Modelling Interactions in Grass-Clover Mixtures

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Modelling Interactions in Grass-Clover Mixtures

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STELLINGEN

1. Heterogeneity in vertical leaf area distribution and variation in leaf dispersion are the basic characteristics of the canopy structure of grass-white clover mixtures.

This thesis

2. Large and small-leaved clover cultivars follow different strategies in response to applied nitrogen, which are regulated by the patterns of allocation of assimilates to leaves and petioles.

This thesis

3. When mixed species have more or less the same height, the height at which the leaf area density is maximal plays a crucial role on the outcome of light competition.

This thesis

4. Clover cultivars have different competitive abilities, which are independent of their yield potentials in monoculture.

This thesis

5. In grass-clover mixtures, the effect of canopy structure on canopy CO_2 assimilation and productivity of species appears to be more important than the effect of the leaf N profile over canopy height.

This thesis

6. Science is concerned with "*verifiability*"; it is not concerned with ultimate "*truth*".

Wigglesworth, V. B. 1967. The religion of science. *Annals of Applied Biology*, 61: 314-321.

8. In any event, it is essential that one does not lose sight of the whole and becomes lost in the complexities within.

7. Attempting to explain everything, explains nothing.

Simberloff, D. and Boecklen W. 1981. Santa Rosalina reconsidered: size ratio and competition. *Evolution*, 35: 1206-1228.

9. If a man will begin with certainties he shall end in doubts; but if he will be content to begin with doubts he shall end in certainties.

Francis Bacon

10. The greatest mistake any ecologist can make is to confuse the concept of statistical significance with that of biological significance.

Krebs, C. J. 1989. *Ecological methodology*

11. This thesis contains a relatively large number of figures.

'What is the use of a book,' thought Alice, 'without pictures or conversations?'

Lewis Carroll, *Alice in Wounderland*

Behorende bij het proefschrift:
Modelling Interactions in Grass-Clover Mixtures

Mehdi Nassiri Mahallati
Wageningen, 27 February 1998

Abstract

Nassiri Mahallati, M. **Modelling interactions in grass-clover mixtures**. PhD thesis, Departments of Agronomy and Theoretical Production Ecology, Agricultural University, Wageningen, The Netherlands, x + 165 pp., English and Dutch summaries.

The study described in this thesis focuses on a quantitative understanding of the complex interactions in binary mixtures of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) under cutting. The first part of the study describes the dynamics of growth, production and the structural characteristics of contrasting grass and clover cultivars under field conditions. This basic information is used in the second part to quantify light absorption, CO_2 assimilation, radiation use efficiency (*RUE*) and light competition of the species using a modelling approach.

Both species showed a seasonal pattern in growth of the dry matter (DM) and leaf area index (*LAI*) during the season so that the grass-dominated swards during spring shifted to clover dominance in summer. Without N fertilisation (-N), this seasonality was mainly controlled by the weather conditions. However, in fertilised mixtures (+N) grass was the dominant component of the mixture during the whole season and clover growth was always limited by light. The competitive ability and persistence of clover were determined by the structural characteristics of the cultivars. In the -N swards, both large and small-leaved clover cultivars had a higher proportion of their leaf area at the top canopy layers than their companion grass. In the +N mixtures, this was observed only in large-leaved clover, whereas the small-leaved cultivar was strongly overtopped. The difference between cultivars was mainly due to the pattern of allocation of the DM into the supporting tissues.

Experimental results showed two sources of vertical heterogeneity within the mixed grass-clover canopies: different patterns of *LAI* distribution and leaf dispersion. The validity of the canopy light partitioning model was considerably improved by introducing these sources of variation into the model. Using this model the *RUE* of species was calculated for different regrowth periods. Grass and clover had a different *RUE* in mixture and monoculture, but overall *RUE* was higher in grass, particularly in spring. The variation in the DM yield of grass under different treatments was due to changes in *RUE* and absorbed radiation. However, in clover these differences were mainly due to the amount of absorbed radiation. Quantification of light competition showed that in spring grass was the strongest competitor. In summer, the competitive ability of clover was related to N level and clover cultivar. Without N, both large and small-leaved clover were better competitors than grass. However, in the +N swards only the large-leaved clover had the same competitive ability as its companion grass. In both species a leaf N profile developed during regrowth, parallel to the light profile within the canopy. The effect of the observed compared to a uniform leaf N profile on canopy CO_2 assimilation of species was low, but it was different between mixtures and monocultures.

It was concluded that the effect of canopy structure on productivity of species was more important than their assimilatory characteristics. The persistence of white clover under cutting may be improved by choosing cultivars with a higher competitive ability, based on canopy structure.

Key words: Perennial ryegrass, white clover, canopy structure, light partitioning, light competition, radiation use efficiency, nitrogen, leaf N profile, CO_2 assimilation, modelling.

To my mother

Contents

Preface

Thanks be to God who gave me the opportunity for the extension of my enlightenment and helped me to finish this programme successfully.

This research was conducted under the auspices of a scholarship awarded by the Islamic Republic of Iran. I appreciate the financial support of the Ministry of Culture and Higher Education, as well as the help of the Embassy of Iran in The Netherlands.

I have to acknowledge my indebtedness to many people who helped me at different stages of this work, in particular to the following persons:

Professor Avaz Koocheki in Iran, who encouraged me and introduced me to my promotor so that I could pursue my study in Wageningen. My promotor Professor Leendert 't Mannetje, for his support and help during the experiment and careful reading of the thesis. My co-promotors Dr. Anjo Elgersma and Dr. Egbert Lantinga, for their support and assistance during all stages of the experiment, the critical reading of different chapters and their great scientific contribution to this research. Professor Martin Kropff, for his comments during the experiment and for his useful suggestions for modelling methods. Drs. Jan Neuteboom, for instructions and help and with the calibration of the inclined point quadrat, as well as his encouraging comments and discussion during the research. Mr. Hans Schlepers and the staff of UNIFARM, for their help and assistance during the two years of field and laboratory work. Ing. Peter van der Putten, for his help and assistance with the photosynthesis measurements and Mr. Hennie Halm for carrying out the chemical analyses. Ms. Marieke Bosman and the other ladies of the secretariat and the library of the Department of Agronomy, for their kind cooperation during my study. Mr. Koop Wind for his help in designing the layout of the thesis, Mr. Hans Romberg, for his help in installation and updating of computer programs, Ms. Elsa Hodgins, for her helpful editorial comments on the final version of the thesis and Mr. B. Loualidi for his help in photocopying and binding of the thesis. Dr. Conny Almekinders, my first and best Dutch friend, for her kindness and hospitality. Mr. Guido van Hasselt, for his kind help during the first days of my arrival in 1994. My Iranian friends in Wageningen, Dr. Mahmoud Mesbah, Mr. Fayez Raiesi and Mr. Mehdi Homaee and their families, as well as Mr. Mehdi Farahpour, for their help and support. My mother, sister and brothers in Iran for their "remote" encouragement and support during my study. Dr. G. N. Dhanapal, Dr. He Wei and Mr. Reuben Muasya for being pleasant roommates at the Department of Agronomy.

This research was conducted in the framework of the C. T. de Wit Graduate School for Production Ecology. I am grateful to the leaders of the discussion group in the Graduate

School, Professors Rudy Rabbinge, Jan Goudriaan and Louise Fresco, as well as to the PhD students in our group for their constructive comments and discussions.

During my study I had the opportunity to visit several research stations of IGER in the United Kingdom. I acknowledge the Graduate School for funding the trip, as well as Dr. Anjo Elgersma and Dr. Susan Schwinning for making all the arrangements. I also thank the Graduate School for subsidising the congress and summer schools which I attended during my study.

Furthermore, I thank all other people who helped me during my study and are not mentioned here.

Finally, I would like to once again pay tribute to my co-promotor Dr. Anjo Elgersma for her kindness and her endless and unforgettable help since the first day of my arrival in The Netherlands.

Chapter 1

General introduction

Sustainability of grassland systems

In practical terms sustainable grassland production should be economically sound, while simultaneously its environmental side effects should be minimised and must not exceed the limits set by society ('t Mannetje, 1994). High rates of nitrogen (N) fertilisation in grasslands have led to increasing levels of N losses through leaching into the groundwater or in gaseous forms into the atmosphere. Therefore, the research in grassland science in North-Western Europe has moved from increasing the quantity of production based on excessive amounts of N fertilisers to a search for alternative methods less dependent on this resource. This has led to a growing interest in ryegrass-white clover associations as a basis for low-input, but highly productive grassland production systems.

It is well established that perennial ryegrass is the most compatible species in association with white clover (Camlin, 1981; Harris, 1987). On the other hand, white clover is the only temperate forage legume species which can persist under both frequent cutting and intensive grazing (Kessler and Nösberger, 1994). In addition, its ability for biological N fixation and its high nutritive value give it an important role in low input grazing systems (Peel and Lloveras, 1994). Thus, white clover is considered as a low cost alternative to industrial N fertilisers, which are manufactured using fossil energy.

The amount of N fixed in mixed swards containing 30-50% clover is estimated to be 150 kg ha^{-1} year^{-1} (Kristensen et al., 1995). Elgersma and Hassink (1997) reported values ranging from 150 to 545 kg N ha^{-1} for mixtures with 40 - 80% clover, respectively. Since the amount of fixed N depends mainly on the proportion of clover in the mixture, these potential benefits can be realized only if clover is present in a sufficient amount in the sward. Management of swards for maintaining a proper balance between grass and clover is very difficult compared with N fertilised grasslands ('t Mannetje, 1996). Harris and Thomas (1973) have suggested that a clover content between 30 to 50% averaged over the year is the desired agronomic level. However, the proportion of white clover fluctuates markedly from year to year and also during the growing season, and depends largely on management practices (Jones and Davies, 1988; Woledge *et al.*, 1992b; Elgersma and Schlepers, 1994, 1997). Since white clover has a high plasticity and is highly sensitive to environmental conditions and management, improvement of its persistence and yield through plant breeding is difficult (Kessler and Nösberger, 1994). So it is necessary to find an appropriate management to support its growth and persistence for a sustainable grass-clover sward. Understanding the factors which influence the composition of mixtures of species under cutting and grazing is a major concern in ecology and agriculture.

Productivity of grass-clover mixtures

There has been a continuing debate concerning whether mixed grass-clover swards are more productive than monocultures. The conclusion of most theoretical studies is that competing populations will tend to diverge in their ecological requirements, so that they increasingly use different resources and thereby reduce or avoid interference. This should be reflected in a higher yield of mixture components than of either of the species grown in a pure stand.

The cyclic growth pattern in grass-clover mixtures has been hypothesised by Turkington and Harper (1979). They showed that in a permanent pasture the first flush of perennial ryegrass growth occurred in March-June, followed by a second flush in August-September. However, clover showed a single peak during June-July. It can be concluded that the peak of production in ryegrass-white clover mixtures is broader than in ryegrass alone, as white clover has a later peak of production than ryegrass. Therefore, ryegrass-white clover mixtures use resources more efficiently than their corresponding pure stands (Menchaca and Connolly, 1990; Turkington and Jolliffe, 1996), because when species have complementary growth rhythms, competition between them can be minimised.

While in natural grasslands environmental conditions (e.g. temperature and water availability) are the main sources of species variation, in intensive pastures management is crucial for the balance between species. Therefore, species interactions in ryegrass-white clover mixtures have been the subject of several decades of research in grassland science. These competitive interactions, which are mainly affected by harvesting (defined by frequency, intensity, uniformity and timing of defoliation), a proper combination of cultivars and N nutrition, determine the stability of mixtures.

Species competition

Parsons *et al.* (1991b) showed that in grazed swards stable persistence of perennial ryegrass and white clover has generally been unachievable, except in the special case where the competition advantage of clover was exactly balanced by the feeding preference of grazing animals. This led to the suggestion that ryegrass-clover mixtures are intrinsically unstable. Thornley *et al.* (1995) and Schwinning and Parsons (1996a) suggested that the key in understanding the intrinsic sources of variability in grass-clover mixtures is the difference in their response to availability of N in the soil. When soil N is low, clover has a greater relative growth rate (*RGR*) than grass, since it can replace inorganic N uptake with N fixation. When soil N is high grass has the greatest *RGR*, because N uptake is more efficient than a combination of N uptake and N fixation (Thornley *et al.*, 1995). Based on these results, Thornley *et al.* (1995) concluded that through N cycling the two species establish an intermediate soil inorganic N level, at which their competitive advantages are balanced, and thus they can coexist.

Camlin (1981), Martin and Field (1981), Harris (1987), and Woledge *et al.* (1992b) as well as other researchers concluded that competition for the aerial resource light is the main component which affects performance and productivity of species in mixed swards. Similarly, Schwinning and Parsons (1996b), using a simulation model, showed that grass benefits from the ability of clover to introduce N into the system, but simultaneously suppresses clover growth through competition for light. These interactions provide the basis for large oscillations of grass and clover densities.

Competition for light depends on the canopy characteristics of species (e.g. spatial distribution of leaf area and angle). Canopy structure in its turn depends on genotypes as well as management practices such as defoliation system and application of N. On the other hand, canopy structure is influenced by variation in environmental conditions during the season. Combination of these variables results in a complex pattern of growth and production. Explanation of this complexity is only possible through a modelling approach (Parsons *et al.*, 1991b).

Competition models for grass-clover mixtures

Several mechanistic models with various levels of resolution have been constructed to address a fundamental basis of growth and production in pastures (Noy-Meir, 1976; Christian *et al.*, 1978; Innis, 1978; Blackburn and Kothman, 1989). However, these models are mainly focused on grassland productivity and hardly deal with species competition.

In general, two types of light absorption models are applied to grass clover mixtures: single layer canopy models (Ross *et al.*, 1972; Johnson *et al.*, 1989; Sinoquet *et al.*, 1990) and multi-layer canopy models (Rimmington, 1984; Faurie *et al.*, 1996), which are more realistic. A more flexible approach for light absorption and CO_2 assimilation between component species is followed in the model INTERCOM (Kropff and van Laar, 1993). The model simulates light absorption and partitioning between species based on the pattern of their leaf area distribution over canopy height. Among several ecophysiology-based models for intercopping reviewed by Caldwell (1995), INTERCOM was the only one that includes a leaf-level CO_2 assimilation function to estimate canopy CO_2 assimilation. In the present study INTERCOM is used as a tool to study the mechanisms of light competition in grass-clover mixtures because of its capability for simulation of light absorption and CO_2 assimilation rate of species within canopy layers.

Aims of research

The objectives of this study are to identify the differences between contrasting perennial ryegrass and white clover cultivars in growth, productivity and in the patterns of investment of their resource into the light absorbing and photosynthetic tissues (total leaf area and its vertical distribution). This basic information is needed to explain changes in the composition of mixtures and to extend the existing simulation models for competition between species under cutting.

The ultimate goal is to gain insight into the mechanisms that determine clover persistence under various management strategies.

Outline of the thesis

The thesis includes the results of two years of field experiments (1995 and 1996) on growth characteristics of perennial ryegrass and white clover in mixture and monoculture. Experiments were carried out in Wageningen, The Netherlands. Interactions between species were studied on the basis of the INTERCOM model.

In **Chapter 2** the effect of cutting frequency on yield and species balance during successive regrowth periods of contrasting grass and clover cultivars in mixtures is presented. **Chapter 3** includes the seasonal growth patterns of species in mixture and monoculture, with and without N fertiliser. The effect of N on canopy structure and distribution of light, biomass and N in mixed canopies is presented in **Chapter 4.** In **Chapter 5** a light absorption and partitioning model for a mixed grass-clover canopy is described. Using this model, the effect of N on the seasonal pattern of canopy development, light absorption, radiation use efficiency and light competition are studied in **Chapters 6** and **7**. **Chapter 8** describes the distribution of N within the mixed canopy and its effect on leaf and canopy CO_2 assimilation. Finally the general discussion of all results and final conclusions are presented in **Chapter 9.**

Chapter 2

Competition in contrasting grass-clover mixtures: dynamics of species composition, light absorption and dry matter production during regrowth

M. Nassiri and A. Elgersma

Abstract

To explain the effect of defoliation interval on differences in growth pattern between clover cultivars the dynamics of leaf area increase, light interception and dry matter (DM) production were studied within successive regrowth periods in contrasting perennial ryegrass-white clover mixtures without N fertiliser at two cutting intervals. During 1995 the mixtures were cut 8 (F1) or 6 times (F2) at a stubble height of 5 cm. After harvest about 50 g DM m^{-2} (with 52 % clover) was present in the stubble, and the *LAI* was 0.5 (38 % clover). There was little variation throughout the growing season. During regrowth the relative growth rate of leaf area and DM was higher in clover than in grass, especially during summer, in both cutting treatments. Therefore, during regrowth the clover content increased in the leaf area and in the DM yield of the mixtures.

By the end of spring regrowth there was about 55 % clover in the *LAI* and 45 % in the DM, with little difference between cutting treatments. In summer, the clover content in *LAI* and DM in both mixtures was about 70-75%, which was much higher than in spring. There was a decline during autumn, especially in F2 and in the mixtures with the small-leaved white clover cv. Gwenda and the medium-leaved cv. Retor.

The percentage of absorbed radiation was 20-30% after harvest and increased during 3 weeks to about 95% (closed canopy). There was not much difference between cutting treatments, except that the canopy of the frequently-cut swards was not yet closed at the moment of spring harvest.

Mixtures with large-leaved clover cv. Alice, had a more rapid increase in *LAI* and DM than mixtures with cvs. Gwenda or Retor, despite their lower initial clover content after harvest. This resulted in a higher clover content in the *LAI* and DM in mixtures with cv. Alice from 2 weeks of regrowth onwards and led to a higher total and clover yield in mixtures with cv. Alice at all harvests throughout the growing season in both cutting treatments. Cutting frequency did affect the change in clover-grass ratio during regrowth, which was significantly higher in mixtures with cv. Alice than in mixtures with cv. Gwenda, but only under infrequent cutting. Large-leaved cv. Alice had a higher *LAI* and DM at harvest, but small-leaved cv. Gwenda had a higher *LAI* and DM than medium-leaved cv. Retor.

Key words: White clover, perennial ryegrass, growth dynamics, leaf size, light interception, cutting frequency, competition.

Introduction

Compatibility in a grass-clover mixture depends on the cultivar combinations and the management imposed. There are differences in productivity and seasonality of growth pattern between clover cultivars. Once species are sown and swards are established, the main management decisions in practice are fertiliser regime and harvesting strategy. The defoliation interval is the main cause of different responses of clover cultivars to management (Curll and Wilkins, 1982).

Leaf size is the most important criterium to distinguish clover cultivars (Caradus *et al.*, 1989). The productivity of each leaf size category is affected by the frequency of defoliation. The better productivity of large-leaved clovers under infrequent cutting and the suitability of small-leaved cultivars for frequent defoliation have been emphasised by many researchers (Wilman and Asiegbu, 1982; Evans and Williams, 1987; Swift *et al.*, 1992; Kang and Brink, 1995). However, results of a long-term experiment on yield response of contrasting clover cultivars to cutting frequency in Wageningen (Elgersma and Schlepers, 1997) did not show such a clear difference. Large-leaved clover cv. Alice was more productive than other cultivars, both when cut at 1200 and at 2000 kg DM ha^{-1}.

The difference in response of clover cultivars to cutting interval is possibly due to morphological or physiological attributes such as difference in petiole length (Wilman and Asiegbu, 1982) and the capacity of cultivars to maintain their leaf area in response to cutting frequency (Kang and Brink, 1995). There is also evidence of the effects of light environment within the canopy on petiole elongation and stolon branching (Thompson, 1995). The amount of leaf area in the stubble after cutting determines the initial light interception and subsequent regrowth.

Information on the dynamics of changes in DM and *LAI* of grass and clover during successive regrowth periods throughout the growing season in response to cutting interval is needed to understand competition in mixed canopies. However, in grass-clover mixtures such data are scarce. Therefore, the dynamics of leaf area increase, light interception and DM production were studied within successive regrowth periods in binary mixtures of diploid and tetraploid perennial ryegrass with a large, medium and small-leaved white clover cultivar in response to cutting interval. The objectives of this research were to study the effect of cutting treatment and mixture composition on the dynamics of leaf area increase, light interception and DM production during regrowth throughout the growing season.

Materials and methods

The experiment was established on clay soil in 1991 in Wageningen, The Netherlands (Elgersma and Schlepers, 1994). The perennial ryegrass (*Lolium perenne*) cultivars Condesa (tetraploid) and Barlet (diploid, erect) were sown in binary mixtures with white clover (*Trifolium repens*) cultivars Alice (large-leaved), Retor (medium-leaved) and Gwenda (small-leaved). The six mixtures will be abbreviated as CA, CR, CG, BA, BR and BG. There were two cutting frequencies, based on approximate target yields of 1200 (F1) and 2000 kg DM ha^{-1} (F2). As yield is related to canopy height, target yield was estimated by measuring sward surface height (SSH).

The experimental design was a split-plot with cutting frequency as the main plot and mixture as subplot; there were three replications. P and K were applied regularly, but no fertiliser N was applied. Experimental details have been reported earlier (Elgersma and Schlepers, 1997).

In 1995 the mixtures were cut 8 and 6 times (F1 and F2, respectively) at 5 cm sward height. Detailed measurements were taken in all mixtures in F2, and in CG and CA in F1. Sampling commenced after the first harvest on May 2 and 9 for F1 and F2, respectively, and continued until October 26 (Table 1).

Sward surface height (SSH) was measured weekly and before each harvest by taking 10 measurements per subplot, using a falling plate (diameter 50 cm, weight 350 g, 0.18 g cm^{-2}).

Table 1. Dates of harvest of whole subplots and regrowth periods in F1 (R1-R7) and F2 (R1-R5) based on taking quadrat samples.

Cutting frequency	F1		F2		
Harvest date	Regrowth period		Harvest date	Regrowth period	
May 2			May 9		
	} R1	2/5-23/5		} R1	9/5-4/6
May 24			June 7		
	} R2	24/5-13/6		} R2	7/6-6/7
June 13			July 7		
	} R3	13/6-4/7		} R3	7/7-1/8
July 4			August 2		
	} R4	4/7-25/7		} R4	2/8-13/9
July 26			September 13		
	} R5	26/7-15/8		} R5	13/9-26/10
August 17			October 26		
	} R6	17/8-15/9			
September 18					
	} R7	18/9-26/10			
October 26					

At weekly intervals, all above-ground plant material in a 10 x 10 cm quadrat was harvested at ground level. The first 5 cm layer from the base was considered stubble, the remaining canopy (> 5 cm) will be termed harvestable herbage. The material in both layers was separated into dead materials and clover flowers (if present), which were excluded from further measurements, and clover leaf blades, clover petioles, grass leaf blades and grass (pseudo) stems plus leaf sheaths (Nassiri *et al.*, 1996a). The area of leaf blades of grass and clover was measured with an electronic leaf area meter (Li-Cor 3100®, Li-Cor Inc., Lincoln, NE, USA). The harvested material was dried at 70 °C during 24 hours and weighed. To obtain leaf areas for mixtures in F1 (CA and CG), estimates were used from inclined point quadrat data, which showed a good relation with *LAI* values measured with the leaf area meter (Chapter 5).

The total absorbed radiation by the mixed canopy was measured weekly at the top of the canopy and at ground level using a linear ceptometer.

In this study two distinct analyses were carried out with subsets of the data. Firstly, to test the effect of the two grass and three clover cultivars, the six mixtures were analysed within F2. Secondly, to test the effect of cutting frequency and clover cultivar the data of CA and CG in F1 and CA, CG and CR in F2 were analysed separately.

Results

Sward surface height

The average rate of increase in sward surface height (SSH) (cm day^{-1}) during regrowth is shown in Figure 1, which illustrates the seasonal pattern during the growing season.

