease α also lower RUE (Sinclair and Muchow, 1999). High values of α maximise RUE particularly when most leaves of the canopy are receiving low irradiance. Canopy architecture determines the distribution of irradiance over the photosynthetic surfaces and hence, relative to the leaf α, the possibility for high canopy RUE. This also becomes important in silvopastoral systems where low irradiance is imposed by the tree shade. Therefore the proposed model could also be used for calibrating models which utilise RUE to predict DM production.

The individual functions for temperature, N%, water status, regrowth duration and shade were empirically derived and summarised into easily transferable coefficients using "broken stick" or non-linear regressions. The success of this approach for predicting α is reliant on these relationships holding in environments outside those from which they were derived. To confer repeatability, they must have a biologically meaningful basis and should be consistent with previous reports based on single factor analysis for cocksfoot.

The maximum value found for α for cocksfoot leaves in non-limiting conditions in this study was 0.036 μmol CO_2/μmol PPFD or 0.0069 mg CO_2 J^{-1} (α_s= 1). This is consistent

with Thornley (1998) who reported for grasslands in general an optimum value of 0.0063 mg CO_2 J^{-1}.

Furthermore, the decline of *Pmax* was always more marked than the decrease in α for all the factors studied, indicating that *Pmax* was affected more by the physical (e.g. reduction in stomatal conductance) and biochemical limitations of the photosynthetic process than α. The differential effects of environmental factors on *Pmax* and α agrees with those values reported by Marshall and Biscoe (1980b) for winter wheat and Thornley (1998) for grasslands in general.

7.4.2 Temperature function for α

The decrease in α of 2.8% per °C above 24 °C (Figure 7.1) was greater than those reported by Thornley (1998) for grasslands in general where α decreased by about 1.5% per °C at temperatures above 15 °C. Ku and Edwards (1978) also reported a decrease of 8% in α for wheat when the temperature was increased from 15 to 25 °C. This inconsistency in the response of α could be caused by a differential rate of photorespiration between species. In general, the photorespiration rate of non-N limited and irrigated leaves increases with temperature (Bull, 1969). This is consistent with Ehleringer and Björkman (1977) and Ehleringer and Pearcy (1983) who reported that photorespiration was the main cause of the reduction in α for C_3 grasses and declined from 0.06 mol CO_2 mol^{-1} PPFD at 20 °C to 0.04 mol CO_2 mol^{-1} PPFD at 36 °C. In addition, Hay and Walker (1989) suggested that high temperature affects the carboxylase activity of the enzyme, which could lead to a decreased in α.

7.4.3 Nitrogen function for α

The response of α to N showed that 4.0% N content was a critical value below which α started to decrease at 0.061 μmol CO_2/μmol PPFD per 1% N (Figure 7.2). In contrast, for *Solidago altissima* L. leaves, Hirose and Werger (1987b) reported that α decreased linearly with a decline in N content at 0.0188 μmol CO_2/μmol PPFD per g N m^{-2}. Connor *et al.* (1993) reported no detectable change of α (mean 0.05 mol CO_2 mol^{-1}) in sunflower (*Helianthus annuus* L.) leaves for a range of N contents between 0.63 and 5.0%. This difference in the responses of α to N could result from a differential concentration of N compounds that affect α. The N compounds likely to cause changes in α are the soluble proteins and predominantly enzymes involved in CO_2 fixation and regeneration of the CO_2

acceptor molecule ribulose 1.5-bisphosphate, and the compounds located in the chloroplast, including chlorophyll, associated with the light reactions (Grindlay, 1997). The carboxylation rate depends on the amount of active enzyme present and any limitation imposed by substrate concentration and therefore of the N content (Seemann *et al.*, 1987; Evans, 1989). In this study, chlorophyll content varied positively with herbage N content and ranged from 0.05 g m^{-2} at 1.5% N to 0.96 g m^{-2} above 5.5% N (Figure 4.4, Section 4.3.3). However, the chlorophyll content at 4% N, when α started to decrease, was 0.60 g m^{-2}. This can be interpreted as giving a greater capacity for light absorption and increasing α per unit leaf area.

7.4.4 Water status function for *α*

There was a negative linear relationship between α and the water status of the plants but only from severe water stressed situations (Figure 7.3). From ψ_{lp}= -10 bar to the maximum water stress measured in this experiment of -16 bar, α decreased 29%. In contrast, Thornley (1998) reported water stress had a theoretical small effect on α with a maximum reduction of 8% at a leaf water potential of –50 bar. Similarly, Jones *et al.* (1980) found only 6% difference between irrigated and water stressed (daily minimum leaf water potential of –20 bar) perennial ryegrass (*Lolium perenne* L.) swards. In the present study, it is likely that ψ_{lp} of –16 bar, at which α was minimum, fell progressively during the day reaching a higher maximum negative value at noon (when the radiation and temperature are highest) than those reported by Jones *et al.* (1980). In addition, a more significant effect of water stress was reported for alfalfa (*Medicago sativa* L.) by Antolín and Sánchez-Díaz (1993) who found that α decreased by 75% in plants with ψ_{lp}= –26 bar.

This reduction in *α* for plants under severe water stress (ψ_{lp}< -10 bar) would represent evidence of non-stomatal limitation. It is likely that recovery from reduced *α* values in severe water stress situations might be slower than when *Pmax* values are reduced without concomitant changes in *α*. Severe levels of water stress can decrease the rate of net photosynthesis per unit leaf area by reducing the activity and concentration of RuBP carboxylase (Section 2.3.1.3). Kaiser (1987) suggested that dehydration (less than 70% relative water content or more than 70% decrease in cell volume) can directly affect *α* by inhibition of two carboxylating enzymes activities (RuBP carboxylase and phospho-enolpyruvate carboxylase). A similar mechanism may explain these results for cocksfoot.

7.4.5 Regrowth duration function for α

No significant decline in α occurred from day 20 to 40 after which α declined by up to 17% after day 60 of regrowth (Figure 7.4). Sheehy (1977) found that α of the youngest fully expanded leaf of perennial ryegrass declined between days 15 and 35 of regrowth from 0.019 to 0.014 mg CO_2 J^{-1}.

The decrease of α with days of regrowth may be related to an ageing effect (Section 5.4.2.1). This was confirmed by Marshall and Biscoe (1980b) who reported that for flag leaves of winter wheat α was unaffected by leaf age from 4 to 40 days after full elongation, but a reduction of 4 μg CO_2 J^{-1} in α was observed during the period 52-57 days after full elongation.

The decrease of α with regrowth time could be related indirectly to variation in N and chlorophyll content in leaves (Section 5.4.2.2). In this experiment leaf N content decreased 36% from 10 to 60 days regrowth (Figure 5.4, Section 5.3.3) and the chlorophyll content per unit of area of consecutive youngest expanded leaves decreased from 0.96 g m^{-2} at day 20 to 0.60 g m^{-2} at day 60 of regrowth (Figure 5.5, Section 5.3.4). However, this corresponded with a decline from 5.9 to 4.0% N, a range over which α was constant (Figure 7.2). Thus, the decline in older leaves is more likely to be associated with other changes taking place in the leaves. Lawlor *et al.* (1989) reported that the decrease in carboxylation activity of non-limiting N flag leaves of winter wheat from 1.5 μmol CO_2 m^{-2} s^{-1} Pa^{-1} at full expansion to 0.1 μmol CO_2 m^{-2} s^{-1} Pa^{-1} after 53 days was due to a decrease in total RuBisCO activity (from 110 to <10 μmol CO_2 m^{-2} s^{-1}) Similarly, Treharne and Eagles (1970) reported a decrease of 60% in RuBisCO activity of cocksfoot leaves from full expansion to 30 days of age and this seems the most likely explanation of the decrease in α observed in the present study.

7.4.6 Shade function for α

The photosynthetic efficiency α of individual cocksfoot leaves from high to low light intensities decreased as a function of the magnitude and duration of the PPFD level previously experienced (Figures 7.5a and 7.5b). The minimum value of α_s for plants grown at 5% of open PPFD was 20% lower than those grown at 50% of open PPFD. This was consistent with data found in controlled environment conditions by Charles-Edwards *et al.*

(1974) who reported for six populations of *Lolium* sp. a mean decrease in α of about 40% from 250 to 60 W m^{-2}.

The magnitude and the period required for reduction in α can depend on the deactivation of enzymes involved in carbon metabolism or on the effect on the pools of intermediates (Sassenrath-Cole and Pearcy, 1994; Pearcy *et al.*, 1996). In the present study, the non-stomatal limitation was 92% greater than the stomatal limitation after 10 minutes of severe shade (Figure 6.9a, Section 6.3.1.5) and for moderate shade there was almost no stomatal limitation after 30 minutes of shade (Figure 6.9b, Section 6.3.1.5). In comparison, the magnitude of the maximum non-stomatal limitation and the time required to reach this maximum value under severe shade was 2.5-fold greater than under moderate shade. This is consistent with the decrease in α_s which in the first 20 minutes after entering shade was 95% faster for plants grown at 5% of open PPFD than for those grown at 50% of open PPFD (Figures 7.5a and 7.5b). Sassenrath-Cole and Pearcy (1994) reported a deactivation of RuBisCO and FBPase (fructose-1,6-bisphosphatase) activities at low PPFD (35 µmol m^{-2} s^{-1}) for soybean leaves. In this work, the authors have reported that after 5 minutes at low PPFD, the FBPase activity was insufficient to support the maximal light-saturated rate of photosynthesis and that RuBisCO activity declined more slowly, retaining half-maximal activity after 20 minutes at low PPFD. A similar mechanism may explain these results for cocksfoot.

For full induction (α_s= 1) of cocksfoot leaves exposed to 180 minutes of severe shade required 37 minutes of full sunlight (Figure 7.5a). Pearcy *et al.* (1996) reported that the period required for full induction is dependent on the need to activate the enzymes involved in carbon metabolism and the need for adequate pools of intermediates to be built up to allow adequate rates of catalysis. A fast phase activates rapidly as PPFD increases, and is associated with limitations in ribulose 1,5-bisphosphate (RuBP) regeneration during the first 1-2 minutes of induction (Sassenrath-Cole and Pearcy, 1992). However, limitations of enzymes in this part of the carbon reduction cycle by the light activation state are most evident after relatively short low-light periods (<5 minutes) when the other limitations have not yet developed. After long periods in low PPFD, this fast phase may be masked by other slower limitations consisting of the light-activation requirement for RuBisCO (Pearcy *et al.*, 1996). The results of this study indicated that the response of α

during induction was rapid (α_s= 0.96 after 3 minutes) which increases the efficiency of use of the period of full sunlight.

In addition, comparisons among species from sun or shade environments have shown no differences in α (Section 2.3.1.6). Thus, at low PPFD, the photosynthetic apparatus appears remarkably capable of using the majority of absorbed photons for photochemistry, independently of the light environment in which plants were grown or any genetic adaptation to sun and shade environments. Therefore, in the absence of stress, the maximum α of sun- and shade-adapted species, or of plants of a species acclimated to different light environments, are similar. This contrasts with results found in this study where cocksfoot plants grown for 10-11 years under shade from radiata pine had a reduced value of α compared with plants grown only in full sunlight over the same period. The implication is that factors other than adaptation affected α under severe shade in this study.

The physiological explanations for the effects of the five factors on α are summarised in Table 7.1.

Table 7.1 Summary of the effect and biological impact of temperature, N, water stress, regrowth duration and shade on α.

Factor	**Function**	**Maximum α range**	**Minimum α values**	**Biological impact**
Air temperature	Two stage linear	10 to 24 °C	31 °C	Increase photorespiration.
Leaf N%	Two stage linear	4.0 to 5.9% N	1.5% N	N increases enzyme activity (RuBisCO), then carboxylation rate. N increases chlorophyll content, then light reactions.
Leaf ψ_{lp}	Two stage linear	-0.1 to –10.0 bar	- 16.0 bar	Severe water stress increases mesophyll resistance and decreases enzyme activity.
Regrowth duration	Quadratic	20 to 40 days	60 days	Ageing process decreases carboxylation activity.
Time under shade	Exponential decay	0 to 1 min	From 60 min	Shade deactivates RuBisCO and FBPase activities.

7.4.7 The degree of convexity θ

The degree of curvature of the leaf response curve θ was unaffected by the range of temperature, N, water status, regrowth duration and shade studied (Figure 7.6) and had a mean value of 0.96 ±0.02. Similarly, Thornley (1998) reported for grasslands in general a constant value of 0.95 and Weir *et al.* (1984) reported a constant value of 0.995 was used for the AFRC wheat model. Marshall and Biscoe (1980b) reported no trend with leaf age of wheat remaining in the range 0.85-0.99. In contrast, Hirose and Werger (1987b) reported that increasing tissue N, θ decreased from 0.9 (leaf N of 0.8 g m^{-2}) to 0.6 (leaf N of 2.0 g m^{-2}) and Stirling *et al.* (1993) reported for maize that θ decreased from 0.95 to 0.75 when temperatures fell below 10 °C.

To understand the θ values presented in this work, it is important to refer to Marshall and Biscoe (1980a) and Thornley and Johnson (2000) who describes θ as the ratio of physical to total resistance to CO_2 transfer.

$$\theta = \frac{r_p}{(r_p + r_x)} \qquad \textbf{Equation 7.11}$$

Where r_p is the physical resistance and r_x is the carboxylation resistance.

Thus, if θ is zero, which implies that carboxylation resistance is much greater than physical resistances, then Equation 7.1 is reduced to a rectangular hyperbola. In contrast, when θ is close to unity the opposite occurs. Thus, for $\theta = 1$, the photosynthetic rate at the reaction sites increases linearly as irradiance increases until photosynthesis is limited by the diffusion of CO_2 from the air (Thornley and Johnson, 2000). As θ is the ratio of r_p to (r_p + r_x) then (1- θ) is the ratio of r_x to (r_p + r_x) and for cocksfoot has a mean value of 0.035 over the range of environment and regrowth duration factors included in this study. This ratio implies that r_x is approximately 3.5% of the total leaf resistance to CO_2 transfer and that it does not change substantially under the changing conditions used in this study. Similarly, Marshall and Biscoe (1980a) reported that r_x for leaves of *Phaseolus vulgaris* L. was approximately 2% of the total leaf resistance to CO_2 transfer.

7.5 Conclusions

• Temperature, herbage N%, leaf water status, regrowth duration and shade of cocksfoot plants modified the utilisation of solar energy for the photosynthetic activity in leaves through their effect on α. Generally, the extent over which α was affected was less than *Pmax*. In contrast, the degree of curvature of the leaf response curve θ was unaffected by for the range of the five factors studied.

• The 'law of the minimum factor' model explained about 88% of the variation in α for individual leaves of cocksfoot when one or more than one factor was constrained. Thus, α as a potential input variable into canopy photosynthesis models to predict growth in pastures in silvopastoral system, was satisfactorily predicted using the five main environmental and management variables examined in this study.

In the next chapter, the values of α and θ derived in this chapter, together with the leaf photosynthesis models based on *Pmax* (Chapters 4, 5 and 6), will be incorporated into a canopy photosynthesis model for predicting cocksfoot production in silvopastoral systems.

CHAPTER 8

Simulation and validation of a canopy photosynthesis model for cocksfoot under different nitrogen, water, temperature, regrowth duration and shade regimes

8.1 Introduction

Canopy photosynthesis models, used to predict growth, have frequently been based on the amount of light intercepted by leaf surfaces (dependent upon LAI and canopy architecture) at different depths in the canopy. Therefore, the resulting level of photosynthesis of those leaves, and the subsequent partitioning of photosynthates to growth and respiration is the basis for DM production (Section 2.3).

In a silvopastoral system, there is an added complication of fluctuating light regimes in addition to the impact of environmental (temperature, N and water stress) and management (regrowth duration) factors on canopy photosynthetic rates. To date, the influence of these factors on cocksfoot canopy photosynthesis and pasture production in silvopastoral systems has not been defined. Therefore, the aim of the research presented in this chapter is to predict pasture growth rates and DM production for the Lincoln University silvopastoral system (Chapter 3) using the physiological basis outlined in Chapters 4 to 7. Specifically, the first part of the chapter reports on simulations of net canopy photosynthesis for cocksfoot under different environmental and management conditions. This is done by integrating the leaf photosynthesis models developed for *Pmax*, α and θ (Chapters 4, 5, 6 and 7) into a canopy photosynthesis model. Initially, the effects of temperature, water status, N%, regrowth duration and shade (intensity and light regimes) on cocksfoot daily canopy photosynthesis is examined when any one of these factors was limiting.

In the second part of this chapter, the canopy photosynthesis model is used to predict DM for the cocksfoot pastures grown under a diverse range of environmental and management situations in the open and under trees. These predicted values are compared with observed

values reported in Chapter 3. To predict DM production, the main canopy characteristics affecting light interception (LAI and leaf angle) from the field measurements (Chapter 3) were incorporated with the leaf photosynthesis models (*Pmax*, α and θ), into the canopy photosynthesis model. The field data provide a framework for testing the primary objective of this thesis, which was to predict actual growth rates and DM production in a silvopastoral system using a semi-mechanistic mathematical model.

Therefore, the objectives of the research outlined in this chapter are to:

1) simulate net daily canopy photosynthesis rates incorporating the leaf photosynthesis models (*Pmax*, α and θ) into a canopy photosynthesis model when one environmental or management factor was limiting.

2) determine the optimum net canopy photosynthesis and LAI for each environmental and management variable enounced in 1;

3) propose biological explanations for the simulated response of net canopy photosynthesis to the factors enounced in 1;

4) validate the canopy photosynthesis model against observed DM data obtained from cocksfoot pastures grown under a diverse range of environmental and management situations in open conditions and in the silvopastoral system.

8.2 General description of the canopy photosynthesis model

The mathematical model of canopy photosynthesis consists of four steps:

1) calculation of leaf light distribution and interception at different canopy depths;

2) calculation of gross canopy photosynthesis incorporating variations in photosynthetic capacity of individual cocksfoot leaves for a wide range of temperature, N, water status, regrowth duration and shade conditions;

3) calculation of total respiration;

4) calculation of net canopy photosynthesis.

The canopy photosynthesis model was based on previous mathematical models developed by other authors (Section 2.3) and adapted by Varella *et al.* (2002) for fluctuating light regimes. This model was improved by incorporating the final multiplicative model for *Pmax* (Section 6.3.2.5) and the 'law of the minimum factor' model for α (Section 7.3.7). A diagrammatic representation of the canopy photosynthesis model used to predict DM production is given in Figure 8.1.

8.2.1 Light interception

The actual PPFD of light received by each individual leaf must be known to estimate its photosynthetic rate. The penetration of direct sun light rays into a canopy is a function of the leaf area and angle, and the solar elevation above the horizon (Equation 8.1). The incident intensity of PPFD on an area of leaf at the level Z in the canopy (Iz) is calculated based on mathematical equations developed by Wilson (1960). Their F'/F ratio, which calculates the probability of contact of a given leaf angle by an inclined needle based on the theory of inclined point quadrats, is also the ratio between the actual area of a leaf (F) and the shadow it would cast (F') in the context of light rays. Consequently, the light from a source (i.e. sun light rays) penetrating a layer of leaves in a canopy would be a function of the area of shadow each leaf can cast. The mathematical equation proposed by Wilson (1960) is then corrected to estimate the sunlit area of the foliage canopy by considering leaf angle and solar elevation angle (Duncan *et al.*, 1967) (Equation 8.1).

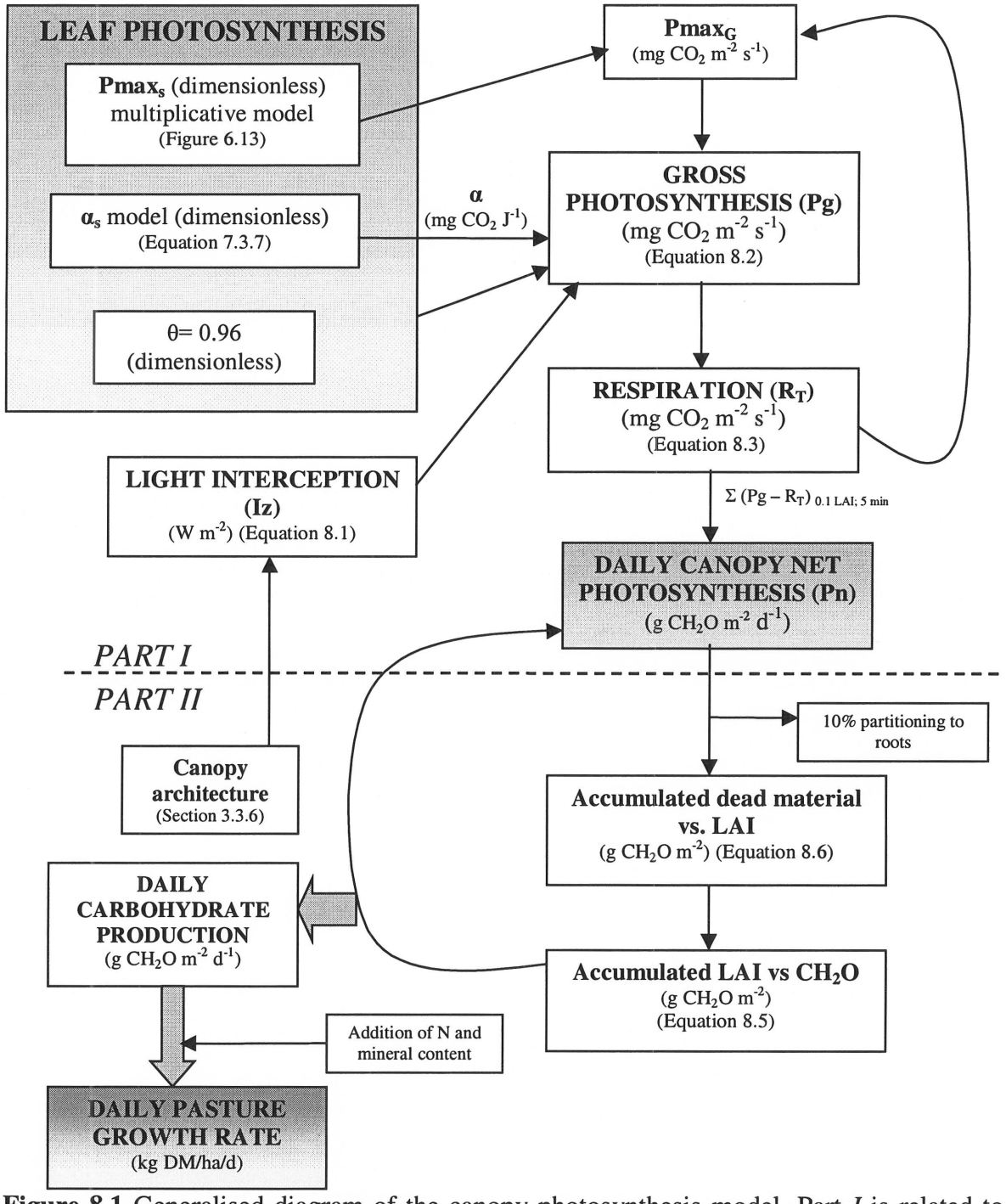

Figure 8.1 Generalised diagram of the canopy photosynthesis model. Part *I* is related to simulations of net daily canopy photosynthesis (*Pn*) for cocksfoot in different environmental and management conditions calculated from canopy gross photosynthesis (*Pg*), light interception (*Iz*) and total respiration (R_T) every 0.1 units of LAI for each 5 minutes during a day. Part *II* (together with Part *I*) is related to validation of simulated values from the canopy photosynthesis model against observed DM data obtained for cocksfoot pastures grown under a diverse range of field conditions. $Pmax_s$ represents the maximum standardised saturated leaf photosynthetic rate and $Pmax_G$ represents values of *Pmax* adjusted by respiration for inclusion in Equation 8.2; α is the initial slope of the light response curve or the photosynthetic efficiency; θ is a dimensionless parameter indicating the degree of curvature of the photosynthesis curve.

$$I(z) = I_o e^{-(LAI*[F'/F]_{\gamma,\beta}/sin\beta)} = I_o e^{(-LAI*K)}$$

$$[F'/F]_{\gamma,\beta} = \cos\gamma * \sin\beta \quad \text{if } \gamma \leq \beta$$

$$[F'/F]_{\gamma,\beta} = \sin\beta * \cos\gamma * [1 + 2/\pi\,(\tan\Phi_o - \Phi_o)] \quad \text{if } \gamma > \beta$$

Equation 8.1

Where *I(z)* is the incident PPFD on the leaf area at the level z in the canopy (W m^{-2}); *Io* is the incident PPFD above the canopy (W m^{-2}); LAI is the cumulative LAI down to level z (dimensionless); *F'/F* is the Wilson-Reeve ratio; γ is leaf angle (degrees); β is the solar elevation above the horizon (0 to 90° expressed in radians); Φ_o is the angle value between 0 and 90° which satisfies the relationship $\cos\Phi = \cot\gamma * \tan\beta$ expressed in radians; k is the extinction coefficient.

Equation 8.1 gives the area of light penetrating each foliage layer within the canopy. It is in the form of the equation for the Bourguer-Lambert-Beer's law and is equivalent to the equation described by Monsi and Saeki (1953) which uses the extinction coefficient 'k'. To calculate the area of sunlit leaves within each layer (*I(z)* above the layer), the area of sunlight emerging from each layer is subtracted from the area entering (*I(z)* below the layer). Equation 8.1 can also be used to calculate the penetration of diffuse light within the canopy, but total flux rather than the area is computed. Furthermore, Equation 8.1 is valid with the assumption that all leaves grow equally in all directions around the individual pseudo-stem, thus all leaves are randomly distributed in the horizontal strata.

For simulations, *I(z)* values for different layers within the canopy were calculated using Equation 8.1 for every 0.1 accumulative LAI, and every 5 minute interval of I_o. Values of I_o were obtained from quantum sensors installed above the coksfoot canopy and recorded every 5 minutes by a datalogger. Solar elevation angles (β) were calculated for a latitude of 43° 38'S and longitude of 172° 28' corresponding to the location of this trial (Lincoln, Canterbury, New Zealand). The maximum sun angle elevation is 69.8° on December 21st and the lowest maximum sun angle elevation is 23° on June 21st.

For all simulations in the first part of this chapter, *I(z)* was calculated by solving Equation 8.1. Input values of I_o were for a summer sunny day (around 21 December) and a canopy leaf angle of 68°. These were used to determine the effect of environmental and

management factors on canopy photosynthesis rate. The daily PPFD integral of a summer sunny day, described in Figure 6.1 (Section 6.2.1.1), had a maximum of 1800-1900 μmol m^{-2} s^{-1} PPFD around midday with positive quanta values from 5:30 to 20:00 h.

8.2.2 Canopy gross photosynthesis

As shown in Figure 8.1, the daily canopy gross photosynthesis was calculated based on the sum of leaf gross photosynthesis of component layers through the canopy. This is described by a non-rectangular hyperbola (Weir *et al.*, 1984; Marshall and Biscoe, 1980a,b; Thornley, 1998) (Equation 8.1).

$$Pg = \frac{[Pmax_G + \alpha I(z)] - \sqrt{[Pmax_G + \alpha I(z)]^2 - 4\theta\alpha I(z) Pmax_G}}{2\theta}$$

Equation 8.2

Where Pg is the gross photosynthesis (mg CO_2 m^{-2} s^{-1}) for each layer of the canopy; $Pmax_G$ represents the maximum saturated gross leaf photosynthetic rate (mg CO_2 m^{-2} s^{-1}); α is the initial slope of the light response curve or the photosynthetic efficiency (mg CO_2 J^{-1}); θ is a dimensionless parameter indicating the degree of curvature of the light response curve.