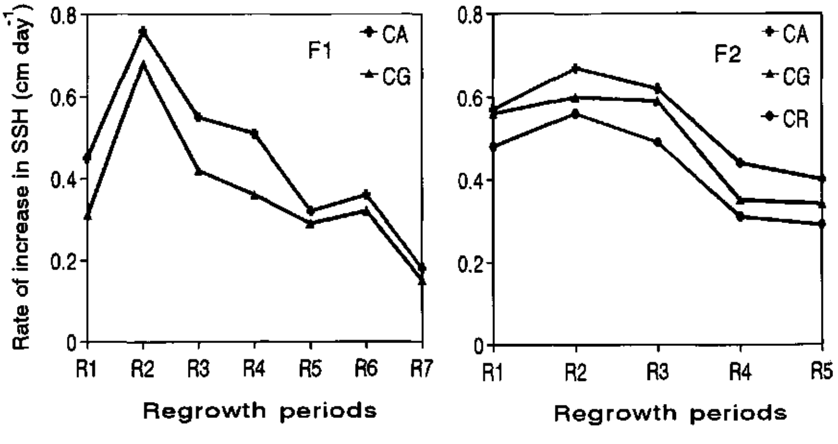

Figure 1. Average rate of increase in sward surface height (SSH) in different grass-clover mixtures (CA, CG, CR) during different regrowth periods in two cutting treatments (F1 and F2).

In both cutting treatments the highest daily increase rate in SSH occurred in June, in R2, and decreased thereafter. This pattern was most pronounced in F1. Mixtures with Alice always had a higher SSH increase rate than mixtures with Gwenda or Retor. Figure 2 shows the SSH of the mixtures at weekly intervals during 3 regrowth periods in F1 (R1, R4, R7) and F2 (R1, R3, R5), representing spring, summer and autumn, respectively. Initial SSH after cutting was 5 cm in all treatments.

In spring and summer SSH increased almost linearly during regrowth. However, in autumn the rate of increase was low (Figure 1) and more days of regrowth were needed to achieve the target SSH for the next harvest (Figure 2). In general, SSH was higher in CA than in CG or CR. Comparison of the mixtures within F2 revealed that there was no effect of grass cultivar on SSH, whereas mixtures with Retor had a lower SSH increase rate than mixtures with Alice and Gwenda during late summer (R3) and autumn (R5) (Figure 2).

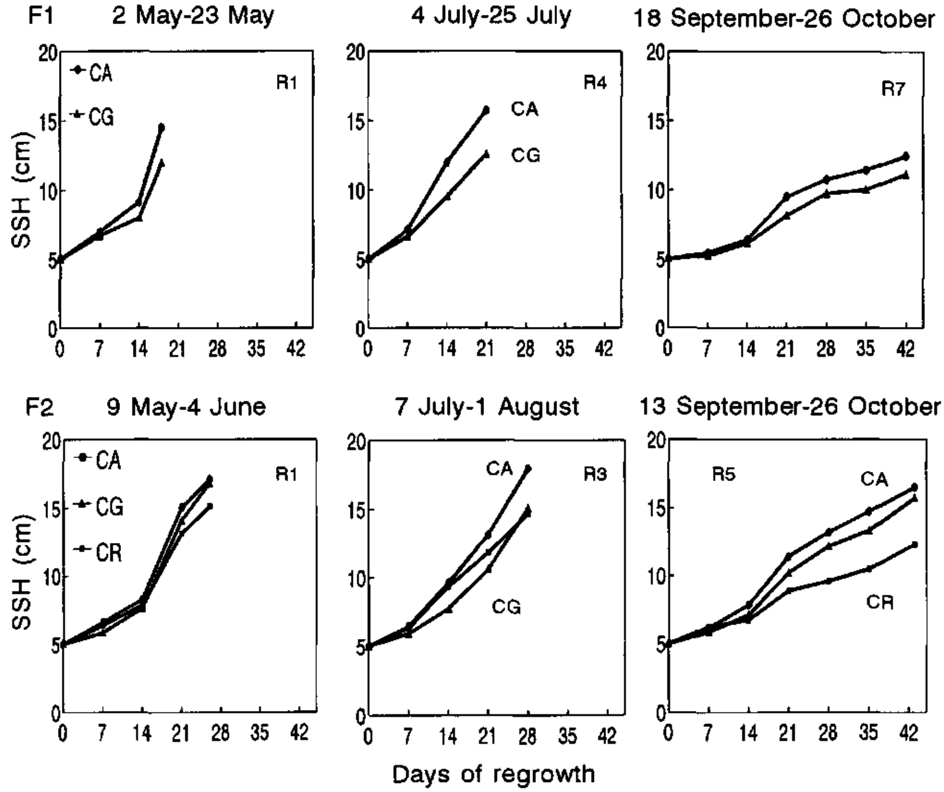

Figure 2. Increase in sward surface height (SSH) during 3 regrowth periods (R1, R4 and R7) in F1 and (R1, R3 and R5) in F2.

Dynamics of leaf area increase

Figure 3 shows the weekly increase in grass and clover *LAI* in the harvestable forage (above 5 cm) during 3 regrowth periods. In general, grass *LAI* was not significantly different between F1 and F2 throughout the growing season.

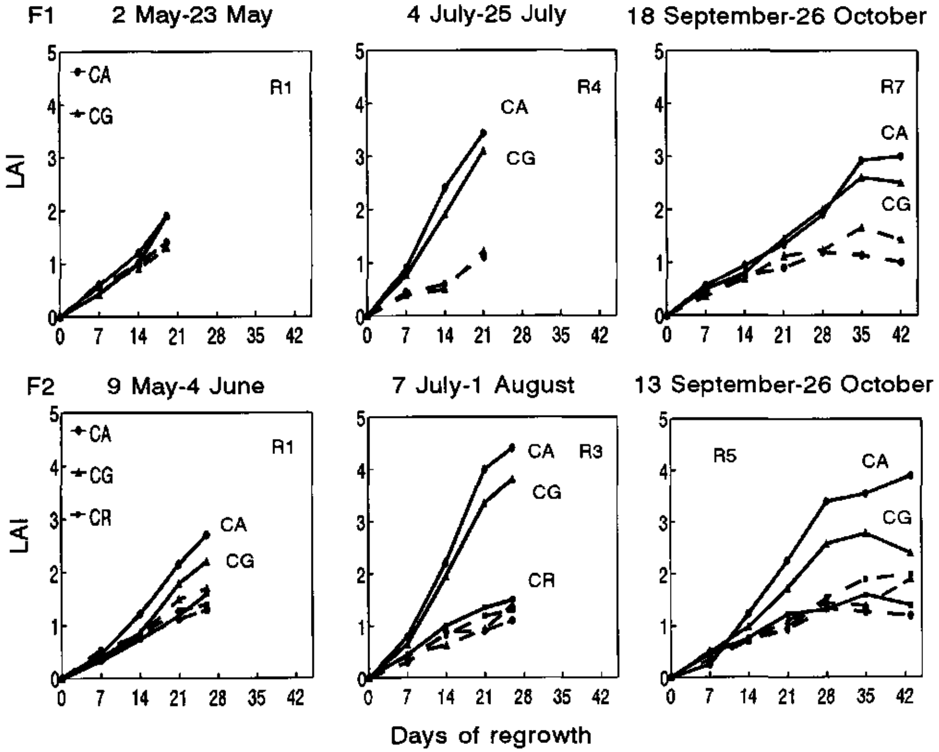

Figure 3. Dynamics of growth of leaf area of grass and clover in harvestable herbage (> 5 cm) during R1, R4 and R7 in F1 and R1, R3 and R5 in F2. (—), clover; (----) grass.

However, in all regrowth periods and in both cutting frequencies grass had a lower growth rate of leaf area than clover. This difference was most pronounced in summer, when the rate of increase of clover *LAI* was maximal and the highest *LAI* was reached. In both cutting regimes small-leaved Gwenda had a lower leaf area than large-leaved Alice, but this difference was not significant in F1. In F2 the long regrowth period in autumn led to a significant reduction in the *LAI* of Gwenda during the final week of regrowth. In this period grass *LAI* was also significantly higher in CG than in CA (Figure 3). In F2 during all regrowth periods Retor had a lower *LAI* compared to the other clovers, particularly from spring onwards. Grass *LAI* was not affected by clover cultivars. However, during the last regrowth (R5) in the mixture with Retor the grass *LAI* was higher than the clover *LAI* (Figure 3). In general, the clover *LAI* was highest in large-leaved Alice and lowest in medium-leaved Retor.

Pattern of light absorption

The weekly change in light absorption by the mixed canopies during regrowth is shown in Fig. 4. After cutting, the stubble absorbed 20 - 30 % of the incoming radiation. In F1 the spring regrowth period was short and at harvest the light absorption was still in the linear phase, indicating that the canopy was still open. During summer and autumn a closed canopy, defined as an absorption level of 95% of the incoming radiation, occurred after 20 days of regrowth in CA and CG in both cutting intervals. The longer regrowth duration in F2 led to a closed canopy at the end of all periods. In autumn the lower leaf area increase rate (Figure 3) resulted in a decrease in the rate of light absorption in the mixtures compared to spring and summer.

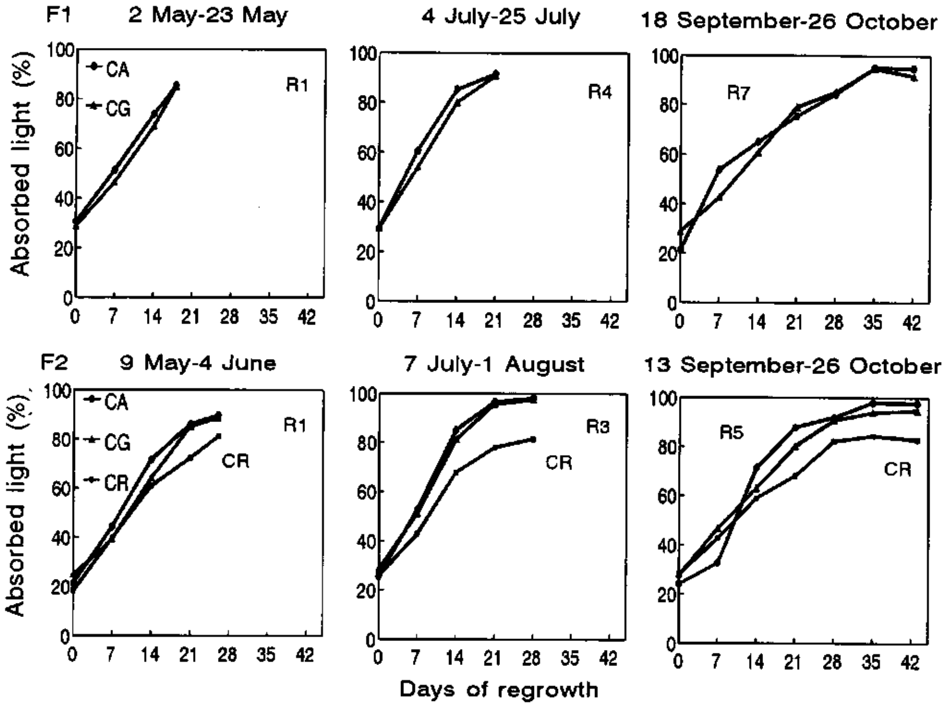

Figure 4. Time course of light absorption by the mixed canopy during R1, R4 and R7 in F1 and R1, R3 and R5 in F2.

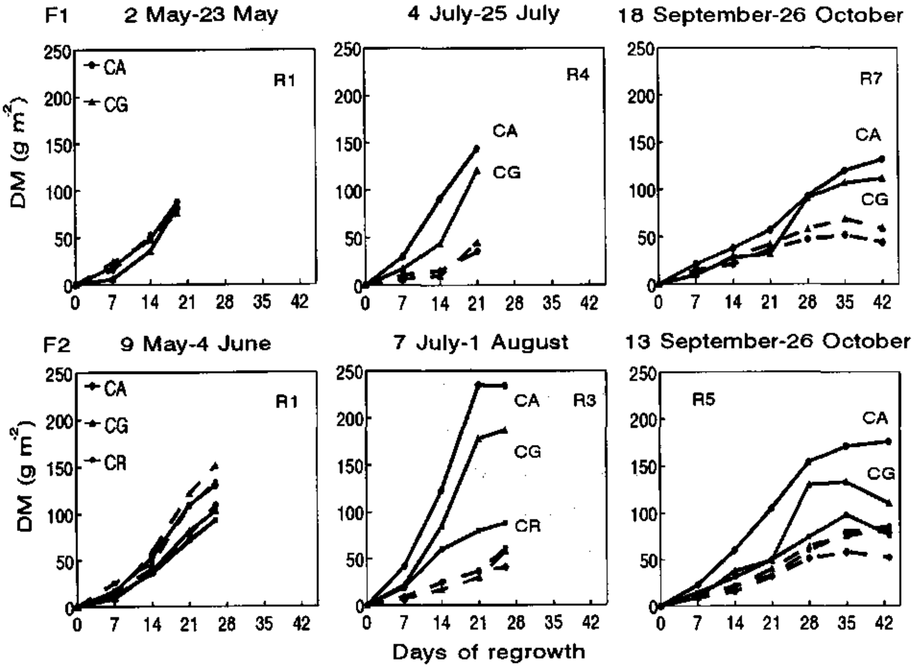

Figure 5. Dynamics of growth of DM of grass and clover in harvestable herbage (> 5 cm) during R1, R4 and R7 in F1 and R1, R3 and R5 in F2. (—), clover; (---) grass.

Therefore, in CA and CG the canopy was closed about 2 weeks later in autumn than in summer (Figure 4). In F2 a closed canopy was not found in the CR mixture and the total absorbed light was lower than in CA and CG, which reflects the lower *LAI* of Retor (Figure 4).

Dynamics of dry matter (DM) increase

Figure 5 shows the increase in harvestable DM during 3 regrowth periods. The pattern was similar to that of the increase in harvestable *LAI* (Fig. 3). In spring, grass and clover DM yields both increased rapidly, with a linear increase from the first week after cutting onwards with no difference between CA, CG and CR. In summer, the increase in grass DM was slower than in spring, whereas the clover DM increased much faster than in spring. In F2, the increase in clover DM during weeks 2 and 3 was linear, but there was only little increase during week 4 in F2. By the end of the summer regrowth period, Alice and Gwenda had the same DM yield in F1, but in F2 the DM yield of Alice was significantly higher. Gwenda and Retor always had a lower DM yield compared to Alice (Figure 5). In autumn, the clover growth rate was comparable to that in spring, and the grass growth rate to that in summer. However, in F2 the greater reduction in DM of Gwenda led to a significant difference between Gwenda and Alice, which was not observed in F1. In F2, the DM ranking of clover cultivars (A > G > R) remained unchanged after spring. For grass DM the opposite was found in autumn, with mixtures containing Alice producing less grass than mixtures with Gwenda. No difference was observed in grass DM between CG and CR (Figure 5).

In Figure 6, grass DM in weekly cut quadrats from all regrowth periods except spring has been plotted against clover DM. This relationship describes the increase in grass DM as a function of clover DM. In other words, it expresses the aggressivity of clover cultivar over grass. Linear regression analyses showed that CG had the same slope in both cutting treatments, but CA had a significantly lower slope in F1 than in F2 (Table 2).

Table 2. The result of linear regression between clover DM and grass DM during regrowth periods. Regression lines are presented in Figure 6.

Cutting frequency	Mixture	Intercept	Slope#	r^2
F2	CA	15.59	0.183 (± 0.029) c	0.755**
	CG	14.32	0.364 (± 0.038) b	0.792**
	CR	6.60	0.692 (± 0.069) a	0.813**
F1	CA	12.80	0.304 (± 0.035) b	0.752**
	CG	11.91	0.389 (± 0.039) b	0.790**

Slopes with the same letter are not significantly different at the 5% level, figures in brackets show the SE of coefficients.

** Significant at P < 0.01

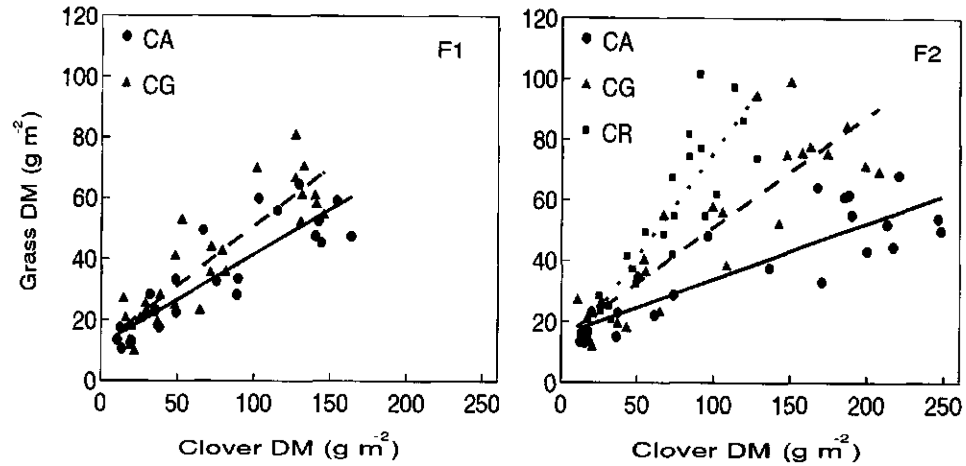

Figure 6. Relationships between clover DM and grass DM in harvestable herbage (> 5 cm) during different regrowth periods in F1 and F2. CA (—); CG (----); CR (·····). For more information see Table 2.

Thus in F2 the slope was significantly lower in CA than in CG, indicating the higher aggressivity of large-leaved Alice (less grass DM at the same clover DM compared to mixtures with Gwenda). In addition, in F2 the slope of the regression line was highest in CR (Table 2 and Figure 6), which shows the lower competitive ability of Retor. In spring no such pattern was observed (not shown).

Clover content in *LAI* and DM

Figure 7 shows the dynamics of the clover content in the total aboveground DM and *LAI*. In spring in F2 the clover content in the DM was higher in CG than in CA and CR at the start (day zero) of regrowth in the stubble, just after cutting. However, the clover content in CR and especially CA increased after 7, 14 and 21 days of regrowth, whereas the clover content in CG remained constant. Therefore, the clover content in the DM at harvest was higher in CA than in CG and CR ($P < 0.05$) (although the difference in DM was not significant (Fig. 5)). During summer the amount of clover in the stubble at day zero was much higher than in spring. During regrowth it increased from about 53 % to about 74 % after 4 weeks, with no significant difference between CA and CG. However, it was significantly lower in CR. In autumn the initial clover content was comparable to that in summer, but increased more slowly during regrowth. In CA, a level of about 70 % clover was reached after 4 weeks of regrowth, which lasted until harvest. At harvest the clover content was significantly different in all mixtures (Figure 5).

A similar pattern was found for the clover content in the *LAI*. However, after harvest (day zero) the clover content in *LAI* was always lower than the clover content in the DM. During the first week of regrowth the increase of clover in the *LAI* was stronger than its increase in the DM.

In F1 similar results were found. The initial clover content was comparable to that in F2, but the increase in clover content during regrowth was somewhat faster in F1.

Discussion

At both cutting frequencies, the SSH achieved by the end of the regrowth periods was higher in spring and summer than in autumn. In spring both mixtures had the same SSH, but in summer SSH was highest in mixtures with Alice (Figure 2). The height of many species is a function of temperature (Spitters, 1989). However, Barthram and Grant (1994) showed that at low temperatures grass had a greater rate of increase in height than clover. Eagles and Othman (1986) suggested that short days and low temperatures result in short petioles in clover. Arnott and Ryle (1982) found that maximum petiole lengths were achieved under long days (16 h) with temperatures of 15-20 °C.

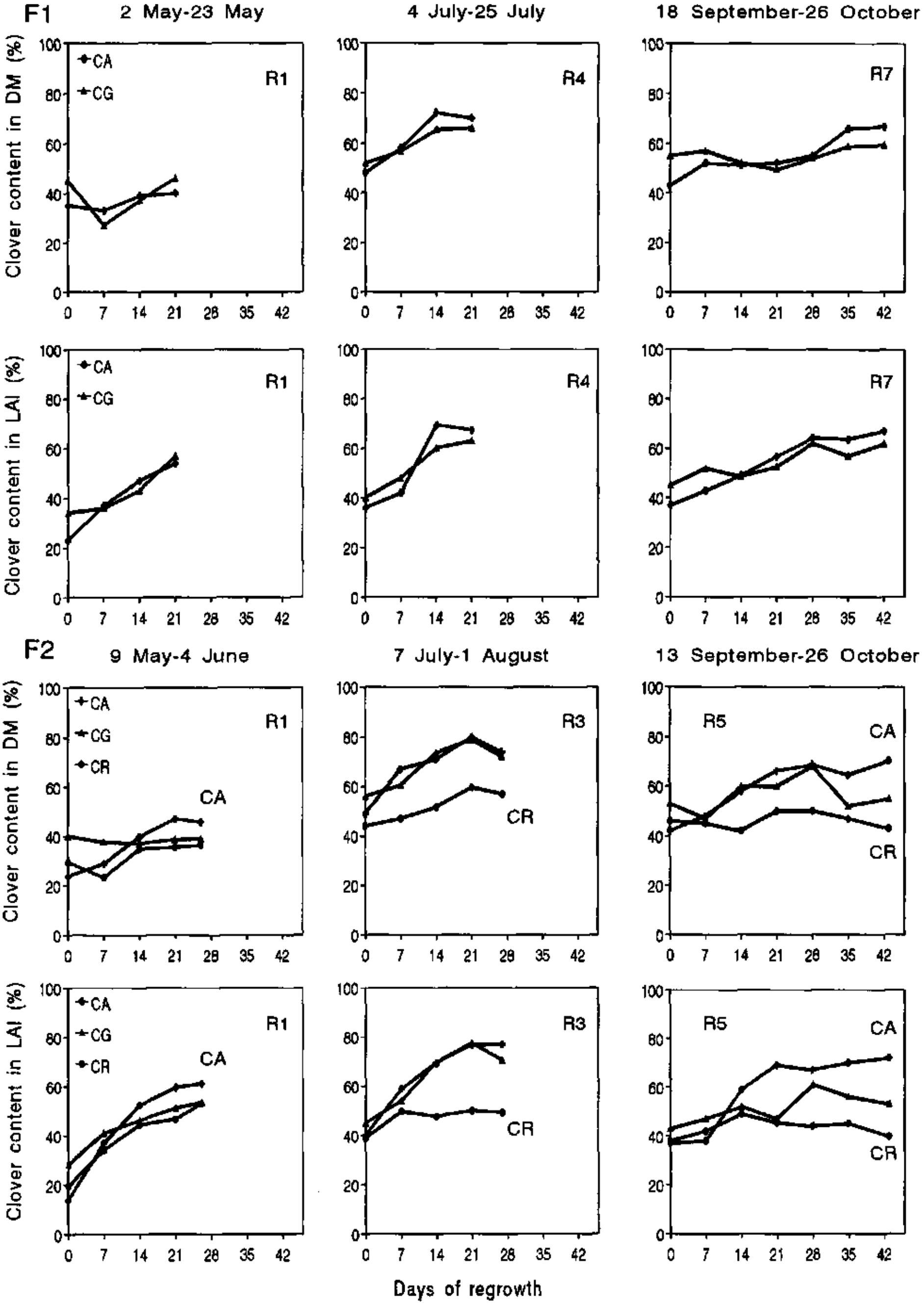

Figure 7. Dynamics of clover content in DM and *LAI* during R1, R4 and R7 in F1 and R1, R3 and R5 in F2.

It can be concluded that in spring the SSH and its rate of increase were determined by grass, but that during summer, when environmental conditions were more favourable for clover compared to unfertilised grass, SSH was controlled by clover. Therefore, only during summer mixtures containing the long-petioled Alice had a higher SSH than mixtures with Gwenda. Gwenda showed less response in petiole length to temperature (Chapter 3).

Evans and Williams (1987) and Swift *et al.* (1992) confirmed that small-leaved clover had a better performance under frequent defoliation than under a less frequent cutting regime. However, our results showed that both large-leaved and small-leaved clover performed better in F2 than in F1. This difference was obvious both in growth of leaf area (Figure 3) and DM (Figure 5). In F2 the growth difference and yield ranking of clover cultivars (A > G > R) was not exactly in accordance to their leaf size, because medium-leaved Retor, which is less winterhardy and more susceptible to diseases, performed less than small-leaved Gwenda. The same ranking order of cultivars was also reported for annual herbage production by Elgersma and Schlepers (1997) and Elgersma *et al.* (in preparation). While in F2 during the summer and autumn regrowth periods the *LAI* and DM were significantly different between clovers cultivars, in F1 there was no difference between Alice and Gwenda. In fact the lower clover *LAI* and DM in F1 compared to F2 was less pronounced in Gwenda than in Alice, indicating that in F1 growth conditions favoured the small-leaved clover (Figures 3 and 5).

Swift *et al.* (1993) emphasized that small-leaved cultivars, with their more stoloniferous growth habit, are much better suited for grazing or frequent cutting. The beneficial effect of frequent cutting to small-leaved clover can be attributed to the higher light intensity at ground level. In F1, where the total canopy *LAI* was lower than in F2, light interception was also lower by the end of regrowth (Figure 6). A higher light intensity in the base of the canopy promotes the development of axillary buds of clover stolons (Solangaarachchi and Harper, 1987; Thompson, 1993), as well as ryegrass tillers (Casal *et al.*, 1985). It has been shown that *LAI* values of 2.5 and 3 in monocultures and mixtures are the respective thresholds above which the development of axillary clover buds stops (Simon *et al.*, 1989). In the present experiment, even in F1 the total *LAI* was higher than 3 at harvest, but still lower than in F2 (Figure 3). While at harvest the growth rate of *LAI* was still in the linear phase in F1, the intercepted light by the canopy was almost maximal (Figure 4) because light interception increases very little with increasing *LAI* above a value of ca. 3 (Belanger *et al.*, 1992). Therefore, it can be concluded that overall clover stolons experienced a shorter duration of shading in F1 than in F2.

It has been suggested that large-leaved clover cultivars allocate more DM to petioles at the expense of stolons (Rhodes and Harris, 1979). Thompson (1995) showed that stolon growth (horizontal) was altered by the light environment at the node, and petiole growth (vertical) by light perceived at the petiole tip. It seems therefore, that the longer defoliation intervals in F2 led to a better light environment at top layers for large-leaved Alice, with its lower stolon density, to elongate its petioles and become a stronger competitor. Under this condition, the longer periods of shading compared to frequent cutting will result in reduction or even death of both clover growing points and grass tillers (Lawson *et al.*, 1997). Thus, a significantly lower DM yield and *LAI* could also be expected in small-leaved Gwenda, with its more stoloniferous growth habit, compared to Alice in summer and autumn regrowth (Figures 3 and 5). However, in F1 both clovers had the same *LAI* and DM. Accordingly, Wilman and Asiegbu (1982) found that the annual yield of small-leaved clover was equivalent to that of large-leaved clover under 3-weekly defoliation, but less than that of large-leaved clover when the cutting interval increased to 16 weeks. Kang and Brink (1995) also found that large-leaved cultivars yielded more DM than small-leaved clovers and concluded that a principal difference between white clover cultivars is their capacity to maintain leaf area in response to defoliation variables. While in the present study the difference between the two cutting frequencies was only 1 week, even this small difference was sufficient to result in a change in aggressivity of large-leaved clover over grass in both cutting treatments.

In F2, the increase in grass DM per increase in clover DM (Figure 6) was lowest in CA and highest in CR. However, in CA frequent cutting (F1) almost doubled the slope of the regression line between grass and clover DM compared to F2. In contrast, in CG it was similar in F1 and F2 (Table 1). In F2, the slope of the regression line was highest in Retor (R > G > A) and showed the inverse ranking order as was observed for the DM and the *LAI* of clover cultivars (A > G > R).

Nearly all clover *LAI* was removed following defoliation, but ryegrass retained a higher proportion of its leaf area after cutting (similar finding by Woledge *et al.*, 1992b), which gave the grass a competitive advantage. However, the dynamics of clover content during regrowth in F1 and F2 (Figure 7) showed that both clover cultivars increased their content by weight and by *LAI*. Woledge (1988) and Woledge *et al.* (1992a) obtained the same results and concluded that clover had a higher relative growth rate (RGR) than grass. In spring, both in F1 and F2 the increase in clover content in DM was less obvious than in other periods, indicating that both species had the same RGR of their DM. However, the RGR of clover *LAI* was always higher than that of grass LAI (Figure 7). Our results also show that in F2 the small-leaved clover had a lower RGR than the large-leaved clover, particularly in spring and autumn, but this difference was not observed in F1. The results of this study, together with previous results on seasonal productivity of contrasting grass-clover mixtures (Elgersma and Schlepers, 1997), showed that there were significant and consistent differences between clover cultivars, whereas grass cultivars had no effect.

Further information about the vertical distribution of leaf area and biomass in the mixed canopy and light interception at various heights will provide more insight into the nature of the response of clover cultivars to cutting intervals.

Chapter 3

The effects of repetitive nitrogen applications on contrasting perennial ryegrass and white clover cultivars in mixtures and monocultures under cutting. I. Seasonal growth and dry matter yield

M. Nassiri and A. Elgersma

Abstract

Binary mixtures and monocultures of the diploid perennial ryegrass cultivars Barlet (erect) and Heraut (prostrate) and the white clovers cvs. Alice (large-leaved) and Gwenda (small-leaved) were established in a cutting experiment on a sandy soil. Grass monocultures received three N levels (0, 150 or 300 kg ha^{-1}) split over the season. Mixtures were supplied with 150 kg N ha^{-1} (+N) or no N (-N). No N was applied to clover monocultures. All plots were cut 5 times during 1996 (from 10 May until 7 October).

Repetitive application of N had a deleterious effect on clover growth. The average clover content declined from 42.5% in the -N mixtures to 11.8% in the +N swards. There was no N x clover cultivar interaction. Grass growth increased significantly in response to N, but there were no differences between cultivars. The annual yield of -N mixtures was close to that of N150 grass monocultures, but N300 pure grass outyielded both the +N and the -N mixtures. In both the +N and the -N mixtures clover lost relatively more leaf area and less DM than grass due to cutting, leading to a lower clover content in the *LAI* of the stubble. In the -N mixtures the clover content in the DM and in the *LAI* increased towards the end of the successive regrowth periods, compared to the initial clover content after cutting in the stubble. Maximum clover contents were found during summer. In the +N mixtures large-leaved Alice maintained its content during summer. However, at each harvest the content of small-leaved Gwenda in the *LAI* and DM was lower than at the start of regrowth. The advantage of Alice was mainly due to its greater petiole length, which increased in response to N, while the opposite was observed for Gwenda. The allocation of DM to the petioles led to a decrease in the LWR of the large-leaved clover in the +N mixtures, while the small-leaved clover had a higher LWR and SLA in the +N mixtures than in the -N mixtures. It is proposed that clover cultivars that have a different pattern of DM allocation follow different strategies in response to applied N.

Key words: White clover, perennial ryegrass, N, competition, mixture, monoculture.

Introduction

White clover is usually grown with companion grasses, in cultivated pastures mainly with perennial ryegrass. White clover is receiving increased attention, because of the growing tendency to develop a more extensive and environmentally safe approach to grassland-animal production systems (Fisher, 1996). The cohabitation of ryegrass and white clover is possibly due to asynchrony in the growth patterns of both species, which has been observed under different management practices (Curll and Wilkins, 1982; Davies, 1992; Barthram and Grant, 1994), and to the beneficial effects of fixed N on ryegrass (Harris, 1987).

Despite the fact that ryegrass is the most compatible species with white clover (Chestnutt and Lowe, 1970), the potential benefits of a legume species, e.g. its ability to fix N, can be realised only if a sufficient amount of clover is present. However, seasonal and annual variability of growth lead to a variable clover content under both a cutting or grazing management (Parsons *et al.*, 1991a). The relative persistence of species in mixtures is partially dependent on genetically controlled differences in their competitive abilities (Aarssen, 1983). However, a proper combination of grass and clover cultivars, availability of inorganic N and defoliation patterns, among other practices, are also important factors in controlling the clover persistence.

Incompatibility of clover persistence with N fertilisation has been frequently reported. Soussana and Arregui (1995) showed that the clover content in a mixed sward was negatively correlated with the ryegrass N nutrition level. Similarly, Woledge (1988) and Fisher and Wilman (1995) reported a significant decline in the clover content in the DM when N fertiliser was applied in spring. The clover response to inorganic N is usually evaluated under a single application of N during spring, when clover growth and N fixing ability are limited due to unfavourable environmental conditions (Marriott, 1988). In a controlled environment, the clover depression in a +N mixture was more pronounced at low than at high temperatures (Davidson and Robson, 1986). Clover which has been treated with spring N will recover during the growing season to the same content and yield as clover which was not treated (Frame, 1987).

Limited information is available regarding the effect of repetitive application of N on clover growth in mixtures. Frame and Boyd (1987), using different N levels applied over the season, showed that at a high N level (360 kg ha^{-1}) the annual clover yield was reduced by 88% compared to a control treatment. On the other hand, Wilman and Asiegbu (1982) concluded that large-leaved clover cultivars were more productive than others at all N rates used in their study. It seems therefore, that under spring-applied N clover yield and persistence are positively correlated to clover leaf size. However, it is not clear if this effect also exists under repetitive N applications. In unfertilised mixed swards, grass growth depends on the symbiotically fixed N by clover, next to soil N mineralisation and deposition (Elgersma and Hassink, 1997). The atmospherically-fixed N in mixed swards containing 30-50% clover was estimated as 157 kg ha^{-1} year^{-1} in the first cropping year by Kristensen *et al.* (1995). The application of this amount of N during the growing season would enable a study of the growth of clover in mixtures, while grass growth would be mainly independent of fixed N.

In this research the effect of repetitive N applications on the yield and performance of species in mixtures of contrasting white clover and ryegrass cultivars, as well as their monocultures, were investigated under cutting. The aim was to study the physiological traits that contribute to the persistence of white clover, and the balance between species in response to an application rate of N equal to the estimated amount of N fixed by clover, throughout the growing season.

Materials and methods

Experimental site, design and management

Three adjacent trials were sown in the autumn of 1995 on an actually N-deficit sandy soil at Wageningen, The Netherlands. A series of monocultures and mixtures ranging from extreme clover dominance to extreme grass dominance was established by application of varying levels of N to grass-clover mixtures in 1996.

In the first trial (experiment 1), two white clover (*Trifolium repens*) cultivars: Alice and Gwenda (large and small-leaved, respectively) and two perennial ryegrass (*Lolium perenne*) cvs. Barlet (diploid, erect) and Heraut (diploid, prostrate) were used to make four different mixtures (the mixtures are hereafter referred to by the first letter of their component cultivar names). The mixtures were grown under two N levels, 0 (-N) and 150 kg N ha^{-1} (+N) during the growing season. The monocultures of the clover cultivars were sown in the plots adjacent to the mixtures (experiment 2). No fertiliser N was applied to the clover monocultures. The plot size in experiments 1 and 2 was 2.8 x 8.5 m. In the third trial, monocultures of both ryegrass cultivars were established in 2.8 x 7 m plots (experiment 3). During the growing season of 1996 the monocultures received 3 levels of N (0 (N0); 150 (N150) and 300 kg N ha^{-1} (N300)). Increasing N doses were applied in pure grass during the growing season (Table 1) to simulate the expected seasonal pattern of clover-derived N in mixtures on relative basis (Elgersma and Schlepers, 1997). In all experiments the seeding rate was 4 and 25 kg ha^{-1} for clover and grass, respectively. In all trials a randomised complete block design with 2 replications was used. Sampling started on 10 May and continued till 7 October 1996. All plots were cut at an approximate average target yield of 2000 kg DM ha^{-1} (which took 5 to 7 weeks, depending on growth conditions). This allowed for 5 cuts (4 regrowth periods, R1-R4) during the growing season (Table 1). However, the poor growth of the unfertilised grass monocultures allowed only 3 cuts on these plots.

Table 1. Timing and amount of N fertilisation and duration of regrowth periods (R) in mixtures and monocultures.

			N treatment (kg ha^{-1})				
		Duration	Mixtures		Grass monocultures		
Regrowth period	R	(days)	-N	+N	N0**	N150	N300
17 April - 10 May		34	0	30*	0	10*	20*
10 May - 17 June	1	39	0	30	0	30	60
17 June - 22 July	2	35	0	30	0	30	60
22 July - 2 Sep.	3	42	0	30	0	40	80
2 Sep. - 7 Oct.	4	35	0	30	0	40	80

* amount of N applied at the start of each regrowth period; spring N was applied on April 17.

** this treatment was harvested on 17 June, 2 September and 7 October.

Measurements

The total leaf area of species was measured with an inclined point quadrat (Warren Wilson, 1963). In all plots the total vegetation in a 10 by 10 cm quadrat was cut at ground level at weekly intervals and carefully transferred to the laboratory. A 5-cm layer from the base of the canopy was cut with a paper cutter and considered as stubble.

The cut material (below and above 5 cm) was separated into grass and clover. The dry weight of grass leaves and stems and clover leaves and petioles was measured after drying for 24 hours at 70° C. At the end of each regrowth period whole plots were machine-harvested at 5 cm sward height. The dry matter of grass and clover was determined from the samples taken from the harvested material after drying. Immediately after each cut, the composition of the stubble was determined from the sample taken by the 10 x 10 cm quadrat. Specific leaf area (SLA, leaf area/leaf dry weight), leaf weight ratio (LWR, leaf weight/ shoot weight) and leaf area ratio (LAR, leaf area/ shoot weight; SLA x LWR) were measured for each species, both in the stubble and above it. By the end of the second regrowth period, clover petiole length and specific petiole length (length per unit dry weight, cm mg^{-1}) were measured in 100 random samples taken from each plot.

Weather data during the growing season (Figure 1) were taken from the Haarweg meteorological station, at 500 m distance from the experiment site.

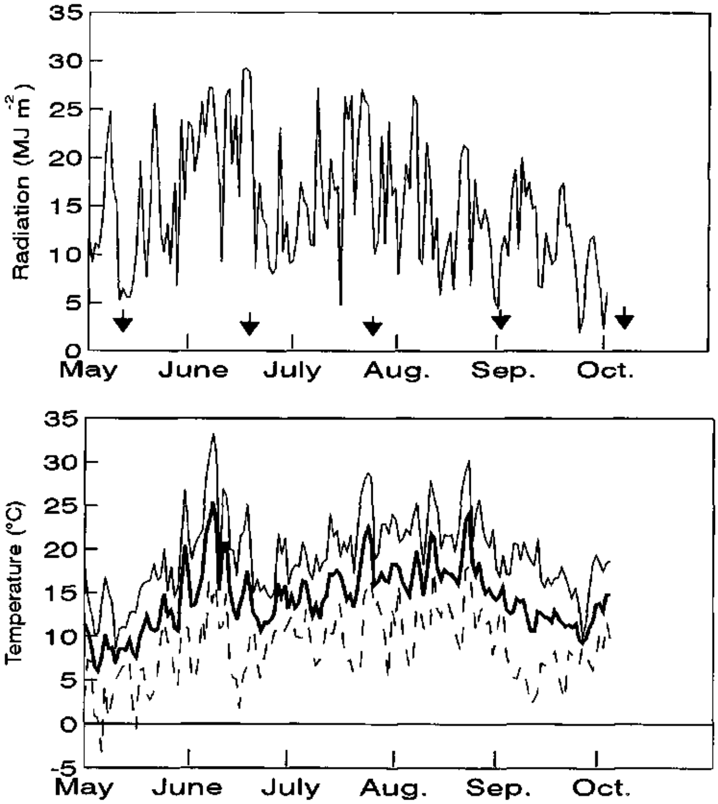

Figure 1. Daily totals of incoming solar radiation and daily values of minimum (----), maximum (—) and average (━) temperatures during the growing season of 1996. Arrows show the cutting dates.

Results

Annual growth of species

Application of N significantly ($P < 0.05$) increased the total annual DM yield averaged over all mixtures (Table 2). In the +N mixtures, the annual grass yield (948 g m^{-2}) was significantly ($P < 0.05$) higher compared to that of the -N mixtures (520 g m^{-2}), but no significant differences were detected between both ryegrass cultivars in their response to N. In both clover cultivars the annual DM yield was significantly ($P < 0.05$) reduced by 67% under repetitive N application compared to unfertilised mixtures. As a result, the overall mean clover content in the DM was 42.5% in the -N mixtures and only 11.8% in the +N mixtures (Table 2). The clover DM in mixture with grass cv. Heraut was lower than that in cv. Barlet, but the difference was only significant for small-leaved clover (Gwenda). The total annual DM yield was always lower in the unfertilised mixtures, but this was only significant ($P < 0.05$) in HA and HG (Table 2). In monocultures no significant differences were observed between clover or grass cultivars. However, grass DM was significantly ($P < 0.05$) increased with increasing N levels. The clover DM in both the +N and the -N mixtures was lower than in monocultures, but the total yield of the mixtures was significantly higher.

Table 2. Annual DM yield (g m^{-2}) of grass and clover in different mixtures and in monoculture. Seasonal variation in DM yield of mixtures are shown as coefficient of variation (cv) calculated for all regrowth periods.

	Annual yield (g m^{-2})			cv (%)		
Treatment	Grass	Clover	Total	Grass	Clover	Total
Experiment 1						
Mixture (-N)						
BA	516	489	1005	55.6	32.3	18.6
BG	566	368	952	65.8	32.8	29.6
HA	473	365	939	70.1	36.8	25.3
HG	525	398	823	48.5	31.7	22.3
Mean	*520*	*384*	*905*	*59.6*	*32.4*	*22.9*
Mixture (+N)						
BA	910	183	1093	52.3	38.9	40.6
BG	983	114	1096	43.3	29.4	36.5
HA	975	133	1108	32.4	21.1	30.0
HG	921	81	1003	36.7	37.9	32.2
Mean	*948*	*128*	*1075*	*40.6*	*24.3*	*34.4*
LSD(5%)	*95*	*97*	*138*	-	-	-
Experiment 2						
Alice	-	765	765	-	15.0	15.0
Gwenda	-	725	725	-	16.1	16.1
Mean	-	*745*	*745*	-	*15.4*	*15.4*
LSD(5%)	-	*73*	*73*	-	-	-
Experiment 3						
Monoculture						
B(N0)	322	-	322	46.1	-	46.1
H(N0)	471	-	471	34.4	-	34.4
Mean	*396*	-	*396*	*39.0*	-	*39.0*
B(N150)	965	-	965	46.1	-	46.1
H(N150)	975	-	975	26.7	-	26.7
Mean	*970*	-	*970*	*35.8*	-	*35.8*
B(N300)	1238	-	1238	28.5	-	28.5
H(N300)	1179	-	1179	28.9	-	28.9
Mean	*1209*	-	*1209*	*28.3*	-	*28.3*
LSD(5%)	*73*	-	*73*	-	-	-

The presence of clover in the -N swards had a drastic effect on grass growth. As a result, the grass DM in these mixtures was significantly ($P < 0.05$) higher than in N0 pure grass. Unfertilised mixtures with grass cultivar Barlet (BA and BG) had the same annual production as the N150 grass monoculture ($P < 0.05$). However, the N300 pure grass outyielded both the +N and the -N mixtures ($P < 0.05$) (Table 2).

The relative distribution of the annual yield of grass and clover over the four regrowth periods (Figure 2) showed maximal grass growth in the first period, declining towards the end of the season. The opposite pattern was observed in clover, however. In grass monocultures the seasonal variation in DM (expressed by the coefficient of variation, cv) decreased with increasing N levels. The same was observed for grass and clover in mixtures. In the +N mixtures the cv of grass and clover DM was lower than in the -N mixtures, but a higher cv of total DM was obtained in the +N mixtures (Table 2). This led to a more even distribution of the total DM in the -N compared to the +N mixtures (Figure 2).

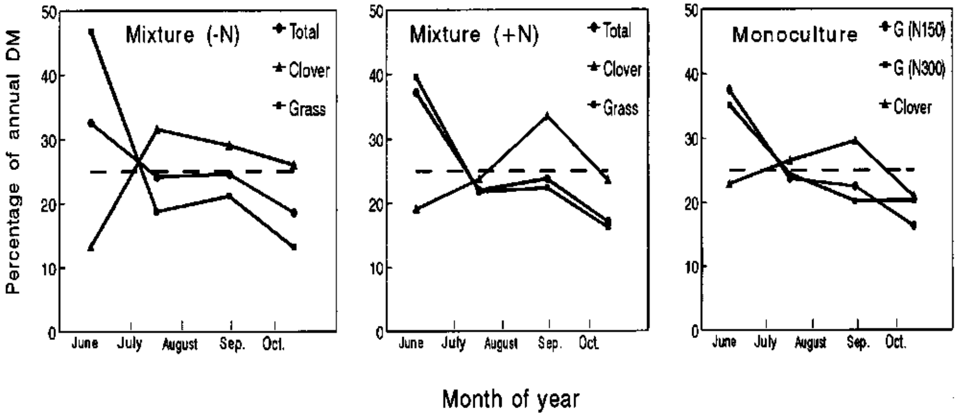

Figure 2. Mean seasonal distribution of grass, clover and total DM, expressed as percentage of the total yield of the 4 regrowth periods in mixtures and in monocultures.

Seasonal growth of species

The time course of the DM production during three regrowth periods is illustrated in Figure 3. Both in monocultures and mixtures the maximum growth rate of the DM of both species was achieved after 4 weeks of regrowth. Maximum grass growth occurred during spring and decreased towards the end of the season. Applied N had a substantial effect on grass growth in both mixtures and monocultures, which was most pronounced during spring.

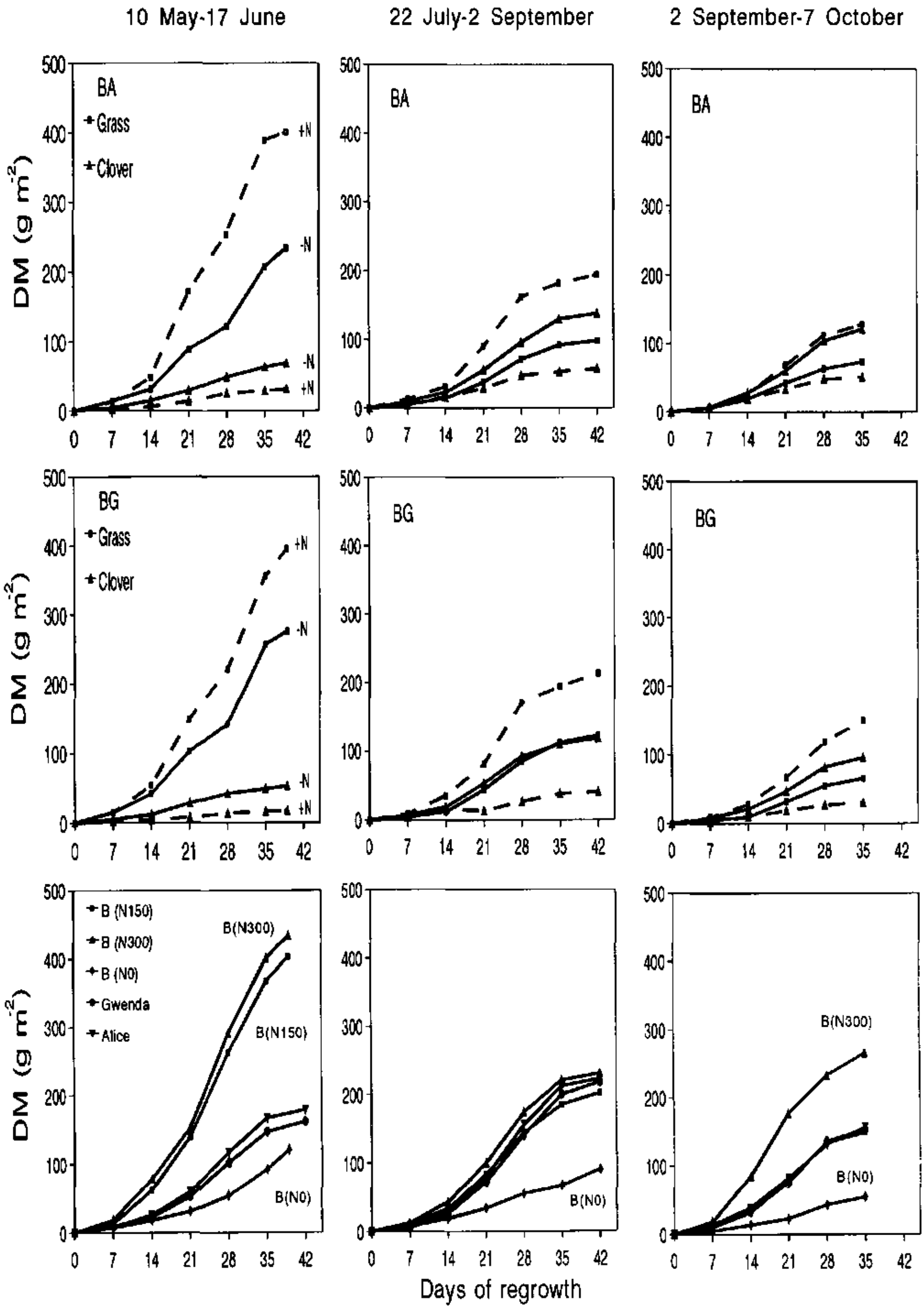

Figure 3. Time course of grass and clover DM in mixtures and in monocultures during different regrowth periods.