For $Pmax_s$ (Figure 8.1) values were obtained from the use of the final modified multiplicative model (Figure 6.14; Section 6.3.2.5). Because $Pmax_s$ values were obtained from net photosynthesis light curves, they were adjusted by respiration for inclusion in Equation 8.2 ($Pmax_G$) (Figure 8.1). Values of α_s were obtained from the use of the 'law of the minimum factor' model (Equation 7.10; Section 7.3.7). The standardised values of $Pmax_s$ and α_s were converted to mg CO_2 m^{-2} s^{-1} and mg CO_2 J^{-1}, respectively. For θ, a mean value of 0.96 (Section 7.3.6) was used for all predictions.

The rate of Pg was calculated for each layer of the canopy (every 0.1 LAI) for each 5 minutes during a day using Equation 8.2 and incorporating the $I(z)$ values calculated from Equation 8.1 (Figure 8.1). These Pg values were multiplied by the LAI of the layer to calculate the contribution to Pg per m^2 of ground and values for each layer were then summed to give canopy gross photosynthesis on a daily basis.

8.2.3 Canopy respiration and canopy net photosynthesis

The canopy respiration was calculated based on an equation proposed by McCree and Troughton (1966) and McCree (1970). Total respiration rate (R_T) was calculated from the sum of growth (R_G) and maintenance (R_M) respiration (Equation 8.3).

$$R_T = R_G + R_M = a\sum_{h=0}^{h=H} Pg_{(5min)} + bW2^{0.05(Tmax+Tmin)}$$

Equation 8.3

Where R_T is the total respiration (mg CO_2 m^{-2} s^{-1}); a is the growth respiration coefficient (dimensionless); H is the number of daylight hours; b is the maintenance respiration coefficient (day^{-1}); *Tmax* and *Tmin* are the daily maximum and minimum temperatures (°C), respectively; W is the dry weight of the canopy expressed in g CO_2 equivalents per m^2.

Growth respiration is a function of daily canopy gross photosynthesis and is expressed in mg CO_2 m^{-2} s^{-1}. The growth respiration coefficient was assumed to be one-quarter of the gross photosynthesis (a= 0.25) according to values reported by McCree and Troughton (1966) for white clover and Thornley (1998) for pastures in general. This value is comparable with the range reported by Robson *et al.* (1988) for perennial grasses (0.20-0.35).

Maintenance respiration is temperature sensitive and is a fraction of the whole pasture dry weight (Equation 8.3). In this experiment, the mean value of W was 6.4 g CO_2 equivalent m^{-2}, according to the conversion 1 g DM= 1.43 g of CO_2 (McCree, 1974).

Values of b have been reported to be dependent on the N content of leaves (Section 2.4). Using the linear relationship between b and N content proposed by Johnson *et al.* (1995), the maintenance respiration coefficient was 0.030 d^{-1} at 5.9% N and declined linearly at a rate of 0.052 d^{-1} per 1% foliage N down to 0.015 d^{-1} at 1.5% N.

The effect of water stress on b was taken into account using a dimensionless correction factor (f_w) proposed by Thornley (1998). Thus, the maximum maintenance respiration coefficient when foliage N content was non-limiting (b= 0.03 d^{-1}) was reduced by

multiplying *b* by the correction factor which decreased exponentially with water stress expressed as leaf water potential (Equation 8.4).

$$b = 0.03 * f_w$$

$$f_w = \left[e^{(18*\psi / 8314*293.15)}\right]^{20}$$

Equation 8.4

Where f_w is the correction factor (dimensionless, 0-1); ψ is the leaf water potential (kPa).

In this study, the coefficient *b* related to water status of the cocksfoot plants and ranged from 0.030 d^{-1} in well irrigated plants (ψ_{lp}= -0.1 to –1.0 bar) to 0.024 d^{-1} at a ψ_{lp} of -16.0 bar or severe drought.

The value of *b* used in Equation 8.3 was 0.03 d^{-1} for predictions of *Pn* at different air temperatures, regrowth durations and shade conditions.

For simulation of canopy photosynthesis, R_G was calculated as a proportion of *Pg* at 5 minute intervals and each 0.1 LAI layer. R_M was constant during a day but dependent on the dry weight of the canopy, temperature, N content and/or water stress status. Both, R_G and R_M were then summed and transformed to a daily basis and expressed as mg CO_2 m^{-2} d^{-1}.

Finally, *Pg* was reduced by subtracting R_T to give the net canopy photosynthetic rate (*Pn*) per day (mg CO_2 m^{-2} d^{-1}) (Figure 8.1). Also, 10% of this find value was subtracted from the daily simulated *Pn* as the contribution of assimilate partitioned to the roots (Johnson and Thornely, 1983).

8.3 Simulations

For all simulations in the first part of this chapter, non-limiting conditions for each individual factor were defined as: a constant temperature of 21 °C throughout the day; 5% N for herbage content; ψ_{lp}= -0.1 bar for leaf water status; and 20 days for regrowth duration. Except for the simulation of the effect of temperature on *Pn* (Simulation 1), R_M was calculated using a maximum and minimum temperature of 21 °C and 4 °C, respectively.

8.3.1 Simulation 1: Effect of temperature on net daily canopy photosynthesis (*Pn*)

The aim of the first simulation was to evaluate the effect of air temperature throughout a day on *Pn* when other factors were non-limiting.

To do this, *I(z)* was calculated by solving Equation 8.1 (Section 8.2.1).

Secondly, values of air temperature recorded by a datalogger at 5 min intervals were used as an input variable to calculate $Pmax_s$ according to Equation 4.7 (Section 4.3.7). Figure 8.2a shows, as an example, the diurnal variation of actual air temperature for three sunny days with a maximum temperature of: (i) 31 °C, which represents a summer day with a limitation in $Pmax_G$ and α due to high temperatures; (ii) 21 °C, which represents a spring day within the optimum temperature range; and (iii) 10 °C, which represents a winter day with a limitation in $Pmax_G$ from low temperatures.

For the spring day, $Pmax_G$ was predicted to increase from 0.15 mg CO_2 m^{-2} s^{-1} at 5:00 h to 1.21 mg CO_2 m^{-2} s^{-1} around midday when incoming radiation was maximal, and then declined during the afternoon (Figure 8.2b). In contrast, $Pmax_G$ for the summer day had a predicted minimum value at 13:00 h (0.57 mg CO_2 m^{-2} s^{-1}) coinciding with high incident radiation. Maximum $Pmax_G$ values were predicted during the morning (from 9:20 to 10:20 h) and again in late afternoon when temperatures were between 20 and 23 °C (Figure 8.2c). For a winter day, predicted $Pmax_G$ reached the highest value of 0.50 mg CO_2 m^{-2} s^{-1} from 12:30 to 13:30 h when temperature was 10 °C (Figure 8.2d).

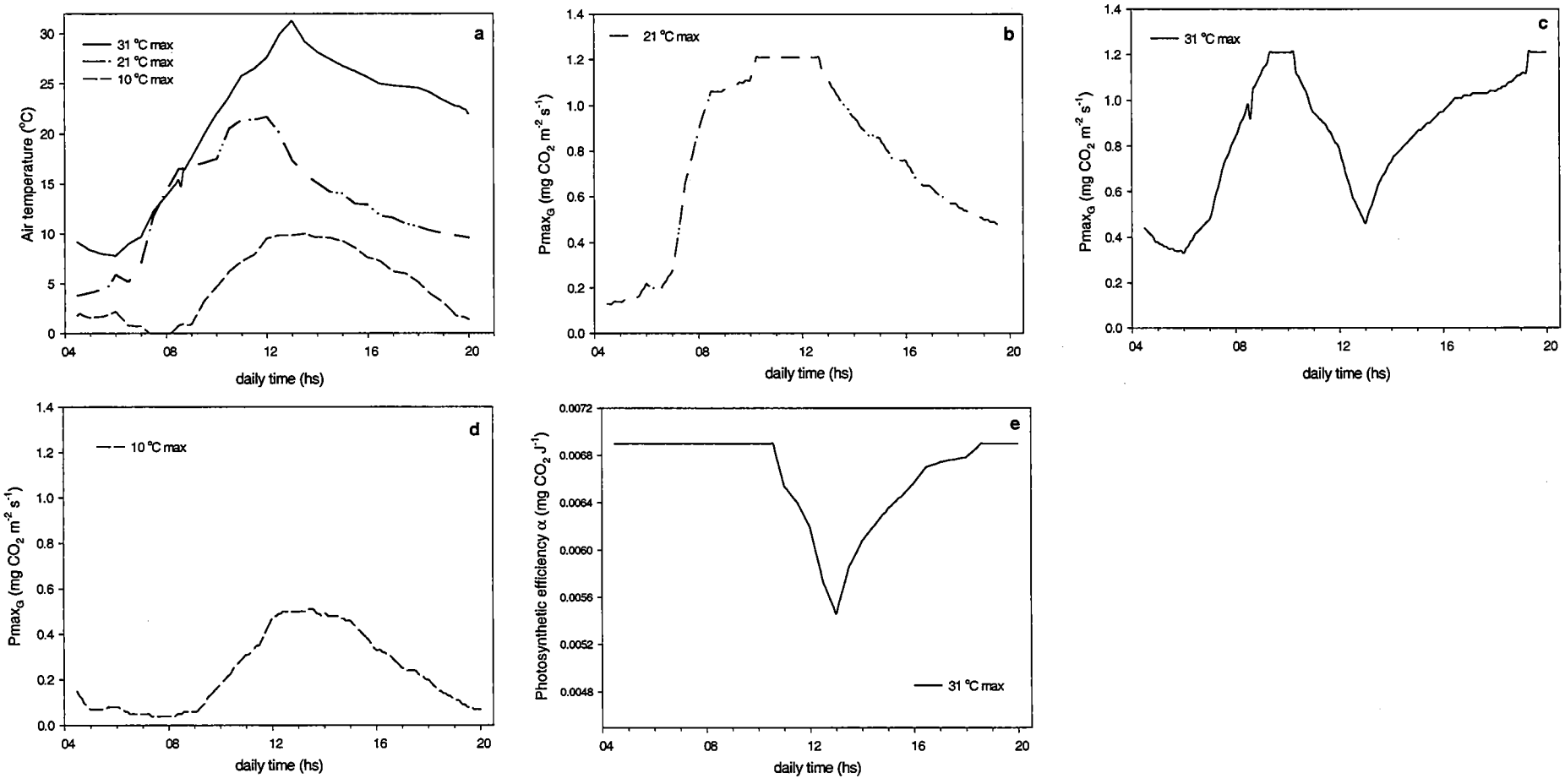

Figure 8.2 a) Diurnal course of air temperature measured on a spring (max. 21 °C), summer (max. of 31 °C) and winter (max. of 10 °C) day in Canterbury, New Zealand, and predicted diurnal course of maximum rate of leaf photosynthesis ($Pmax_G$) for cocksfoot grown under field conditions in response to those temperatures in spring (b), summer (c) and winter (d). (e) Change in photosynthetic efficiency (α) of cocksfoot leaves due to the changes in temperature shown in (a) for a summer day. Note: α did not vary for the spring or winter days with a constant value of 0.0069 mg CO_2 J^{-1}.

Similarly, the recorded values of air temperature at 5 minute intervals were used to calculate α_s using Equation 7.2 (Section 7.3.1). For air temperatures during a sunny spring and winter day (Figure 8.2a) α remained constant with an optimum value of 0.0069 mg CO_2 J^{-1}. In contrast, for the summer day, α decreased from 0.0069 mg CO_2 J^{-1} at 10:25 h to 0.0054 mg CO_2 J^{-1} at 13:00 h when temperature was 31 °C, and then increased reaching the maximum value again at 18:30 h when temperature was 24 °C (Figure 8.2e).

The calculated values of $Pmax_G$ and α were incorporated into Equation 8.2 to predict Pg for each 5 minute interval and for each 0.1 accumulated LAI.

Thirdly, the maximum and minimum temperature for each day were used to calculate R_M (Equation 8.3).

A total of 11 simulations were run with different maximum temperatures at midday on sunny days to evaluate the effect of actual air temperature on net canopy photosynthesis. The days were selected from sunny days throughout the years of this experiment (Section 3.2.3.1). This gave a range of maximum temperatures of 10, 12, 14, 16, 18, 21, 23, 25, 27, 29 and 31 °C. For each simulation the actual temperatures recorded for the day at 5 minute intervals were used as input data.

Simulations showed that *Pn* was affected by air temperature. In Figure 8.3, *Pn* values predicted from the canopy photosynthesis model that correspond to the three temperature regimes (Figure 8.2a), are presented. The *Pn* response was parabolic against LAI and increased to reach a maximum and then declined as LAI increased further. The changes in air temperature affected the maximum value and shape of the *Pn* response. For example, for the spring day (max. 21 °C) the maximum *Pn* was 30.8 g CO_2 m^{-2} d^{-1} at LAI= 3.8, and *Pn* was 16.3 g CO_2 m^{-2} d^{-1} at LAI= 9.5. In contrast, the maximum *Pn* for a summer day (max. 31 °C) was 15.5 g CO_2 m^{-2} d^{-1} at LAI= 2.3, and *Pn* was zero at LAI= 6.3. For the winter day (max. 10 °C), the maximum *Pn* value (18.3 g CO_2 m^{-2} d^{-1}) was reached at LAI= 5.

For every canopy LAI value, *Pn* varied according to temperature. For example, at LAI= 3, which represents a mean value during grazing periods in spring (Section 3.3.5.1), *Pn* was

30.3 g CO_2 m^{-2} d^{-1} for a maximum temperature of 21 °C compared with only half this at maximum temperatures of 10 and 31 °C.

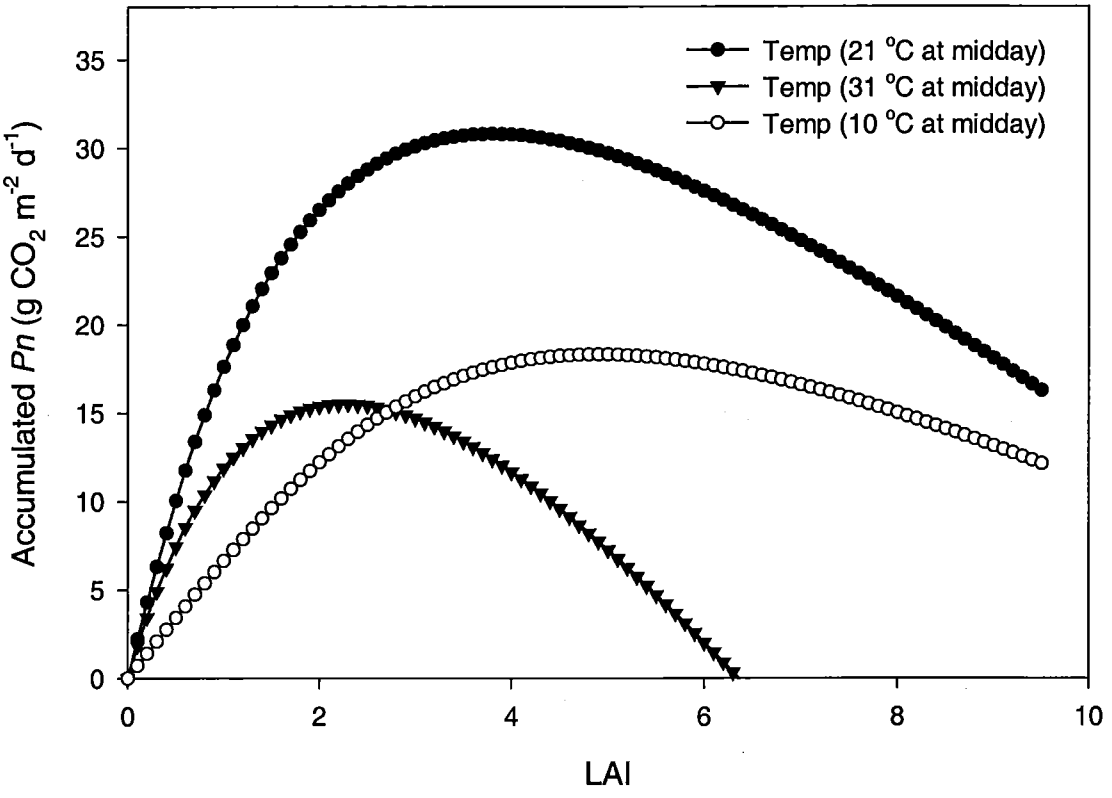

Figure 8.3 Predicted accumulated daily net canopy photosynthesis (*Pn*) against leaf area index (LAI) for measured changes in air temperature (presented in Figure 8.2a) for a cocksfoot pasture where other factors were non-limiting. Simulations of *Pn* were analysed for maximum diurnal temperature of 21 °C (spring day), 31 °C (summer day), and 10 °C (winter day).

The effect of the measured daily temperature regime on the maximum *Pn* (Pn_{max}) and the optimum LAI (LAI at Pn_{max}) is shown in Figure 8.4. Pn_{max} increased approximately linearly by 1.4 g CO_2 m^{-2} d^{-1} per °C, from 10 to 19 °C, then plateaued at 30.8 g CO_2 m^{-2} d^{-1} from 19 to 22 °C and finally declined by 1.0 g CO_2 m^{-2} d^{-1} per °C from 22 °C to 27 °C, and by 2.7 g CO_2 m^{-2} d^{-1} per °C from 27 to 31 °C. The optimum LAI declined with increasing air temperature from LAI= 5 at 10 °C to LAI= 2.3 at 31 °C, which gave a reduction rate for the optimum LAI of approximately 0.13 units of LAI per °C (Figure 8.4).

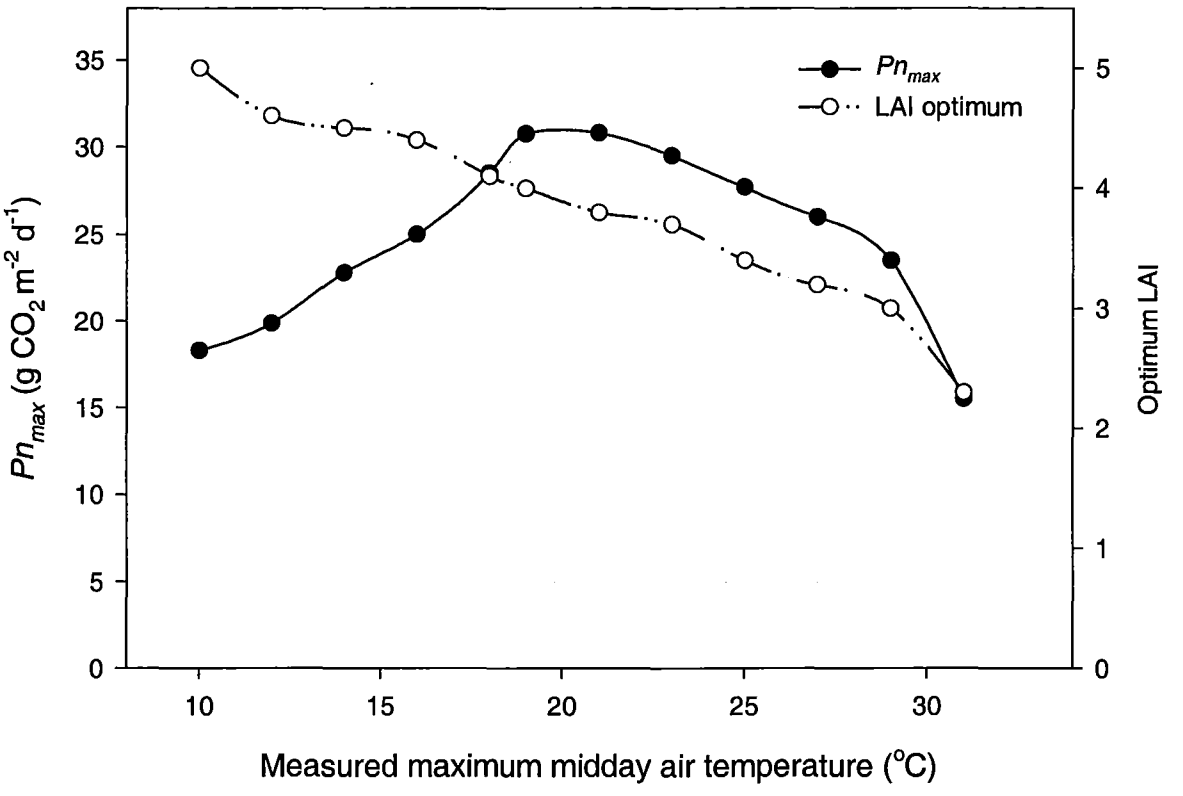

Figure 8.4 Predicted maximum daily net canopy photosynthesis (Pn_{max}) and optimum leaf area index (LAI) against measured maximum midday air temperature for a cocksfoot pasture where other factors were non-limiting.

8.3.2 Simulation 2: Effect of foliage N content on *Pn*

The second simulation evaluated the effect of foliage N content on *Pn* when other factors were non-limiting.

Values of *I(z)* were calculated as given in Section 8.3.1. $Pmax_s$ was calculated according to Equation 4.7 (Section 4.3.7) and α_s was calculated according to Equation 7.3 (Section 7.3.2). $Pmax_G$ and α were assumed to be state variables for each run, thus both variables were constant during the day. For example, simulated $Pmax_G$ values declined according to the three stage linear Equation 4.7 from 1.21 mg CO_2 m^{-2} s^{-1} at 5.2-5.9% N to 0.3 mg CO_2 m^{-2} s^{-1} at 1.5% N. Similarly, simulated α values decreased in a two stage linear way (Equation 7.3) from 0.0069 to 0.0039 mg CO_2 J^{-1} for 5.9-4.0% N and 1.5% N, respectively. The calculated values of $Pmax_G$ and α were then incorporated into Equation 8.2 to predict *Pg* at 5 minute intervals and for each 0.1 accumulated LAI.

The R_M was affected by foliage N content by changing the coefficient *b* in Equation 8.3

according to the negative slope of 0.052 d^{-1} per 1% N described in Section 8.2.3.

Twelve simulation runs corresponding to different measured foliage N% were simulated: 1.5, 1.8, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 4.7, 5.0, 5.5 and 5.9% N. In Figure 8.5, a representative selection of seven of these *Pn* values predicted from the canopy photosynthesis model, are presented. As for temperature, *Pn* followed a parabolic response against LAI. From 1.5 to 4.0% N the curves were almost parallel, but from 4.0 to 5.9% N *Pn* was higher until it reached the maximum value (at LAI< 2) and then declined more sharply than it did for the other family of curves. At LAI= 3, *Pn* ranged from 10.9 g CO_2 m^{-2} d^{-1} at 1.5 %N to 32.3 g CO_2 m^{-2} d^{-1} at 5.9 %N.

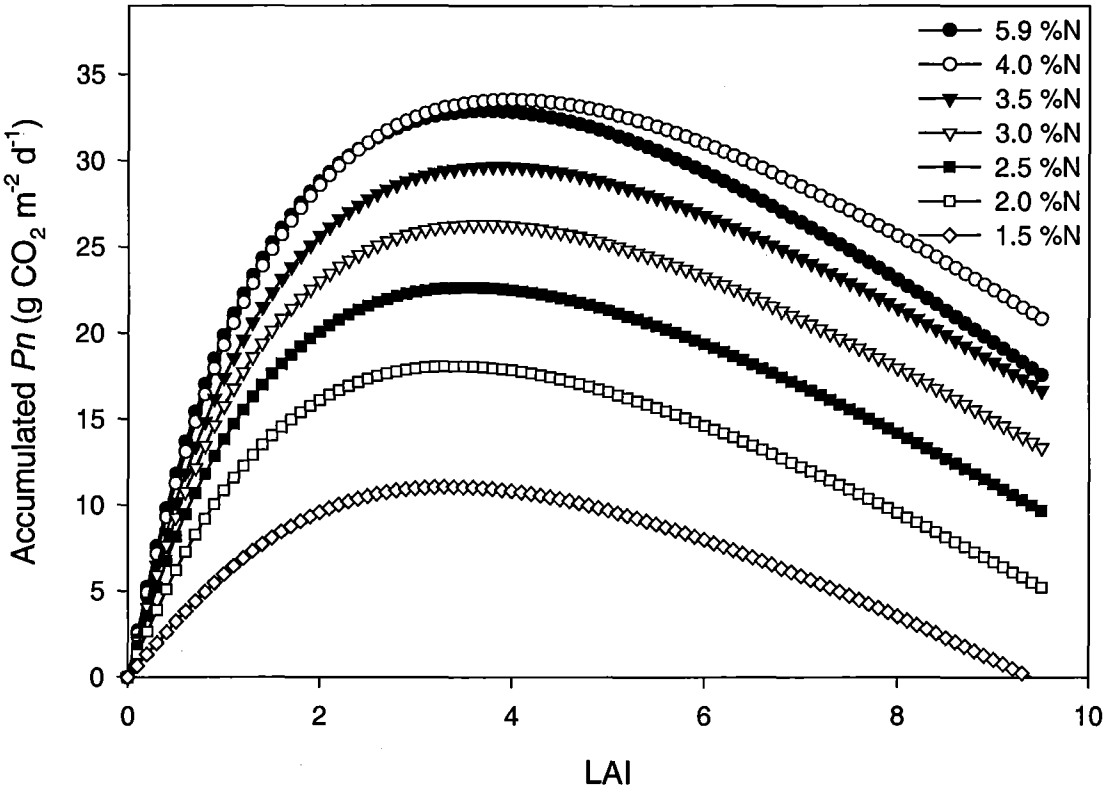

Figure 8.5 Predicted accumulated daily net canopy photosynthesis (*Pn*) against leaf area index (LAI) for different measured foliage nitrogen percentages for a cocksfoot pasture where other factors were non-limiting.

The main effect of foliage N content on *Pn* was through changes in Pn_{max} values (Figure 8.6) which increased linearly by 9.05 g CO_2 m^{-2} d^{-1} per 1% N content from 1.5 to 4.0% N and then remained constant. This was followed by a slight decline of 0.3 g CO_2 m^{-2} d^{-1} per 1% N. In contrast, foliage N content had a small effect on optimum LAI. The optimum LAI increased from 3.4 units at 1.5% N to 4.0 units at 4.0% N, and from this point declined to LAI= 3.7 at 5.9% N (Figure 8.6).