During the season, grass growth in the +N mixtures was lower than in N300, but similar to that of the N150 grass monoculture. Clover growth showed a seasonal pattern with the lowest growth in spring and the highest growth during summer (Figure 2). There were no significant differences between cultivars. While this seasonality was found both in +N and -N mixtures, due to the much higher clover content in the +N mixtures the effect on DM yield was much larger (Figure 3). As a result, the +N mixtures were grass-dominated during all regrowth periods.

Table 3. Leaf area index (*LAI*) of grass and clover in mixture and in monoculture by the end of different regrowth periods (R1-R4).

	R1		R2		R3		R4	
Treatments	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover
Experiment 1								
Mixture (-N)								
BA	2.8	1.4	2.5	3.0	2.5	2.3	2.0	3.2
BG	2.6	1.0	2.4	2.5	2.7	2.2	2.0	2.5
HA	2.35	1.0	2.1	2.7	2.3	2.0	1.5	2.7
HG	2.1	1.2	2.3	2.3	2.7	2.1	2.0	2.1
Mean	*2.5*	*1.1*	*2.3*	*2.6*	*2.5*	*2.2*	*1.9*	*2.6*
Mixture (+N)								
BA	3.9	0.7	3.8	1.0	4.3	0.9	3.2	0.6
BG	3.7	0.5	4.4	0.6	4.2	0.7	4.0	0.5
HA	3.1	0.5	4.6	0.8	4.6	0.6	4.1	0.5
HG	3.2	0.5	4.2	0.7	4.1	0.3	4.1	0.3
Mean	*3.5*	*0.6*	*4.2*	*0.8*	*4.3*	*0.6*	*3.8*	*0.5*
LSD(5%)	*0.36*	*0.32*	*0.62*	*0.49*	*0.77*	*0.66*	*0.70*	*0.54*
Experiment 2								
Alice	-	2.4	-	3.9	-	3.8	-	3.5
Gwenda	-	2.3	-	3.7	-	4.0	-	3.9
Mean	-	*2.4*	-	*3.8*	-	*3.9*	-	*3.7*
LSD(5%)	-	*0.28*	-	*0.31*	-	*0.39*	-	*0.40*
Experiment 3								
Monoculture								
B(N0)	1.3	-	-	-	-	1.3	-	1.1
H(N0)	1.2	-	-	-	-	1.6	-	1.5
Mean	*1.3*	-	-	-	-	*1.5*	-	*1.3*
B(N150)	3.5	-	-	5.0	-	4.5	-	3.9
H(N150)	3.1	-	-	5.2	-	5.0	-	4.2
Mean	*3.3*	-	-	*5.1*	-	*4.8*	-	*4.1*
B(N300)	4.8	-	-	6.1	-	5.8	-	5.0
H(N300)	4.2		-	5.9	-	6.0	-	5.2
Mean	*4.5*		-	*6.0*	-	*5.9*	-	*5.1*
LSD(5%)	*0.61*		-	*0.56*	-	*0.68*	-	*0.50*

Leaf area index

Grass *LAI* increased significantly ($P < 0.05$) in response to applied N, both in mixtures and monocultures. There was no difference between Barlet and Heraut (Table 3). N300 pure grass had the heighest *LAI* compared to N150 and N0 monocultures ($P < 0.05$). In the +N mixtures grass had the same *LAI* as in N150, but in the -N mixtures grass produced a significantly ($P < 0.05$) higher *LAI* than in the N0 monoculture. The grass *LAI* in the +N mixtures and N-fertilised monocultures were stable during the season. In the -N mixtures grass had a lower *LAI* in the last harvest than during spring and summer (Table 3).

No significant difference in *LAI* was found between the large and small-leaved clovers in monoculture or in mixture. In both clover cultivars the *LAI* was significantly ($P < 0.05$) lower in the +N mixtures compared to the -N mixtures (Table 3) and there was no clover × N interaction. In the -N mixtures the clover *LAI* was low in spring, but increased thereafter. This seasonal variation in clover *LAI* was not observed in the +N mixtures.

Stubble composition

No significant differences between regrowth periods were observed ($P > 0.05$) for the DM yield and *LAI* in the stubble. The total DM and *LAI* of the stubble, averaged over the season, was the same in the +N and the -N mixtures and amounted to 55.3 g m^{-2} and 0.62, respectively (Table 4). The contribution of grass to total DM and *LAI* of the stubble was higher than that of clover and increased in the +N mixtures. As a result, the average clover content in the +N mixtures in the DM (36.5) and *LAI* (26.5) was reduced ($P < 0.05$) in the -N mixtures to 19.8 and 12.4%, respectively.

This reduction was most pronounced in large-leaved Alice. Small-leaved Gwenda had a higher residual *LAI* than Alice, which was obvious in mixtures as well as monocultures. The lower leaf content in the clover stubble led to a lower clover LWR compared to that of grass. The clover LWR was lower in the +N swards than in the -N mixtures, but the reduction was only significant for large-leaved Alice (Table 4). Overall, the regrowth of mixtures started from a stubble in which grass had a higher *LAI* and LWR, but a lower SLA than clover.

Table 4. Seasonal mean values of DM (g m^{-2}), *LAI*, specific leaf area (SLA, cm^2 g^{-1}) and leaf weight ratio (LWR, g leaf g^{-1} shoot DM) for grass and clover in the stubble (5 cm height).

	DM			*LAI*			SLA		LWR	
Treatments	Grass	Clover	Total	Grass	Clover	Total	Grass	Clover	Grass	Clover
Experiment 1										
Mixture (-N)										
BA	38.9	18.6	57.5	0.45	0.12	0.57	257	358	0.45	0.18
BG	37.9	23.9	61.8	0.46	0.19	0.65	248	346	0.49	0.23
HA	31.0	19.6	50.6	0.41	0.13	0.54	265	349	0.50	0.19
HG	32.7	18.6	51.3	0.45	0.20	0.65	272	347	0.51	0.31
Mean	*35.1*	*20.2*	*55.3*	*0.44*	*0.16*	*0.60*	*261*	*349*	*0.49*	*0.23*
Mixture (+N)										
BA	43.7	13.6	57.3	0.55	0.05	0.60	253	368	0.51	0.10
BG	46.5	17.1	63.6	0.53	0.12	0.65	265	351	0.43	0.20
HA	51.4	11.6	63.0	0.55	0.07	0.62	256	355	0.42	0.17
HG	61.4	8.0	69.4	0.56	0.07	0.63	246	365	0.37	0.24
Mean	*50.8*	*12.6*	*63.3*	*0.55*	*0.08*	*0.63*	*255*	*347*	*0.43*	*0.18*
LSD(5%)	*10.4*	*7.1*	*11.2*	*0.18*	*0.04*	*0.17*	*16*	*10*	*0.11*	*0.06*
Experiment 2										
Alice	-	48.1	48.1	-	0.24	0.24	-	356	-	0.14
Gwenda	-	51.3	51.3	-	0.41	0.41	-	347	-	0.23
Mean	-	*49.7*	*49.7*	-	*0.33*	*0.33*	-	*353*	-	*0.19*
LSD(5%)	-	*6.6*	*6.6*	-	*0.12*	*0.12*	-	*14*	-	*0.08*
Experiment 3										
Monoculture										
B(N0)	53.2	-	53.2	0.45	-	0.45	201	-	0.42	-
H(N0)	54.1	-	54.1	0.47	-	0.47	207	-	0.42	-
Mean	*53.7*	-	*53.7*	*0.46*	-	*0.46*	*204*	-	*0.42*	-
B(N150)	63.5	-	63.5	0.56	-	0.56	250	-	0.35	-
H(N150)	58.8	-	58.8	0.47	-	0.47	247	-	0.32	-
Mean	*61.2*	-	*61.2*	*0.52*	-	*0.52*	*248*	-	*0.34*	-
B(N300)	63.0	-	63.0	0.60	-	0.60	250	-	0.38	-
H(N300)	47.2	-	47.2	0.58	-	0.58	256	-	0.48	-
Mean	*55.2*	-	*55.2*	*0.59*	-	*0.59*	*251*	-	*0.43*	-
LSD(5%)	*9.3*	-	*9.3*	*0.11*	-	*0.11*	*13*	-	*0.10*	-

Changes in clover content in DM and *LAI*

The mean clover content in the DM and *LAI* (averaged over the four regrowth periods and the four mixtures) was 42.5 and 42.8% in the -N mixtures, which declined to 11.8 and 12.4% in the +N swards, respectively (Figure 4). At each harvest clover lost more *LAI* and less DM than grass (Figure 5). No significant differences were found in the reduction of DM or *LAI* due to cutting between clover cultivars in monoculture or in the -N swards. In the +N mixtures, however, Gwenda lost significantly less *LAI* and DM than Alice. The reduction in grass DM and *LAI* was the same in mixtures and monocultures, except in the N0 grass where significantly less *LAI* and DM were lost after cutting (Figure 5b). However, the absolute grass *LAI* was lower in N0 than in fertilised grass monocultures (Table 4) at the start of each each regrowth period.

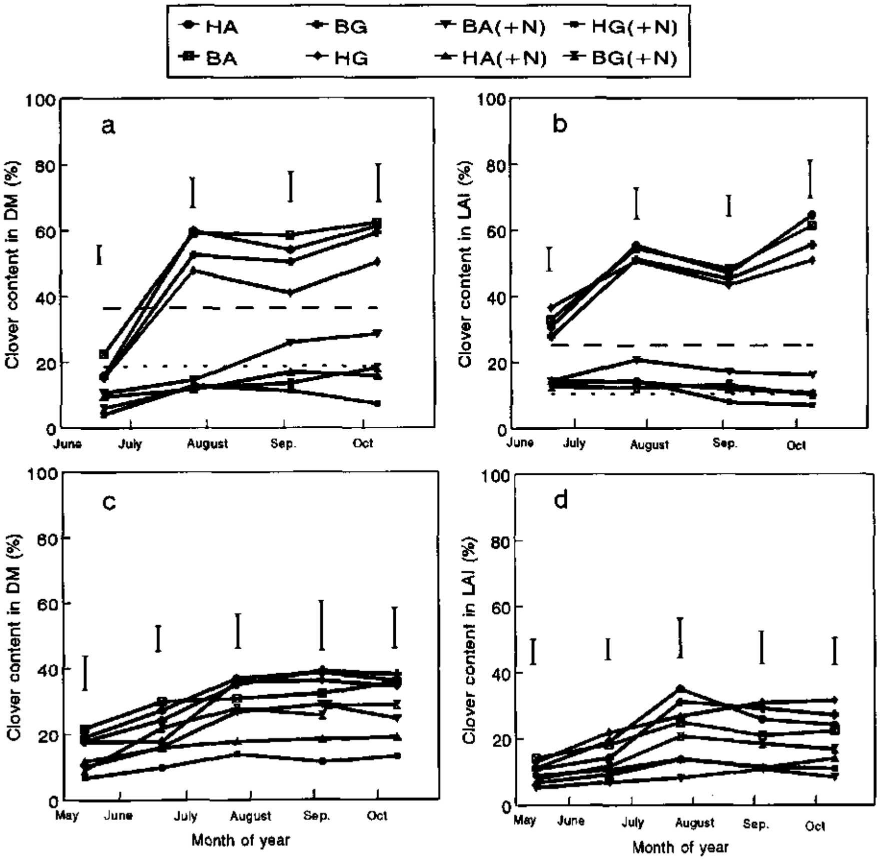

Figure 4. Clover content in the DM a) at the end of each regrowth period, c) at the start of each regrowth period and clover content in *LAI*, b) at the end and, d) at the start of regrowth. (----) and (....) in a and b show the annual mean clover content at the start of regrowth. Bars are LSD (5%).

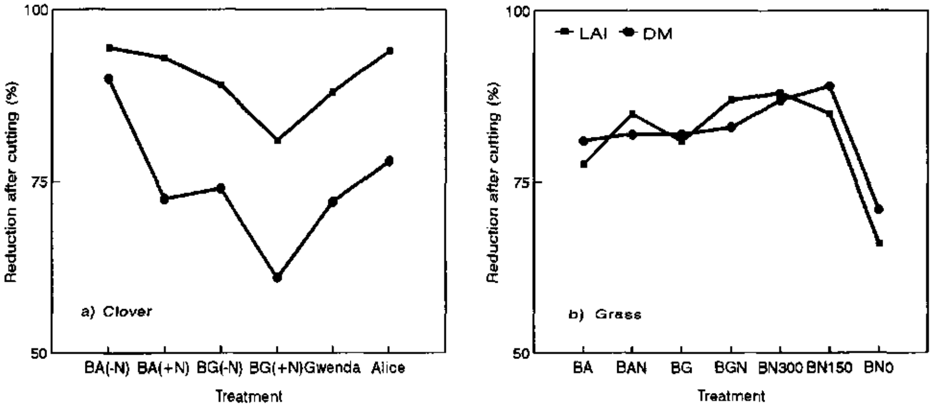

Figure 5. Mean reduction in grass and clover *LAI* and DM due to cutting in mixtures and monocultures.

Comparison of the clover content by the end of each regrowth period to the clover content at the start of regrowth (Figures 4 and 6) showed that in the -N mixtures both clovers increased their content in DM and in *LAI* (Figure 6) during successive regrowth periods, with a maximum in the third cut. In the +N mixtures, Gwenda maintained its content in the DM and in the *LAI* during the season and a significant increase in clover content by weight was observed only in the BA mixture during the last 2 cuts (Figures 4a and 6).

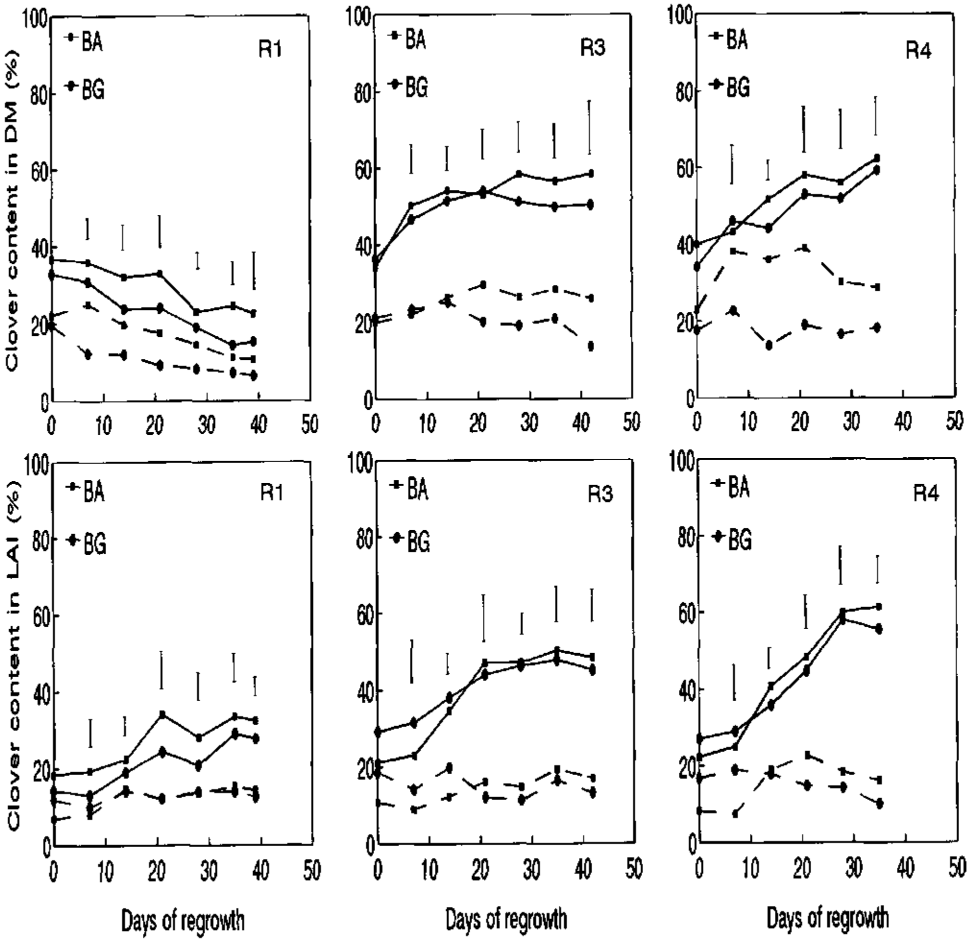

Figure 6. Change in clover content in DM and *LAI* during three regrowth periods (R1, R3 and R4) for BA and BG in +N (----) and -N (—) mixtures, vertical bars are LSD (5%).

Table 5. SLA ($cm^2\ g^{-1}$), LWR (g leaf g^{-1} shoot DM) and LAR (cm^{-2} leaf g^{-1} shoot DM) of grass and clover in mixture and in monoculture, averaged over different regrowth periods (R1-R4).

	R1						R2					
	SLA		LWR		LAR		SLA		LWR		LAR	
Treatments	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover
Experiment 1												
Mixture (-N)												
BA	233	322	0.45	0.52	105	190	231	322	0.79	0.50	183	161
BG	231	324	0.48	0.57	111	168	229	335	0.80	0.53	183	178
HA	230	320	0.50	0.58	115	185	221	330	0.76	0.46	168	152
HG	237	329	0.43	0.50	102	164	234	326	0.71	0.48	166	157
Mean	*233*	*324*	*0.47*	*0.55*	*108*	*177*	*229*	*328*	*0.77*	*0.49*	*175*	*161*
Mixture (+N)												
BA	244	345	0.42	0.39	103	169	256	329	0.81	0.30	207	99
BG	246	361	0.46	0.56	113	209	261	362	0.83	0.42	216	152
HA	241	340	0.39	0.52	94	176	255	341	0.76	0.39	194	133
HG	248	357	0.45	0.57	112	203	259	369	0.79	0.50	205	185
Mean	*245*	*351*	*0.43*	*0.54*	*105*	*189*	*258*	*361*	*0.80*	*0.40*	*206*	*145*
LSD(5%)	*13*	*19*	*0.13*	*0.11*	*17*	*10*	*16*	*22*	*0.13*	*0.11*	*13*	*17*
Experiment 2												
Alice	-	327	-	0.43	-	141	-	341	-	0.47	-	160
Gwenda	-	336	-	0.50	-	168	-	331	-	0.54	-	179
Mean	-	*339*	-	*0.47*	-	*154*	-	*336*	-	*0.51*	-	*170*
LSD(5%)	-	*12*	-	*0.09*	-	*14*	-	*13*	-	*0.10*	-	*15*
Experiment 3												
Monoculture												
B(N0)	200	-	0.56	-	112	-		-		-		-
H(N0)	198	-	0.59	-	117	-		-		-		-
Mean	*199*	-	*0.58*	-	*114*	-		-		-		-
B(N150)	243	-	0.47	-	114	-	247	-	0.78	-	193	-
H(N150)	239	-	0.51	-	122	-	255	-	0.72	-	184	-
Mean	*241*	-	*0.49*	-	*118*	-	*251*	-	*0.75*	-	*188*	-
B(N300)	254	-	0.39	-	99	-	266	-	0.77	-	205	-
H(N300)	261	-	0.43	-	112	-	271	-	0.79	-	214	-
Mean	*258*	-	*0.41*	-	*106*	-	*269*	-	*0.78*	-	*209*	-
LSD(5%)	*11*	-	*0.09*	-	*14*	-	*14*	-	*0.10*	-	*11.4*	-

Table 5. (Continued)

	R3						R4					
	SLA		LWR		LAR		SLA		LWR		LAR	
Treatments	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover
Experiment 1												
Mixture (-N)												
BA	234	330	0.86	0.48	201	158	231	331	0.81	0.49	187	162
BG	231	321	0.74	0.50	171	160	228	324	0.84	0.53	192	172
HA	229	339	0.87	0.47	199	159	230	327	0.83	0.50	191	163
HG	236	336	0.78	0.52	184	174	227	331	0.88	0.56	199	185
Mean	*233*	*332*	*0.81*	*0.49*	*189*	*163*	*229*	*328*	*0.84*	*0.52*	*192*	*171*
Mixture (+N)												
BA	238	321	0.81	0.33	193	103	264	319	0.86	0.38	227	121
BG	228	372	0.86	0.40	196	219	257	366	0.87	0.45	224	164
HA	246	330	0.79	0.39	194	129	258	326	0.89	0.39	230	127
HG	248	361	0.88	0.61	214	220	247	358	0.76	0.53	187	189
Mean	*243*	*352*	*0.84*	*0.36*	*199*	*125*	*257*	*353*	*0.85*	*0.44*	*216*	*154*
LSD(5%)	*16*	*18*	*0.10*	*0.12*	*14*	*12*	*17*	*15*	*0.09*	*0.11*	*13*	*18*
Experiment 2												
Alice	-	334	-	0.49	-	164	-	336	-	0.51	-	171
Gwenda	-	321	-	0.58	-	186	-	331	-	0.59	-	195
Mean	-	*328*	-	*0.54*	-	*175*	-	*334*	-	*0.55*	-	*183*
LSD(5%)	-	*15*	-	*0.10*	-	*13*	-	*10*	-	*0.12*	-	*15*
Experiment 3												
Monoculture												
B(N0)	200	-	0.26	-	52	-	225	-	0.79	-	178	-
H(N0)	207	-	0.45	-	93	-	204	-	0.77	-	157	-
Mean	*204*	-	*0.36*	-	*72*	-	*215*	-	*0.78*	-	*167*	-
B(N150)	239	-	0.92	-	219	-	245	-	0.88	-	215	-
H(N150)	243	-	0.81	-	197	-	263	-	0.85	-	224	-
Mean	*241*	-	*0.87*	-	*209*	-	*254*	-	*0.87*	-	*220*	-
B(N300)	265	-	0.81	-	215	-	279	-	0.73	-	204	-
H(N300)	260	-	0.89	-	231	-	274	-	0.80	-	219	-
Mean	*263*	-	*0.85*	-	*223*	-	*277*	-	*0.77*	-	*212*	-
LSD(5%)	*11*	-	*0.12*	-	*13*	-	*16*	-	*0.18*	-	*15*	-

Specific leaf area, leaf weight ratio, leaf area ratio and petiole length

The specific leaf area of species remained unchanged during the season and no significant differences were observed between grass or clover cultivars. Clover had a higher SLA than grass, both in mixed and pure stands (Table 5). The seasonal averages of grass and clover SLA were 334 and 245 cm^2 g^{-1}, respectively. Except during the first regrowth period, when grass had many flowering stems, grass had a higher LWR than clover (Table 5). This led to a decrease in grass LAR in spring compared to the other regrowth periods. In Alice the higher petiole length and a higher specific petiole length (SPL, Table 6) resulted in a lower LWR compared to Gwenda (Table 5). This difference was most pronounced in the +N mixtures, where the length of petioles in Alice was significantly increased in response to applied N (Table 6).

Table 6. Frequency of petiole length of large and small-leaved clover cultivars in mixture and monoculture during summer. Specific petiole length (SPL, cm g^{-1}) for each petiole length class is also given.

Petiole length class	Small (5-10 cm) f (%)	Small (5-10 cm) SPL	Medium (10-15 cm) f (%)	Medium (10-15 cm) SPL	Large (15-20 cm) f (%)	Large (15-20 cm) SPL	> 20 cm f (%)	Mean SPL
Treatment								
BA(-N)	6.5	0.72	41.3	0.70	46.2	0.63	6.0	0.68
BA(+N)	4.1	1.14	23.2	0.86	62.7	0.81	10.0	0.94
Alice	4.0	0.96	30.5	0.67	63.4	0.57	2.1	0.73
BG(-N)	16.6	1.08	67.2	0.87	19.2	0.74	0.0	0.90
BG(+N)	16.8	1.72	78.3	1.20	4.9	1.22	0.0	1.38
Gwenda	12.1	1.09	69.9	0.81	18.0	0.53	0.0	0.81
LSD(5%)	4.5	0.11	9.3	0.14	12.8	0.12	-	0.13

Discussion

The annual yield of grass monocultures increased significantly with increasing N levels. There were no differences between cultivars. Our results for the annual yield of the N300 grass monoculture was in agreement with the value of 11.3 to 12 t ha^{-1} per annum reported for high N grass on soils with a low capacity of N delivery (Reid, 1970 and Morrison *et al.*, 1980). Agronomic data on the DM yield of clover monocultures are scarce. Cowling (1961) reported the value of about 8 t ha^{-1} $year^{-1}$, which is close to our result of 745 g m^{-2} per year, averaged over cultivars. The clover monocultures outyielded the N0 grass monocultures.

However, the annual DM yield of the N150 and N300 grasses exceeded those of pure clovers. Pure clover yielded 76.4 and 61.6% relative to N150 and N300 grasses, respectively. The latter was in agreement with a value of 59% reported for high N grass monoculture under controlled conditions (Davidson and Robson, 1986). In accordance with Frame and Boyd (1987), repetitive N fertilised mixtures outyielded grass N150 monoculture and -N mixtures. The annual yield of both small and large-leaved clovers was significantly reduced in the +N swards. There was no N x clover cultivar interaction. This is in agreement with findings of Laidlaw (1984) and Frame and Boyd (1987), but in contrast with Wilman and Asiegbu (1982) who reported that the adverse effects of N are higher in small-leaved cultivars.

Unfertilised mixtures had similar annual DM yields as N150 grass. Morrison *et al.* (1980) reported that grass-clover mixtures with a clover content of about 20% produced the same annual yield as grass monoculture receiving 200 kg N per season. Our results, however, are closer to Reid (1970), who obtained the same DM yield from an unfertilised mixture and pure grass with 150 kg N year^{-1}.

The distribution of seasonal yield of grass and clover (Figure 2) showed similar patterns in mixtures and monocultures. Grass had the highest proportion of its annual DM in the first cut (corresponding to its reproductive growth). This declined remarkably during summer. However, for clover the opposite pattern was observed.

This asynchrony in production patterns has an important role in the cohabitation of species (Harris, 1987). In the -N swards, during the first regrowth, the clover effects on DM yield were mostly indirect. However, during summer the lower grass productivity was compensated by a higher clover productivity. Increasing direct effects of clover from spring to autumn have also been observed by Evans *et al.* (1990). As a result, the total DM yield in the -N mixtures showed little variation (Table 2). This harmony was disturbed by N in the +N swards, where grass still had a high seasonal variation, while the clover content (11.8%) was not sufficient to overcome this variation. Therefore, the seasonal distribution of the total yield in the +N swards followed the variable pattern of the grass component (Table 1 and Figure 2). The growth of DM during each regrowth period (Figure 3) showed a similar trend as seasonal yield. In all mixtures and monocultures grass had higher growth during spring, coinciding with its reproductive stage. The opposite was observed for clover. This reflects the well-known relationships between temperature and growth of grass (Harris, 1987) and clover (Kleter, 1968; Davies, 1992) and the higher growth rate of grass during reproductive development (Parsons and Robson, 1982; Taube, 1990). It has been found that grass allocates more DM to above-ground tissues during the reproductive stage (Parsons and Robson, 1982), and N drastically increases this allocation (Davidson *et al.*, 1986). These results explain the higher yield of grass in the fertilised monocultures and the +N mixtures observed in this study (Figures 3a, b and c). Clover had a low growth rate during the first cut, particularly in mixtures. The same results were reported by Davies and Evans (1982) and Marriott (1988). This could be the result of a lower CO_2 assimilation rate of clover (Woledge, 1988) and consequently a lower radiation use efficiency in spring (Chapter 6). During summer and autumn the clover DM and *LAI* increased, both in monocultures and -N mixtures (Figure 3), which could be the result of a more favourable temperature for clover growth (Harris, 1987; Davies, 1992), and of a reduction in grass competitive ability after flowering (Rhodes, 1970; Collins and Rhodes, 1990).

Due to cutting, clover lost more *LAI* than grass, especially in the large-leaved cultivar, but the reduction in DM was greater in grass than in clover (Figure 4). This resulted in a lower clover content at the start of regrowth. Woledge (1988) and Woledge *et al.* (1992b) suggested that an increasing clover content in the harvested yield, in spite of its lower contribution at the start of regrowth, could be the result of a higher RGR of clover than that of grass.

Our results showed that in both -N and +N mixtures the clover content decreased during the spring regrowth. In the -N mixtures both large and small-leaved clovers increased their content by *LAI* and DM during summer regrowth. This supports the conclusion of Woledge *et al.* (1992a) that clover had a higher RGR of leaf area and DM than grass during summer. In the -N mixtures both clover cultivars maintained their contribution in the total *LAI* and DM during summer. Large-leaved Alice maintained its content in the DM during the last 2 cuts, indicating that it had the same RGR as grass in fertilised mixtures. It seems that the conclusion of Woledge (1988), based on work with a large-leaved clover and spring-applied N is also valid for small-leaved clover, as well as for repetitive N applications.

During the first regrowth, grass had a lower LAR compared to other periods, both in mixtures and in monocultures. This was mainly due to a decrease in its LWR at the flowering stage (Wilkins, 1995). This pattern in the first regrowth period, when the growth rate of clover was low (Figure 3), led to overtopping of clover. Consequently the clover content decreased, both in the +N and -N mixtures (Figures 4 and 5). During the second and other regrowth periods both clovers, particularly the large-leaved, had a lower LAR (decrease in LWR) in the -N mixtures compared to monocultures. The lower LAR, which was due to an increase in supporting organs (petioles, Table 5) at the expense of leaf area, gave a better position to clover leaves to avoid shading by grass. On the other hand, in accordance to Parsons *et al.* (1991a) and Davidson and Robson (1986), clover had a greater SLA than grass. Therefore, the lower LAR of clover was compensated to some extent by its higher SLA to enhance the area available for light interception without a concomitant increase in leaf mass. As a result, the clover content in the DM and in the *LAI* increased during the successive cuts (Figures 4 and 5). Although both clovers had a higher petiole length in mixture than in monoculture, the increase in petiole length (and therefore the decrease in LAR) was greater in the long-petioled cultivar Alice. Elgersma *et al.* (1996) also reported a higher growth rate of internodes in Alice compared to Gwenda. Wilman and Asiegbu (1982) showed that the length of clover petioles increased in response to N. We found the same results, but only in large-leaved Alice. In the +N mixtures Alice, with its larger petioles (Table 6) and its higher biomass invested in petioles (lower LWR, Table 5), avoided shading by grass. However, its LAR was significantly reduced (Table 5).

Small-leaved Gwenda, with smaller and thinner petioles (Table 6), had a higher LAR compared to the -N swards and monocultures and was therefore overtopped by grass in the +N mixtures. However, Gwenda had a significantly higher SLA in the +N mixtures (Table 5) and therefore increased the efficiency of its biomass for light absorption under shading. Thus the large-leaved clover avoided shading in the +N mixtures by decreasing its LAR and increasing the amount of light absorbed per unit of leaf area. However, the small-leaved clover tolerated shading in the +N swards by increasing its SLA and enhancing the amount of light absorbed per unit of biomass. It seems that the avoiding strategy was more efficient, because the large-leaved cultivar was even able to increase its content in the +N sward (Figure 5). Biere (1987) concluded that small differences in RGR and/or its components (net assimilation rate and LAR) may be crucial for the resulting competitive advantages. In the present study small-leaved Gwenda increased its RGR in the +N mixture by increasing its LAR (higher LWR and SLA compared to the -N mixtures). However, in large-leaved Alice the LAR was decreased. Such a decrease in clover LAR in fertilised mixtures was in contrast to the same LAR of clover in +N and -N mixtures reported by Woledge (1988) and Davidson and Robson (1986). However, Soussana *et al.* (1995a) also reported a decrease in clover LAR at high N rates.

It has been suggested that a 25% decrease in the allocation of DM to leaves could drastically reduce the clover RGR (Parsons *et al.*, 1991a). On the other hand, Porter (1989) showed a strong negative correlation between the net assimilation rate and the LAR. It can be hypothesised that large-leaved clover, despite its lower LAR in the +N swards, was able to maintain its RGR as high as that of grass through a increased CO2 assimilation rate. It has been shown that a non-uniform pattern of leaf N distribution within the canopy will lead to a significant increase in canopy CO_2 assimilation (Hirose and Werger, 1987a; Chapter 8). To test this hypothesis, more information about the distribution of leaf area, biomass and N within the mixed grass-clover canopy is needed.

Introduction

Ryegrass-white clover mixtures are important in forage-animal production systems in temperate climates. Successful coexistence of species mainly depends on the ability of species to avoid direct competition for limited resources throughout temporal and spatial niche divergence (Hill and Michaelison-Yeates, 1987; Collins and Rhodes, 1989).

Successive peaks of grass and clover growth, due to seasonality in growth of the two species (Harris, 1987), lead to a better performance of mixtures compared to pure stands. Although both species have a high seasonal variation in growth, the temporal difference in the timing of growth peaks leads to a more stable total DM yield in mixture than in monocultures (Chapter 3).

Several studies on cut grass-clover mixtures showed that clover leaves occupy the higher parts of the canopy, whereas grass leaves are positioned in lower layers (Woledge, 1988; Woledge *et al.*, 1992a; Nassiri *et al.*, 1996a). This spatial divergence in leaf area distribution has an important role in light competition and avoidance from overtopping, even in N fertilised swards (Woledge *et al.*, 1992b; Chapter 3). However, to place the higher proportion of its *LAI* at the top layers, clover has to increase the petiole length by allocating more DM to petioles.

It has been confirmed that petiole length increases in response to shading (Wilman and Asiegbu, 1982; Thompson, 1993). However, this response is genotype-related (Gautier *et al.*, 1995). Our earlier results also showed that in fertilised mixtures, where clover overtopping was expected due to extra growth of grass, large-leaved clover cv. Alice had higher and thicker petioles than small-leaved ccv. Gwenda (Chapter 3).

The DM cost of larger petioles in turn results theoretically in a lower LAR and therefore also in a lower RGR, because of the strong relationship between these two growth indices (Porter, 1989). On the other hand, Woledge (1988) showed that clover had the same RGR as grass in +N mixtures. The only possibility for maintaining the RGR in spite of a decrease in the LAR, is increasing the net assimilation rate (RGR = LAR x NAR). It has been shown that a non-uniform pattern of N distribution within the canopy will lead to a significant increase in canopy CO_2 assimilation (Hirose and Werger, 1987a). However, no information is available on the vertical distribution of DM, LAR and N in grass-clover mixtures.

This paper reports the effect of repetitive N applications on the vertical distribution of leaf area, light, biomass and N in perennial ryegrass and a large and small-leaved white clover cultivar in mixtures and monocultures, during spring and summer regrowth.

Material and methods

Two adjacent trials were established in autumn 1995 on a sandy soil in Wageningen, The Netherlands. In the first trial two white clover (*Trifolium repens*) cultivars; Alice and Gwenda (large and small-leaved, respectively), and perennial ryegrass (*Lolium perenne*) cv. Barlet (diploid, erect) were sown in mixture (mixtures hereafter referred to by the first letter of their component cultivar names). The experimental design and management were described in Chapter 3.

The mixtures were grown under 2 N levels, 0 (-N) and 150 kg N ha^{-1} (+N) during the growing season. The monocultures of the clover cultivars were sown in a second trial. In a third trial monocultures of ryegrass were established, which received 3 levels of N, i.e. (0 (N0), 150 (N150) and 300 kg N ha^{-1} year $^{-1}$ (N300). N was applied after each harvest. In all trials a randomised complete block design with two replicates was used. Sampling was done from May till October 1996. All plots were cut at an approximate target yield of 2000 kg DM ha^{-1}. There were 5 cuts (4 regrowth periods, R1-R4) over the whole growing season; the N0 treatment was cut 3 times.

Measurements

The absorbed PAR (photosynthetically active radiation) by mixed and pure canopies was measured weekly using a linear ceptometer at successive 5-cm layers from the top. During each regrowth period the vertical distribution of leaf area of species was measured weekly using an inclined point quadrat with the inclination angle 32.5° (Warren Wilson, 1963). In each plot all contacts with points in 35 to 40 descents were recorded for grass and clover laminae.

The total aboveground biomass was sampled weekly by cutting a 10 x 10 cm area. The harvested materials were cut into layers of 5 cm with a paper cutter, while the leaf inclination was kept as natural as possible. The materials in each layer were separated into grass leaf, grass sheath (or flowering stem), clover leaf and clover petiole. The weight of each component was determined after drying for 24 hours at 70° C. The first 5 cm layer from base was considered as stubble and the layers above it as harvestable material. Dried leaves of both species were ground and their N content (g N g^{-1} DM) measured by Kjeldahl analysis, using an autoanalyser with a continuous-flow analysing system. The leaf N concentration was divided by species SLA (g m^{-2}) to calculate the leaf N concentration on a leaf area basis (g N m^{-2} leaf) for each layer.

Results

Vertical distribution of leaf area

The application of N had a large effect on the development of the canopy in grass monocultures. (Figure 1). The N fertilised monocultures, N150 and N300, had the same height, with a more or less similar pattern of leaf area distribution over height, and differed mainly in density of leaf area. Grass had a well-developed canopy in the -N mixtures compared to that of the N0 monocultures (Figures 1 and 2). The distribution of grass *LAI* in the +N mixtures was similar to that in the N150 monocultures, but the grass *LAI* was significantly denser in the N300 monoculture compared to N150 and the +N and -N mixtures (Figures 1 and 2). In clover monocultures, however, a significant difference was observed in the leaf area distribution between cultivars. Large-leaved Alice had a higher proportion of its *LAI* above 15 cm height, while in small-leaved Gwenda the leaves were positioned in lower parts of the canopy. Both in mixtures and monocultures the height of the maximum leaf area was a logistic function of the total height of species (Figure 3). In grass this height was not affected by N and was around 10 cm in closed canopies. In clover it was lower in Gwenda than in Alice. At the end of the first regrowth period, both in mixtures and fertilised monocultures, the maximum grass *LAI* was located at the 10-15 cm layer. In this period the maximum leaf density in Alice was found in the layer 10-15 cm, but in Gwenda in the layer 5-10 cm height (Figure 2). In the mixtures with Alice despite the fact that the maximum *LAI* of grass and clover occurred in the same layer, the total grass *LAI* was higher than the total clover *LAI*. The high amount of grass *LAI* present above maximum clover height resulted in overtopping of clover during spring in both +N and -N mixtures. This was more pronounced in mixtures with small-leaved Gwenda (Figure 2).

During summer the maximum density of grass leaves occurred at 10 cm. However, clover leaves were positioned in higher layers. Therefore, in the -N mixtures the clover *LAI* in the upper layers of the canopy was greater than that of grass, and clover was not overtopped during summer regrowth (Figure 2). However, in the +N mixtures clover was overtopped as a result of the vertical dominance of grass (Figure 2). The degree of overtopping was different in the large and small-leaved clovers and was controlled by the height at which the maximum *LAI* occurred. Small-leaved Gwenda had a lower height of maximum *LAI* than large-leaved Alice, both in mixture and monoculture (Figures 1 and 2). This was less pronounced in the +N mixtures. Therefore, by the end of the summer (2 September) and autumn regrowth periods (7 October), overtopping of clover was only visible in the +N mixtures with small-leaved Gwenda (Figure 2).

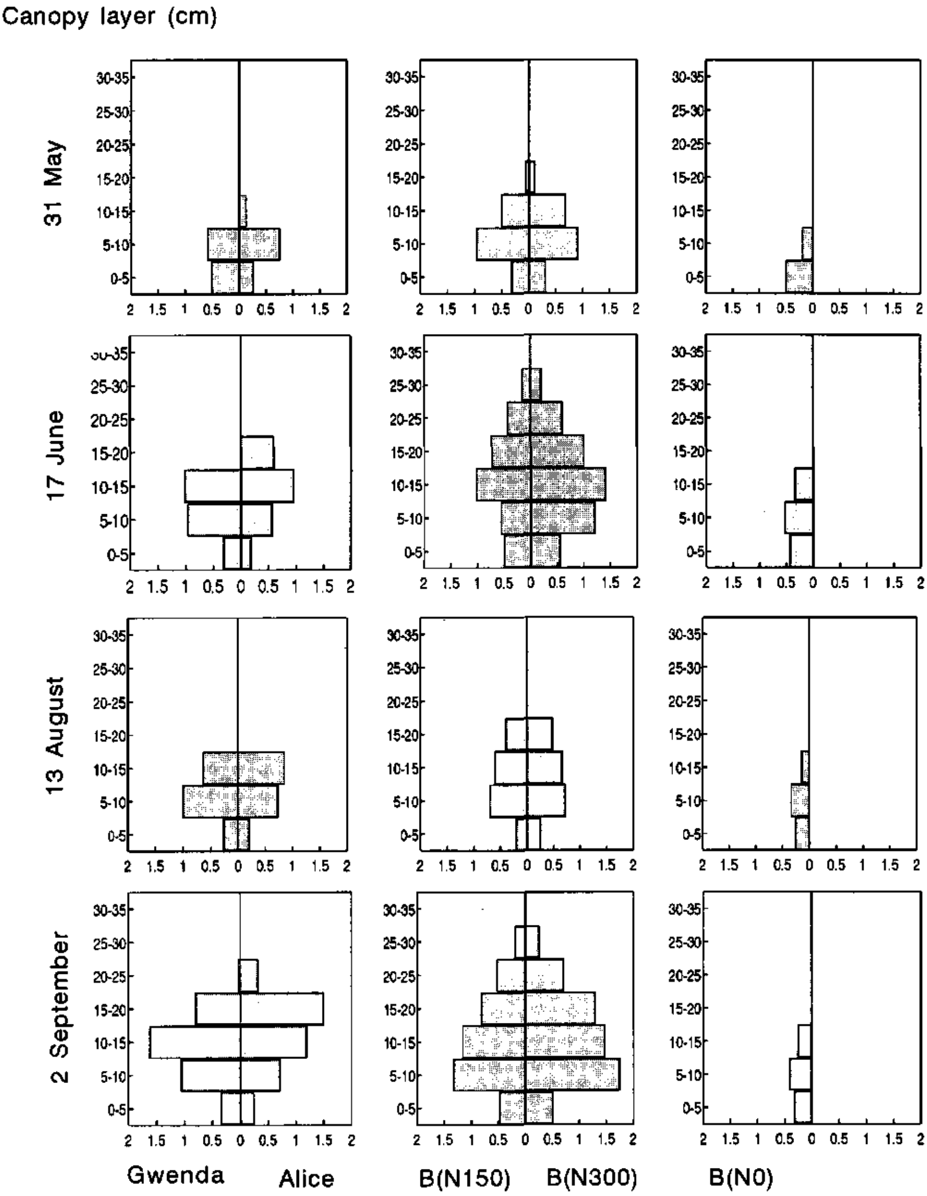

Figure 1. Vertical distribution of leaf area of perennial ryegrass (cv. Barlet) and white clover (cvs. Gwenda and Alice) in monoculture. Data are presented for the spring regrowth period (R1), 3 weeks (31 May) and 5 weeks (17 June) after cutting, as well as for the summer period (R3), after 3 weeks (13 August) and 6 weeks (2 September) of regrowth.

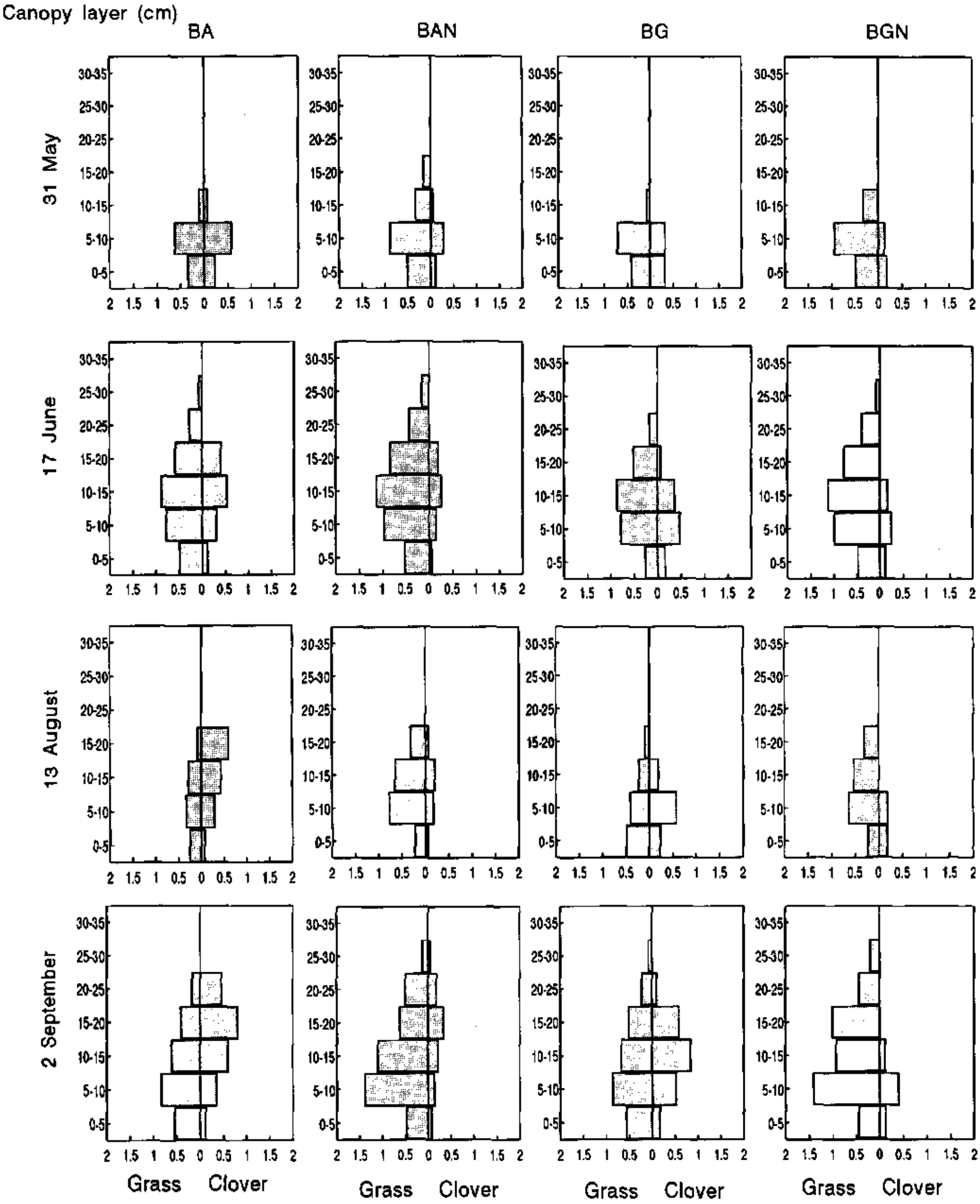

Figure 2. Vertical distribution of leaf area of grass and clover in different mixtures (B: Barlet; A: Alice; G: Gwenda). Data are presented for the spring regrowth period (R1), 3 weeks (31 May) and 5 weeks (17 June) after cutting, as well as for the summer period (R3), after 3 weeks (13 August) and 6 weeks (2 September) of regrowth.