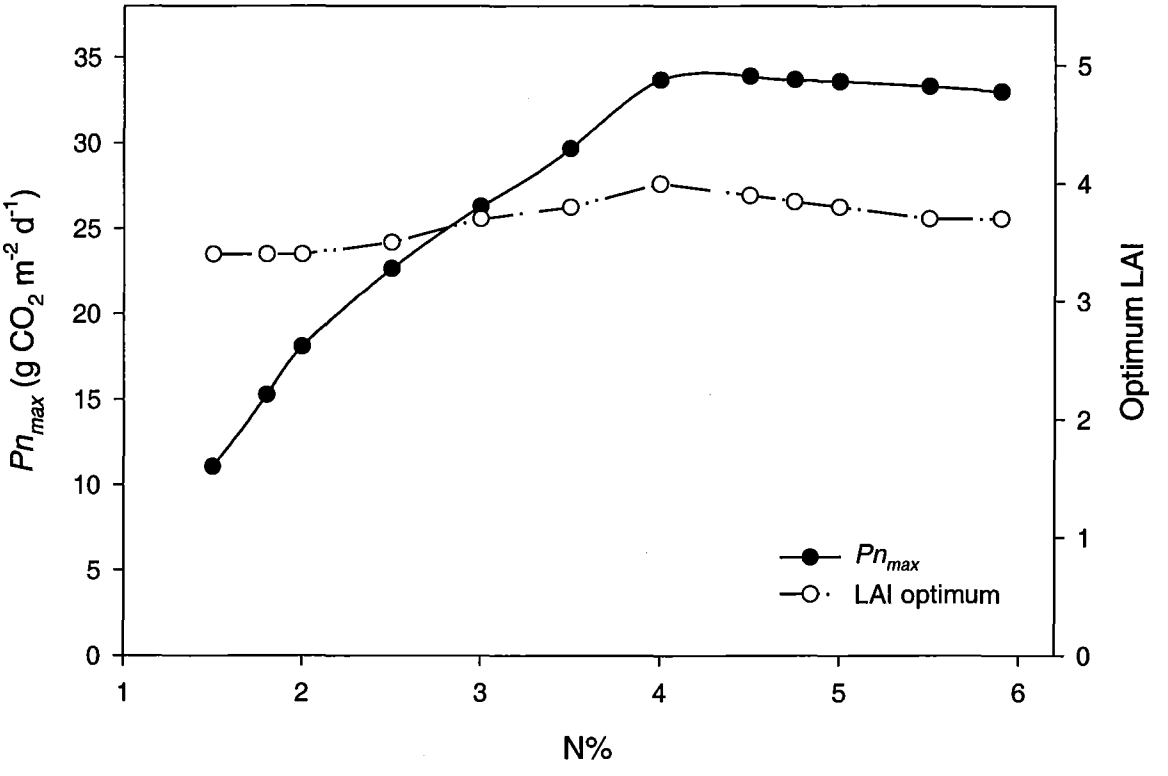

Figure 8.6 Predicted maximum daily net canopy photosynthesis (Pn_{max}) and optimum leaf area index (LAI) against measured foliage nitrogen percentage (N%) for a cocksfoot pasture where other factors were non-limiting.

8.3.3 Simulation 3: Effect of water stress on *Pn*

In this third simulation, the effect of the water status on *Pn* only was evaluated when other factors were non-limiting.

Values of *I(z)* were calculated as given in Section 8.3.1. $Pmax_s$ was calculated according to Equation 4.7 (Section 4.3.7) and α_s was calculated according to Equation 7.4 (Section 7.3.3). As for N, $Pmax_G$ and α were assumed to be state variables for each run, thus both variables were constant during the day. For example, $Pmax_G$ declined from 1.21 mg CO_2 m^{-2} s^{-1} for well irrigated plants (ψ_{lp}= -0.1 to –1.0 bar) to 0.02 mg CO_2 m^{-2} s^{-1} at a ψ_{lp} of -13.5 bar. Values of α decreased from 0.0069 mg CO_2 J^{-1} for the range of ψ_{lp}= -0.1 to 10.0 bar to 0.0049 mg CO_2 J^{-1} for plants under a severe water stress of ψ_{lp}= -16.0 bar. The calculated values of $Pmax_G$ and α were then incorporated into Equation 8.2 to predict *Pg* at 5 minute intervals and for every 0.1 of accumulated LAI. The R_M was affected by water status by changing the coefficient *b* in Equation 8.3 according to Equation 8.4 (Section 8.2.3).

A total of 17 runs that correspond to plants with measured ψ_{lp} values of -0.1, -1.0, -2.0, -3.0, -4.0, -5.0, -6.0, -7.0, -8.0, -9.0, -10.0, -11.0, -11.5, -12.0, -13.0, -14.0, and –16.0 bar were used to evaluate the effect of water stress on *Pn*.

In Figure 8.7 the output from the canopy photosynthesis model for eight representative water status situations is presented. From ψ_{lp}= -0.1 bar to ψ_{lp}= -11.5 bar, water stress mainly affected the maximum *Pn* with a consistent parabolic pattern of response across LAI. However, for ψ_{lp}=–12.0 bar, the optimum LAI also declined and *Pn* was zero at LAI= 4.0.

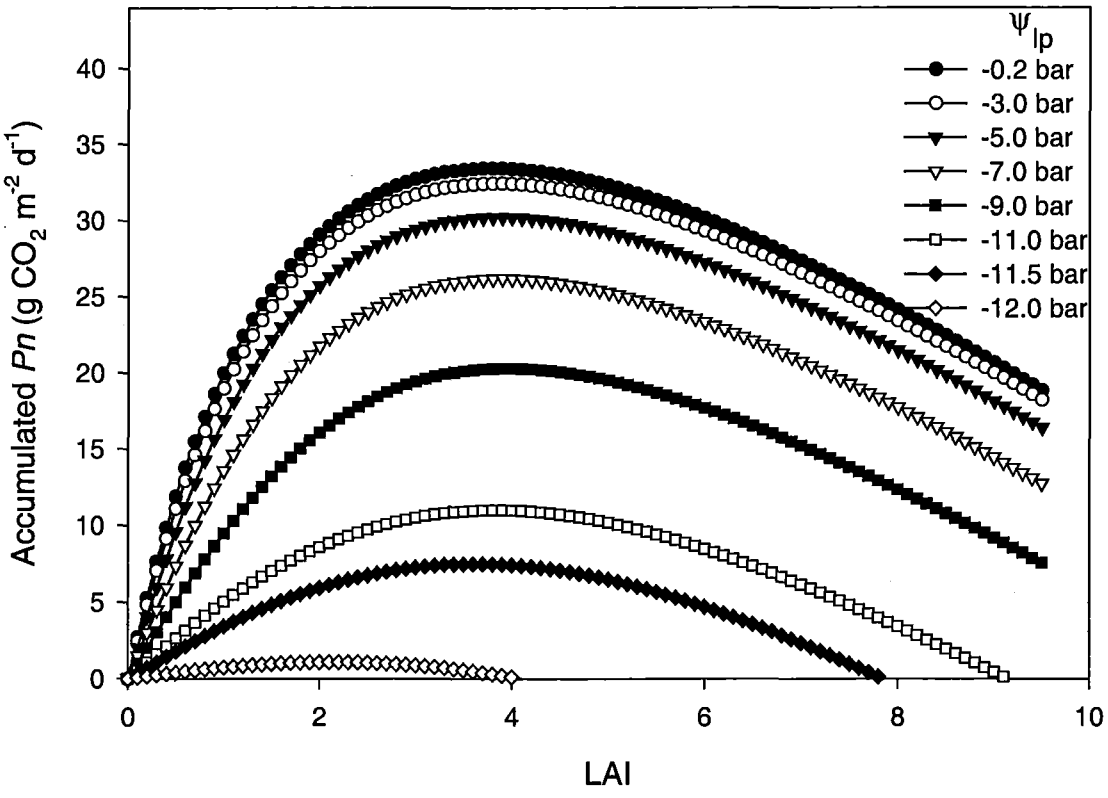

Figure 8.7 Predicted accumulated daily net canopy photosynthesis (*Pn*) against leaf area index (LAI) for different measured water status expressed as pre-dawn leaf water potential (ψ_{lp}) for a cocksfoot pasture where other factors were non-limiting.

The effect of water status on Pn_{max} and optimum LAI is summarised in Figure 8.8. Pn_{max} decreased non-linearly with water stress. From ψ_{lp}= -0.1 to –1.5 bar, Pn_{max} remained constant (33.5 g CO_2 m^{-2} d^{-1}) and from this point Pn_{max} decreased at a rate of 2.2 g CO_2 m^{-2} d^{-1} per bar of ψ_{lp}. This was followed by a further decline of 7.5 g CO_2 m^{-2} d^{-1} per bar of ψ_{lp} down to zero Pn_{max} at ψ_{lp}= -12.5 bar.

In contrast, the optimum LAI was stable at about 3.8 from ψ_{lp}= -0.1 to –11.0 bar, but then declined by 2.4 units of LAI per bar of ψ_{lp} (Figure 8.8).

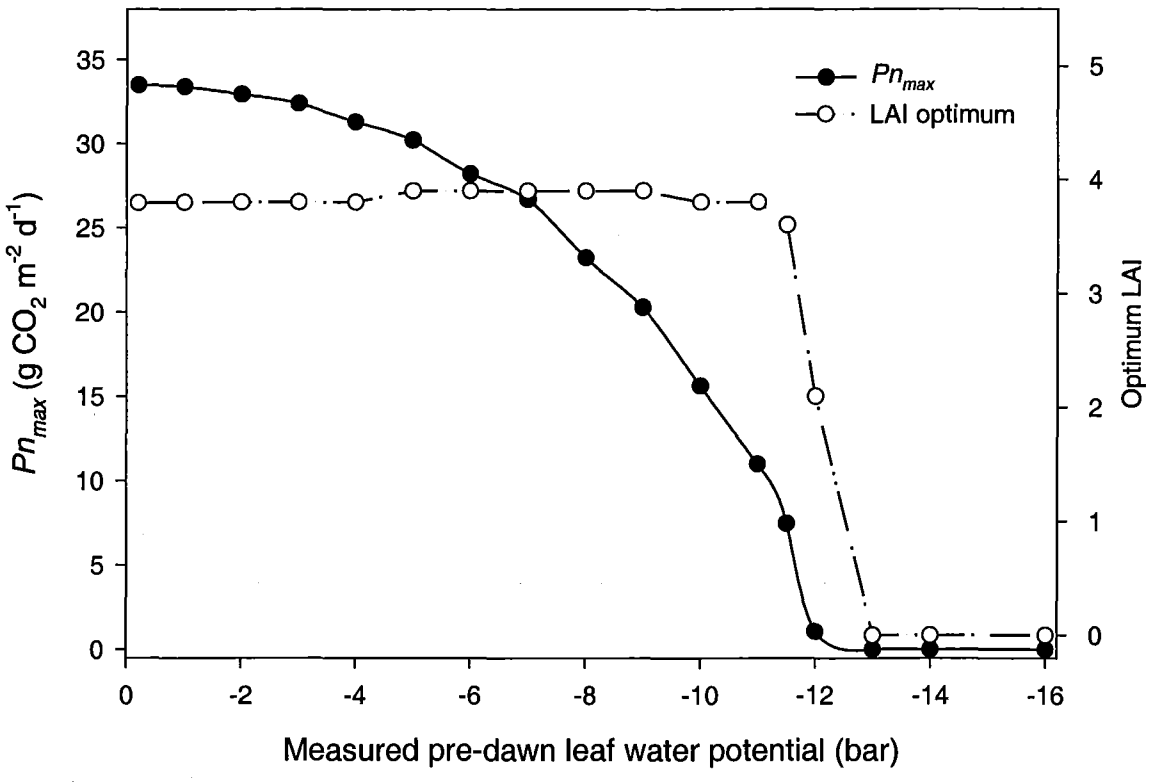

Figure 8.8 Predicted maximum daily net canopy photosynthesis (Pn_{max}) and optimum leaf area index (LAI) against measured water status, expressed as pre-dawn leaf water potential (ψ_{lp}), for a cocksfoot pasture where other factors were non-limiting.

8.3.4 Simulation 4: Effect of regrowth duration on *Pn*

The fourth simulation evaluated the effect of regrowth duration on *Pn* when other factors were non-limiting.

Values of *I(z)* were calculated as given in Section 8.3.1. $Pmax_s$ was calculated according to Equation 5.1 (Section 5.3.1) and α_s was calculated using Equation 7.5 (Section 7.3.4) but in two theoretically different ways:

i) considering the effect of regrowth on $Pmax_G$ and α as a state variable over a day and within the canopy layers. Thus, the maximum value of $Pmax_G$ was 1.21 mg CO_2 m^{-2} s^{-1} considering that the whole canopy (all LAI layers) had 20 days regrowth. Then, $Pmax_G$ declined according to the quadratic Equation 5.1 to 0.68 mg CO_2 m^{-2} s^{-1} assuming that the whole canopy had 60 days of regrowth. Similarly, α decreased in a quadratic way (Equation 7.5) from 0.0069 mg CO_2 J^{-1} for 20 days regrowth to 0.0058 mg CO_2 J^{-1} for 60 days regrowth. Six runs for this simulation were carried out for 20, 30, 40, 50 and 60 days

regrowth.

ii) considering the effect of regrowth on $Pmax_G$ and α as a state variable changing within the canopy layers every 0.1 LAI. Because Equations 5.1 (*Pmax*) and 7.5 (α) are quadratic functions with time of regrowth (days) as an independent variable, the relationship between days of regrowth and LAI of the summer 60-day cage (January-February 2000) (Section 3.3.5.2) was used.

The calculated values of $Pmax_G$ and α were then incorporated into Equation 8.2 to predict *Pg* for each 5 minute interval and for every 0.1 accumulated LAI.

Assuming the whole canopy had 21 days regrowth, *Pn* reached a maximum value of 33.8 g CO_2 m^{-2} d^{-1} at LAI= 3.8 and then declined to 18.9 g CO_2 m^{-2} d^{-1} at LAI= 9.5 (Figure 8.9a). In contrast, for plants with 60 days regrowth, *Pn* reached a maximum value of 22.1 g CO_2 m^{-2} d^{-1} at LAI= 3.4 and then *Pn* declined to 5.9 at LAI= 9.5. The main effects of regrowth duration on Pn_{max} and optimum LAI are shown in Figure 8.10. Pn_{max} decreased by 0.17 g CO_2 m^{-2} d^{-1} per day of regrowth from 20 to 40 days and by 0.41 g CO_2 m^{-2} d^{-1} per day of regrowth from 40 to 60 days. The optimum LAI only decreased 0.4 units from 20 to 60 days regrowth (Figure 8.10).

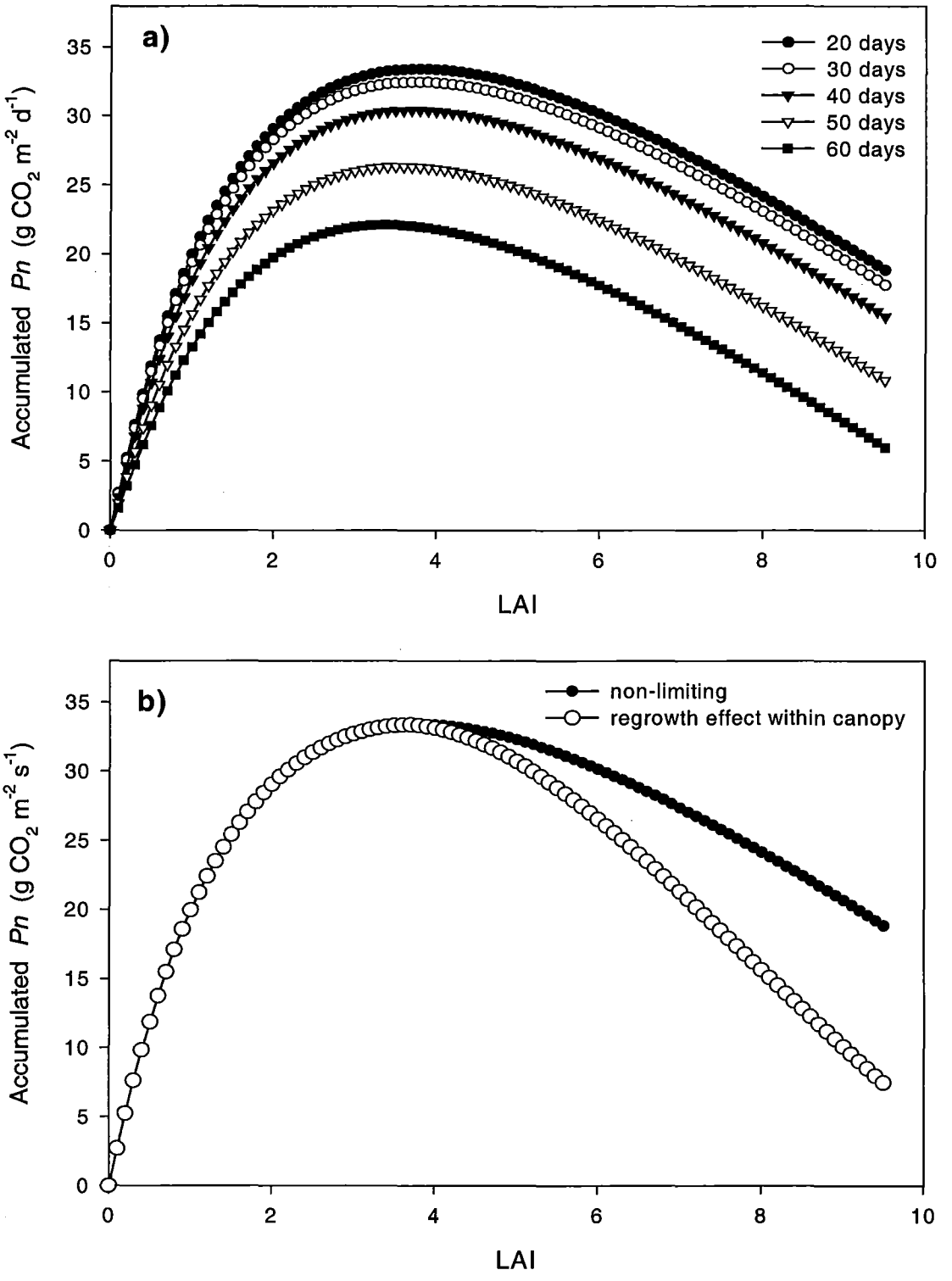

Figure 8.9 Predicted accumulated daily net canopy photosynthesis (*Pn*) against leaf area index (LAI) for different days of regrowth for a cocksfoot pasture where other factors were non-limiting. Simulations of *Pn* were analysed from two different perspectives: a) simulation of *Pn* considering the effect of regrowth on $Pmax_G$ and α as a state variable within the canopy layers. Thus, $Pmax_G$ and α considering the whole canopy (at all LAI layers) had 20, 30, 40, 50 and 60 days of regrowth; b) considering the effect of regrowth on $Pmax_G$ and α as a state variable changing within the canopy layers every 0.1 LAI compared with a non-limiting situation.

On the other hand, considering that $Pmax_G$ and α changed within the canopy layers, *Pn* reached the maximum value at LAI= 3.8 and then declined to 7.5 g CO_2 m^{-2} d^{-1} at LAI= 9.5 compared with 18.9 g CO_2 m^{-2} d^{-1} for the non-limiting condition (20 days regrowth) (Figure 8.9b).

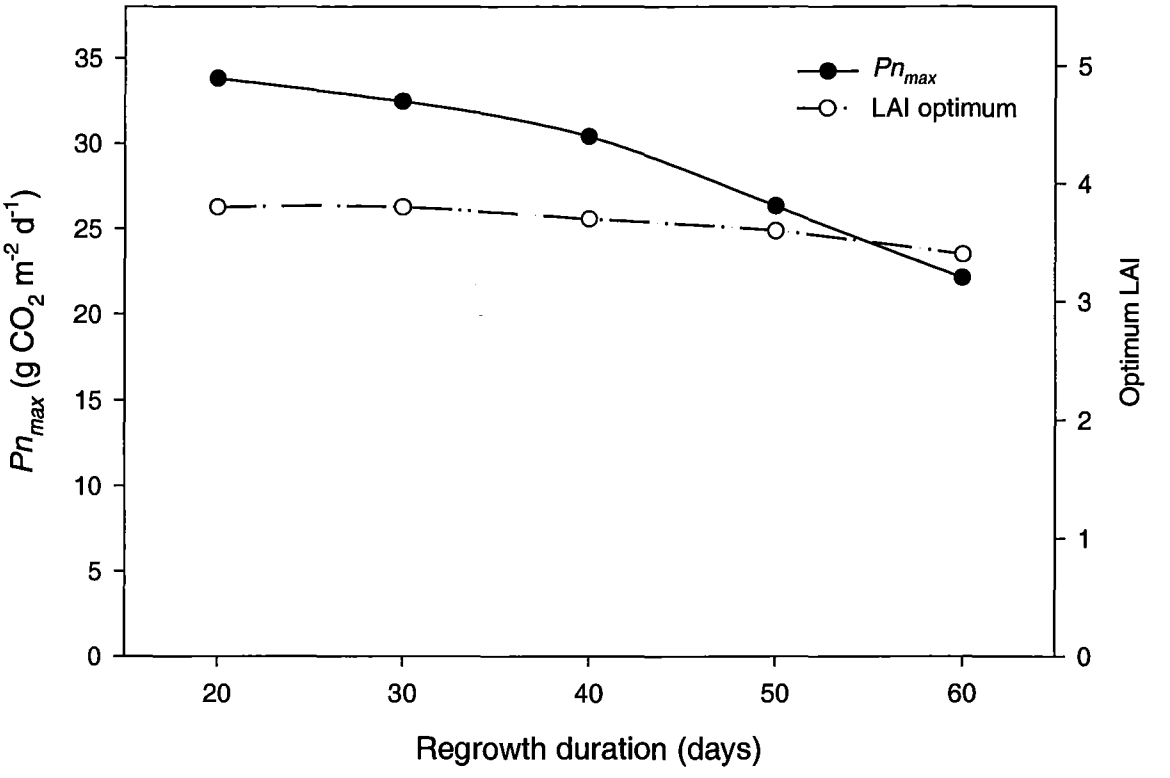

Figure 8.10 Predicted maximum daily net canopy photosynthesis (Pn_{max}) and optimum LAI against regrowth duration (days) for a cocksfoot pasture where other factors were non-limiting. Data predicted from Figure 8.9a.

8.3.5 Simulation 5: Effect of light regime and light intensity on *Pn*

The aim of this simulation was to evaluate the effect of light regime and light intensity on *Pn* when other factors were non-limiting.

Firstly, *I(z)* was calculated by solving Equation 8.1 incorporating input values of I_o for each 5 minute interval over a summer sunny day (around 21 December) under the following light regimes and intensities:

i) full sunlight (100% transmissivity),

ii) continuous moderate cloth shade (50% PPFD of the open or 50% transmissivity);

iii) a fluctuating light regime with alternating periods of full sunlight and severe shade (5% of the open PPFD) from slat shade at intensities of: 10, 20, 30, 40, 50, 60, 70, 80 and 90% transmissivity. This range is used to represent overstorey canopies of different density or size.

The light regime measured from shade cloth, which provided a continuous 50% of open PPFD (850-950 μmol m^{-2} s^{-1} PPFD at midday) throughout a day, was presented in Figure 6.1c (Section 6.2.1.1). The measured light regime from the slatted structure (Section 3.2.3.3) provided a fluctuating full sunlight/severe shade regime with a total of 45% of open PPFD throughout a day, as presented in Figure 6.1b (Section 6.2.1.1). From the slatted measurements, additional daily PPFD integral values were interpolated to generate a range of fluctuating light intensities from 10 to 90% transmissivity. Figure 8.11 shows the open light regime contrasted with that for three severe shade intensities: (a) 20% (which is equivalent to the treatment Trees+slats described in Section 3.2.3.3), (b) 50% (which is equivalent to the treatment Open+slats described in Section 3.2.3.3) and (c) 80% PPFD of the full sunlight regime.

The interval of full sunlight and shade periods around midday was approximately: 2 h full sunlight and 2 h shade for the 50% transmissivity regime (Figure 8.11a), 45 min full sunlight and 3 h shade for the 20% transmissivity regime (Figure 8.11c) and 3 h full sunlight and 45 min shade for the 80% transmissivity regime (Figure 8.11b). For 90% transmissivity, plants would experience only 100 min of shade per day compared with only four periods of 10 min of full sunlight for the 10% transmissivity treatment.

Secondly, values of I_o were used as an input variable to calculate $Pmax_s$ under severe shade using Equation 6.2 and under moderate cloth shade using Equation 6.3 (Section 6.3.1.1). The linear equations presented in Table 6.2 (Section 6.3.1.3) were used to calculate *Pmax* during induction (recovery from slat shade). The saturation point for $Pmax_G$ was considered to be from 1000 μmol CO_2 m^{-2} s^{-1} for non-limiting conditions (Section 4.3). This saturated value of $Pmax_G$ during summer is reached from 9:00 to 17:00 h (Figure 8.11). Before and after those times, $Pmax_G$ was reduced in proportion to the incoming PPFD. As an example of the methodology, $Pmax_G$ in the severe shade regime of 50% transmissivity reached a maximum value (1.21 mg CO_2 m^{-2} s^{-1}) for almost 1.5-2 h around midday, and under the shade period $Pmax_G$ declined exponentially during 2 h to a value of

0.50 mg CO_2 m^{-2} s^{-1} (Figure 8.11d). The time required for full induction of $Pmax_G$ was 20 minutes after the increase of PPFD (full sun) occurred. In contrast, for 80% transmissivity the maximum $Pmax_G$ value remained for 2.7 h during the full sunlight period and only declined to 0.6 mg CO_2 m^{-2} s^{-1} (Figure 8.11e). For the 20% transmissivity regime, $Pmax_G$ reached the maximum value of 1.21 mg CO_2 m^{-2} s^{-1} for only 30 min over the day and $Pmax_G$ declined down to the steady-state value of 0.44 mg CO_2 m^{-2} s^{-1} (Figure 8.11f).

The time required for full induction of $Pmax_G$ was 35 min after the increase of PPFD (full sun) occurred. $Pmax_G$ for the regime of 90% transmissivity had the maximum value for 6.5 h over the day. In contrast, $Pmax_G$ never reached the maximum value for the extreme regime of 10% transmissivity. For the continuous cloth shade regime $Pmax_G$ also never reached the maximum value, but most of the time $Pmax_G$ was 0.9 mg CO_2 m^{-2} s^{-1} (or 74% of that achieved in full sunlight).

Similarly, values of I_o for every 5 minute interval were used to calculate α_s according to Equation 7.6 for plants under slat shade and according to Equation 7.7 for plants under cloth shade (Section 7.3.5). To calculate α during the induction process Equation 7.8 (Section 7.3.5) was used. The maximum value of α during the full sunlight period was 0.0069 mg CO_2 J^{-1} and the minimum value of α was 0.0051 mg CO_2 J^{-1} after 3 h under the slat shade for the regime with 10% transmissivity. For the continuous cloth shade regime, α was stable at about 0.0063 mg CO_2 J^{-1}.

The calculated values of $Pmax_G$ and α were incorporated into Equation 8.2 to predict Pg for every 5 minute interval and for every 0.1 accumulated LAI.

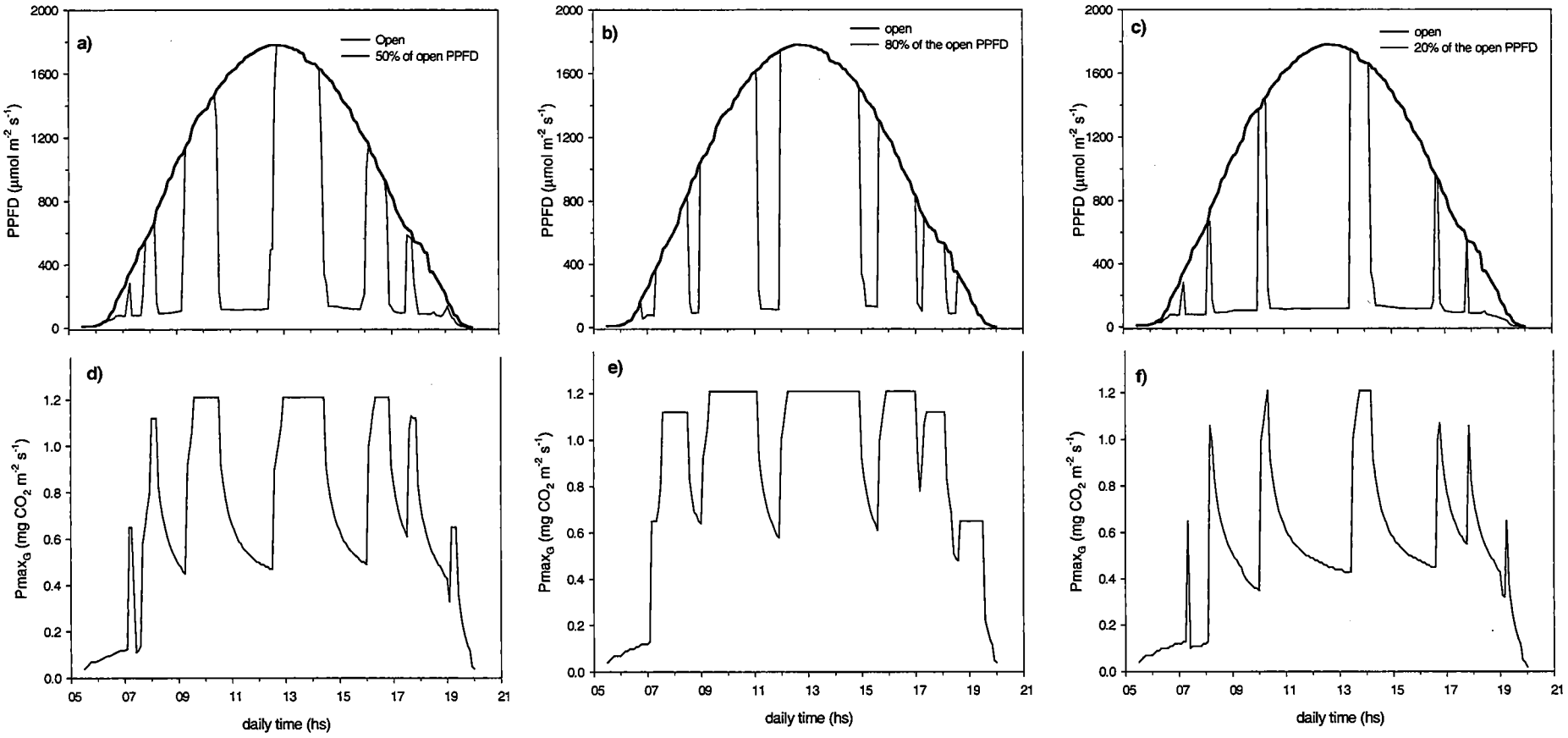

Figure 8.11 Simulated photosynthetic photon flux density (PPFD) (a, b, c) and predicted maximum rate of leaf photosynthesis ($Pmax_G$) (d, e, f) on a typical summer sunny day (Canterbury, New Zealand) for cocksfoot plots under different fluctuating light regimes contrasted with an open situation (—) and where other factors were non-limiting.

Figure 8.12 shows the canopy *Pn* for a combination of the three light regimes (full sunlight, continuous shade and fluctuating light regime) and six light intensities. In all simulations *Pn* followed a parabolic shape against LAI, but as light intensity decreased, the maximum *Pn*, optimum LAI and values of *Pn* after its maximum value also decreased. For example, under full sunlight conditions *Pn* reached a maximum value of 33.4 g CO_2 m^{-2} d^{-1} at LAI= 3.7 and then declined to 18.8 g CO_2 m^{-2} d^{-1} at LAI= 9.5. In contrast, for plants under a fluctuating light regime of 20% transmissivity, *Pn* reached a maximum value of 0.75 g CO_2 m^{-2} d^{-1} at LAI= 0.7 and then declined to zero *Pn* at LAI= 1.6.

In addition, it was predicted that the continuous light regime of 50% transmissivity would produce more DM than the same intensity but for a fluctuating light regime (10.4 vs 8.4 g CO_2 m^{-2} d^{-1}), and the maximum *Pn* (2.5 vs 2.2 LAI units) and *Pn*= 0 (6.9 vs 6.1 LAI units) occurred at a higher LAI for the continuous light regime.

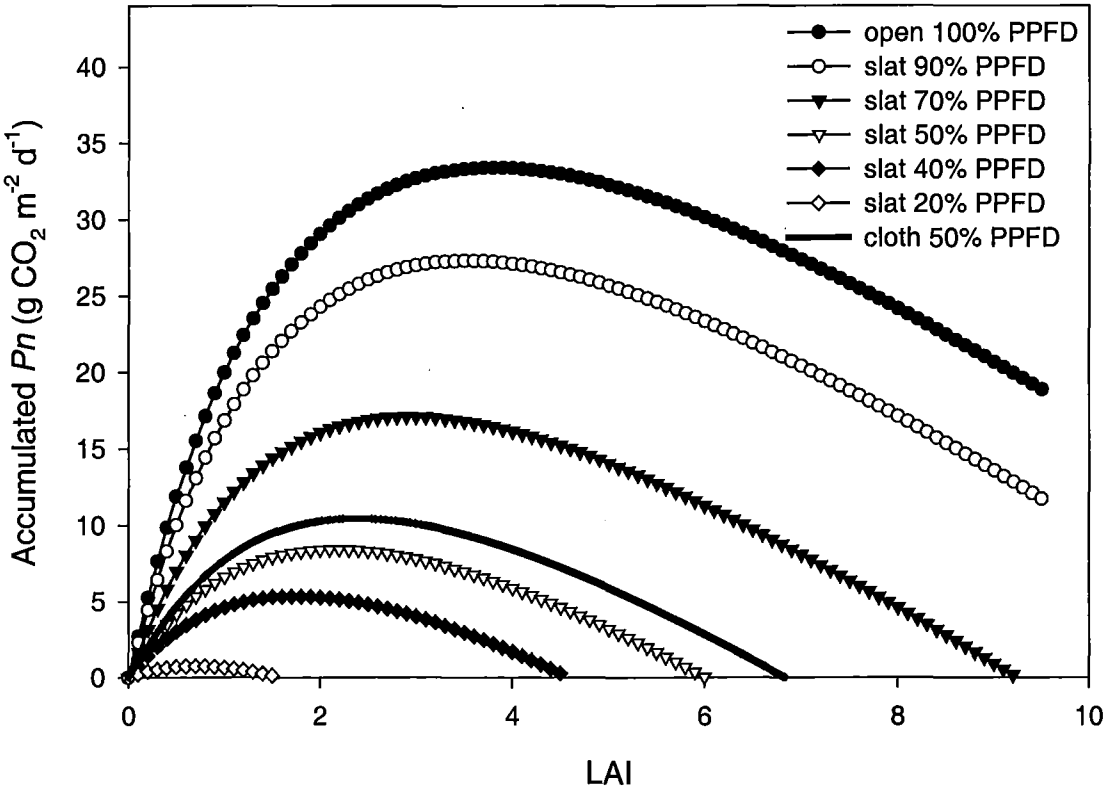

Figure 8.12 Predicted accumulated daily net canopy photosynthesis (*Pn*) against leaf area index (LAI) for different light regimes (full sunlight or open, continuos cloth shade and fluctuating severe shade or slat) and light intensities (100, 90, 70, 50, 40 and 20% transmissivity) for a cocksfoot pasture where other factors were non-limiting.

The changes in light intensity on Pn_{max} and optimum LAI for the fluctuating and full sunlight regimes are shown in Figure 8.13. Pn_{max} decreased from 33.4 g CO_2 m^{-2} d^{-1} under the full sunlight to zero under the 10% transmissivity regime. Similarly, the optimum LAI decreased linearly from 3.7 under a full sunlight regime to zero under 10% transmissivity.

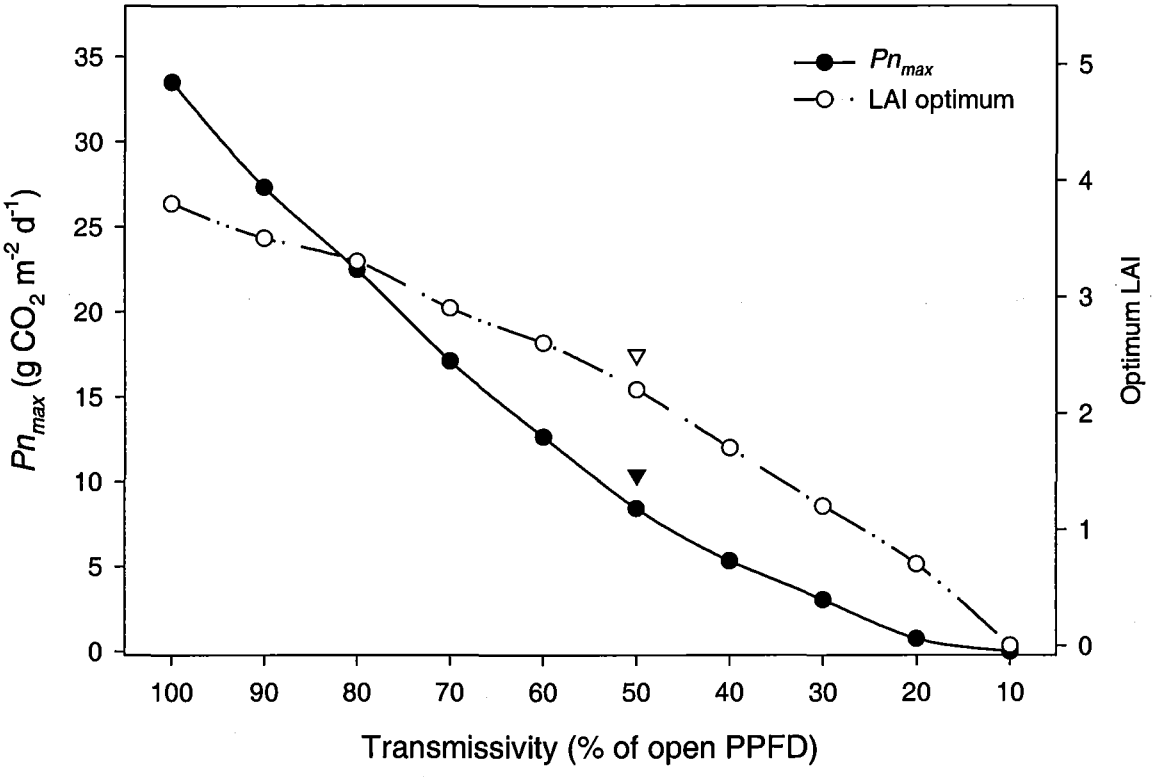

Figure 8.13 Predicted maximum daily net canopy photosynthesis (Pn_{max}) and optimum leaf area index (LAI) against different intensities of fluctuating light regime for a cocksfoot pasture when other factors were non-limiting. Pn_{max} (▼) and optimum LAI (▽) values for a continuous 50% transmissivity light regime are also indicated.

8.3.6 Validation of the canopy photosynthesis model

The aim of the research reported in this section was to validate the canopy photosynthesis model comparing the predicted DM values from simulations with observed DM values obtained for cocksfoot pastures grown under a diverse range of environmental and management situations in the open and under trees.

To do this, it was necessary to transform the output of the model, expressed in g CO_2 m^{-2} d^{-1} of *Pn*, to carbohydrate equivalents (CH_2O) by multiplying by 0.65 (Hay and Walker, 1989). Secondly, to relate CH_2O to DM and vice versa, the N and minerals (P, K, Ca, S and Mg) content were added from CH_2O or discounted from DM (Figure 8.1). Thus,

1 g CH_2O= 1 g DM – x g N – y g Minerals.

Where *x* and *y* are the measured values for these components.

The N content was obtained for each harvest as described in Section 4.2.3. The mineral content of foliage from a 0.2 m^2 quadrat, cut to 25 mm height, was analysed each season. The results and techniques used for evaluation of mineral content are given in Appendix 6.

Thirdly, a relationship between LAI and CH_2O was used to determine the foliage developed after each day of growth (Figure 8.1). To do this, LAI and kg DM/ha (transformed to g CH_2O m^{-2}) data from vegetative cocksfoot pastures (Figure 3.11; Section 3.3.7) were analysed using linear and non-linear regression analysis. Because there were no significant differences in the slope of the relationship between LAI and g CH_2O m^{-2} for each environmental factor, a single function could be used. This relationship was described by a rectangular hyperbola function (Equation 8.5), which resulted in an R^2 of 0.93 and ESE of LAI of 0.51.

$$LAI = \frac{10.9 * C}{239 + C}$$

Equation 8.5

Where C is the accumulated carbohydrate equivalent (g CH_2O m^{-2}).

From 0.5 to 3.0 units of LAI, the relationship was approximately linear and increased at a rate of 30.3 g CH_2O m^{-2} per unit of LAI. From this point to LAI= 8 the relationship became curvilinear with a rate of 114 g CH_2O m^{-2} (Figure 8.14).

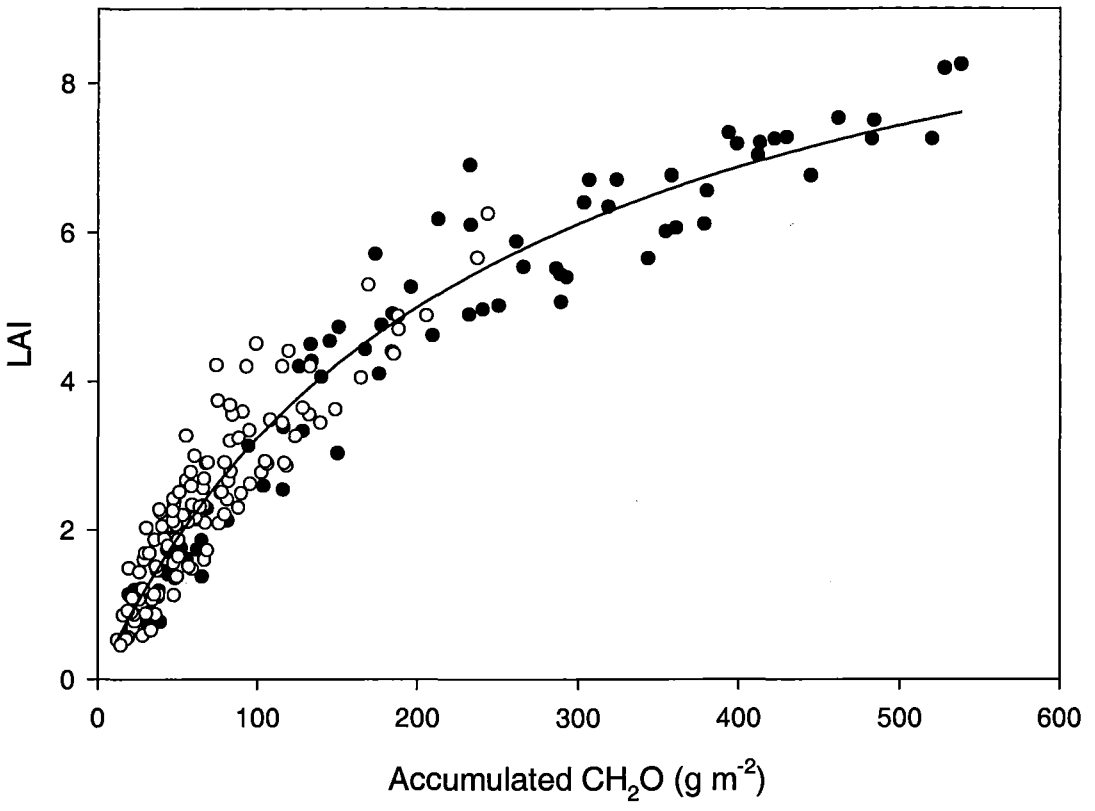

Figure 8.14 Leaf area index (LAI) against accumulated carbohydrate (CH_2O) for vegetative cocksfoot pastures. The line is for the fitted single rectangular hyperbola function (Equation 8.5). Observed data with (•) and without (○) 300 kg N/ha.

The fourth relationship was between LAI and accumulated dead material expressed as g CH_2O m^{-2}. This was used to determine the loss of growth from senescent foliage after each day of growth (Figure 8.1). To do this, from each harvest total LAI and the proportion of senescent material (transformed to g CH_2O m^{-2}) from vegetative cocksfoot pastures in the main plots and exclosure areas (Section 3.3.4) were analysed using linear and non-linear regression analysis. The proportion of senescent material (dead and yellow leaves) was obtained from the botanical composition in cocksfoot pastures by dissecting a sub-sample (Section 3.2.4). This data is presented in Appendices 2 and 3.

From LAI= 0.5 to 4.0, the senescent material only increased at a rate of 2.0 g CH_2O m^{-2} per unit of LAI, and from this point to LAI= 8 the rate was 17 g CH_2O m^{-2}. At LAI=4 the accumulated proportion of senescent material represented only 5% of the total accumulated growth, and at LAI=8 this proportion was 12% of the total accumulated growth (Figure 8.15).

This relationship was described by a single exponential function (Equation 8.6), which resulted in an R^2 of 0.94 and ESE of accumulated CH_2O of 3.5.

$$Sm = e^{0.54*LAI} \qquad \textbf{Equation 8.6}$$

Where *Sm* is the amount of senescent material (g CH_2O m^{-2}) accumulated per unit of LAI.

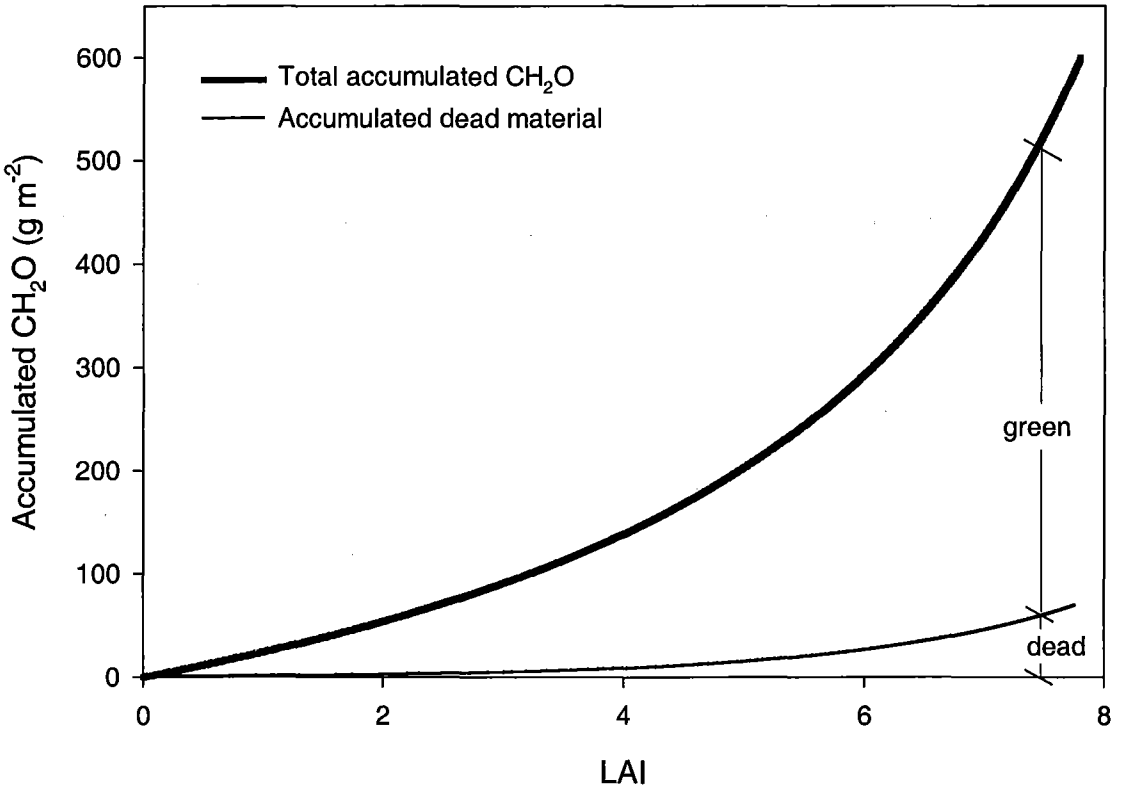

Figure 8.15 Accumulated dead and green dry matter (DM) expressed as carbohydrate (CH_2O) against leaf area index (LAI). The fitted single rectangular hyperbola for total accumulated carbohydrate function (Equation 8.5) and the single exponential function for accumulated dead material (Equation 8.6) are indicated.

For the validations, *I(z)* was calculated by solving Equation 8.1 incorporating input values of actual I_o for each particular day of the validation period which had been recorded by a datalogger at 5 minute intervals. A single canopy leaf angle was used for each treatment as described in Section 3.3.8.

To quantify the daily gross canopy photosynthesis, values of $Pmax_G$ for each day of the validation period were calculated using the final modified multiplicative model (which includes three interaction functions) proposed in Figure 6.14 (Section 6.3.2.5) and values of α from the 'law of the minimum factor' model presented in Equation 7.10 (Section

7.3.7). To incorporate the effect of the state variables N content, water status (expressed as pre-dawn water potential, ψ_{lp}) and regrowth duration on $Pmax_G$ and α, actual values from field measurements were used. During the simulation these variables remained constant for a particular day. Diurnal changes in N and ψ_{lp} were obtained from linear interpolation between two measurement days. In contrast, $Pmax_G$ and α were calculated for every 5 minute interval throughout the day for the dynamic variables air temperature and light regime. For validations under the tree shade situations, canopy temperature was used during the shade period using the exponential equation presented in Figure 6.10 (Section 6.3.2.1) and the air temperature during the sunny period.

The total canopy respiration (R_T) for each day of validation was calculated based on Equation 8.3. The daily maximum and minimum temperature were used to calculate maintenance respiration (R_M) which was affected by water status by changing the coefficient b according to Equation 8.4 and N content according to the negative slope of 0.052 d^{-1} per 1% N (Section 8.2.3). The gross photosynthesis produced was then reduced by subtracting R_T to give Pn, expressed as CH_2O m^{-2} d^{-1}.

The start point of each validation was at LAI=0.5-0.6 which represents the LAI of the 20 mm stubble height left after grazing of the cocksfoot pasture. After simulating day 1 of the validation period, the increase in dead material associated with the increase in total DM was subtracted from Pn (g CH_2O m^{-2} d^{-1}) according to Equation 8.6. Also, subtracted from the simulated Pn was 10% due to partitioning to the roots (Johnson and Thornely, 1983). The resulting net growth value was then incorporated into Equation 8.5 to determine the foliage developed (LAI) after day 1 of growth. This gave the LAI to use for day 2 of the simulation and iteratively until the end of the simulation period.

A total of 13 validation periods were simulated. These corresponded to a diverse range of environmental and management situations for cocksfoot pastures in the open and under trees. Results were compared with observed DM values obtained over the same period from field conditions. None of the DM values had been used in model development. The main environmental and management conditions considered, and the main objective of each validation, are shown in Table 8.1.

Table 8.1 Main environmental and management conditions and the main objective of each validation period corresponding to cocksfoot pastures in the open and under trees. These field data were used as input data for in the canopy photosynthesis model for simulation of DM production.

Validation	Pasture	Period	Regrowth days	N (%)	ψ_{lp} (bar)	Td (°C)	Tmax (°C)	Leaf angle	Observed (kg DM/ha)	Objectives validation
1	Open W+N	6 Jan to 14	10	5.8	-0.8	14.3	17.7	68	660	To validate the potential growth in open cocksfoot pastures.
2		Feb 00	20	5.4	-0.9	16.8	22.1	68	2180	
3			30	4.8	-0.7	13.9	18.7	64	4010	
4			40	4.0	-1.0	17.0	22.6	55	5100	
5	Trees W+N	6 Jan to 26	10	5.7	-0.9	14.6	17.9	65	550	To validate the potential growth of cocksfoot pasture in the silvopastoral system.
6		Jan 00	20	5.4	-1.0	17.0	22.3	65	1650	
7	Open control	23 Sep to 13 Oct 00	21	3.4	-0.8	9.6	15.0	68	1150	To validate the spring growth. Water non-limiting.
8	Trees control	23 Sep to 13 Oct 00	21	3.5	-1.0	9.9	15.2	65	870	To validate the spring growth under moderate shade. Water non-limiting.
9	Trees+slats	23 Sep to 13 Oct 00	21	3.6	-0.9	9.9	15.2	59	320	To validate the spring growth under severe shade. Water non-limiting.
10	Open control	27 Jan to 16 Feb 01	21	2.6	-8.5	15.6	22.1	68	260	To validate severe water stress and N limiting.
11	Trees control	27 Jan to 16 Feb 01	21	2.2	-9.2	16.1	22.3	65	190	To validate severe water stress and N limiting under moderate shade.
12	Open W	15 Feb to 6	50	3.0	-0.9	15.4	19.0	41	2010	To validate the regrowth duration.
13		Mar 00	60	2.8	-1.1		22.2	40	2230	

Td= mean daily temperature for the validation period; *Tmax*= mean maximum temperature for the validation period.
W+N= irrigated pastures with application of 300 Kg N/ha. W= pastures only irrigated.
Open pasture= 100% transmissivity; Trees pastures= 60% transmissivity; Trees+slat pastures= 25% transmissivity.

Figure 8.16 shows a typical simulated output from the canopy photosynthesis model expressed as daily growth (g CH_2O m^{-2} d^{-1}) including the discount for dead material and with or without considering partitioning to roots for an irrigated cocksfoot pasture in an open situation and with the application of 300 Kg N/ha (open W+N). This corresponds to the validation points 1, 2, 3 and 4 in Table 8.1.

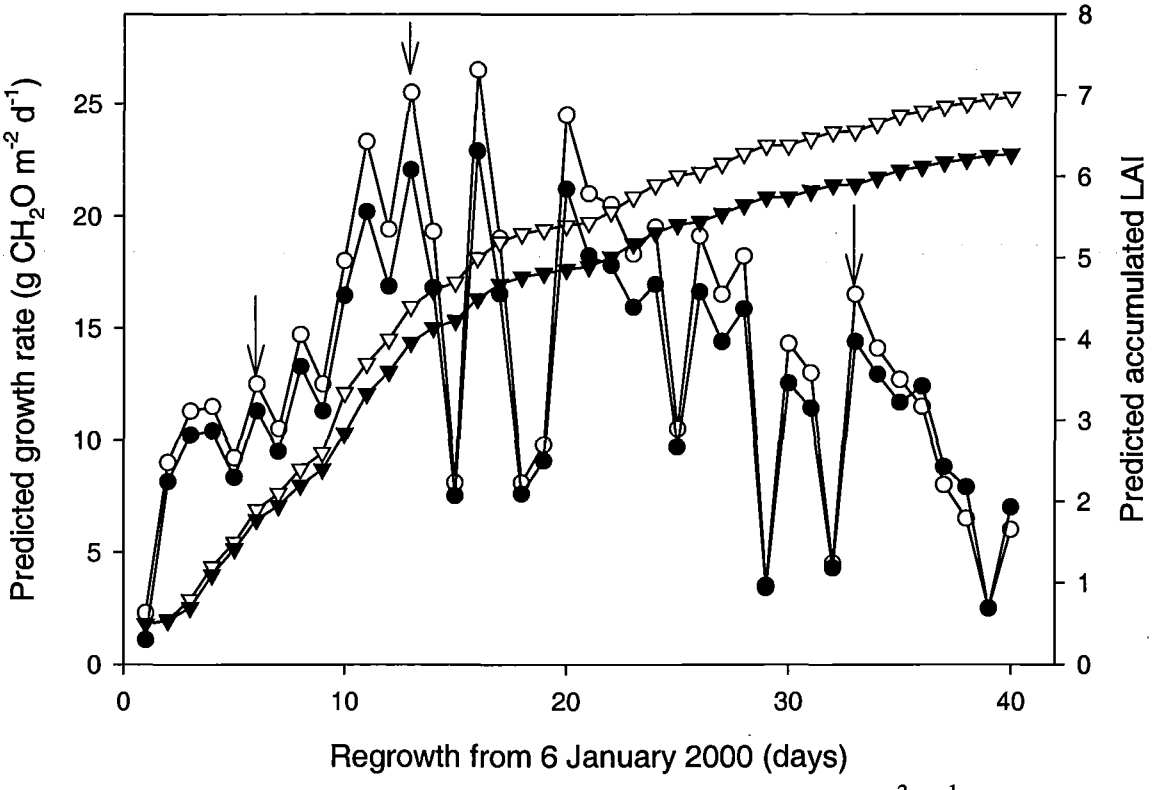

Figure 8.16 Predicted growth rate (●,○) expressed as g CH_2O m^{-2} d^{-1} and predicted accumulated leaf area index (LAI) (▼,▽) for cocksfoot pastures in the open, including (●,▼) and excluding (○,▽) partitioning. Characteristics of the pasture and environmental factors over this regrowth period are summarised in Table 8.1 (Validations 1, 2, 3 and 4). Arrows indicate optimum days for photosynthesis (sunny days and with maximum air temperatures 21 ±2 °C).