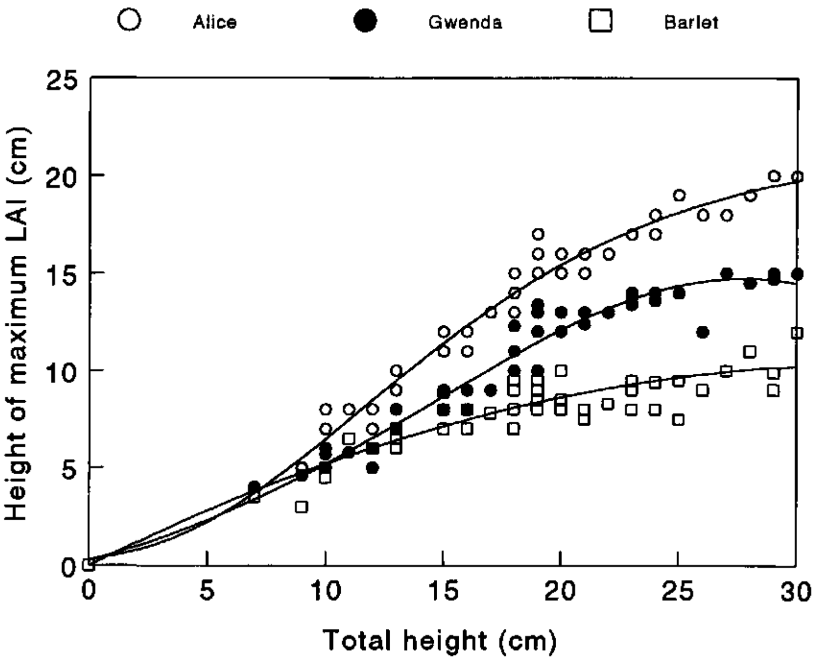

Figure 3. Relation between total height and height of maximum *LAI* in perennial ryegrass (cv. Barlet) and large-leaved (cv. Alice) and small-leaved (cv. Gwenda) white clover. Data are taken from all treatments during the whole growing season and fitted to a logistic function.

Vertical profile of light

The profile of PAR within the canopy was in accordance with the distribution of leaf area over height (Figure 4). Except for clover monocultures during the first regrowth period, a closed canopy (described by absorption of 95% of the total PAR) was achieved by the end of the regrowth period. In clover monocultures light was mainly absorbed in the top layers. This led to a rapid extinction of PAR between 25-15 cm height in the large-leaved clover, and 20-10 cm height in the small-leaved clover (Figure 4). In grass monocultures, however, the light absorption increased slowly with height, so that it reached its maximum at 5 cm height. The pattern of the light profile in the -N mixtures, where clover had vertical dominance in *LAI*, was similar to the PAR profile in the corresponding clover monocultures. However, in the +N mixtures, where grass dominated, the light profile was comparable to that of the N150 pure grass (Figure 4).

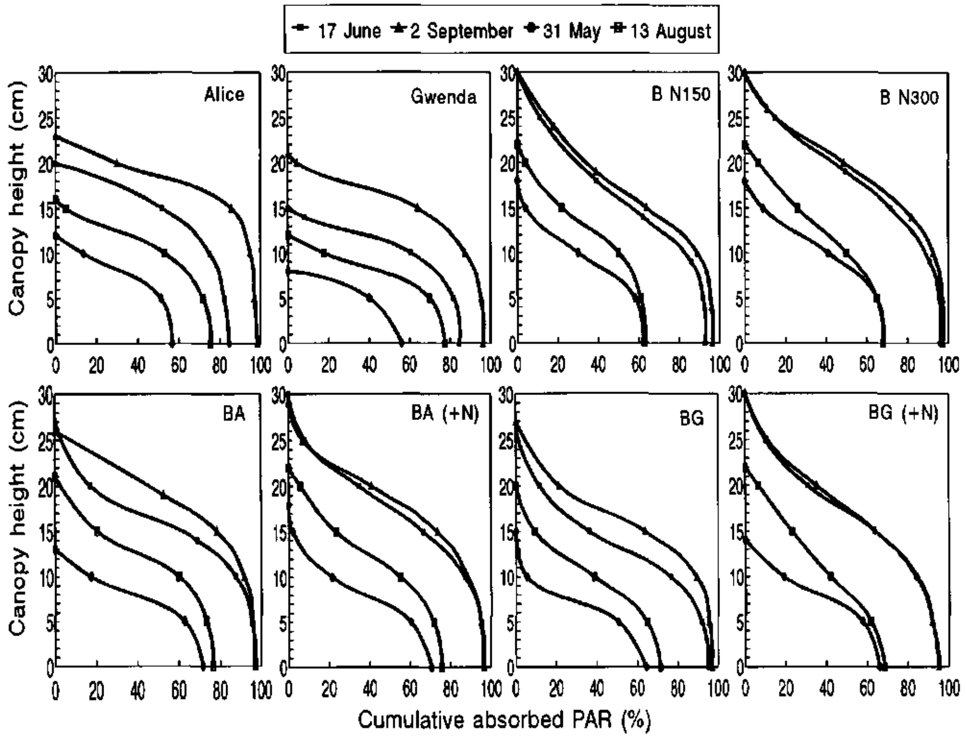

Figure 4. Profile of total absorbed PAR over canopy height in monocultures (a-d) and mixtures (e-h). Data points are shown for dates corresponding to leaf area distribution presented in Figures 3 and 4.

Vertical distribution of biomass

During the first regrowth period both in mixtures and monocultures the contribution of flowering stems to the grass DM yield was high in all canopy layers. Later in the season only grass pseudostems appeared in the lower layers (Figures 5 and 6). Similar to the *LAI* distribution, the clover leaf mass was mainly situated at the top layers, but the inverse was observed for grass. The position of the clover leaf mass and leaf area within the canopy was determined by the distribution of petiole DM. Comparison of clover cultivars (Figure 5) indicated that the large-leaved clover elevated its leaf area and mass to the upper parts of the canopy by allocating more DM to the production of longer and thicker petioles. This difference in DM distribution between clover cultivars was more pronounced in mixtures than in monocultures and was crucial in the +N swards (Figure 6). Large-leaved Alice escaped overtopping by allocating relatively more DM to its petioles compared to Gwenda. The shorter petioles of small-leaved Gwenda allowed more grass leaf mass and leaf area above the clover canopy in the +N mixtures.

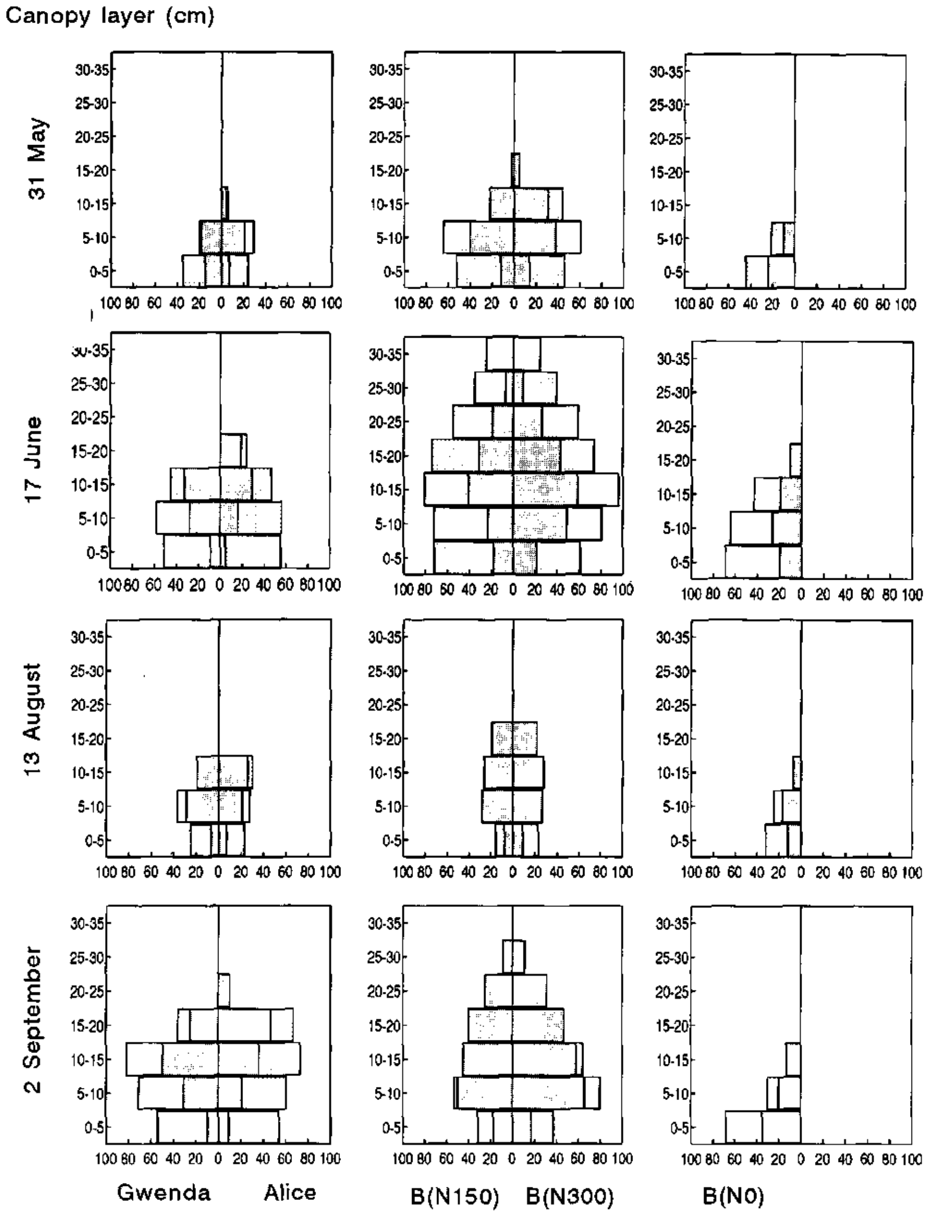

Figure 5. Vertical distribution of DM (g m^{-2}) of grass and clover in monoculture. Data are shown for the dates corresponding to leaf area distribution presented in Figure 1. Filled bars indicate leaf DM and open bars clover petiole or grass (pseudo) stem plus sheath DM.

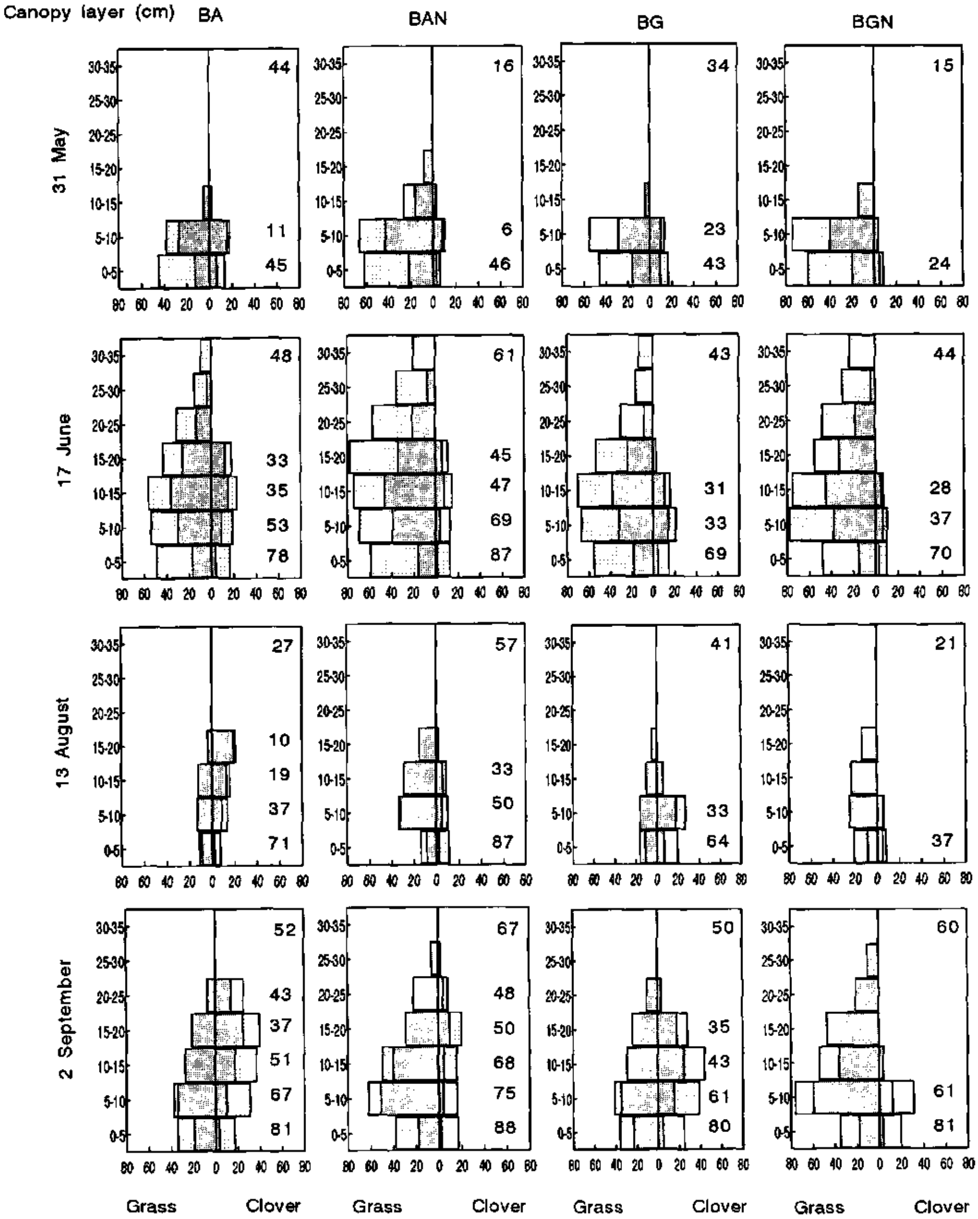

Figure 6. Vertical distribution of DM (g m^{-2}) of grass and clover in mixtures. Data are shown for the dates corresponding to leaf area distribution presented in Figure 2. Filled bars indicate leaf DM and open bars clover petiole or grass (pseudo) stem plus sheath DM. For each layer petiole content (%) in clover DM is shown. Figures at the top right corner show the petiole content (%) in total clover DM.

The vertical pattern of the leaf area ratio (LAR, cm^2 leaf g^{-1} shoot DM) showed the combined effects of changes in leaf weight ratio (LWR, g leaf DM g^{-1} shoot DM) and specific leaf area (SLA, cm^2 leaf g^{-1} leaf DM) over canopy height. Grass had a more uniform LAR distribution over height than clover, particularly in summer (Figure 7).

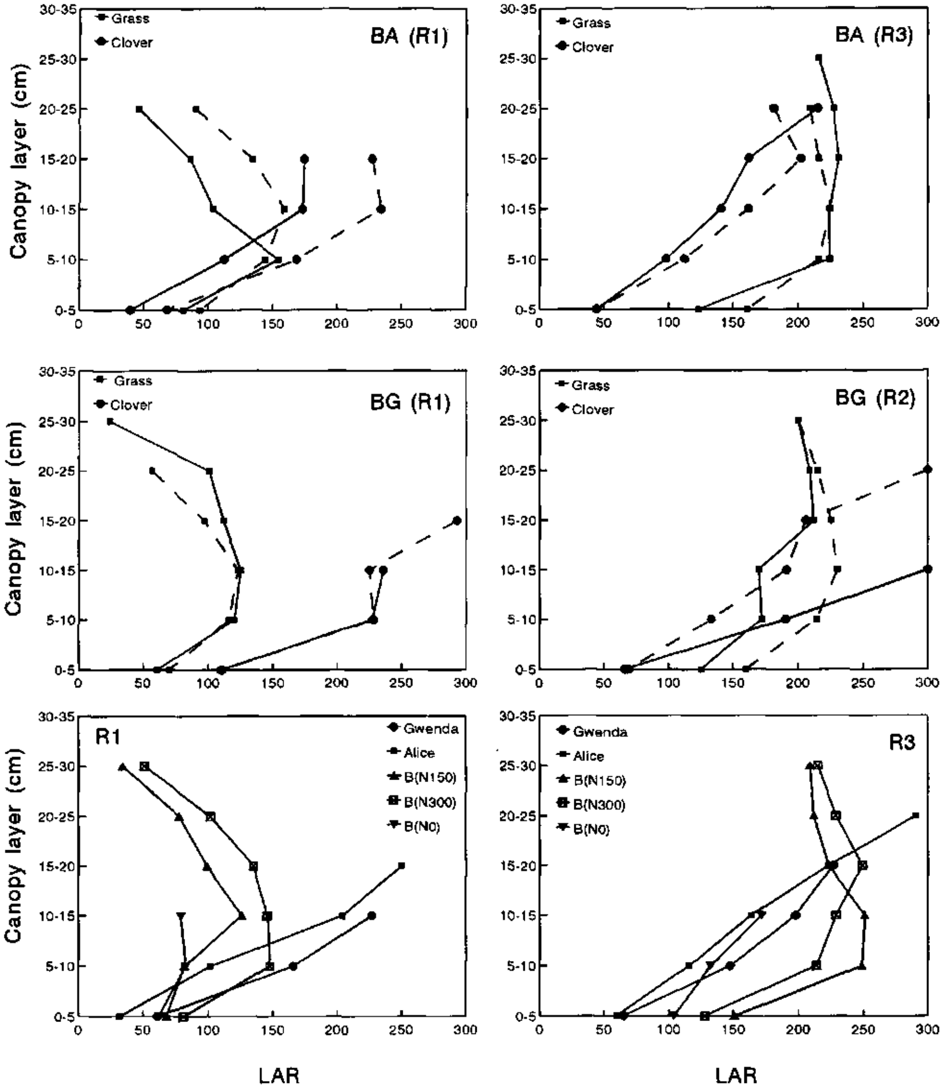

Figure 7. Vertical pattern of leaf area ratio (LAR, cm^2 leaf g^{-1} shoot DM) for grass and clover in mixtures and in monocultures. Data are shown for the end of the first (R1, 17 June) and third (R3, 2 September) regrowth periods. (—) +N, (----) -N mixtures.

In grass the allocation of more DM to the flowering stems during spring resulted in a lower LWR in all layers, which was obvious both in monocultures and mixtures. Therefore, grass had a lower LAR at top layers during flowering compared to the vegetative stage (Figure 7). In summer, particularly in the +N swards, the grass LWR above 10 cm was unity, which led to a higher LAR at top layers. This was sharply reduced at the base of the canopy with a lower LWR.

In clover the vertical variation of LWR and LAR was determined by the distribution of petiole DM. In monocultures the small-leaved clover had a higher LAR than the large-leaved cultivar in all canopy layers (Figure 7). This difference became more obvious in mixtures. In the +N mixtures, Gwenda maintained its LAR in different layers, similar to -N swards (Figure 7). This was achieved by increasing its SLA over height in the +N mixtures, where it was strongly overtopped (not shown).

Alice, however, had a significantly lower LAR in the +N than in the -N mixtures, both in spring and summer (Figure 7). In the +N swards Alice had a high LAR at the top, which sharply declined in lower layers as a result of the higher petiole content.

Vertical distribution of leaf N

Both species showed a gradient of SLN (g N m^{-2} leaf) over canopy height with a higher SLN at top layers (Figure 8). In grass monocultures the profile of SLN was affected by N level (Figures 8c and f) and no N profile was found in the N0 treatment (Figure 8f, dashed line). Clover monocultures had the same leaf N profile at the end of the summer regrowth (Figure 8f). However, in spring the small-leaved clover showed a sharper profile (Figure 8c). In mixture the application of N had no significant effect on the gradient of SLN within the grass canopy, both in spring and summer. However, grass leaves contained more N per unit leaf area in summer (Figures 8a, b, d and e).

The profiles of grass leaf N in the +N and the -N mixtures were comparable with that of the N150 monoculture. In spring both clovers had the same N profile in the +N and the -N mixtures, with a lower gradient compared to grass (Figures 8a and b). In summer, Alice maintained its N profile in the +N mixture (Figure 8d), but in Gwenda SLN had a uniform distribution in the +N compared to the -N mixture (Figure 8e).

Discussion

Competition for light is considered to be important in determining whether grass or clover dominates in the mixed sward (Haynes, 1980; Rhodes and Stern, 1978).

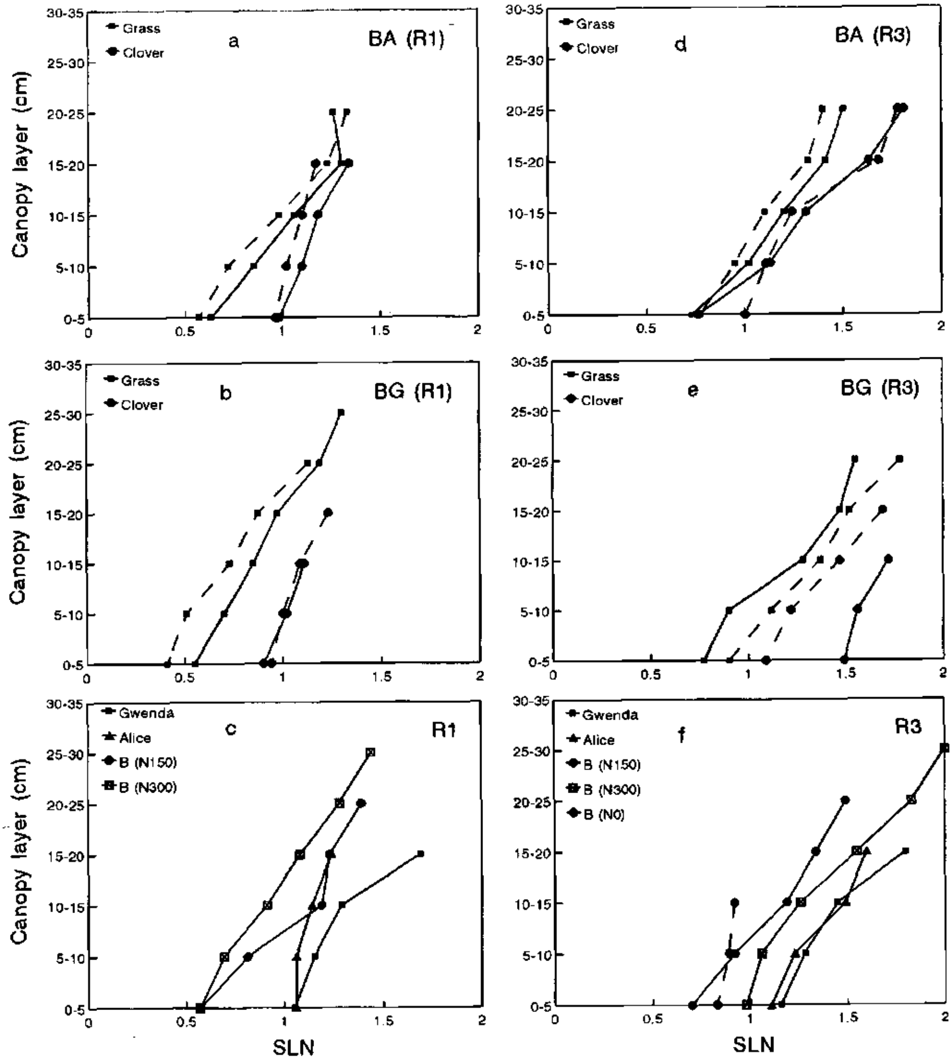

Figure 8. Vertical distribution of specific leaf N (SLN, g N m^{-2} leaf) for grass and clover grown in mixture and in monoculture. Data points shows the N profile by the end of the first (R1, 17 June) and third (R3, 2 September) regrowth periods in (—) +N and (----) -N mixtures.

In mixed canopies the differences between species in carbon gain are associated with structural features, rather than with assimilatory characteristics (Barnes *et al.*, 1990). Therefore, the outcome of competition depends mainly on the distribution of leaves, which determines the pattern of light availability within the canopy. Johnson *et al.* (1989) suggested that in grazed grass-clover canopies the leaf distribution of species is approximately homogeneous throughout canopy depth. However, our results, in accordance to previous observations in infrequently cut swards (Woledge, 1988; Woledge *et al.*, 1992a; Nassiri *et al.*, 1996a) showed that species had different patterns of leaf distribution, both in mixture and monoculture (Figure 1).

Both species showed some departure from a symmetrical leaf distribution (with 50% *LAI* at the half of total height). Clover leaves were mainly situated at the top layers of the canopy, while the grass canopy displayed an inverse pattern. In both species the height of the maximum *LAI* was a logistic function of the total height. In clover the maximum *LAI* occurred on average at 75 and 60% of the total height in large and small-leaved clover, respectively, while in grass it was at 30% of the total height.

Large leaved-clover has longer petioles than small-leaved clover (Chapter 3). This results in a difference in *LAI* distribution and in the degree of overtopping by grass, as was observed in this study. During spring, both clovers were overtopped by grass, especially in the +N mixtures (Figure 1). This can be explained by the lower spring temperature, which results in short petiole lengths of clover (Eagles and Othman, 1986) and the greater height of the maximum grass *LAI* during the reproductive stage, due to stem elongation (Figure 2). Osbourn (1980) reported that in spring up to 80% of the total DM can be made up by the elongated stems of reproductive tillers, which agrees with our results.

Woledge (1978, 1979) showed that the elongation of stem internodes during the flowering stage of grass elevates the growing points, so that young leaves develop at a greater height in the canopy. Therefore, the pattern of light absorption in mixtures was close to that of fertilised pure grasses, indicating grass dominance during spring. In summer and other regrowth periods clover was not overtopped in the -N mixtures. This was achieved by a higher proportion of clover leaf area at top layers, as a result of allocation of more DM to petioles in different canopy layers (Figures 4 and 5).

In the -N mixtures the light profile was similar to that of clover monocultures, displayed by a higher absorption rate at the top which sharply decreased with depth. In fertilised mixtures large-leaved Alice had a greater height of maximum *LAI* compared to monocultures or unfertilised mixtures. However, such a response was not observed in small-leaved Gwenda. Therefore, after the first regrowth period in the +N mixtures overtopping of clover was only observed in mixtures with the small-leaved cultivar (Figure 1).

It has been shown that grass adapts to a tall canopy by increasing the length of its leaves, but clover by increasing the length of its petioles (Wilman and Asiegbu, 1982). In the shade stolon branching will be suppressed, but stolon and petiole elongation are stimulated (Thompson, 1995). Gautier *et al.* (1995) and Thompson (1993) suggested that the response is different between clover genotypes. It is likely that the responses are coupled by the reallocation of resources to petiole or stolon elongation (Thompson, 1995). Rhodes and Harris (1979) suggested that in large-leaved clover cultivars assimilates appeared to be partitioned to form a tall canopy at the expense of the development of the stolon system. It can be concluded that in the +N mixtures the small-leaved cultivar had a lower petiole length because of allocation of DM to stolon elongation. This is also in agreement with Jahufer *et al.* (1994), who found a strong positive correlation between leaf size and plant height within white clover genotypes.

Unfortunately no data are available from the present study about the stolon length of clover cultivars. In raising its leaves to the upper parts of the canopy, large-leaved clover needs more supporting tissues. As a result the fraction of biomass in leaves decreases with increasing height. In large-leaved Alice the clover leaves represented a lower proportion of the shoot DM, but the highest density of leaf mass appeared at higher layers of the canopy than in small-leaved Gwenda. These differences were most pronounced in the +N mixtures (Figure 5). The leaves at the top of the canopy also had a lower SLA, which increased with depth of the canopy (not shown).

It is well-known that leaves that develop in a high light environment have a lower SLA as a result of increased leaf thickness and increased leaf mesophyl cell density (Chabot *et al.*, 1979; Witkowski and Lamont, 1991). The combined effect of vertical variation of LWR and SLA led to a variable LAR over canopy height in both species in the mixtures and monocultures (Figure 7). Based on previous results (Chapter 3) we concluded that during summer small-leaved Gwenda maintained its content in the +N mixtures (the same RGR as grass) by increasing its LAR compared to that in -N mixtures.

The distribution of LAR over canopy height indeed showed that during late summer the small-leaved cultivar had a significantly higher LAR in the +N than in the -N mixtures at the same canopy height (Figure 7). The large-leaved clover was also able to maintain its content in the +N swards (Chapter 3), despite its lower LAR over all canopy layers in fertilised mixtures (Figure 7). Alice avoided overtopping by raising its leaves to the upper parts of the canopy (Figure 2) with a higher light opportunity. These leaves had a lower SLA and therefore a higher N/area content (SLN; Figure 8). This results in higher CO_2 assimilation rates, because the C uptake should be maximal when the leaves receiving the highest irradiance have the highest N concentration (Hirose and Werger, 1987a). Our results showed that the small-leaved clover, which was overtopped in the +N mixtures during summer (Figure 2), had a uniform SLN distribution (Figure 8e) due to the lower light intensity in the lower parts of the canopy. However, the large-leaved clover, by raising its leaves to the upper parts of the canopy (Figure 4), was able to develop a profile of SLN (Figure 8d). It can be concluded that small-leaved clover responded to strong overtopping by grass in +N mixtures through regulation of its RGR by a significant increase in LAR over canopy layers. The large-leaved cultivar avoided overtopping at the expense of a decrease in LAR. Therefore, it can be concluded that the RGR was regulated by an increase in CO_2 assimilation rate through a non-uniform leaf N distribution over canopy height.

Grass and clover had different types of leaf area distribution and there was a profile of leaf N concentration within the canopy. Modelling light partitioning between species, to improve understanding of the relations between the absorbed light, the leaf N concentration and the pattern of CO_2 assimilation at different canopy positions would provide valuable insight into the interactions between grass and clover in mixed canopies (Chapter 8).

Chapter 5

Modelling light partitioning and CO_2 assimilation in grass-clover mixtures: effects of variation in leaf area distribution, extinction coefficient and type of leaf dispersion

M. Nassiri, E. A. Lantinga and M. J. Kropff

Abstract

The vertical profile of leaf area, leaf dispersion, and light partitioning and absorption were studied during two regrowth periods in mixtures without fertiliser nitrogen of perennial ryegrass and two white clover cultivars differing in leaf size under cutting management. Field observations during early June in mixtures with a height of 25 cm revealed that clover had a higher proportion of its leaf area in the top layers of the canopy than grass. Maximum leaf area density of the small- and large-leaved clover occurred at 10 and 18 cm height (h_m), respectively, while in grass, regardless of the companion clover, it was approximately 5 cm. A triangle leaf area density function with height gave a good approximation for this heterogeneity in both species, also for the other measuring dates. Leaf dispersion was studied by analysing inclined point quadrat data. Calculated leaf dispersion factors of the species were linearly correlated with downward cumulative total leaf area index (*LAI*) and appeared to be the principal reason of variation in the extinction coefficient (k) with canopy height. This relationship was negative for clover and positive for grass, indicating a shift from regular leaf dispersion in the top layers to a clumped dispersion in the bottom layers for clover and the reverse pattern for grass. Estimated k-values of the species by means of non-linear multiple regression, using all data of the two successive regrowth periods, showed on average no difference between the apparent and the dispersion-free k of both species, indicating overall random leaf dispersion. Modelling light partitioning and absorption in the mixed canopy with a general multi-layer competition model for mixed canopies (M.J. Kropff & H.H. van Laar, 1993. Modelling crop-weed interactions, CAB International, Wallingford, 274 pp.) revealed, however, that the total absorbed *PAR* was underestimated significantly in the mixture with the large-leaved white clover in case of using apparent k-values derived from the fitting procedure and thus assuming random leaf distribution (model 1). The measured light profile could be satisfactorily mimicked with a modified version of the model by incorporating for both species leaf dispersion as a function of cumulative *LAI* in combination with fitted dispersion-free values of k, thus only reflecting the leaf-angle distribution (model 2). Moreover, there were marked differences between the two models in the simulated profile of light partitioning between the two species. At an incoming *PAR* of 200 J m^{-2} s^{-1}, absorbed *PAR* by clover was about 6% lower with model 1 compared to model 2 (162.4 vs. 173.0 J m^{-2} s^{-1}), whereas for grass a higher amount of absorbed *PAR* was calculated with model 1 (23.2 vs. 16.0 J m^{-2} s^{-1}).

However, the relative difference between the two models in total CO_2 assimilation by clover was only about 3% due to light saturation of the clover leaves in the top layers of the canopy.

Sensitivity analysis on model parameters showed that h_m has an important role in light absorption. Halving the *LAI* of one species can be compensated to a great extent by doubling its h_m. Competitive success of clover in this study was, next to a more planofile leaf-angle distribution, related to its higher h_m and also to regular leaf dispersion in the top layers of the canopy.

Key words: canopy structure, multi-layer light absorption model, leaf area distribution, leaf dispersion, extinction coefficient, clustering, leaf-angle distribution, white clover, perennial ryegrass, CO_2 assimilation

Introduction

Models for canopy CO_2 assimilation are usually structured in two parts. The first part describes light absorption by leaves within the canopy and the second part calculates the rate of CO_2 assimilation of the leaves, based on their assimilatory characteristics. Integration of this rate over canopy height and day gives the total daily rate of canopy CO_2 assimilation (Goudriaan, 1986). Application of light absorption models to mixed canopies is not possible without describing the structure of the mixture and its effect on light absorption by the leaves of the different species in detail. In this paper, the mechanisms of light competition in grass-clover mixtures are studied.

The geometrical structure of the mixed canopy can be described by spatial distribution of assimilatory organs (mainly leaves) and leaf-angle distribution of the species involved. To describe light absorption patterns in grass-clover mixtures, the vertical area distribution of leaves of both species has been studied by many researchers (e.g. Stern and Donald, 1962; Johnson *et al.*, 1989; Woledge *et al.*, 1992a,b; Nassiri *et al.*, 1996a). This spatial distribution of leaf area (leaf area density, *LAD*) shows the density of leaf area around a given location in the canopy. *LAD* is used in multi-layer mixed canopy models for calculating light profiles and absorption of light by the species (e.g. Spitters and Aerts, 1983; Kropff, 1993). In these models, often the same *LAD* functions are defined for both species, i.e. a homogeneous or a parabolic leaf area distribution over total height of the plants. According to Johnson *et al.* (1989) the leaf distribution in continuously-grazed grass-clover swards is approximately homogeneous through the depth of the canopy for each of the species. However, there is experimental evidence that in cut or infrequently-grazed grass-clover mixtures the leaf area distribution can not be described satisfactorily with a homogeneous or a parabolic leaf area distribution, since clover in that case has a greater proportion of its leaf laminae in the upper layers of the canopy than grass (Woledge *et al.*, 1992b; Faurie *et al.*, 1996; Nassiri *et al.*, 1996a,b). Therefore, for such a mixture other relationships are needed between plant height and *LAD*.

The efficiency at which the foliage in the canopy absorbs light is dependent on leaf inclination and the composition of the incoming radiation (diffuse vs. direct flux). In the models mentioned above a fixed extinction coefficient (*k*) is assumed for the species involved over plant height and in time. However, variation in *k* with height and time has been reported for monocultures (Kropff, 1993; Meinke, 1996) as well as mixtures (Inoue, 1995). Skewed *k*-distribution functions have also been observed in perennial ryegrass-white clover mixtures by Nassiri *et al.* (1996a).

For accurate calculations of the absorption of light and CO_2 assimilation in mixed canopies, leaf dispersion also has to be taken into account. Leaf dispersion accounts for the spatial relation between leaves and the pattern of leaf location relative to the adjacent foliage (Andrieu and Sinoquet, 1993). In the case of random dispersion, which is assumed in many competition models, light absorption by leaves follows the Beer-Lambert law. The probability of finding a leaf element within the canopy then follows a Poisson distribution with the variance : mean ratio (relative variance, *RV*) equal to unity (Warren Wilson, 1965). If *RV* exceeds unity, a clustering effect or clumping is present and if the ratio is less than unity, leaves tend to "repel" each other (regular dispersion). In case of non-random dispersion, leaves intercept more or less light than average. In regularly dispersed leaves, leaves intercept more light. Besides, Acock *et al.* (1970) demonstrated that in a model canopy with a leaf area index (*LAI*) of 1, sunlit *LAI* in regularly dispersed leaves was 10% higher than in randomly dispersed leaves. When leaves are clustered, less light is intercepted because of mutual shading. In simulation models the effect of clustering is sometimes treated through correction of *k* by introducing a cluster factor (Spitters *et al.*, 1989; Goudriaan and Van Laar, 1993, Kropff and Van Laar, 1993, Kropff *et al.*, 1994). Baldocchi and Collineau (1994) presented data on leaf dispersion in perennial ryegrass showing all three types of leaf dispersion. In grass-clover mixtures the species generally have different types of leaf dispersion (Nassiri *et al.*, 1996c) and should, therefore, be treated separately.

In general, two types of light absorption models have been applied to grass-clover mixtures: single layer canopy models (Johnson *et al.* 1989; Sinoquet *et al.*, 1990) and multi-layer canopy models (Rimmington, 1984; Faurie *et al.*, 1996). In principle, the latter models are more realistic. Sinoquet *et al.* (1990) applied a light competition model to a white clover-tall fescue mixture. Although they mentioned the importance of variation in leaf dispersion, it was not included in their model because of difficulties in measuring it. Faurie *et al.* (1996) modified the single layer model of Sinoquet *et al.* to a multi-layer model where each layer is characterised by the *LAI*, mean leaf inclination and leaf scattering coefficient of the species present. However, also in this model random leaf dispersion is assumed.

To our knowledge, the inclined point quadrat method (Warren-Wilson, 1960, 1963) is the best available method of obtaining an accurate description of spatial variation of leaf elements throughout the depth of the canopy at a low cost (see e.g. Acock *et al.*, 1970). With this non-destructive method, values can be obtained for leaf area density, leaf dispersion, leaf-angle distribution and extinction coefficient for each of the species present in 5-cm crop layers (Rhodes and Collins, 1993). Therefore, detailed measurements were done in infrequently cut perennial ryegrass-white clover mixtures and simulations were carried out with the well-established light competition and CO_2 assimilation subroutines of the ecophysiological model INTERCOM for interplant competition (Kropff, 1993). The successive topics in this paper are:

- quantification of the vertical dispersion of leaf elements;
- introduction of new *LAD* functions for the two species;
- evaluation of the effect of leaf dispersion on the variation in *k* with canopy height;
- modelling light partitioning and CO_2 assimilation in grass-clover mixtures including variation in leaf dispersion;
- analysis of the sensitivity of the model to different parameters.

Materials and methods

Data collection

The experiment was conducted during two consecutive regrowth periods in late spring and early summer of 1995 on perennial ryegrass-white clover mixtures which were established in April 1991 on heavy river clay at Wageningen, The Netherlands (Elgersma and Schlepers, 1997). Three white clover (*Trifolium repens*) cultivars were grown in mixture with two perennial ryegrass (*Lolium perenne*) cultivars under two cutting regimes and in three replicates. No fertiliser nitrogen was applied. For the purpose of this paper, two clover cultivars with different leaf sizes (Alice, large-leaved and Gwenda, small-leaved) in mixtures with perennial ryegrass cultivar Condesa (tetraploid) were chosen. Mixtures are henceforth referred to by the first letters of their component cultivar names, i.e. CA for Condesa-Alice and CG for Condesa-Gwenda.

During each regrowth period the vertical distribution of leaf area in the canopy was measured weekly using an inclined point quadrat with an inclination angle of 32.5° (Warren Wilson, 1963). All contacts with the point in 40 and 32 descents for CA and CG mixtures, respectively, were recorded for clover and grass laminae. In addition, the results obtained by this indirect, non-destructive method were compared with the direct, destructive method at the same time. An area of 50 x 50 cm from each plot was used for stratified clipping by harvesting 5-cm layers (vertically over the height of the canopy). Harvested material was separated into grass and clover laminae, and area of leaf surfaces were measured with a Li-Cor 3100 Leaf-Area Meter® (Li-Cor Inc., Lincoln, NE, USA). Absorbed *PAR* (photosynthetically active radiation) was measured weekly using a linear ceptometer at successive 5-cm layers in the canopy from top to ground level. Most of the presented experimental data were collected on June 6, after 4 weeks of regrowth, just before cutting the sward.

Data analysis and models

From the data of the inclined point quadrat, *LAI* was calculated for each species in 5-cm layers over canopy height (Warren-Wilson, 1960, 1963). *RV* was calculated to characterize the leaf dispersion using the following equation:

$$RV = \sum_{i=0}^{\infty} \frac{(i-L)^2 P_i}{L} \qquad (1)$$

where i is the number of hits per descent, L is leaf area index and P_i is the proportion of hits with i contacts.

Table 1. Important characteristics of three distributions applied to leaf dispersion (Mototani, 1968).

Leaf dispersion: Distribution	Regular Binomial	Random Poisson	Clumped Negative binomial
Parameters	n,p	L	n,p
Relative variance (RV)	1-p (<1)	1	$\frac{1}{(1-p)}$ (>1)
LAI (L)	np	L	$\frac{np}{(1-p)}$
ξ	$\frac{-\ln(1-p)}{p}$	1	$\frac{-(1-p)\ln(1-p)}{p}$
Sunlit LAI	$1-(1-p)^n$	$1-e^{-L}$	$1-(1-p)^n$
Probability of i contacts (P_i)	$\frac{n!}{(n-i)!i!}(1-p)^{n-i}p^i$	$\frac{e^{-L}L^i}{i!}$	$\frac{(n+i-1)!}{(n-i)!i!}(1-p)^n p^i$

Based on values of RV, three types of leaf dispersion can be distinguished, i.e. regular dispersion (RV<1), random dispersion (RV=1) and clumped dispersion (RV>1) (Warren Wilson, 1960). Nilson (1971) discussed Poisson, binomial and negative binomial probability distributions for random, regular and clumped dispersions, respectively. To test the deviation from randomness, expected values of P_i were calculated for Poisson, binomial and negative binomial probability functions using the equations given in Table 1. The expected values of P_i were tested against observed values using the χ^2 goodness of fit test (Snedecor and Cochran, 1989). This method was applied to the 5-cm layers of the canopy for each species. The type of dispersion and its corresponding probability model were determined based on the results of the test. Parameter ξ and total sunlit LAI can be calculated from RV and total LAI using intermediate parameters n and p (Table 1). Light absorption and partitioning was modelled by means of the light competition subroutines used in the INTERCOM model (Kropff, 1993) with some modifications. Based on earlier observations (Nassiri *et al.*, 1996a), the parabolic LAD function was replaced by a triangle function (Pereira and Shaw, 1980):

$$LAD_h = LAD_m \frac{h}{h_m} \; ; \qquad 0 \le h \le h_m \tag{2a}$$

$$LAD_h = LAD_m \frac{(H-h)}{(h-h_m)} \; ; \qquad h_m \le h \le H \tag{2b}$$

where LAD_h is the leaf area density at height h (m^2 leaf m^{-2} ground cm^{-1} height), LAD_m is the maximum LAD, h_m is the height of maximum LAD and H is the canopy height (cm). LAD_m can be calculated from

$$LAD_m = \frac{2LAI}{H} \tag{3}$$

Downward cumulative LAI at any height in the canopy (LAI_h) can be calculated by integration of Eq. (2) over depth of the canopy:

$$LAI_{h,i} = \int_0^H LAD_{h,i} dh \tag{4}$$

where $LAI_{h,i}$ and $LAD_{h,i}$ are cumulative leaf area index and leaf area density of species i at depth h in the canopy, which gives

$$LAI_{h,i} = \left(1 - \frac{h_i^2}{\left(H_i h_{m,i}\right)}\right) LAI_i \;; \qquad 0 \le h_i \le h_{m,i} \tag{5a}$$

$$LAI_{h,i} = \left(\frac{1-(h_i/H_i)^2}{1-(h_{m,i}/H_i)}\right) LAI_i \;; \qquad h_{m,i} \le h_i \le H_i \tag{5b}$$

For a random leaf dispersion, the exponential extinction of *PAR* in a mixed canopy is described by:

$$I_h = (1\text{-}\rho)I_0 \exp(-\Sigma k_i L_{h,i}) \tag{6}$$

where I_h and I_0 are *PAR* flux (J m^{-2} ground s^{-1}) at height h and at the top of the canopy respectively, ρ the reflection coefficient of the canopy, $L_{h,i}$ the cumulative *LAI* of species i above height h and k_i the extinction coefficient of species i. For grass-clover mixtures the extinction coefficient of both species varies with depth of the canopy. Therefore the above equation can be applied to such a mixture after introducing a variable extinction coefficient with height for each species (Nassiri *et al.*, 1996b):

$$I_h = (1\text{-}\rho)I_0 \exp(-\Sigma k_{h,i} L_{h,i}) \tag{7}$$

where $k_{h,i}$ is the extinction coefficient of species i at height h. The effect of leaf dispersion on light absorption is quantified by introducing a new parameter ξ in Eq. (6) under the assumption of a constant species-dependent k, thus only reflecting the leaf-angle distribution:

$$I_h = (1\text{-}\rho)I_0 \exp(-\Sigma k_{d\text{-}f,i} \xi_{h,i} L_{h,i}) \tag{8}$$

where $\xi_{h,i}$ is a parameter which shows the effect of leaf dispersion of species i above height h and $k_{d\text{-}f,i}$ is the dispersion-free extinction coefficient of species i.

Ross *et al.* (1972)) calculated k of grass and clover using the log-transformed form of Eq. (6). In the present study, non-linear fits of measured values of per layer relative *PAR* and *LAI* and estimated ξ for species were carried out with Eqs (6) and (8). The extinction coefficient derived from Eq. (6) can be seen as an apparent k (k_a) that reflects the combined effect of leaf angle-distribution and leaf dispersion. All regression analyses were performed using Version 1.03 SigmaStat for Windows (Jandel Scientific Software, San Rafael CA). The consequences of using k_a or $k_{d\text{-}f}$ and ξ for the pattern of light partitioning and absorption are evaluated with modified versions of the INTERCOM model.

Derivatives of Eqs (6)-(8) give the absorbed *PAR* by leaves of species *j* at depth *h* in the canopy ($I_{a,h,j}$; J m^{-2} leaf s^{-1}) for the three options:

$$I_{a,h,j} = k_j\,(1-\rho)I_0\,\exp(-\Sigma k_i L_{h,i}) \tag{9}$$

$$I_{a,h,j} = k_{h,j}\,(1-\rho)I_0\,\exp(-\Sigma k_{h,i} L_{h,i}) \tag{10}$$

$$I_{a,h,j} = k_j\,\xi_{h,j}\,(1-\rho)I_0\,\exp(-\Sigma k_{d\text{-}f,i}\,\xi_{h,i} L_{h,i}) \tag{11}$$

Gross CO$_2$ assimilation/light-response for leaf layers can be approximated by (Kropff and van Laar, 1993):

$$A_h = A_m(1-\exp(-\varepsilon I_a / A_m)) \tag{12}$$

where A_h is the gross CO$_2$ assimilation rate (kg CO$_2$ ha^{-1} h^{-1}), A_m is the gross CO$_2$ assimilation rate at light saturation (kg CO$_2$ ha^{-1} h^{-1}), ε the initial light use efficiency (kg CO$_2$ ha^{-1} leaf h^{-1} / (J m^{-2} leaf s^{-1})) and I_a is the amount of absorbed *PAR* (J m^{-2} s^{-1}). Gross canopy CO$_2$ assimilation rate of each species was calculated according to the procedure used in the INTERCOM model including the Gaussian integration method (Kropff and van Laar, 1993). With this method, canopy CO$_2$ assimilation of the species is calculated as the weighted average of the CO$_2$ assimilation rates at five selected heights *h* in the canopy (Goudriaan and van Laar, 1994). For each species, A_m at each height can be calculated from the estimated absorbed *PAR* at that specific canopy height [$I_{a,h,j}$ in Eqs (9)-(11)]. Woledge and Dennis (1982) have found that grass and white clover have similar assimilatory characteristics. In the model the values of ε and A_h for both species were set at 0.36 kg CO$_2$ ha^{-1} leaf h^{-1} / (J m^{-2} leaf s^{-1}) and 40 kg CO$_2$ ha^{-1} leaf h^{-1}, respectively.