Similarly, in Figure 8.17 the output data for 20 days regrowth of cocksfoot pasture in the silvopastoral site including partitioning (Validations 5 and 6; Table 8.1) are shown. The fluctuation in daily growth was due to differences in air temperature and incoming radiation between days. However, for optimum days (sunny days and with maximum air temperatures 21 ±2 °C) the net growth was different depending on the canopy development stage. For example, after 6 days regrowth in the open W+N pasture the net growth (considering partitioning) was 11.3 g CH_2O m^{-2} d^{-1} at LAI= 1.8 (Figure 8.16). After 13

days regrowth the net growth increased to 22.1 g CH_2O m^{-2} d^{-1} at LAI= 3.9. It then decreased to 14.4 g CH_2O m^{-2} d^{-1} when the accumulated LAI was 5.9 (Figure 8.16). Similarly, for the pasture trees W+N the net growth at 6 days (8.3 g CH_2O m^{-2} d^{-1}) with a LAI= 0.9 was lower than at 13 days (Figure 8.17).

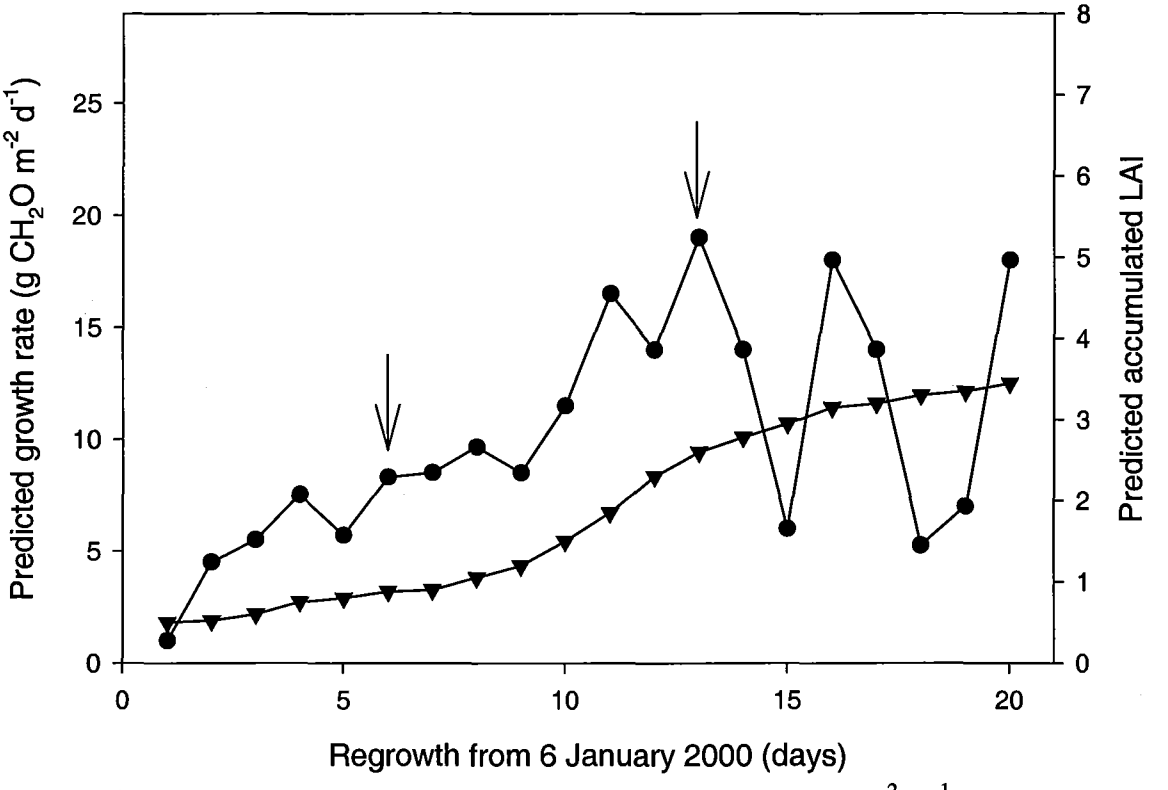

Figure 8.17 Predicted growth rate (•) expressed as g CH_2O m^{-2} d^{-1} and predicted accumulated leaf area index (LAI) (▼) for cocksfoot pastures under trees, including partitioning. Characteristics of the pasture and environmental factors over this regrowth period are summarised in Table 8.1 (Validations 5 and 6). Arrows indicate optimum days for photosynthesis (sunny days and with maximum air temperatures 21 ±2 °C).

The accumulated growth after 40 days from the simulation of the open W+N pasture was 5500 (LAI= 6.2) and 6260 kg DM/ha (LAI= 6.9) with and without partitioning, respectively (Figure 8.16). This indicated that by using a daily 10% discount for partitioning, the model overestimated growth by 8%. However without partitioning the overestimation was 22% of the observed value. For this reason, a 10% partitioning coefficient was incorporated into all other validations.

The remaining simulated results for canopy photosynthesis were compared with the 13 observed dry matter values obtained from harvest for the same period under field conditions (Figure 8.18). The average RMSD (250) was about 14.5% of the mean observed

DM values. However, cocksfoot growth was overestimated by the model for all validation points in the observed range of 190 – 5100 kg DM/ha. (Figure 8.18).

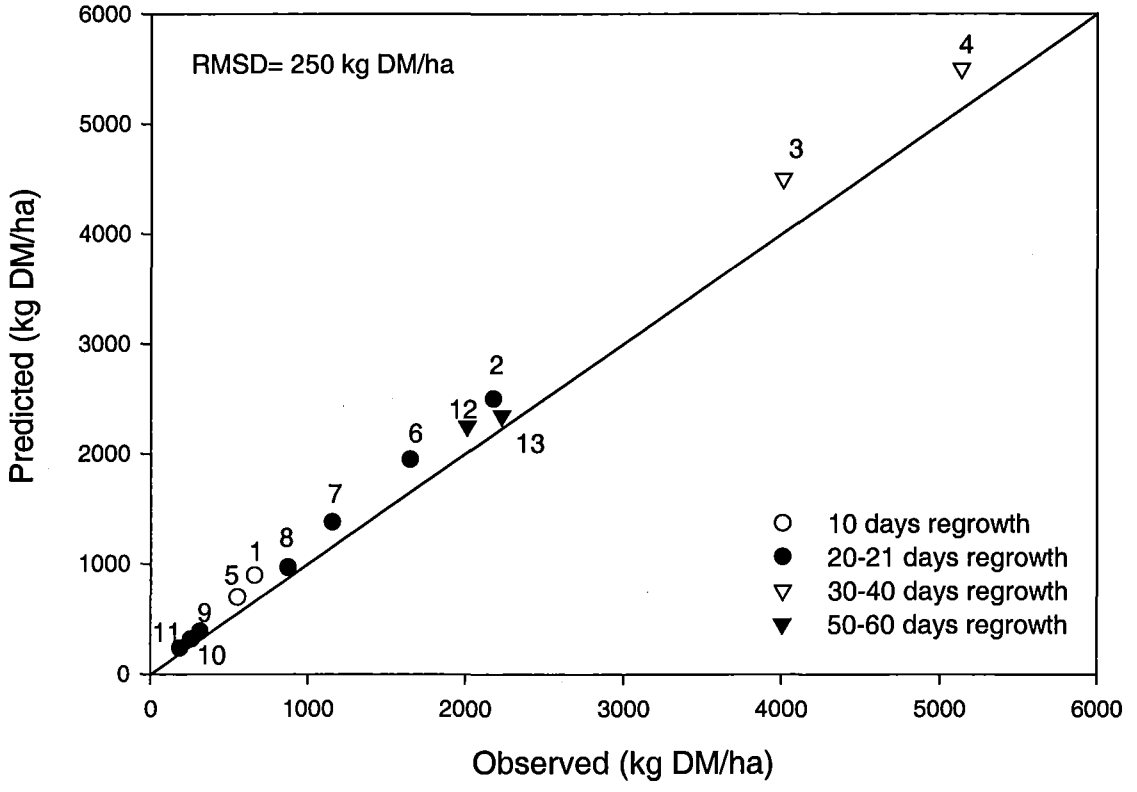

Figure 8.18 Predicted versus observed accumulated dry matter production (kg DM/ha) for cocksfoot grown in field conditions sorted by days of regrowth: 10 days (○), 20-21 days (●), 30-40 days (▽) and 50-60 days (▼). Numbers for each validation, which were presented in Table 8.1, are indicated. Simulated data were based on the complete canopy photosynthesis (including a discount for dead material) and considering partitioning. Details of the environmental conditions experienced during each period are given in Table 8.1. The line indicates a 1:1 relationship between predicted and observed values.

8.4 Discussion

8.4.1 Canopy photosynthesis model performance

The canopy photosyntheis model was used (Figure 8.1) successfully to predict cocksfoot DM production for a wide range of temperature, N, soil moisture, regrowth duration and shade environments (Figure 8.18). The use of the canopy photosynthesis model (Equations 8.1, 8.2 and 8.3) included the modified multiplicative model for *Pmax* (Section 6.3.2.5) and the 'law of the minimum factor' model for α (Section 7.3.7) as input variables, together with the canopy LAI development (Equation 8.5) and leaf senescence functions (Equation 8.6). The validation runs indicated approximately 86% of the variation in cocksfoot growth could be accounted for using the canopy photosynthesis model proposed. Thus, the model accurately simulated daily growth for the range of 9 to 134 kg DM/ha/d and for total production from 190 to 5100 kg DM/ha (Table 8.1).

The success of this approach for predicting cocksfoot growth is reliant on these relationships holding in environments outside those from which they were derived. To confer repeatability, they must have a biologically meaningful basis and should be consistent with previous reports based on canopy data for cocksfoot.

In this study, the daily respiration loss of CO_2 from the model was equal to 25% of the daily gross photosynthesis plus a variable respiration between 1.2 to 3% of the existing dry weight depending on temperature, N content and water stress. The physiological reasons for the sensitivity of maintenance respiration to temperature and N were presented in Section 2.4.

8.4.2 Limitations of the canopy photosynthesis model

The accuracy in the prediction of DM from the canopy photosynthesis model consistently overestimated the observed data (Figure 8.18). This indicates model improvement is possible.

i) *Partitioning*

In the present study partitioning was assumed to be constant (10% to the roots) for all simulations. An increase in the partitioning factor (e.g. from 10% to 12%) may improve the prediction of cocksfoot DM growth by correcting the overestimation. However, the

partitioning process is complex and it has been reported to change with different environmental and management conditions. For example, Whitehead (1995) reported that when N is deficient, grasses maximise their exploration of the soil by allocating a relatively large proportion of their photosynthate to root growth, and changes in root growth rate following changes in N supply can occur. For example, when fertiliser N at a rate of 336 kg N/ha was supplied to cocksfoot grown in soil in a glass-sided box, the rate of root growth was reduced by about 18% (Oswalt *et al.*, 1959). However, when plants are severely deficient in N, the application of fertiliser N may result in some increase in root growth (Hilbert, 1990). Caradus and Evans (1977) reported a seasonal variation in cocksfoot root growth reaching almost zero in winter due to low temperatures (mean minimum temperature < 2°C) and in summer coinciding with a lowering of soil moisture. However in this study it was predicted that at 2 °C leaf photosynthesis is zero (Section 4.3.1) and canopy photosynthesis was predicted to be zero at ψ_{lp}= -12.5 bar. Therefore it is likely that the reported lack of root growth was not due to a lack of partitioning. In addition, Evans (1973) and Davidson and Milthorpe (1966) reported that defoliation regime and intensity affected the root growth of cocksfoot. Shading can reduce the carbohydrate supply to the root system. For example, Wilson and Ludlow (1991) reported a change in shoot/root ratio of 12 tropical grasses from 2.5 at 100% light to 6.7 at 27% light. Robson *et al.* (1988), using a ^{14}C-labelled technique, reported that the percentage of photo-assimilates from the youngest mature leaf of *Lolium temulentum* to root was 17.4% at 188 W m^{-2} irradiance to 4.2% at 47 W m^{-2}. Butler *et al.* (1959) also reported that a 75% reduced light intensity caused a partial or complete stoppage of root growth for white and red clovers and lotus. However, severe shade conditions may have no effect on partitioning to roots. For example, canopy photosynthesis of cocksfoot plants grown at 10% transmissivity was predicted to be zero, so no partitioning would occur. In addition, Hilbert *et al.* (1991), using a cost-benefit model, reported an interaction response effect between daily PPFD and leaf N on the root/shoot ratio. These antecedents indicate the need for modelling partitioning for the five factors studied to quantify the amount of photosynthates derived from leaves going to the roots. A partitioning sub-model could easily be incorporated into the general canopy model.

ii) *Leaf age*

Another reason for the overestimation in cocksfoot growth from the model could be due to a difference in the photosynthetic capacity between leaves in different positions on one

tiller for any regrowth time or any accumulated LAI. The vegetative grass sward usually has three green leaves per tiller of different ages (growing leaves, first and second fully expanded leaves, and senescing leaves). The youngest expanded leaf (first fully expanded leaf) has been reported to correspond with the maximum photosynthetic capacity in a tiller (Section 2.3.1.5). In this study the effect of environmental and management factors on *Pmax* and α was carried out only on this youngest expanded leaf (first fully expanded leaf) which corresponds with the maximum photosynthetic capacity in the tiller. Therefore, it is likely that the predicted canopy photosynthesis was higher than would be obtained from the full canopy of different aged leaves. The influence of leaf age on leaf photosynthesis may have less impact on canopy photosynthesis when other factors, such as N, are limiting.

8.4.3 Uses of the canopy growth model

In addition to the prediction of DM production, the daily prediction of growth could be used for practical purposes such as to determine the optimum time to graze. For example, in Figure 8.16 it was indicated that for the non-limiting pasture situation (temperature, N, water and radiation non-limiting), the 95% light interception (which agrees with the mean maximum daily growth) occurred at a LAI= 5.4 (or 20 days of regrowth). Therefore, from the pasture productivity point of view, the optimum moment for grazing could be assumed when the mean growth rate reaches the maximum value. In contrast, after 20 days of regrowth for the same period of time under trees (Figure 8.17), the 95% light interception had not occurred (*Pn* still remained at 19 g CH_2O m^{-2} d^{-1}). In addition, as a result of the rapid turnover of leaves, any tissue that remains unharvested (cutting or grazing) would be lost through senescence. For example, the proportion of senescent and dead material increased exponentially after LAI= 4 (Figure 8.15). Thus, management decisions for grazing are a compromise between the need to retain leaf area to maximise photosynthesis but as a consequence accept a greater loss due to leaf death, and the need to remove a substantial proportion of the leaf produced for animal productivity.

Factors that decrease canopy photosynthesis also lower RUE (Sinclair and Muchow, 1999). The methodology of using the single-leaf photosynthesis functions (which were summarised into easily transferable coefficients) to predict net canopy photosynthesis, incorporating canopy architecture variables (LAI and leaf angles) and solar elevations, also provides a basis for varying the RUE response across a range of environmental conditions.

Therefore, the proposed canopy growth model could also be used for calibrating models which use RUE to predict DM production.

8.4.4 Effect of temperature on net canopy photosynthesis

There were three stages in the temperature response of maximum net canopy photosynthesis (Pn_{max}) with an optimum temperature range of 19 to 22 °C (Figure 8.4). This optimum range for Pn_{max} was lower than that for *Pmax* (19-23 °C) of a youngest expanded leaf (Section 4.3.1). This difference was caused by the maintenance respiration at the canopy level which increased with temperature according to Equation 8.3 as has been shown for other species (McCree, 1974; Woledge and Dennis, 1982).

The reduction in *Pn* at low temperatures was a consequence of a decrease in *Pmax* (Section 4.4.2). In this study, Pn_{max} at 10 °C midday air temperature (mean daily diurnal temperature of 5.8 °C) was 40% lower than the optimum situation at a maximum temperature of 21 °C (mean daily diurnal temperature of 14.8 °C). Similarly, Johnson and Thornley (1983) predicted, for grasses in general, an increase in growth from 0.16 kg carbon m^{-2} at 5 °C (mean daily diurnal) to 0.22 kg carbon m^{-2} at 15 °C after 30 days regrowth.

The decline in Pn_{max} with temperatures above 22 °C was probably caused by: (i) a decrease in *Pmax* (Section 4.3.1) and α (Section 7.3.1) due to an increase in the photorespiration rate with temperature of non-N limited and irrigated cocksfoot leaves (Section 4.4.2); and (ii) an increase in maintenance respiration with temperature (Equation 8.3). Similarly, Knievel and Smith (1973) showed that temperatures above 28 °C greatly reduce cocksfoot growth.

The effect of maintenance respiration on *Pn* is likely to be the main reason for the faster decline rate from Pn_{max} at high LAI values (Figure 8.3). For example, for the spring day (21 °C at midday) *Pn* was 16.3 g CO_2 m^{-2} d^{-1} at LAI= 9.5 but *Pn* was zero at LAI= 6.3 for a summer day of 31 °C at midday.

The optimum LAI declined with increasing air temperature from 5 units at maximum daily temperature of 10 °C to 2.3 units at 31 °C (Figure 8.4). Again, the increase of maintenance respiration with temperature may lead to reaching Pn_{max} at low LAI values as temperature increased. In practical terms, during summer regrowth periods, when maximum

temperatures often are higher than 22 °C, the rotation length should be shorter (or at a lower LAI) to maximise canopy photosynthesis and avoid loss of DM by respiration.

Also, it is important to highlight the effect of the measured intra-day variation in air temperature on canopy photosynthesis. A whole 'optimum day' rarely occurred in the field. For example, during the spring, the measured optimum temperature range for canopy photosynthesis (19-22 °C) occurred only from 10:30 to 12:30 h (Figure 8.2a) when incoming radiation was maximal. In contrast, for the summer day (maximum of 31 °C) the optimum range of temperature for canopy photosynthesis occurred only in the morning from 9:00 to 10:00 h. As a result of the diurnal variation in air temperature, canopy photosynthesis is reduced from its potential growth. For example, simulated Pn_{max} decreased from 33.9 g CO_2 m^{-2} d^{-1} with a constant optimum temperature of 21 °C over a day to a simulated value of 30.8 g CO_2 m^{-2} d^{-1} with the measured temperature pattern of the spring day (maximum of 21 °C around midday) shown in Figure 8.2a.

8.4.5 Effect of N on net canopy photosynthesis

There was a strong positive relationship between foliage N content and canopy net photosynthesis. For cocksfoot, this response showed that 4.0% N content was a critical value, below which, Pn_{max} were restricted (Figure 8.6). Thus, from 4.0 to 1.5% N content, Pn_{max} decreased by 67%. In contrast, at a leaf level, the measured *Pmax* response had a higher critical N content with a value of 5.2% N (Section 4.3.2). The effect of foliage N on canopy maintenance respiration through an increase in the maintenance coefficient *b* determined that after a N content of 4.0% Pn_{max} decreased 2.5% at 5.9% N (Figure 8.6). Thus, the critical N content for canopy photosynthesis was simulated to be lower than *Pmax*.

Duru *et al.* (1995) reported that the net canopy photosynthesis for 'Lude' cocksfoot pasture grown in a clay loam soil in Toulouse (France) with the application of 120 kg N/ha, during a period of 5 weeks and a mean daily temperature of 18.4 °C, was 3.97 g CO_2 m^{-2} h^{-1} compared with 1.94 g CO_2 m^{-2} h^{-1} for the control. Davidson and Robson (1986) reported that the gross canopy photosynthesis of a low-N (nitrate at 40 μg g^{-1}) perennial ryegrass sward had only half the *Pn* rates than those of high-N (nitrate at 200 μg g^{-1}) swards grown in a controlled environment at 20 °C and 400 J m^{-2} s^{-1} PAR. In these two studies it was found that canopy photosynthesis was doubled with the application of N. Because leaf N

content was not reported, it was not possible to compare directly with the simulations presented in this study. However, their results were consistent with the present study where simulated Pn_{max} doubled when herbage N content increased from 1.9 to 4.0% N.

Pn was affected by foliage N content due to a combination between the photosynthetic capacity of individual leaves and the influence of canopy respiration. The effect of N on photosynthetic capacity of individual leaves (*Pmax* and α) per unit leaf area can be explained by the increment of chloroplast content and the amount and activity of RuBisCO as was discussed in Section 4.4.3 and Section 7.4.3.

Also, at the highest N content (5.9%) the decline of *Pn* after it reached the maximum value was faster due to an increase in *b* values (Figure 8.5). Robson and Parsons (1978) reported that the high-N (solution 300 ppm of N) swards of perennial ryegrass had a 30% higher gross canopy photosynthesis rate at complete light interception (LAI= 5) than the low-N sward (solution 3 ppm of N). This was because the high-N sward had a higher rate of respiration per unit of dry weight (*b*= 0.029) than the low-N sward (*b*= 0.016) and the photosynthesis was partly offset by respiration. Consequently the N sufficient communities accumulated only about 15% more dry matter than those that were deficient.

In addition, in the present study, foliage N content had a small effect on optimum LAI (Figure 8.6). Thus, respiration affected by N content had a small influence at LAI< 3 when *Pn* was maximum. This indicates that similar LAI should be achieved for grazing independent of the herbage N content.

8.4.6 Effect of water status on net canopy photosynthesis

There was a negative curvilinear relationship between net canopy photosynthesis and plant water status (Figure 8.8). Thus, Pn_{max} decreased from 33.5 g CO_2 m^{-2} d^{-1} in irrigated plants to zero at a ψ_{lp}= -12.5 bar. Maintenance respiration also decreased with water stress, therefore the effect of total respiration (i.e. including photorespiration) on net photosynthesis was the main reason for the decrease of the positive range for Pn_{max} at the canopy level (ψ_{lp}= -0.1 to –12.5 bar) compared with the positive range for *Pmax* in the youngest expanded leaf (ψ_{lp}= -0.1 to –14.0 bar) (Section 4.3.4).

Jones *et al.* (1980) reported that canopy photosynthesis of a perennial ryegrass sward under field water stress conditions (daily minimum leaf water potential of –16 bar) was reduced by about half compared with an irrigated sward (daily minimum leaf water potential of –12 bar) at a similar LAI of 2.5. The same authors reported that, in controlled conditions, a sward which developed a rapid water stress (daily minimum leaf water potential of –20 bar) had reduced canopy photosynthesis from 10.0 g CO_2 m^{-2} h^{-1} in irrigated plants to only 2.2 g CO_2 m^{-2} h^{-1} in the severe water stress simulated sward. These values of canopy photosynthesis are higher than the simulated *Pn* values reported in this study. However, in the present study, ψ_{lp} rather than daily minimum leaf water potential was the input variable to predict *Pn* under different water status conditions. Therefore, no direct comparison can be made between the data reported by Jones *et al.* (1980) and simulated values from the model proposed in this study. It is likely, that ψ_{lp} of –12.5 bar, at which water stress level *Pn* was zero, fell progressively during the day reaching a more maximum negative value at noon (when radiation and temperature were highest) than those reported by Jones *et al.* (1980).

Effects of water stress on canopy net photosynthesis can be caused by stomatal and non-stomatal factors affecting the photosynthetic capacity of leaves (*Pmax* and α) and by modifying canopy respiration. In this study, the linear reduction in stomatal conductance to water vapour was the main factor that reduced *Pmax* in the youngest expanded leaf (Section 4.3.6). More severe levels of water stress can decrease the rate of net photosynthesis per unit leaf area by increasing the mesophyll resistance and by reducing the RuBP carboxylase activity in water-stressed leaves (Section 4.4.4). These effects, in addition to stomatal resistance, were confirmed in this study by the negative linear relationship between α and the water status for severe water stressed situations (ψ_{lp}< -10 bar) (Section 7.3.3).

In addition, the combined effect of low *Pn* and high R_T at severe water stress determined that the optimum LAI decreased sharply from ψ_{lp}= -11.5 to –13.0 bar (Figure 8.8) which indicates that a high LAI can not be sustained under such conditions.

8.4.7 Effect of regrowth duration on net canopy photosynthesis

The predicted *Pn* was affected by regrowth duration. For example, Pn_{max} decreased 45% from a non-limiting condition of 20 days regrowth to considering the whole canopy with 60 days regrowth (Figure 8.10). When *Pmax* and α were state variables that changed within the canopy layers (i.e. using individual actual leaf age), *Pn* decreased from the maximum value of 33.3 g CO_2 m^{-2} d^{-1} at LAI= 3.5 to 7.5 g CO_2 m^{-2} d^{-1} at LAI= 9.5 (Figure 8.9b). Similarly, Woledge and Leafe (1976) reported that for an irrigated and fertilised ryegrass sward the gross canopy photosynthesis reached a maximum value (6.5 g CO_2 m^{-2} h^{-1}) after 21 days of regrowth when the canopy achieved more than 75% light interception (LAI= 3.0) and then declined down to 4.5 g CO_2 m^{-2} h^{-1} after approximately 40 days of regrowth (LAI= 4.2) when light interception was virtually complete. Robson (1982) and Sheehy (1977) have reported similar results for perennial ryegrass.

In field situations, the effect of regrowth duration on *Pn* would be a combination of the outputs from Figures 8.9a and 8.9b. This is because, for grasses, there is a combined effect of leaf age, both in different positions on one tiller and as an ageing process of leaves in a particular position on a tiller, leading to a decrease in total canopy photosynthesis (Section 2.3.1.5). A decrease in leaf photosynthetic capacity during regrowth may be reflected in a decline in total canopy photosynthesis. The effect of regrowth duration on *Pmax* and α was discussed in Section 5.4.2 and Section 7.4.5, respectively.

However, the photosynthetic capacity of an individual leaf is only one of the factors, which controls the photosynthetic rate of the whole sward. Other important factors are the area and geometry of the leaf canopy. In this study the mean leaf canopy angle declined from 68° at LAI= 2 to 41° at LAI=7.5 (Section 3.3.8). The architecture of the canopy determines the light interception characteristics of the sward and thus the light intensity which each individual leaf receives. As the cocksfoot sward grew, LAI increased and more light was intercepted, but at the same time, shading of the lower leaves increased so that the mean leaf photosynthetic rate decreased (Section 5.4.2.1). Also, the simulated maintenance respiration increased with increasing LAI, reducing the net canopy photosynthesis.

In practical terms, in an infrequently cut or rotationally grazed cocksfoot sward, for example, a fully light intercepting canopy may be maintained throughout most of the year but the progressive impairment of the photosynthetic capacity of successive leaves over

each vegetative regrowth period would be the main limitation to yield. In contrast, the opposite may occur for continuous grazing which maintains a high leaf photosynthetic capacity, but a low LAI. This may permit light to be wasted on bareground and photosynthetically less effective leaf sheaths. Parsons *et al.* (1988) reported that the maximum gross canopy photosynthesis of a perennial ryegrass sward (irrigated and fertilised) under continuous grazing (maintained at an LAI≈ 1) was less than half that the photosynthesis measured under rotational grazing after 21 days regrowth (9 g CO_2 m^{-2} h^{-1}).

8.4.8 Effect of light intensity and light regime on net canopy photosynthesis

The net canopy photosynthesis rate of cocksfoot plants from high to low light intensities decreased as a function of the intensity and time of the PPFD level experienced. Pn_{max} decreased approximately linearly from 33.4 g CO_2 m^{-2} d^{-1} to zero as PPFD fell from full sunlight to 10% of open PPFD in a fluctuating light regime (Figure 8.13).

In summary, Pn_{max} was predicted to be reduced depending on the duration of shade at which leaves were exposed. Thus, Pn_{max} of cocksfoot plants exposed to a fluctuating regime of 90% transmissivity was 97% higher than plants exposed to 20% transmissivity (Figure 8.13). This is because the time at maximum $Pmax_G$ (1.21 mg CO_2 m^{-2} s^{-1}) and α (0.0069 mg CO_2 J^{-1}) was longer and the time required for full induction after shade for $Pmax_G$ and α was shorter for the individual leaves experiencing 90% transmissivity (Figure 8.11). A decrease in the photosynthetic capacity of leaves under shade would be responsible for the decrease in canopy photosynthesis. In this study, stomatal and non-stomatal factors were jointly responsible for the reduction and induction of *Pmax*, with their relative importance dependent on the duration and intensity of shade (Sections 6.4.3 and 6.4.4). Also, the magnitude for reduction in α depended on the duration and intensity of shade (Section 7.4.6).

In addition, it was predicted that the continuous light regime of 50% transmissivity had higher Pn_{max} than the same intensity but for a fluctuating light regime (10.4 vs 8.4 g CO_2 m^{-2} d^{-1}) (Figure 8.12). The main reasons for the differential response between light regimes would be caused by differences in the leaf photosynthetic rate ($Pmax_G$ and α):

(i) the higher Pn_{max} under continuous 50% transmissivity light regime would result also from the induction process. This does not occur in the continuous regime, and consequently no time was required to reach maximum photosynthesis upon the return to

full sunlight (which does not occur) under this level of light (Section 6.2.3.1). In contrast, cocksfoot plants under fluctuating light regime of the same intensity required 20 minutes for full induction after the increase of PPFD from severe shade (5% of open PPFD) to full sunlight (Section 8.3.5).

(ii) a faster decrease in *Pmax* during periods of severe shade. In this study, the decrease in *Pmax* in the first 30 minutes after entering shade was 92% faster for plants grown at 5% of open PPFD than those grown at 50% of open PPFD (Section 6.3.1.1). This was consistent with a faster closure in stomata (Section 6.3.1.2) and a 2.5-fold greater non-stomatal limitation for plants grown at 5% of open PPFD than at 50% of open PPFD (Section 6.3.1.5).

(iii) the minimum value of α_s for plants grown at 5% of open PPFD was 20% lower than those grown at 50% of open PPFD.

Therefore, to accurately predict understorey responses of species in silvopastoral systems, slatted rather than cloth structures are required. A decline in canopy photosynthesis related to a decrease in light under a continuous light regime (cloth shade) has previously been reported by Frank and Barker (1976). They found a decrease in the rate of net photosynthesis of about 80% when light intensity was reduced from 1160 to 200 μmol m^{-2} s^{-1} PAR for a whole cocksfoot plant in controlled environment conditions. However, in silvopastoral systems understorey plants experience frequent fluctuations in irradiance from full sun to shade caused by tree canopy shading. The physiological controls on photosynthesis rate that operate during such fluctuations are different from those that operate under steady-state conditions (Section 2.3.1.1). There have been few reports referring to the effect of fluctuating light regimes on canopy photosynthesis. Rabinowitch (1956) stated that the rate of photosynthesis of plants under intermittent light could only approach, and not exceed, the rate of photosynthesis under continuous light of identical mean light flux densities (Section 2.3.1.1.2). Similarly, Varella *et al.* (2002) predicted for lucerne in a non-limiting situation, and for a leaf angle of 45°, that an intermittent light regime of 50% PPFD produced slightly less net canopy photosynthesis than the same continuous light PPFD. Thus, these results indicate that the cloth structure did not accurately simulate the *Pn* response of cocksfoot in silvopastoral systems. To date, most of the shade experiments have been carried out using artificial structures which provided continuous light regime (Eagles and Treharne, 1969; Eagles, 1973; Singh *et al.*, 1974; Frank and Barker, 1976; Devkota *et al.*, 1997, 2001).

In addition, $Pmax_G$ may be slightly overestimated, mainly in the full sunlight particularly at high LAI values (LAI> 5). This is because deep in the canopy a leaf may be acting like it is in partial shade due to the movement of the leaves above. There is evidence that *Pmax* depends on the average light level experienced by a leaf, so leaves lower down the canopy have lower *Pmax* values (Robson *et al.*, 1988; Sands, 1995).

The simulated optimum LAI decreased linearly from 3.7 under a full sunlight regime to 0.7 under 20% transmissivity (Figure 8.13). This was because *Pn* decreased with a decline in daily PPFD due to the influence of maintenance respiration which caused a decrease in the optimum LAI. The maintenance respiration also defined the level of *Pn* after the optimum LAI. For example, under full sunlight conditions *Pn* was 18.8 g CO_2 m^{-2} d^{-1} at LAI= 9.5, under a fluctuating light regime of 20% transmissivity *Pn* was zero at LAI= 1.6. This was consistent with Varella *et al.* (2002) who predicted for lucerne (leaf angle 45°) that *Pn* was zero at LAI> 4 for 20% canopy transmissivity and *Pn* was 35 g CO_2 m^{-2} d^{-1} at LAI= 8.

8.5 Conclusions

- Temperature, foliage N content, water status, regrowth duration and light regimes of cocksfoot plants modified the utilisation of solar energy for net canopy photosynthesis.

- The range of optimum net canopy photosynthesis for temperature and N changes over the conditions tested was less than the range over which leaf net photosynthesis (*Pmax*) was optimum. Also net canopy photosynthesis fell to zero at a level of water stress less than at which *Pmax* reached zero.

- The light regime of the silvopastoral system, characterised by periods of full sunlight and severe shade, was accurately simulated using slat structures. Continuous light regime from shade cloth overestimated *Pn* by 20% compared with the slatted structures of the same light intensity over a day but with a fluctuating light regime. This indicated that the artificial slatted structure is more suitable for simulating the response of understorey pasture species in silvopastoral systems.

• The linking of leaf photosynthesis models (*Pmax*, α, and θ) into a canopy model together with respiration, partitioning and the main canopy characteristics affecting light interception (LAI and leaf angle) was a successful approach to predict cocksfoot growth under different temperature, N, water, regrowth duration and shade situations. The model explained about 85% of the variation in cocksfoot growth. However, the canopy model overestimated growth in all validations tested in this study. Thus, a further *Pmax* function for different leaf ages in different positions on tillers and particularly a partitioning sub-model for the five factors studied may be needed to improve the model applicability for DM prediction for cocksfoot pastures.

• The canopy photosynthesis model, through a daily prediction of DM growth, can also be used for practical purposes such as to determine the optimum moment for grazing by evaluating when the actual *Pn* of the sward falls below the expected mean *Pn* for environment and management situations.

In the next chapter, the results from previous chapters are drawn together. The results from this study are compared with those previously reported in the literature and practical implications for predicting cocksfoot DM production in silvopastoral systems are discussed.

CHAPTER 9

General discussion

9.1 Effect of environmental and management changes on DM production in the silvopastoral system

For this study, a wide range of environmental and management conditions were created through changes in light intensity and light regime, temperature, soil moisture, N and regrowth duration. The purpose was to create the range of understorey conditions experienced in a silvopastoral system and then determine the DM production response of cocksfoot to these conditions. This also provided a framework to generate and validate a semi-mechanistic mathematical model of understorey DM production, based on the photosynthetic capacity of leaves and canopy characteristics that affect light interception.

The mean daily temperature during this experiment ranged from 6 °C in winter to 16 °C in summer with a mean minimum temperature of 1.4 °C and mean maximum temperatures of 22.6 °C (Section 3.2.3.1). As a result of the tree competition, irrigation, the application of synthetic urine (300 kg N/ha), regrowth duration and seasonal effects, the mean soil VWC in the top 500 mm varied from 33 to 8.5% (Section 3.2.3.2) and the herbage N content in cocksfoot plants ranged from 1.5 to 5.9% (Section 4.3.2).

The specific component unique to silvopastoral systems compared with open pastures is the light regime. The tree canopy and slatted structures used in this study reduced and modified the light available to the understorey. Specifically, the daily PPFD integral was reduced by 38% under trees and 74% under the slatted structures in the silvopastoral system (Section 3.2.3.3). The temporal pattern of available PPFD also changed in silvopastoral systems. The daily PPFD under the 10-11-year-old trees had alternating periods of full sunlight (~1800 μmol m^{-2} s^{-1} PPFD at midday) and severe shade (~130 μmol m^{-2} s^{-1} PPFD) with intervals of full sunlight and shade that changed from 45-60 minutes (8:00 to 11:00 and 17:00 to 20:00) to 90–120 minutes around midday (Section 6.2.1.1). A similar light pattern was obtained from the slatted structures, which provided a bimodal light regime analogous to the silvopastoral system (Figure 6.1b; Section 6.2.1.1).

In silvopastoral systems the period of full sunlight and shade may change over time according to the development of tree crowns and silvicultural practices applied during the rotation length. This contrasts with a continuous the light regime throughout a day (Figure 6.1c), provided by the shade cloth, which is the most widely used artificial shade to evaluate the effect of shade in a silvopastoral system (Section 2.3.1.1).

The variations in light regimes, together with the environmental and management factors over time, provided a range of DM growth rates from 2 to 154 kg DM/ha/d. This variation in DM growth was in the range previously reported in the literature (Section 2.2). The differences in pasture production rates consequently affected the annual DM production. Particularly, from February 2000 to February 2001 the mean annual total DM production was 8.2 t DM/ha/yr in open, 7.3 t DM/ha/yr in open pasture under slat shade (~43% of open PPFD), 6.3 t DM/ha/yr under trees shade (~58% of open PPFD) and 3.8 t DM/ha/yr in trees+slat situation (~24% of open PPFD). The potential annual yield recorded for the Canterbury sub-humid temperate environment in open pastures, irrigated and fertilised with N as synthetic urine was 28.6 t DM/ha/yr (Section 3.3.1.3). The interaction of DM growth rate and time indicated the range and pattern of DM production required to be predicted by the canopy photosynthesis model developed for the silvopastoral system.

9.2 Structural changes leading to DM changes

The variation in cocksfoot DM production was related to changes in canopy LAI from 0.5 to 8.2 units (Section 3.3.5). The main changes in the morphological development of cocksfoot plants under shade, which affected LAI, were a reduction in tiller population and canopy etiolation (Appendix 4) probably caused by the decrease in the R:FR ratio (Section 3.2.3.4). However, a single relationship between DM production and LAI (Equation 3.4; Section 3.3.7) accounted for the differences in cocksfoot canopy development over time in morphological aspects of the sward caused by environmental and management factors. This was consistent with Duru *et al.* (1997) who reported, for cocksfoot, a single exponential function between LAI and DM for different N levels. Thus, no modelling of tiller and canopy height dynamics was needed for DM predictions into a canopy growth model. The relationship between DM production and LAI was then incorporated into a canopy photosynthesis model to determine the foliage increment for each day of growth.

Another change in the canopy architecture of cocksfoot plants grown at low light level was the mean canopy leaf angle which was 9° more horizontal under severe shade (~24% of open PPFD) than in full sunlight (Section 3.3.8). This difference resulted in significant differences in the mean extinction coefficient (k) of the canopy. Full sunlight pastures had a k value of 0.38 compared with 0.48 of the pastures under ~24% of the open PPFD (Table 3.10; Section 3.3.8). Changes in canopy leaf angle, or k, for cocksfoot with fluctuating light regimes have not been previously reported and therefore it provided new knowledge. Regrowth duration also affected the canopy architecture of cocksfoot pastures when fertilised with 300 kg N/ha and irrigated. The mean canopy leaf angle decreased from 68° at day 20 to 40° at day 60 during the January-February regrowth period. This was consistent with Pearce *et al.* (1967) and Sheehy and Peacock (1977) (Section 2.3.2.6). The mean canopy leaf angles, or values of k, found in this study were then incorporated into the canopy photosynthesis model for DM growth prediction.

9.3 Mechanistic changes leading to DM changes

The prediction of DM production was based firstly on the creation of an integrated leaf photosynthesis model which predicted the response of net photosynthesis to different environmental and management factors.

9.3.1 Leaf photosynthesis models

Leaves are the functional units of pasture photosynthesis and their efficiency of capture and utilisation of solar energy determines pasture productivity (Section 2.3.1). Leaf photosynthesis has frequently been described as a function of PPFD, using a non-rectangular hyperbola function. From this, three parameters are derived to predict growth in pastures through a canopy photosynthesis model: the light-saturated rate which represents the asymptote or maximum saturated leaf photosynthetic rate (*Pmax*), the initial slope of the light response curve or photosynthetic efficiency (α) and a dimensionless parameter indicating the degree of curvature (θ) (Section 2.3.1). Therefore, the first step to develop the predictive model of cocksfoot growth in silvopastoral systems was the determination of the individual relationships between *Pmax*, α and θ and the main environmental and management variables that influence DM production.

9.3.1.1 *Pmax* model

For *Pmax*, a multiplicative model was proposed (Figure 6.14; Section 6.3.2.5) that integrated the light regime response of *Pmax* with temperature, N, soil moisture and regrowth duration. From this, *Pmax* can be predicted for silvopastoral systems where a single factor, two, three, four or all five are limiting or in non-limiting situations for the range:

(i) air temperatures from 2 to 37 °C (including extrapolation of Equation 4.2),

(ii) water status from ψ_{lp} –0.1 to –16.0 bar (corresponding to a soil VWC in the top 500 mm of 8.5 to 34%),

(iii) foliage N content from 1.5 to 5.9%,

(iv) regrowth duration from 20 to 60 days,

(v) the time course of shade (severe or moderate) from 1 to 180 minutes (which can be calculated for different tree canopy cover) and the correspondent induction process from 30, 60 and 180 minutes of severe shade.

This model has five individual functions of *Pmax* for each variable studied and three interaction functions for situations of low N ($\leq$ 2%) and high temperature (> 23 °C), regrowth duration ($\geq$ 40 days) and water status (ψ_{lp} –0.1 to –16.0 bar), and time under severe shade (1 to 180 minutes) and water stress (ψ_{lp}= –4 to –13 bar). Validation of this model (objective 5 of this study) indicated 78% of the variation in *Pmax* could be accounted for using these five factors by the addition of the interaction functions.

In its simplest form, using temperature, water status, N and regrowth duration, the *Pmax* model provides a basis for predicting DM production of cocksfoot in any open pasture situation where these four variables are known. Thus, validation of the open pasture could occur in any temperate environment.

Results from the present study showed that the fluctuating light regimes influenced the net leaf photosynthesis rate of cocksfoot plants depending on the time and intensity of the full sunlight/shade periods (Figures 6.3 and 6.5). Plants exposed to longer periods under severe shade (5% of the open PPFD), reached lower levels of *Pmax* and required a longer duration to reach full induction than plants exposed to a shorter shade period. Thus, trees with a larger crown could be expected to reach lower levels of *Pmax* and take longer to return to maximum *Pmax*. The result of these differences in the response to the temporal pattern of

shade would be an overall change in canopy carbon gain over a day for cocksfoot plants. Therefore, the time course of shade affecting leaves was incorporated into the canopy photosynthesis model for the silvopastoral system.

The only interaction for *Pmax* in the silvopastoral system was between severe shade and water stress, and it was characterised by three distinct aspects (Section 6.3.2.3): (i) *Pmax* did not decrease in a multiplicative way when both factors were limiting; (ii) the decrease in *Pmax* during the initial period under severe shade was faster for plants grown with water stress than those grown without water stress. Therefore, cocksfoot plants that experienced water stress during alternating light/shade intervals appeared to have a more sensitive response pattern in *gs* than plants grown in full sunlight; (iii) as occurred with irrigated plants, the reduction of *gs* in water stressed plants occurred slower than the reduction in *Pmax* under severe shade. This indicates that factors other than stomatal closure caused the reduction in *Pmax* during the first minutes under shade of these water stressed plants (Sassenrath-Cole and Pearcy, 1994; Pearcy *et al.*, 1996). The relative importance of stomatal and non-stomatal limitations for the reduction in *Pmax* of plants exposed under severe and moderate shade derived in the present study (Section 6.3.1.5) provided a mechanistic explanation and also improves the predictive ability of the model. This interaction represents new information for silvopastoral systems because previously the effects of shade and water stress on cocksfoot and grasses in general, have only been reported on a seasonal dry matter basis (Braziotis and Papanastasis, 1995; Devkota *et al.*, 1997, 1998; Joshi *et al.*, 1999).

Another important aspect for the prediction of *Pmax* in silvopastoral systems was the use of canopy temperatures under severe shade rather than air temperature. For irrigated plants air temperature was higher than canopy temperature under shade (Section 6.3.2.1). The difference between air and canopy temperature under severe shade (T_{a-c}) against air temperature varied according to an exponential function (Figure 6.10). From 10 to 31 °C air temperature, the mean T_{a-c} ranged from 1.8 to 7.4 °C under the shade. These differences probably resulted from the energy balance of leaves through a reduction in the incoming radiation (Section 2.2.2). The differences between air and canopy temperatures indicate that canopy temperature needs to be used directly for predicting canopy photosynthesis in a silvopastoral system. Alternatively, if only air temperatures are available, a predictive

function for canopy temperatures is required as a function of different shade intensities and temperature levels.

The success of using the five factors and the interaction factor for predicting *Pmax* showed that predictions were transferable from open to shade conditions and suggests a similar approach could be used in other silvopastoral environments (outside those in which these equations were derived).

9.3.1.2 α model and θ

The use of the 'law of the minimum factor' model (Equation 7.10; Section 7.3.7) resulted in the development of an empirical model, which accurately predicted α for a wide range of temperature, N, water status, regrowth duration and shade environments created in this study (objective 4 of this study). The integrated influence of these factors on α of cocksfoot leaves in a silvopastoral system has not been defined previously (Section 2.3.1.6). Validation of the model indicated approximately 88% of the variation in α could be accounted for using these five factors as single functions without recourse to interactions (objective 5 of this study). This confirms that the rate of α was controlled by the most limiting factor when temperature, N, water status, regrowth duration and/or shade were limiting and not by the multiplicative effect of factors as occurred with *Pmax*.

A depression of α may result in a reduced capability of leaves to operate efficiently under low light. Values of α are determined by the efficiency with which absorbed photons are used for CO_2 assimilation and are related to the RuBisCO activity and photorespiration (Section 2.3.1.6). This was confirmed in this study because there were indications that factors other than stomatal conductance (*gs*) affected photosynthesis (e.g. N and shade) (Sections 4.3.6 and 6.3.1.5) and consequently it was likely that these factors also affected α.

The decline of *Pmax* was always more marked than the decrease in α for all the factors studied, indicating that *Pmax* was affected more by the physical (e.g. reduction in stomatal conductance) and biochemical limitations of the photosynthetic process than α. These differential effects of environmental factors on *Pmax* and α agree with those values reported by Marshall and Biscoe (1980b) for winter wheat and Thornley (1998) for temperate grasslands in general.

In contrast, θ was unaffected by the range of temperature, N, water status, regrowth duration and shade created (Figure 7.6) presenting a mean value of 0.96. Based on Marshall and Biscoe (1980a) and Thornley and Johnson (2000) who describe θ as the ratio of physical to total resistance to CO_2 transfer, it was estimated that the carboxylation resistance represented 3.5% of the total leaf resistance to CO_2 transfer and that it did not change substantially under the changing conditions used in this study.

9.3.2 What is shade-tolerance

The rate of decrease in *Pmax* under different shade intensities and the responses during induction could be used as physiological indicators to define shade-tolerant species from a physiological perspective in silvopastoral systems. Thus, species with a slow decrease in *Pmax* when exposed to shade, or fast responses to induction (higher values of IS_1 and IS_{10}), would be classified as more shade-tolerant because they would increase the carbon photosynthetic gain. It could be expected that species with faster stomatal opening during the slow phase of induction would allow greater sun utilisation of fluctuating light in silvopastoral systems. Cocksfoot showed a fast induction response (IS_1= 67 after 60 minutes under shade of 85-95 μmol m^{-2} s^{-1} PPFD). In comparison, Chazdon and Pearcy (1986) reported an IS_1 value of approximately 45 for leaves of the shade-tolerant *Alocasia macrorrhiza* after 60 minutes under shade (7-10 μmol m^{-2} s^{-1} PPFD). Kursar and Coley (1993) reported that the induction of CO_2 assimilation to 50% of eight shade-tolerant species of Panama's rainforest occurred very quickly ranging from 1 to 3 minutes after 15 minutes at < 10 μmol m^{-2} s^{-1} PPFD. Thus, cocksfoot on the basis of a comparative slow decrease rate in *Pmax* and fast responses to induction, would be classified as a 'shade tolerant' species from a physiological perspective. However, under grazing regimes the concept of 'shade tolerance' may be more appropriate if the morphological response of the pasture to changes in light quality and quantity under tree shade, together with changes in feeding value, are considered. A more universal criteria to evaluate the tolerance to shade of pastures under grazing regimes would be the ecological stability and persistence of a pasture together with animal production per hectare.

9.3.3 Advances in predicting leaf photosynthesis

The success of the approach used for predicting *Pmax* using the multiplicative model and α using the 'law of the minimum factor' model was that they can be used in environments

outside those in which the equations were derived. The individual photosynthesis functions of the models were empirically derived and summarised into easily transferable coefficients using "broken stick" or non-linear regressions. These physiological variables provided a theoretically explanation of a proportion of the variation in DM growth found in this study. They can be used to assist the prediction of pasture growth through their incorporation into a canopy photosynthesis model. These models extend our knowledge of pasture growth prediction in silvopastoral systems because the integrated relationships between shade limitation in fluctuating light regimes and other environmental (temperature, N and water stress) and management (regrowth duration) factors affecting photosynthetic rate of cocksfoot leaves have not been defined (Section 2.3.1.7). Thornley (1998), who quantified the important abiotic and biotic factors necessary to develop a comprehensive mechanistic simulation model of grassland ecosystems, did not take into account limitations from regrowth duration and light regimes.

Furthermore, the individual factor responses also provide a basis for varying the RUE response across a range of environmental conditions. Factors that decrease *Pmax* and α also lower RUE (Sinclair and Muchow, 1999). The important consequence is that the highest α for foliage canopies occurs when most leaves receive low irradiance and operate near maximum RUE. Canopy architecture determines the distribution of irradiance over the photosynthetic surfaces and hence, relative to the leaf α, the possibility for high canopy RUE. This also becomes important in silvopastoral systems where low irradiance is imposed by the tree shade. Therefore, the proposed models could also be used for calibrating models which utilise RUE to predict DM production.

A major simplification in the model was that there is no prediction for the reproductive stage of the pasture. The main reasons for this is that, in well-managed pastures, there is little reproductive growth. Thus, grazing and cutting regimes are applied for farmers to avoid reproductive pastures because they have a low digestibility and palatability. This simplification is consistent with Thornley (1998).

9.4 Canopy photosynthesis model

A physiologically based description of pasture growth operates through changes in the efficiency of conversion of energy to DM and the total amount of energy available for this conversion. This in turn is influenced by the combination of light interception and

photosynthetic activity of individual leaves within the canopy which are influenced by environmental and management changes (Section 2.3). Consequently, the canopy photosynthesis model used to predict growth in this study was based on the amount of light intercepted by leaf surfaces (dependent upon LAI and canopy architecture) at different depths in the canopy and the resulting level of photosynthesis of those leaves. The subsequent partitioning of photosynthates to growth and respiration is the basis for DM production (Section 8.2). Therefore, simulations of net canopy photosynthesis (*Pn*) for cocksfoot in different environmental and management conditions were carried out using the leaf photosynthesis models developed for *Pmax*, α and θ into a canopy photosynthesis model according to objective 6 of this study. The effects of temperature, water status, N%, regrowth duration and shade (intensity and light regimes) on cocksfoot daily canopy photosynthesis, when only one of these factors was limiting, was examined.

For all simulations, the *Pn* response was parabolic against LAI and increased to reach a maximum and then declined as LAI increased further. The environmental and management factors affected the maximum *Pn* (Pn_{max}) and the optimum LAI (LAI at Pn_{max}). For example, Pn_{max} ranged from ~34 g CO_2 m^{-2} d^{-1} (irrigated, >4 %N, 21 °C, 20 days regrowth and full sunlight condition) to zero at water stress of ψ_{lp}= –13.0 bar. Optimum LAI values ranged from 5 units at 10 °C (only temperature limiting) to 0.1 units for water stressed plants (ψ_{lp}= –12.9 bar).

The range of optimum net canopy photosynthesis for temperature and N changes over the conditions tested was less than the range over which leaf net photosynthesis (*Pmax*) was optimum. Also net canopy photosynthesis fell to zero at a level of water stress less than at which *Pmax* reached zero. These differences were due to a combination between the reduction in photosynthetic capacity of individual leaves within the canopy and the influences of canopy respiration.

From simulations of *Pn* at different light intensities of the fluctuating light regime, Pn_{max} was reduced depending on the duration of shade at which leaves were exposed. Pn_{max} decreased approximately linearly as light intensity declined from 33.4 g CO_2 m^{-2} d^{-1} under a full sunlight regime to zero under 10% of open PPFD in a fluctuating light regime (Figure 8.13; Section 8.3.5). Thus, Pn_{max} of cocksfoot plants exposed to a fluctuating light regime of 90% transmissivity was 97% higher than plants exposed to 20% transmissivity

because the time at maximum $Pmax_G$ (1.21 mg CO_2 m^{-2} s^{-1}) and α (0.0069 mg CO_2 J^{-1}) of individual leaves was longer and the time required for full induction after shade for $Pmax_G$ and α was shorter (Figure 8.11; Section 8.3.5). Consequently, a decrease in the photosynthetic capacity of leaves under shade would be responsible for the decrease in canopy photosynthesis for different times under severe shade. In this study, stomatal and non-stomatal factors were considered to be jointly responsible for the reduction and induction of *Pmax*, with their relative importance depending on the duration and intensity of shade (Sections 6.4.3 and 6.4.4). Also, the magnitude for reduction in α depended on the duration and intensity of shade (Section 7.4.6).

By calculating the time course of shade for a particular tree canopy (from different tree planting density, age, pruning and thinning intensities, crown size, etc.) in a silvopastoral system, it was possible to predict *Pn* for up to 180 minutes of severe shade and the correspondent induction process (from low to high light levels).

9.4.1 Fluctuating versus continuous light regime

In addition to light intensity, light regime is an important aspect for predicting daily canopy photosynthesis and growth in silvopastoral systems. It was predicted that the continuous light regime of 50% transmissivity throughout a day had higher Pn_{max} than the same intensity but for a fluctuating light regime with periods of 90-120 minutes of full sunlight and severe shade (10.4 vs 8.4 g CO_2 m^{-2} d^{-1}) (Figure 8.12; Section 8.3.5). There has been little research reporting the effect of fluctuating light regimes on canopy photosynthesis. The results of this study agree with Rabinowitch (1956) who stated that the rate of photosynthesis of plants under intermittent light could only approach, and not exceed, the rate of photosynthesis under continuous light of identical mean light flux densities (Section 2.3.1.1.2). Similarly, Varella *et al.* (2002) predicted that lucerne, in a non-limiting situation and for a leaf angle of 45° that an intermittent light regime of 50% PPFD would produce slightly less net canopy photosynthesis than the same continuous light PPFD.

The reasons for the overestimation in *Pn* under a continuous light regime compared with a fluctuating light regime were: (i) the decrease in *Pmax* in the first 30 minutes after entering shade was 92% faster for plants grown at 5% of open PPFD than those grown at 50% of open PPFD (Section 6.3.1.1) and values of α for plants grown at 5% of open PPFD were

20% lower than those grown at 50% of open PPFD (Section 7.3.5); (ii) under continuous 50% transmissivity light regime the induction process did not occur, consequently no time was required to reach maximum photosynthesis under this level of light (Section 6.2.3.1). In contrast, cocksfoot plants under a fluctuating light regime of the same intensity required 20 minutes for full induction after the increase of PPFD from severe shade (5% of open PPFD) to full sunlight (Section 8.3.5).

Consequently, these results strongly indicate that artificial cloth structure, which has been used widely to simulate light reduction (Section 2.3.1.1.1), is not suitable to represent the response of understorey pastures in temperate silvopastoral systems.

9.5 Validation and use of the canopy photosynthesis model

Following the primary objective and specifically objective 7 of this thesis, the simulated values from the canopy photosynthesis model were validated against observed DM data (Section 8.3.6) obtained for cocksfoot pastures grown under a diverse range of environmental and management situations in the open and under trees (Chapter 3).

This canopy photosynthesis model (Equations 8.1, 8.2 and 8.3) included the multiplicative model for *Pmax* (Section 6.3.2.5) and the 'law of the minimum factor' model for α (Section 7.3.7), together with a canopy LAI development function (Equation 8.5) and the proportion of senescent leaf function (Equation 8.6). Combined this resulted in the development of a complete model which predicted cocksfoot growth for a wide range of temperature, N, soil moisture, regrowth duration and shade environments. The relationship between carbohydrate and LAI (Equation 8.5), used to determine the foliage increment after each day of growth, was indirectly derived from the empirical relationship between accumulated DM production and LAI (Equation 3.4) by subtracting the N and minerals content. An independent function derived from actual measurements of carbohydrates and LAI may be needed for model improvement and independent validation.

Validation from 13 observed DM data points obtained from different environmental and management conditions indicated approximately 86% of the variation in cocksfoot growth was accounted for using the complete model proposed in Figure 8.1. Thus, the simulated growth was close to the observed data.

The canopy photosynthesis model proposed in this study provides a powerful and valuable tool for understanding and predicting the pasture understorey DM production in silvopastoral systems. To date, net canopy photosynthesis models have been used for different crops and for grasslands under full sunlight regimes (Section 2.3). Therefore, the model derived in this study of fluctuating light regimes contributes by filling gaps in our knowledge of general pasture growth models. This power to predict the effects of changes in DM production may also have immediate application in pasture management or in helping agronomists to improve practices in silvopastoral systems.

Prediction of pasture production on a farm basis is an important part of feed planing. Feed profiling (for appropriate stocking rate), feed budgets (for seasonal planning) and grazing plans (short-term planning to achieve desired intakes and rotation length) need an accurate assessment of DM production (Lucas and Thompson, 1990). Using the model proposed in this study different seasonal scenarios affecting DM production (e.g. dry summer or cool spring), may provide different strategies for farmers. Also, it is possible to simulate the potential increase in DM production (or the equivalent of animal performance) from N fertiliser or irrigation interacting with shade in silvopastoral systems.

Also, the daily prediction of growth can be used for practical purposes such as to determine the optimum moment for grazing. For example, it was indicated that for the non-limiting pasture situation (temperature, N, water and radiation non-limiting), the 95% light interception (which agrees with the mean maximum daily growth) occurred at LAI= 5.4 (or 20 days of regrowth). Therefore, from the pasture productivity point of view, the optimum moment for grazing could be assumed when the mean growth rate reaches the maximum value. In contrast, after 20 days of regrowth for the same period of time but under trees, the 95% light interception had not occurred (*Pn* still remained at 19 g CH_2O m^{-2} d^{-1}). This indicates that long grazing rotations may be used under trees to provide time to accumulate sufficient pasture mass. However, problems from longer spelling times would be: i) taller pasture and increased reproductive development that result in reduced bulk density, and ii) older forage of lower nutritive value.

9.6 Dynamic features of the cocksfoot model

An important characteristic of models is the dynamic component (Section 2.3). A dynamic model describes the time-course of various state variables, such as nitrogen content, or leaf water potential and driving environmental variables such as temperature or radiation. In this study, *diurnal time-scale* for predictions of DM production in silvopastoral systems arises from the diurnally varying components of the environment such as temperature and radiation. Air temperature and radiation are readily available field measurements. For silvopastoral systems, canopy temperature needs to be used directly for prediction of canopy photosynthesis. Alternatively, if only air temperatures are available, the function to predict canopy temperatures from air temperatures fitted (Section 6.3.2.1), for pine tree shade in this study, gave a practical solution. To quantify available radiation or PPFD for understorey pasture requires the installation of quantum sensors under trees.

In contrast, water status expressed as pre-dawn leaf water potential (ψ_{lp}) and foliage N content are state variables which are difficult to obtain from field measurement on a daily basis for input in a model. Because ψ_{lp} is difficult to measure, the strong relationship between ψ_{lp} and soil VWC (0-500 mm depth) (Figure 4.6; Section 4.3.5) provides an alternative method to predict growth from the soil VWC in this experiment. A solution to this practical limitation would be the incorporation of a water module. For example, Thornley (1998) reported a complete water basis sub-model which predicts plant water potential for pastures in general based on the masses of water in soil, root and shoot, and processes of rainfall, rainfall interception and evaporation from the canopy, drainage, movement of water from the soil to root, root to shoot, and evapotranspiration. Also, Coughenour (1984) described a grassland water sub-model which includes a plant water pool, substrate pool, water movement driven by a water potential gradient for a multi-layered soil.

Similarly, leaf N was estimated from bulked leaf dry matter samples in the present study. Additional measurements by SPAD meters may provide a rapid and acceptable level of accuracy for field prediction. Other alternatives, to obtain the N content on a daily basis as an input for the model, would be incorporating a N module into the canopy photosynthesis model proposed in this study. Thornley (1998) described a sub-model to predict the N content in pastures over time, which is influenced by seasonal variation (temperature, soil moisture, etc.). The shoot N pool arises from transport of substrate N from the root which

includes the N uptake from the soil mineral N pool, recycling from litter, utilisation of substrate N for shoot growth and flux of substrate N with senescence to the soil. Also, Greenwood and Barnes (1978) reported a theoretical model for predicting the decline in N content in plants during growth.

These water and N sub-models could be easily incorporated into the canopy photosynthesis model to provide dynamic values of ψ_{lp} and herbage N content.

9.7 Model improvements

Overall the predictions obtained from the cocksfoot model, compared with the 13 observed DM data points, showed an overestimation in growth. This indicated that the complete model had limitations and still needs to be improved indicating the need for future work. In order of importance these are as follows:

i) The main limitation for predicting DM production was that in the present study partitioning to roots was assumed to be constant (10%). It has been reported that partitioning changes with N, temperature, defoliation regime and shade (Section 8.4.2). These antecedents indicate the need for modelling partitioning for the five factors studied to quantify the amount of photosynthates derived from leaves going to the roots. A partitioning sub-model could easily be incorporated into the general canopy model.

ii) The variation in leaf angle, with four levels of light intensity and the reduction in the R:FR ratio, indicates the need for a sub-model that predicts variation in leaf angle or k for a continuous range of shaded environments.

iii) Another reason for the overestimation in cocksfoot growth from the model could be due to the photosynthetic capacity between leaves in different positions on one tiller being different for any regrowth time or any accumulated LAI. The youngest expanded leaf has been reported to correspond with the maximum photosynthetic capacity in a tiller (Section 2.3.1.5). In this study the effect of environmental and management factors on *Pmax* and α was carried out only on the youngest expanded leaf. Therefore, it is likely that photosynthesis was overestimated at a canopy level. A leaf age function for leaves in different positions on a tiller would reduce photosynthesis of the canopy.

iv) In this study, the total foliage N content was used for growth prediction. However, there is evidence that N content varies as a function of relative depth in the canopy. Therefore, the carbon gain for a whole canopy should be maximised when leaf N is distributed in such a way that the leaves in the microenvironments receiving the highest PPFD have the highest N concentrations (Field, 1983; Hirose and Werger, 1987a). Therefore, a sub-model to calculate the distribution of leaf N within the canopy may improve the growth prediction.

9.8 Conclusions

The canopy photosynthesis model proposed in this study to predict DM production in silvopastoral systems is a semi-mechanistic model based on the photosynthesis process (mechanistic component) with individual functions empirically derived and summarised into easily transferable coefficients (empirical component). To date, growth models have been used for different crops and for grasslands under full sunlight regimes. Therefore, the model proposed in this study provides an improvement in knowledge for pasture growth prediction because it integrates relationships between shade limitation in fluctuating light regimes and other environmental (temperature, N and water stress) and management (regrowth duration) factors affecting photosynthetic rate of cocksfoot pasture in a silvopastoral system. Also, the semi-mechanistic canopy model provides a powerful and valuable tool for understanding and predicting the pasture understorey DM production in silvopastoral systems. This model needs to be tested outside the environment in which it was derived. Thus, independent validations using a quantitative comparison with measured DM data points obtained from other environment/management scenarios (lower or higher temperatures, different soil textures) or contrasting silvopastoral sites (other tree species, a more dense stand) will provide a strong evaluation of the model. Such validations can stimulate further work, experimental or theoretical, and lead to valuable progress in the management and utilisation of silvopastoral systems.

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APPENDIX 1

Silvicultural schedule used in the Lincoln University silvopastoral experiment for cocksfoot plots.

PRUNING

	First pruning[1] (1994)	Second pruning[1] (1995)	Third pruning[1] (1996)	Fourth pruning[2] (1997)
Age (years)	4.5	5.5	6.5	7.5
Prune height (m)	1.8	2.2	4.1	6.0

1. Pruning to a crown length of 4 m (biological criteria)
2. Pruning to 6 m (silvicultural criteria)

Note: There were no follower trees. Stocking pruned: 400 stems/ha first and second pruning and 200 stems/ha third and fourth pruning.

THINNING

	First thinning (1992)	Second thinning (1993)	Third thinning (1994)	Fourth thinning (1996)
Age (year)	2.5	3.5	4.5	6.5
Remnant stocking (stems/ha)	800	600	400	200
Mean crop height (m)	1.5	2.9	4.6	9.1

Summary of the main dasometric variables of radiata pine grown in coksfoot plots in the Lincoln University silvopastoral experiment at age 9 (1999) and 10 (2000).

Year	DBH (mm)	H (m)	BA (m^2/ha)	MCL (m)	TCL (m/ha)
1999	230	11.6	8.3	5.6	1120
2000	260	13.3	10.6	7.3	1450
SD	5.6	1.15	0.89	0.45	109.1

DBH= diameter at breast height (1.4 m from soil); H= total height; BA= basal area; MCL= mean crown length; TCL= total crown length per hectare; SD= standard deviation.

Note: mean crown length (MCL) was derived by subtracting pruned from total height. Total crown length (TCL) per hectare, reflecting changes in the canopy after thinning operations, was derived from MCL.

APPENDIX 2

Seasonal changes in pasture botanical composition (%) for the experiment with four light regimes (Section 3.2.2.1). Data determined by dissecting a 50 g fresh weight sub-sample from each DM cut after 21 days regrowth before grazing. Senescent component represents only cocksfoot senescent and dead material.

Rotation	Treatment	Cocksfoot	Senescent material	Weeds	Clover
October 1999	Open	81.3 a	3.7 a	4.0 a	11.0 a
	Open + slat	84.4 a	1.9 a	6.1 a	7.6 b
	Trees	84.5 a	3.0 a	2.9 ab	9.7 b
	Trees + slat	93.2 a	3.3 a	0.8 b	2.6 c
November 1999	Open	79.1 a	2.9 a	6.0 a	12.0 a
	Open + slat	90.0 a	2.0 a	4.1 a	3.9 ab
	Trees	84.2 a	3.2 a	3.5 ab	9.1 a
	Trees + slat	94.5 a	3.0 a	0.7 b	1.8 b
December 1999	Open	81.3 a	6.6 a	5.6 a	4.2 a
	Open + slat	89.3 a	5.4 a	3.5 a	1.7 ab
	Trees	85.5 a	7.2 a	4.9 a	1.8 ab
	Trees + slat	89.6 a	6.7 a	3.2 a	0.6 b
January 2000	Open	76.2 a	12.8 a	4.0 a	7.0 a
	Open + slat	83.5 a	12.0 a	1.8 a	2.7 b
	Trees	82.5 a	15.0 a	2.0 a	0.6 c
	Trees + slat	80.3 a	14.0 a	4.4 a	1.3 c
February 2000	Open	75.5 a	8.0 a	3.3 a	13.2 a
	Open + slat	86.6 a	6.8 a	4.8 a	1.8 b
	Trees	87.0 a	8.0 a	2.5 a	2.4 b
	Trees + slat	86.0 a	11.5 a	1.3 a	1.2 b
March 2000	Open	81.0 a	12.0 a	3.1 a	3.9 a
	Open + slat	86.0 a	10.2 a	0.9 b	2.9 a
	Trees	84.3 a	15.0 a	0.2 b	0.1 b
	Trees + slat	82.7 a	16.0 a	0.9 b	0.4 b
April 2000	Open	73.8 a	15.0 a	3.0 a	8.2 a
	Open + slat	78.3 a	12.2 a	5.2 a	4.3 ab
	Trees	76.0 a	18.0 a	0.7 b	5.3 ab
	Trees + slat	81.6 a	17.0 a	0.5 b	0.9 b
May 2000	Open	69.7 b	14.3 a	3.2 a	12.8 a
	Open + slat	81.2 a	12.7 a	4.0 a	2.1 b
	Trees	73.6 ab	13.7 a	1.1 b	11.7 a
	Trees + slat	83.0 a	16.0 a	0.4 b	0.6 c
June 2000	Open	76.7 a	13.0 a	1.2 ab	9.1 a
	Open + slat	85.0 a	11.2 a	1.4 ab	2.3 c
	Trees	80.1 a	14.2 a	0.3 b	5.4 b
	Trees + slat	81.8 a	14.2 a	2.7 a	1.3 c
July 2000	Open	80.3 a	8.0 a	1.4 a	10.3 a
	Open + slat	90.2 a	7.1 a	0.5 b	2.2 b
	Trees	86.7 a	8.1 a	0.2 b	5.0 b
	Trees + slat	92.8 a	6.7 a	0.1 b	0.5 c

Note: Values with different letters within a column and rotations are significantly different based on lsd test at 0.05 probability.

Rotation	Treatment	Cocksfoot	Senescent material	Weeds	Clover
August 2000	Open	80.5 a	8.0 a	0.5 a	11.0 a
	Open + slat	88.2 a	8.5 a	0.8 a	2.4 c
	Trees	86.0 a	9.0 a	0.4 a	4.6 b
	Trees + slat	88.9 a	9.6 a	0.3 a	1.2 c
September 2000	Open	78.2 a	8.4 a	1.1 a	12.3 a
	Open + slat	87.2 a	5.5 a	0.8 a	6.5 b
	Trees	84.4 a	7.0 a	0.0 b	8.6 b
	Trees + slat	89.6 a	6.5 a	0.6 a	3.3 c
October 2000	Open	80.4 a	6.8 a	1.3 a	11.5 a
	Open + slat	90.4 a	6.6 a	0.6 b	2.3 b
	Trees	87.1 a	4.8 a	0.6 b	7.4 ab
	Trees + slat	92.0 a	4.5 a	0.6 b	3.0 b
November 2000	Open	70.7 b	8.0 a	1.7 a	11.7 a
	Open + slat	86.7 ab	6.5 a	0.6 b	2.8 b
	Trees	87.3 ab	7.1 a	0.0 c	3.4 b
	Trees + slat	92.3 a	6.6 a	0.0 c	0.6 c
December 2000	Open	78.5 a	7.7 a	2.2 a	6.8 a
	Open + slat	90.2 a	4.8 b	1.0 b	3.3 b
	Trees	85.7 a	9.2 a	0.1 c	3.8 b
	Trees + slat	94.2 a	4.5 b	0.6 bc	0.8 c
January 2001	Open	88.3 a	7.7 b	1.1 a	3.0 a
	Open + slat	92.2 a	4.0 b	0.8 a	3.0 a
	Trees	88.7 a	10.5 a	0.3 b	0.4 b
	Trees + slat	91.1 a	8.2 ab	0.4 b	0.4 b
February 2001	Open	87.6 a	11.0 a	0.3 a	1.2 a
	Open + slat	91.7 a	6.0 b	0.4 a	1.9 a
	Trees	84.1 a	15.5 a	0.1 a	0.2 b
	Trees + slat	86.9 a	13.0 a	0.1 a	0.0 b
March 2001	Open	80.5 a	18.9 a	0.2 a	0.4 b
	Open + slat	92.9 a	5.7 b	0.3 a	1.0 a
	Trees	76.3 a	23.7 a	0.0 a	0.0 b
	Trees + slat	79.9 a	20.1 a	0.0 a	0.0 b
April 2001	Open	73.8 a	26.0 a	0.0 a	0.2 b
	Open + slat	83.3 a	15.8 b	0.2 a	0.6 a
	Trees	66.2 a	33.8 a	0.0 a	0.0 b
	Trees + slat	73.1 a	26.9 a	0.0 a	0.0 b
May 2001	Open	84.4 a	14.1 a	0.8 a	0.7 a
	Open + slat	92.1 a	7.1 b	0.0 b	0.8 a
	Trees	84.2 a	15.2 a	0.3 b	0.3 a
	Trees + slat	82.5 a	17.3 a	0.0 b	0.2 a

Note: Values with different letters within a column and rotations are significantly different based on lsd test at 0.05 probability.

APPENDIX 3

Seasonal changes in pasture botanical composition (%) for the exclosure experiment (Section 3.2.2.2). Data determined by dissecting a 50 g fresh weight sub-sample from each DM cut after 10 days regrowth. Senescent component represents only cocksfoot senescent and dead material.

Regrowth period from 1 September to 30 October 1999

Regrowth day	Treatment	Cocksfoot	Senescent material	Weeds	Clover
10	Open C	95.1 a	2.5 b	0.9 b	1.6 a
	Open N	95.2 a	1.7 b	0.8 b	2.4 a
	Trees C	91.8 a	4.2 a	2.5 a	1.5 a
	Trees N	89.9 a	3.0 a	5.5 a	1.6 a
20	Open C	93.5 a	3.0 a	1.0 a	2.5 a
	Open N	94.3 a	2.9 a	1.6 a	1.2 a
	Trees C	92.0 a	4.3 a	2.1 a	1.6 a
	Trees N	92.4 a	4.1 a	1.7 a	1.8 a
30	Open C	84.8 a	4.6 a	6.7 a	3.9 a
	Open N	93.3 a	3.0 a	1.8 b	2.0 b
	Trees C	89.1 a	4.5 a	2.9 b	3.5 a
	Trees N	91.9 a	3.0 a	3.4 ab	1.7 b
40	Open C	80.5 a	5.7 a	4.2 a	9.6 a
	Open N	93.6 a	3.1 a	1.1 b	2.3 b
	Trees C	83.0 a	4.6 a	4.4 a	8.0 a
	Trees N	94.3 a	3.1 a	1.1 a	1.5 b
50	Open C	82.7 ab	6.2 a	3.3 a	7.9 a
	Open N	93.8 a	4.0 b	0.8 b	1.5 b
	Trees C	78.9 b	5.9 a	5.9 a	9.3 a
	Trees N	93.3 a	3.0 b	2.2 ab	1.5 b
60	Open C	83.0 ab	6.6 a	3.9 a	6.5 a
	Open N	92.2 a	6.8 a	0.2 b	0.7 b
	Trees C	80.7 b	8.2 a	4.9 a	6.2 a
	Trees N	93.7 a	5.2 b	0.1 b	1.0 b

Note: Values with different letters within a column and rotations are significantly different based on lsd test at 0.05 probability.

Continuation Appendix 3

Regrowth period from 1 November to 30 December 1999

Regrowth day	Treatment	Cocksfoot	Senescent material	Weeds	Clover
10	Open C	96.6 a	1.6 a	0.7 cd	1.1 e
	Open W	89.5 a	1.0 a	7.5 b	2.0 de
	Open N	93.6 a	1.3 a	0.0 d	5.1 cd
	Open W+N	98.3 a	1.2 a	0.0 d	0.5 e
	Trees C	81.8 b	1.3 a	7.5 b	9.4 b
	Trees W	76.5 b	1.1 a	8.2 ab	14.2 a
	Trees N	76.9 b	1.1 a	11.0 a	11.0 ab
	Trees W+N	92.2 a	1.4 a	2.8 c	3.6 d
20	Open C	89.8 ab	2.8 a	1.1 d	6.3 bc
	Open W	78.3 c	1.4 a	9.2 ab	11.1 b
	Open N	95.5 a	2.0 a	1.5 d	1.0 c
	Open W+N	94.4 a	1.3 a	1.0 d	3.3 c
	Trees C	78.8 c	1.4 a	10.5 a	9.3 ab
	Trees W	80.2 bc	1.8 a	7.8 bc	10.2 a
	Trees N	85.8 b	1.2 a	6.1 c	6.9 bc
	Trees W+N	84.0 b	2.4 a	10.4 a	3.2 c
30	Open C	87.3 ab	3.0 a	4.4 c	5.3 cd
	Open W	84.6 b	1.7 bc	6.2 bc	7.5 b
	Open N	96.3 a	2.8 ab	0.5 d	0.4 e
	Open W+N	90.0 a	1.5 c	8.2 b	0.3 e
	Trees C	81.4 c	2.2 b	4.8 c	11.6 ab
	Trees W	79.9 c	1.3 c	8.8 a	10.0 a
	Trees N	85.5 b	1.5 c	8.7 b	4.3 d
	Trees W+N	89.4 a	3.5 a	5.3 bc	1.8 de
40	Open C	87.2 b	3.5 ab	0.8 d	8.5 b
	Open W	81.8 bc	4.4 a	2.3 cd	11.5 a
	Open N	94.4 a	3.5 ab	1.6 d	0.5 d
	Open W+N	90.7 ab	2.9 b	3.2 bc	3.2 c
	Trees C	79.5 c	2.2 b	8.2 a	10.1 ab
	Trees W	84.0 b	2.3 b	4.6 b	9.1b
	Trees N	87.9 b	2.8 b	5.3 b	4.0 bc
	Trees W+N	94.6 a	4.7 a	0.3 d	0.4 c
50	Open C	86.2 a	4.9 ab	0.7 ef	8.2 ab
	Open W	77.0 b	5.5 a	4.5 cd	13.0 a
	Open N	94.1 a	5.0 ab	0.0 f	0.9 c
	Open W+N	86.9 a	4.8 ab	7.8 b	0.5 c
	Trees C	75.6 b	4.6 b	9.5 a	10.3 a
	Trees W	82.3 a	5.4 a	5.2 c	7.1 b
	Trees N	89.6 a	5.8 a	3.8 d	0.8 c
	Trees W+N	87.0 a	5.9 a	6.8 b	0.3 c
60	Open C	78.5 b	8.4 a	1.5 d	11.6 ab
	Open W	83.6 ab	5.6 b	4.3 bc	6.5 b
	Open N	90.6 a	6.7 ab	1.8 d	0.9 cd
	Open W+N	87.4 a	5.1 b	7.3 a	0.2 d
	Trees C	81.2 b	6.3 b	2.3 c	10.2 b
	Trees W	86.4 a	5.4 b	3.1 cd	5.1b
	Trees N	90.6 a	7.5 a	0.5 e	1.4 c
	Trees W+N	93.5 a	6.4 ab	0.0 e	0.1 d

Values with different letters within a column and rotations are significantly different based on lsd test at 0.05 probability.

Continuation Appendix 3

Regrowth period from 6 January to 6 March 2000

Regrowth day	Treatment	Cocksfoot	Senescent material	Weeds	Clover
10	Open C	89.8 a	3.2 ab	2.9 bc	4.1 bc
	Open W	84.2 a	3.5 ab	4.2 b	8.2 a
	Open N	95.0 a	1.3 b	0.8 c	2.9 c
	Open W+N	85.5 a	1.8 b	10.5 a	2.2 c
	Trees C	92.0 a	5.0 a	1.1 c	1.9 c
	Trees W	87.0 a	2.4 b	2.7 bc	7.8 a
	Trees N	92.1 a	2.4 b	0.4 c	5.2 b
	Trees W+N	94.1 a	1.9 b	1.4 c	2.6 c
20	Open C	73.9 b	7.8 a	7.6 a	10.7 ab
	Open W	74.6 b	5.6 ab	6.1 a	13.7 a
	Open N	81.9 b	2.4 b	6.7 a	9.0 b
	Open W+N	86.0 ab	2.8 b	2.7 b	5.5 c
	Trees C	87.0 ab	7.0 a	2.9 b	3.1 c
	Trees W	90.8 a	3.8 b	2.1 b	3.3 c
	Trees N	91.8 a	3.0 b	0.6 b	4.5 c
	Trees W+N	93.7 a	3.4 b	0.6 b	2.3 c
30	Open C	71.4 c	8.2 a	7.6 a	12.8 b
	Open W	72.5 c	5.7 b	6.7 a	15.1 a
	Open N	88.0 ab	2.2 b	2.1 b	7.7 c
	Open W+N	88.1 ab	5.4 b	2.1 b	4.4 d
	Trees C	81.7 b	8.4 a	4.2 b	5.8 d
	Trees W	86.7 ab	3.9 ab	3.9 b	5.4 d
	Trees N	93.3 a	4.2 b	1.4 b	1.2 e
	Trees W+N	94.1 a	5.1 b	0.2 c	0.5 e
40	Open C	76.9 bc	9.0 b	3.9 b	10.2 a
	Open W	74.3 c	6.5 c	4.8 ab	14.4 a
	Open N	74.6 bc	14.8 a	3.0 b	7.6 b
	Open W+N	84.8 b	10.9 a	0.8 c	3.5 b
	Trees C	76.3 bc	9.2 b	7.3 a	7.2 b
	Trees W	78.8 b	9.1 b	3.6 b	8.5 b
	Trees N	88.9 a	10.2 ab	0.0 d	0.9 c
	Trees W+N	93.5 a	6.5 c	0.0 d	0.0 c
50	Open C	76.8 c	11.3 ab	4.7 a	7.3 b
	Open W	73.4 c	9.8 b	1.8 b	15.0 a
	Open N	82.5 b	16.3 a	0.0 c	1.2 c
	Open W+N	87.5 a	11.3 ab	0.8 b	0.4 d
	Trees C	83.6 ab	10.1 b	4.2 a	2.1 c
	Trees W	75.9 c	9.9 b	4.0 a	10.2 b
	Trees N	87.9 a	11.9 ab	0.0 c	0.2 d
	Trees W+N	91.6 a	8.4 b	0.0 c	0.0 d
60	Open C	72.4 b	19.7 a	2.0 b	5.9 b
	Open W	71.7 b	10.0 c	1.7 b	16.6 a
	Open N	79.6 ab	20.1 a	0.0 c	0.3 d
	Open W+N	79.8 ab	20.1 a	0.0 c	0.1 d
	Trees C	83.4 a	13.2 c	2.4 b	0.9 c
	Trees W	71.6 b	10.4 c	9.4 a	8.7 b
	Trees N	87.2 a	12.8 bc	0.0 c	0.0 e
	Trees W+N	84.8 a	15.2 b	0.0 c	0.0 e

Values with different letters within a column and rotations are significantly different based on lsd test at 0.05 probability.

Regrowth period from 8 March to 7 May 2000

Regrowth day	Treatment	Cocksfoot	Senescent material	Weeds	Clover
10	Open C	87.5 a	5.2 a	3.2 ab	4.1 b
	Open W	90.4 a	2.0 bc	2.4 b	5.2 a
	Open N	92.4 a	1.6 c	0.0 d	6.0 a
	Open W+N	90.7 a	1.8 c	0.8 c	6.7 a
	Trees C	90.9 a	6.4 a	2.5 b	0.2 c
	Trees W	87.8 a	4.2 ab	3.2 ab	4.7 b
	Trees N	93.1 a	3.1 b	0.3 cd	3.5 b
	Trees W+N	86.5 a	2.6 b	4.2 a	6.7 a
20	Open C	80.8 b	9.4 a	3.0 a	6.8 a
	Open W	87.7 a	4.7 b	2.4 a	5.2 b
	Open N	88.4 a	3.5 b	0.4 b	7.8 a
	Open W+N	89.0 a	2.2 b	3.5 a	5.3 b
	Trees C	85.2 ab	10.7 a	1.9 b	2.2 c
	Trees W	87.6 a	6.3 ab	2.5 a	3.6 bc
	Trees N	89.0 a	4.2 b	0.8 b	6.0 ab
	Trees W+N	89.2 a	3.0 b	1.5 b	6.3 ab
30	Open C	77.7 b	12.2 a	2.5 ab	7.7 a
	Open W	84.8 ab	6.0 b	1.7 b	7.5 a
	Open N	90.9 a	4.3 bc	2.2 b	2.5 b
	Open W+N	88.6 a	2.7 c	3.9 a	4.7 b
	Trees C	84.0 ab	11.6 a	0.5 c	3.9 b
	Trees W	82.9 b	6.5 b	3.4 a	7.2 a
	Trees N	88.2 a	5.0 b	0.8 bc	6.0 ab
	Trees W+N	86.7 a	3.8 c	1.9 b	7.6 a
40	Open C	75.5 c	13.5 a	2.4 c	8.6 a
	Open W	80.7 b	6.8 b	0.6 d	11.9 a
	Open N	91.5 a	4.6 c	1.5 cd	2.4 c
	Open W+N	86.0 ab	5.0 b	7.7 a	1.4 c
	Trees C	79.7 b	13.7 a	1.0 d	5.7 b
	Trees W	74.7 c	7.7 b	3.7 b	13.8 a
	Trees N	87.9 a	5.0 bc	4.1 ab	3.0 c
	Trees W+N	88.8 a	6.4 b	1.2 d	3.7 c
50	Open C	73.0 c	15.5 a	1.5 b	10.0 b
	Open W	76.5 bc	11.3 a	0.1 c	12.1 a
	Open N	88.9 ab	7.2 b	2.4 ab	1.5 d
	Open W+N	94.6 a	5.2 b	0.0 c	0.2 d
	Trees C	78.4 b	14.8 a	0.8 bd	5.9 c
	Trees W	77.6 b	7.9 b	3.2 a	11.3 b
	Trees N	89.3 a	7.1 b	1.5 b	2.0 d
	Trees W+N	88.2 ab	8.4 b	1.5 b	1.9 d
60	Open C	72.2 c	16.3 ab	0.5 ab	11.0 a
	Open W	78.4 b	12.7 b	0.2 b	8.7 a
	Open N	91.4 a	8.4 c	0.0 b	0.2 c
	Open W+N	88.1 a	11.6 b	0.0 b	0.3 c
	Trees C	73.5 bc	19.9 a	0.6 ab	6.0 b
	Trees W	79.0 b	8.9 c	1.8 a	10.3 a
	Trees N	86.5 a	10.7 b	1.5 a	1.2 c
	Trees W+N	86.9 a	10.5 b	1.8 a	0.9 c

Values with different letters within a column and rotations are significantly different based on lsd test at 0.05 probability.

Continuation Appendix 3

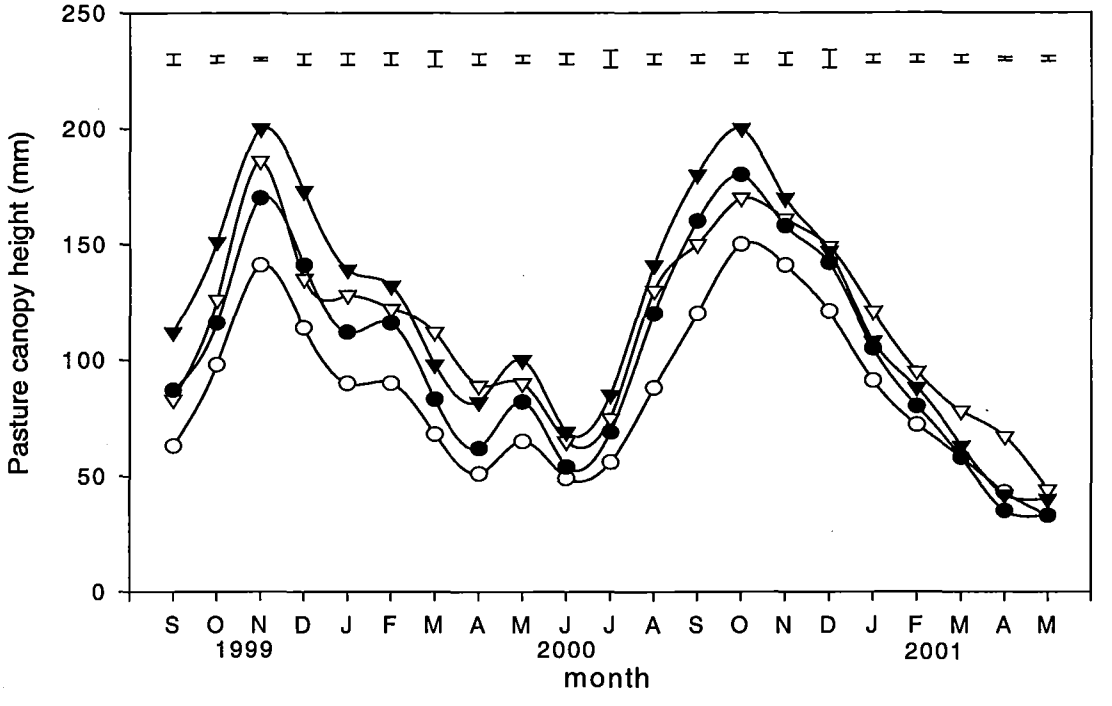
Regrowth period from 8 May to 16 August 2000

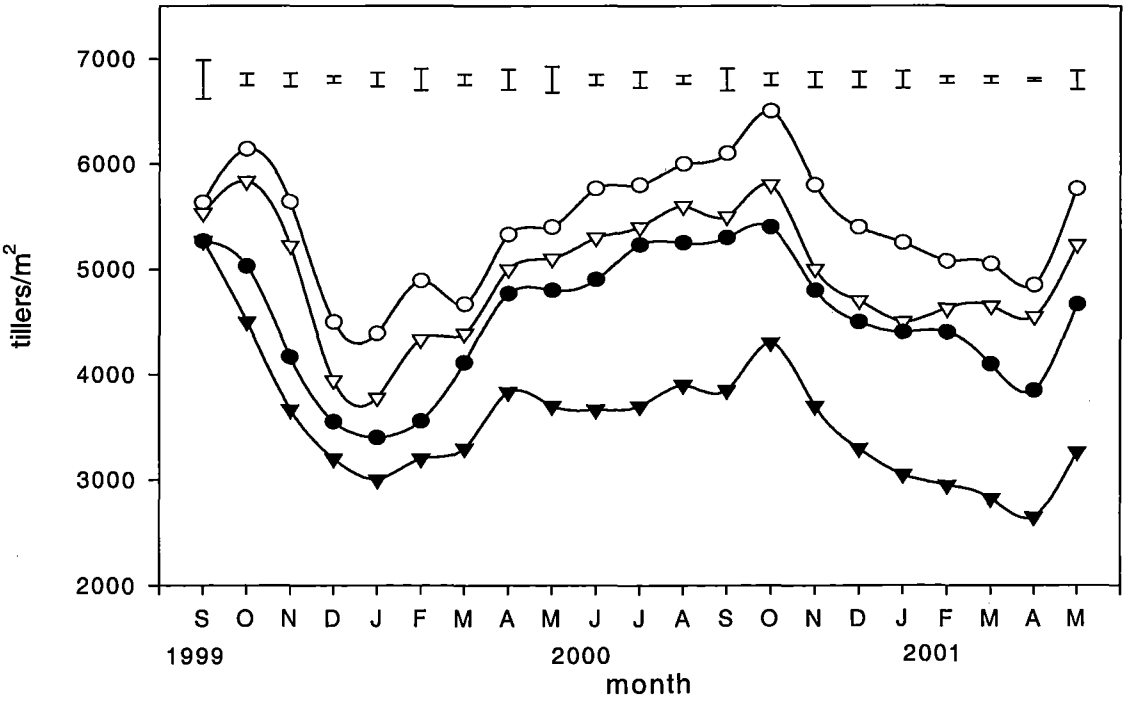
Regrowth day	Treatment	Cocksfoot	Senescent material	Weeds	Clover
15	Open C	84.5 a	5.3 a	0.3 bc	9.9 a
	Open N	92.7 a	2.3 b	0.0 c	5.0 ab
	Trees C	90.5 a	5.0 a	0.0 c	4.5 ab
	Trees W	91.1 a	3.2 ab	2.2 a	3.5 b
	Trees N	94.1 a	2.8 b	0.5 b	2.6 bc
	Trees W+N	95.5 a	2.2 b	0.7 b	1.7 c
30	Open C	81.7 b	8.6 a	0.4 ab	9.4 a
	Open N	93.4 a	4.6 b	0.2 b	1.9 c
	Trees C	88.1 a	7.2 a	0.2 b	4.5 b
	Trees W	88.6 a	6.5 ab	0.8 a	4.2 b
	Trees N	92.5 a	4.6 b	0.0 b	2.9 b
	Trees W+N	93.8 a	5.0 b	0.0 b	1.2 c
50	Open C	78.8 b	10.3 a	0.8 ab	10.1 a
	Open N	92.7 a	5.2 b	0.2 b	1.9 c
	Trees C	84.9 ab	9.0 a	1.9 a	4.3 b
	Trees W	85.1 ab	8.6 a	0.3 b	6.0 b
	Trees N	90.7 a	5.9 b	0.0 b	3.5 bc
	Trees W+N	93.5 a	5.5 b	0.0 b	1.0 c
70	Open C	78.7 b	11.9 a	0.3 b	9.1 a
	Open N	90.0 a	9.0 a	0.0 b	1.0 c
	Trees C	83.7 ab	10.5 a	0.5 a	5.3 b
	Trees W	84.2 ab	11.1 a	0.1 b	4.5 b
	Trees N	92.6 a	6.8 ab	0.0 b	0.6 c
	Trees W+N	94.1 a	5.8 b	0.0 b	0.1 c
90	Open C	77.5 b	15.9 a	0.2 b	6.3 a
	Open N	90.0 a	9.7 ab	0.0 b	0.3 c
	Trees C	85.2 ab	11.5 a	1.8 a	1.5 b
	Trees W	84.1 ab	13.1 a	0.5 b	2.4 b
	Trees N	91.4 a	8.5 b	0.0 b	0.1 c
	Trees W+N	93.3 a	6.7 b	0.0 b	0.0 c
110	Open C	76.2 b	17.4 a	0.6 a	5.7 a
	Open N	88.9 a	10.8 b	0.0 b	0.3 c
	Trees C	83.5 a	14.3 a	0.9 a	1.3 b
	Trees W	83.3 a	14.8 a	0.5 a	1.4 b
	Trees N	89.5 a	10.5 b	0.0 b	0.0 c
	Trees W+N	88.6 a	11.4 b	0.0 b	0.0 c

Values with different letters within a column and rotations are significantly different based on lsd test at 0.05 probability.

APPENDIX 4

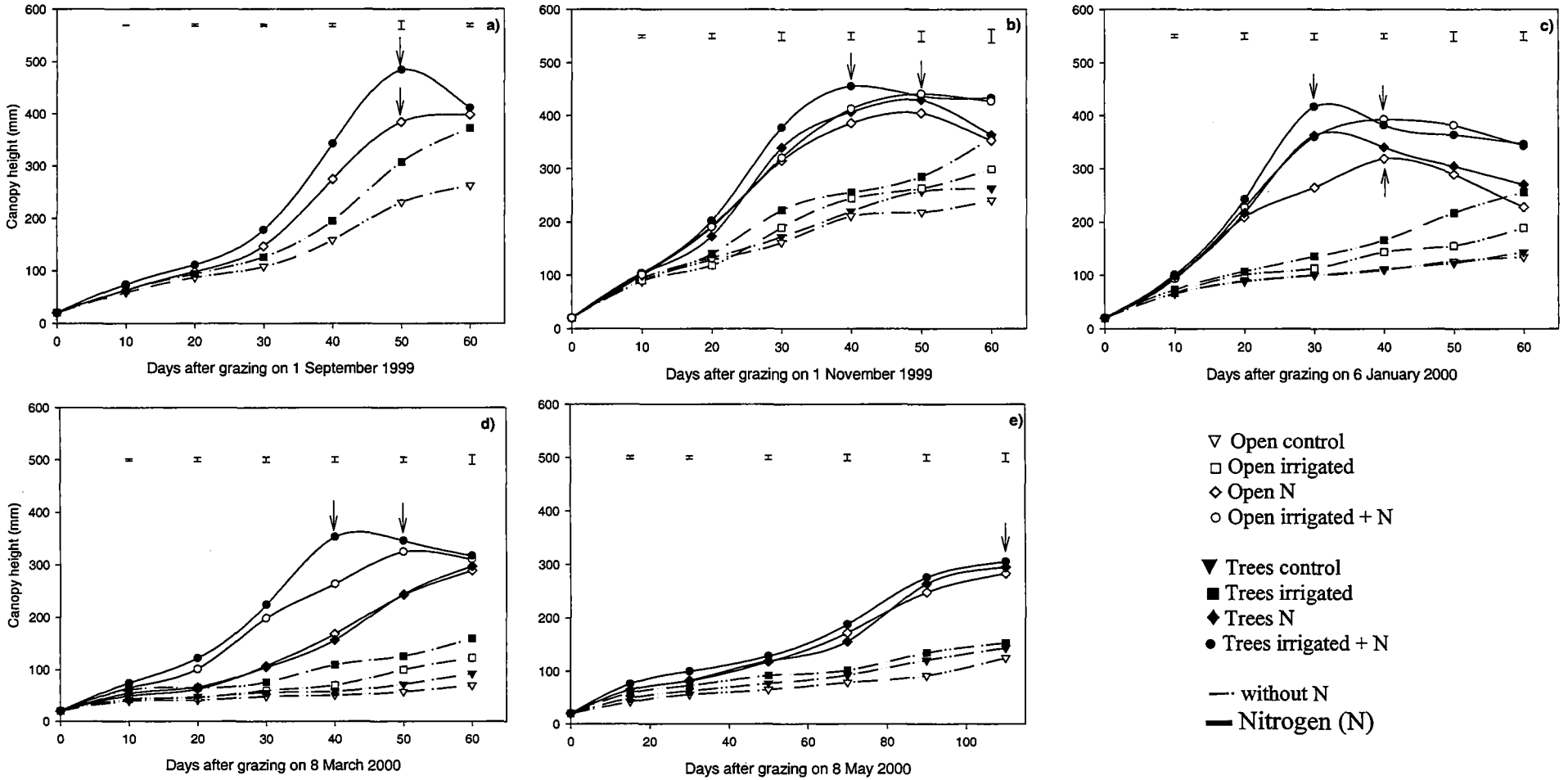
Cocksfoot canopy height and tiller population (21±1 days regrowth) over time for four shade treatments: open (○) (100% transmissivity), open+slat (▽) (~43% transmissivity), under trees (●) (~58% transmissivity) and trees+slat (▼) (~24% transmissivity). Bars indicate standard error of the mean (sem).





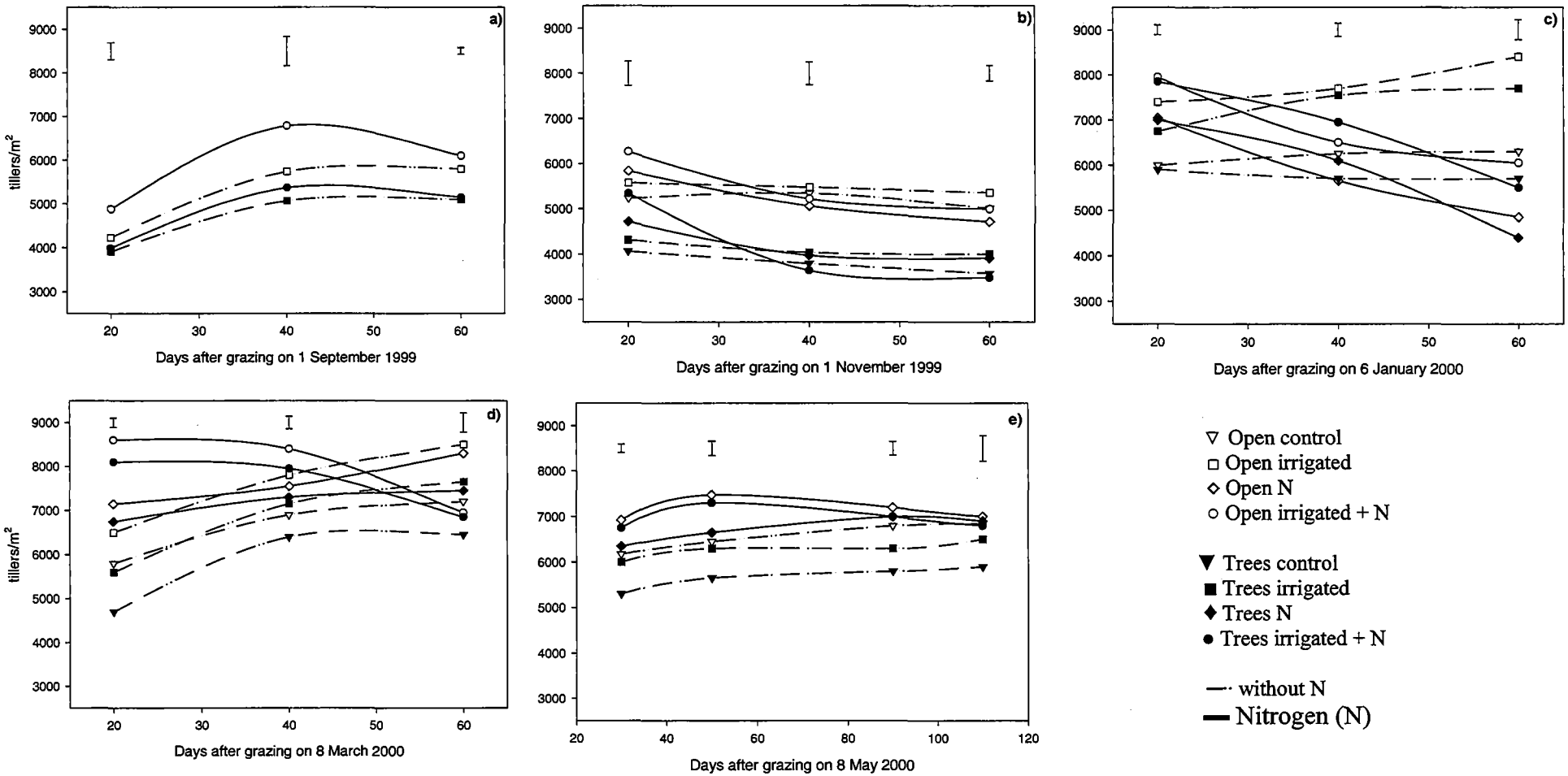
APPENDIX 5

Cocksfoot canopy height (mm) over time for two levels of light intensity (open pasture: 100% transmittance or pasture under tree shade: ~58% transmissivity), two levels of irrigation (0 or fully) and two levels of nitrogen (0 or 300 kg N/ha). Four 60-day regrowth periods were used during spring, summer and autumn (a-d), and a 110-day regrowth period (e) was used during winter. Bars indicate standard error of the mean (sem). Arrows indicate the start of lodging.



Continuation Appendix 5

Cocksfoot total tiller population over time for two levels of light intensity (open pasture: 100% transmittance or pasture under tree shade: ~58% transmissivity), two levels of irrigation (0 or fully) and two levels of nitrogen (0 or 300 kg N/ha). Four 60-day regrowth periods were used during spring, summer and autumn (a-d), and a 110-day regrowth period (e) was used during winter. Bars indicate standard error of the mean (sem). Note: The differences in total tiller population during the November-December regrowth period was influenced by variations in reproductive tiller population.



APPENDIX 6

Mean mineral content in herbage cocksfoot pasture for different seasons at 21 days regrowth used for the calculation of carbohydrate content in Section 8.3.6 (Chapter 8).

Date	Treatment	P (g/kg DM)	Mg (g/kg DM)	K (g/kg DM)	Ca (g/kg DM)	S (g/kg DM)
Oct-99 (spring)	Open	4.5	1.6	36.0	3.9	2.2
	Open + Slats	4.6	1.8	39.9	3.9	3.2
	Trees	5.0	1.8	42.4	3.9	3.5
	Trees + Slats	4.9	2.4	46.3	4.0	3.1
Jan-00 (summer)	Open	4.3	1.6	35.2	4.5	2.3
	Open + Slats	4.4	1.6	34.3	4.3	2.8
	Trees	5.3	1.9	36.3	4.6	3.4
	Trees + Slats	7.1	1.8	39.1	4.6	3.3
Apr-00 (autumn)	Open	5.1	1.6	32.1	4.6	2.5
	Open + Slats	5.3	1.8	36.3	4.2	3.1
	Trees	5.4	2.1	35.1	4.7	3.6
	Trees + Slats	5.7	2.2	39.1	4.9	3.4
Jun-00 (winter)	Open	4.4	1.7	26.7	5.0	3.5
	Open + Slats	4.8	1.8	28.6	5.4	3.4
	Trees	5.5	2.0	29.0	5.8	3.7
	Trees + Slats	5.5	2.3	30.1	6.5	3.5
Oct-00 (spring)	Open	4.8	1.4	29.3	4.0	2.9
	Open + Slats	4.8	1.7	28.9	3.9	4.1
	Trees	5.6	1.7	33.0	4.5	4.0
	Trees + Slats	5.2	1.8	34.0	4.7	3.5
Jan-01 (summer)	Open	3.3	1.8	25.9	5.9	2.4
	Open + Slats	3.9	2.0	25.8	5.3	3.6
	Trees	5.0	2.1	28.3	6.4	3.7
	Trees + Slats	4.6	2.3	30.2	6.6	3.5
Apr-01 (autumn)	Open	3.1	1.6	20.4	5.2	2.3
	Open + Slats	3.2	1.8	22.1	5.4	2.8
	Trees	2.8	2.1	14.9	6.5	3.0
	Trees + Slats	2.6	1.9	18.8	5.5	3.0

Note: Phosphorus was analysed according to the method No. 964.06 described by Padmore (1990). Calcium and magnesium analysis was done according to the atomic absorption method (Padmore, 1990). Potassium analysis was done according to the atomic emission method (Padmore, 1990). Sulphur analysis was carried out according to methodology proposed by Quin and Woods (1976).

APPENDIX 6

Mean mineral content in herbage cocksfoot pasture for different seasons at 21 days regrowth used for the calculation of carbohydrate content in Section 8.3.6 (Chapter 8).

Date	Treatment	P (g/kg DM)	Mg (g/kg DM)	K (g/kg DM)	Ca (g/kg DM)	S (g/kg DM)
Oct-99 (spring)	Open	4.5	1.6	36.0	3.9	2.2
	Open + Slats	4.6	1.8	39.9	3.9	3.2
	Trees	5.0	1.8	42.4	3.9	3.5
	Trees + Slats	4.9	2.4	46.3	4.0	3.1
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	Open + Slats	4.4	1.6	34.3	4.3	2.8
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	Trees	5.5	2.0	29.0	5.8	3.7
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	Open + Slats	3.9	2.0	25.8	5.3	3.6
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	Trees + Slats	4.6	2.3	30.2	6.6	3.5
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Note: Phosphorus was analysed according to the method No. 964.06 described by Padmore (1990). Calcium and magnesium analysis was done according to the atomic absorption method (Padmore, 1990). Potassium analysis was done according to the atomic emission method (Padmore, 1990). Sulphur analysis was carried out according to methodology proposed by Quin and Woods (1976).

APPENDIX 7

List of publications related to the present study

Peri P.L., Lucas R.J., Moot D.J., Varella A.C., and McNeil D.L. (2001) Optimising yield and quality of orchardgrass pasture in temperate silvopastoral systems. *Proceedings of the XIX International Grassland Congress*, pp. 657-658. Sao Paulo, Brazil.

Peri P.L., Varella A.C., Lucas R.J. and Moot D.J. (2001) Cocksfoot and lucerne productivity in a *Pinus radiata* silvopastoral system: a grazed comparison. *Proceedings of the New Zealand Grassland Association*, 63, 139-147.

Peri P.L., Moot D.J., McNeil D.L., Varella A.C. and Lucas R.J. (2002) Modelling net photosynthetic rate of field grown cocksfoot leaves under different nitrogen, water and temperature regimes. *Grass and Forage Science*, 57, 61-71.

Peri P.L., McNeil D.L., Moot D.J., Varella A.C. and Lucas R.J. (2002) Net photosynthetic rate of cocksfoot leaves under continuous and fluctuating shade conditions in the field. *Grass and Forage Science*, 57 (2): In press.