Results

Vertical distribution of leaf area

Linear regression between number of hits and *LAI* during a number of regrowth periods resulted in coefficients of 1.13 and 1.16 for grass and clover, respectively, which were close to the value of 1.1 reported by Grant (1993).

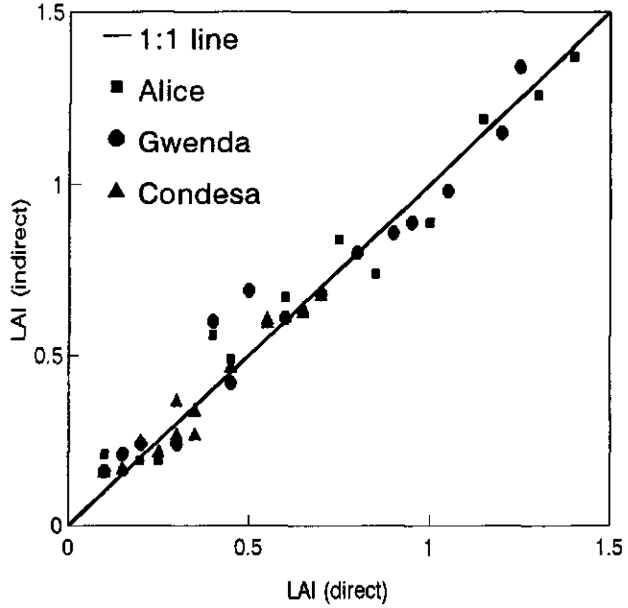

Figure 1. Relationship between direct (stratified clipping) and indirect (inclined point quadrat) measurement of LAI for grass and clover. Data are from two regrowth periods.

Comparison between direct and indirect measurement of *LAI* of species showed that the inclined point quadrat data used in this study gave a good estimation of *LAI* for both species (Figure 1). The triangle leaf area density function reasonably approximated the heterogeneity in vertical distribution of leaf area of species (Figure 2). Leaf area index produced per cm height, i.e. leaf area density (*LAD*), was different between species as well as between clover cultivars (Figure 2a). Both white clover cultivars had a higher LAD_m than their companion ryegrass. The difference between maximum *LAD* of clover cultivars was not significant. However, in the large-leaved clover cv. Alice maximum *LAD* occurred at 18 cm height (at 72% of total height), which was significantly ($P< 0.05$) higher than the height of maximum *LAD* in the small-leaved clover (10 cm, at 40% of total height) (Figure 2a). In both mixtures the maximum *LAD* of grass occurred at 5 cm height (at 20% of total height) and was therefore not affected by clover cultivar. The difference in total *LAI* of clover cultivars was not significant. However, total *LAI* of grass was higher in the mixture with Gwenda than with Alice ($P< 0.05$). Clover had a higher proportion of its leaf area in the upper layers than grass and it was affected by leaf size. In the large-leaved clover, 53% of total *LAI* was above 15 cm height, while the small-leaved clover had only 30% of its total *LAI* above this height. The difference in *LAI* of clover cultivars was not significant ($P< 0.05$) below the height of 10 cm (Figure 2b).

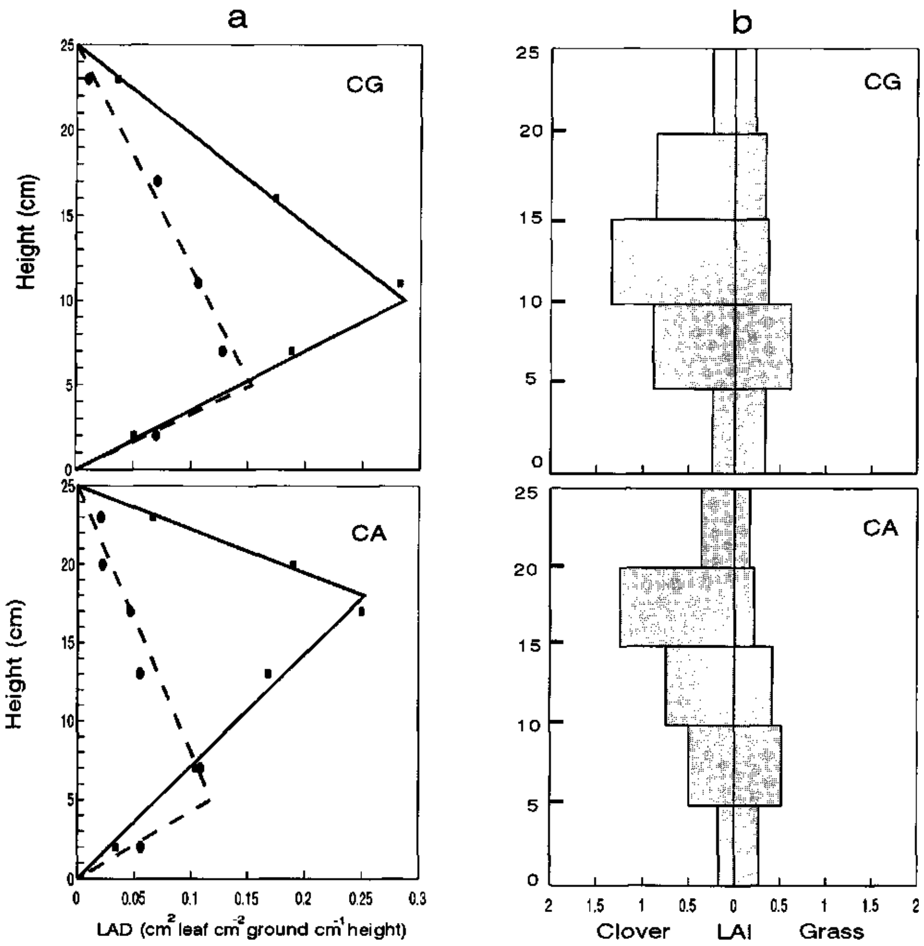

Figure 2. Patterns of leaf area distribution over height in Condesa-Alice (CA) and Condesa-Gwenda (CG) mixtures on 6 June; a) leaf area density fitted to triangle functions for grass (-----) and clover (—). Symbols show measured values obtained by stratified clipping; b) leaf area distribution measured by inclined point quadrat.

Leaf dispersion over height of the canopy

The analysis of point quadrat data measured on 6 June 1995 showed a different type of dispersion over height for both species and both clover cultivars (Table 2). The large-leaved clover (cv. Alice) had a regular dispersion in the first and second 5-cm layers from the top (*RV* = 0.87 and 0.62, respectively). In the third layer, clover leaves were dispersed almost randomly (*RV* = 1.04) and clumping occurred in the lowest two layers (*RV* = 1.72 and 1.38, respectively).

Table 2. Analysis of the data obtained by inclined point quadrat for grass and clover in the two mixtures in 5-cm layers on 6 June. Proportion of quadrats for a given number of hits was calculated from 80 and 64 descents in Condesa-Alice and Condesa-Gwenda mixtures, respectively (pooled data from two replicates).

Height (cm)	No. of hits per descent	Clover (Alice) Prop. of quadrats	Grass (Condesa) Prop. of quadrats	Clover (Gwenda) Prop. of quadrats	Grass (Condesa) Prop. of quadrats
	0	0.725	0.925	0.813	0.875
	1	0.225	0.025	0.156	0.063
20-25	2	0.050	0.025	0.031	0.031
	3	0.000	0.025	0.000	0.031
	4	0.000	0.000	0.000	0.000
	LAI	0.38	0.17	0.25	0.25
	RV	0.85	1.93	0.93	1.72
	ξ	1.08	0.70	1.03	0.75
	0	0.300	0.875	0.531	0.813
	1	0.400	0.075	0.250	0.094
15-20	2	0.225	0.025	0.156	0.063
	3	0.075	0.025	0.063	0.031
	4	0.000	0.000	0.000	0.000
	LAI	1.25	0.23	0.87	0.35
	RV	0.68	1.61	1.02	1.49
	ξ	1.21	0.77	1.00	0.81
	0	0.575	0.825	0.250	0.750
	1	0.175	0.125	0.375	0.125
10-15	2	0.200	0.050	.0281	0.063
	3	0.050	0.000	0.094	0.031
	4	0.000	0.000	0.000	0.031
	LAI	0.84	0.25	1.41	0.53
	RV	1.09	1.09	0.63	1.78
	ξ	0.96	0.96	1.24	0.74
	0	0.763	0.650	0.531	0.594
	1	0.100	0.225	0.250	0.281
5-10	2	0.075	0.125	0.125	0.094
	3	0.025	0.000	0.063	0.031
	4	0.025	0.000	0.031	0.000
	LAI	0.52	0.54	0.94	0.64
	RV	1.63	0.94	1.25	1.00
	ξ	0.78	1.03	0.89	1.00
	0	0.900	0.800	0.844	0.719
	1	0.050	0.150	0.094	0.250
0-5	2	0.050	0.050	0.063	0.031
	3	0.000	0.000	0.000	0.000
	4	0.000	0.000	0.000	0.000
	LAI	0.17	0.28	0.25	0.35
	RV	1.31	1.02	1.17	0.79
	ξ	0.87	0.99	0.92	1.12

In the small-leaved clover (cv. Gwenda), dispersion was random in the two top layers (RV=1) and regularity occurred at 10-15 cm height (*RV*<1). Below this height, the leaf dispersion was clumped (*RV*>1). The dispersion of grass leaves was deviating. In grass, clumping was observed in the top layers and this changed to a random dispersion in the lower layers. The dispersion of grass leaves in the mixture with the small-leaved clover showed more or less the same pattern as described for grass in mixture with the large-leaved clover.

Variation in leaf dispersion and extinction coefficient

Analysis of the point quadrat data shows the different types of leaf dispersion for both species over canopy depth on one specific measuring date (Table 2). When depth was expressed as downward cumulative *LAI*, a linear relation was found between the dispersion factor (ξ) and *LAI* for grass ($r^2 = 0.85$) and clover ($r^2 = 0.72$), although with inverse slopes (Figure 3). Note that Figure 3 refers to eight sampling events during the last three weeks of two regrowth periods of five weeks showing no significant differences between the two clover cultivars. Grass had the lowest dispersion factor in the top layers which increased with depth in the canopy, whereas the reverse pattern was observed for both clover cultivars.

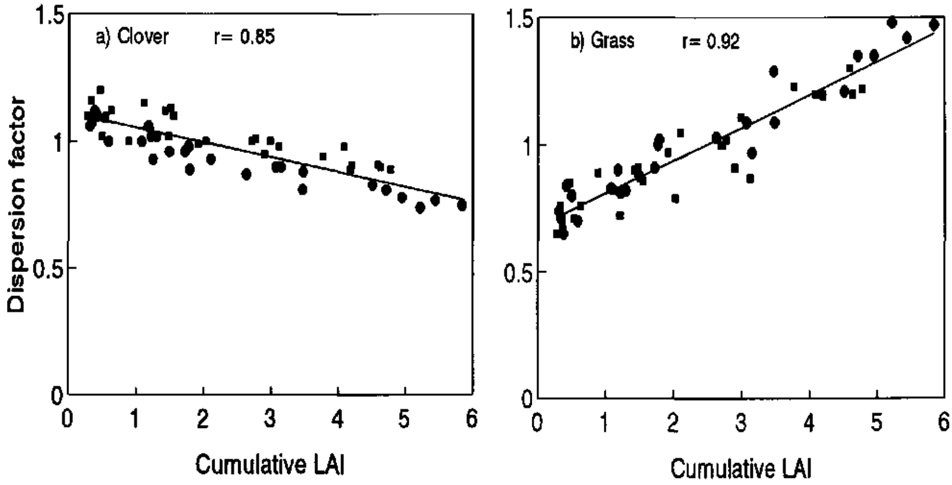

Figure 3. Fitted linear relationship between dispersion factor and cumulative downward *LAI* in (a) clover and (b) grass; measured values are also shown (●, Condese-Alice; ■, Condesa-Gwenda). Data are from two regrowth periods.

For both species the estimated extinction coefficients derived from Eqs (6) and (8) are shown in Table 3. The dispersion-free *k* ($k_{d\text{-}f}$) in Eq. (8) for each of the species was not significantly different between the two mixtures with mean values of 0.52 and 1.02 for grass and clover, respectively. In addition, the estimated apparent *k* (k_a) of the species, which reflects the combined effects of leaf-angle distribution and leaf dispersion, was not significantly different from $k_{d\text{-}f}$ (Table 3).

Table 3. Dispersion-free and apparent extinction coefficient (*k*) of grass (*g*) and clover (*c*) estimated by non-linear regression with standard errors between brackets.

	Dispersion-free k^1			Apparent k^2		
Mixture	Grass	Clover	r^2	Grass	Clover	r^2
CG	0.50 (0.10)	1.09 (0.08)	0.99	0.50 (0.08)	1.00 (0.07)	0.99
CA	0.55 (0.09)	0.96 (0.05)	0.98	0.52 (0.08)	0.97 (0.05)	0.99
Mean	0.52	1.02		0.51	0.98	

1) Calculated from non-linear fit of data to: relative PAR = exp $(-(LAI_c\, k_c \xi_c + LAI_g\, k_g\, \xi_g))$

2) Calculated from non-linear fit of data to: relative PAR = exp $(-(LAI_c\, k_c + LAI_g\, k_g))$

This indicates that the overall leaf distribution of the species was random and variation in leaf dispersion had no significant effect on the average extinction coefficient. It is therefore, concluded that the lower leaf dispersion factor of grass in the top layers was compensated by its higher value at lower layers, whereas in clover the compensation effect was reversed. This observed variation in ξ results in a variable value of the product $\xi.k_{d\text{-}f}$ over cumulative *LAI* or height as described in Eq. (8).

Comparison between models

Total absorbed light

Total absorbed *PAR* by the mixed canopy was simulated by the default model with a fixed apparent *k* and random leaf dispersion but including triangle leaf area density functions (model 1) as well as a modified version with non-random leaf dispersion in combination with a dispersion-free *k* (model 2).

The models were validated against measured total absorbed *PAR*. Simulated total absorbed *PAR* in both mixtures and its partitioning are shown in Table 4 and Figure 4. In the mixture with the large-leaved clover (CA; Figure 4a) model 1 showed a sigificant underestimation of the total absorbed *PAR* by about 2%. However, using a dispersion parameter as a correction factor for a fixed *k* gave perfect estimations of the pattern of measured total absorbed *PAR*. In the mixture with the small-leaved clover (CG; Figure 4b) the effect of dispersion was smaller and no significant deviation appeared from the model without dispersion. The results clearly show that only after the introduction of a parameter for leaf dispersion the observed light profile could be mimicked for the two mixtures. When total absorbed *PAR* density was plotted over canopy height, again the models gave similar results for the CG mixture (Figure 5a). However, for the CA mixture, a difference between models was found at 15-25 cm height where the clover leaves were regularly dispersed (Table 1) and *LAI* per leaf layer was highest at 15-20 cm height (Figure 2b).

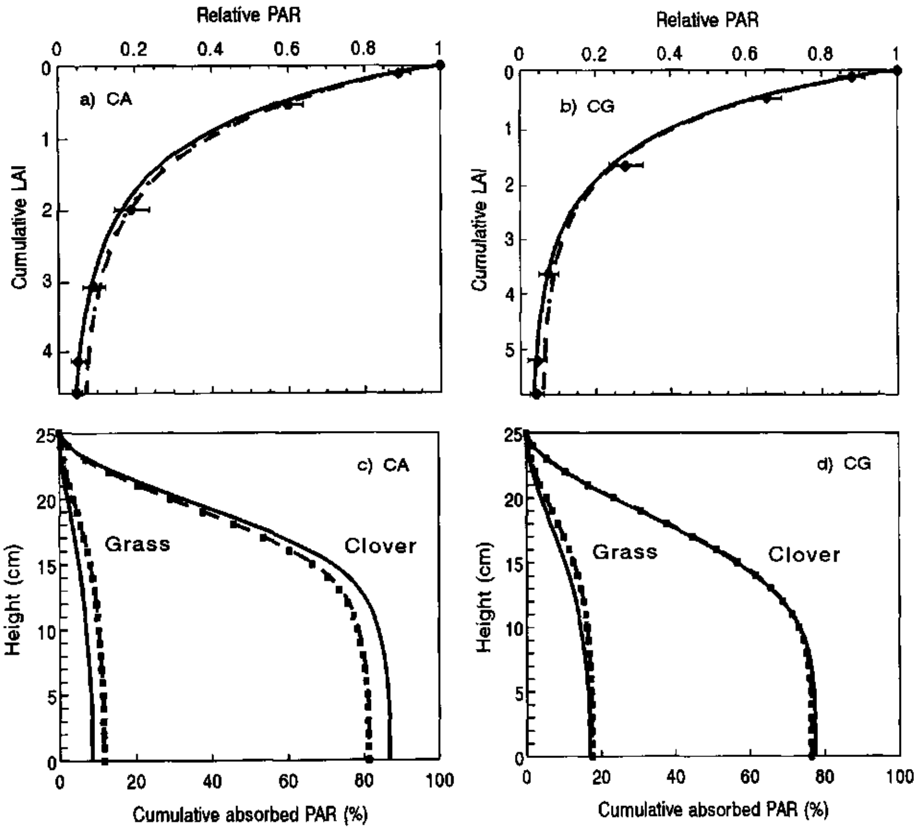

Figure 4. Light extinction with cumulative downward *LAI* in the mixtures on 6 June. Comparison of observed values (●), model 1 with fixed dispersion-free *k* (.....) and with fixed apparent *k* (----), and model 2 with fixed dispersion-free *k* and variable leaf dispersion factor (—): (a) Condesa-Alice; (b) Condesa-Gwenda. Cumulative absorbed *PAR* over height by each species: (c) Condesa-Alice; (d) Condesa-Gwenda.

Note that in all cases there is hardly any effect of using either the value for dispersion-free *k* or apparent *k* in model 1 since they are not significantly different (Table 3).

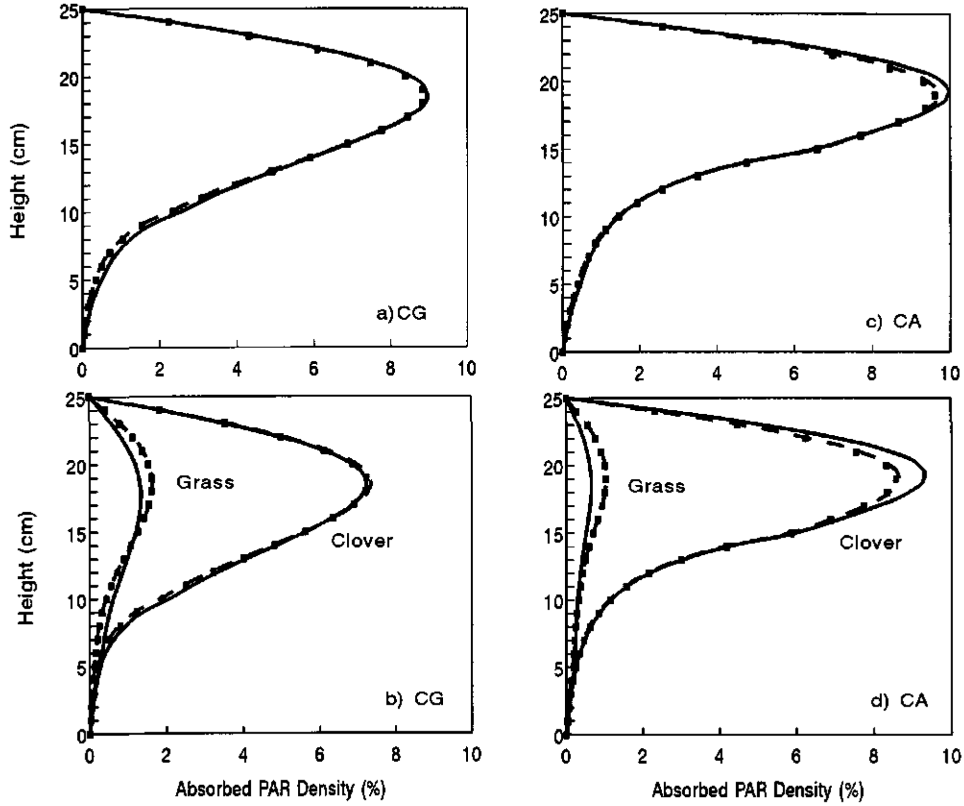

Figure 5. Density of total absorbed *PAR* (a,b) and absorbed *PAR* by each species (c,d) simulated by model 1 with fixed dispersion-free *k* (----) and with fixed apparent *k* (▪▪▪▪), and by model 2 with fixed dispersion-free *k* and variable leaf dispersion factor (—) for Condesa-Gwenda (a,b) and Condesa-Alice (c,d). Absorbed *PAR* density (%) indicates the percentage of incoming *PAR* absorbed per cm canopy height; total area of the individual curves yields the total percentage of incoming *PAR* absorbed.

Light partitioning between species and CO_2 assimilation rate

In the CG mixture, where no deviation from Poisson distribution was observed for the clover leaves in the 15-25 cm layer (Table 2), assumption of randomness yielded the nearly same light partitioning pattern as variation in leaf dispersion (Table 4 and Figure 4d). At an incoming *PAR* of 200 J m^{-2} s^{-1} and taking model 2 as a reference, absorbed *PAR* by clover in the CA mixture was about 6% lower with model 1 (162.4 vs. 173.0 J m^{-2} s^{-1}) whereas for grass model 1 yielded a higher amount of absorbed *PAR* (23.2 vs. 16.0 J m^{-2} s^{-1}). The difference between the models occurred mainly in the 15-25 cm layer (Figure 5d). Simulated profile of light partitioning between species (Figures 5b and d) clearly shows the effect of leaf dispersion. While in the CG mixture the same light profile was achieved by all models (Figure 5b), in the mixture with the large-leaved clover (CA) discrepancies appeared (Figure 5d). For grass, overestimation of absorbed *PAR* with model 1 occurred in the region where grass leaves were clumped (15-25 cm layer; Table 2). For clover, underestimation of absorbed *PAR* occurred in the same region as a result of regular dispersion in the top layers.

Table 4 Simulated absorbed *PAR* (J m^{-2} s^{-1}) and rate of gross canopy CO_2 assimilation (P_c, kg CO_2 ha^{-1} h^{-1}) by the mixtures and partitioning of absorbed *PAR* between the species at an incoming radiation of 200 J m^{-2} s^{-1} *PAR*.

Mixture		Model 1 (random leaf dispersion)				Model 2 (variable leaf dispersion)[c]	
		PAR_{abs} [a]	P_c	PAR_{abs} [b]	P_c	PAR_{abs} [a]	P_c
	Total	188.3	65.1	188.2	65.1	189.2	64.0
CG	Clover	153.2	52.6	152.5	52.4	155.4	52.6
	Grass	35.1	12.5	35.7	12.7	33.8	11.4
	Total	186.2	63.7	185.6	63.8	189.0	63.2
CA	Clover	163.0	55.3	162.4	55.5	173.0	57.3
	Grass	23.2	8.4	23.2	8.3	16.0	5.9

[a] Calculated with dispersion-free *k* (Table 3)

[b] Caculated with apparent *k* (Table 3)

[c] Leaf dispersion factor for each mixture calculated separately (*L* = total cumulative *LAI*):

CG mixture: clover: $\xi = 1.076 - 0.553 \times L$ ($r^2 = 0.96$)
grass: $\xi = 0.715 + 0.113 \times L$ ($r^2 = 0.95$)
CA mixture: clover: $\xi = 1.136 - 0.507 \times L$ ($r^2 = 0.91$)
grass: $\xi = 0.568 + 0.122 \times L$ ($r^2 = 0.88$)

The models gave nearly the same results for total gross canopy CO_2 assimilation rate (P_c) in both mixtures (Table 4). In the CG mixture, where the simulated absorbed *PAR* by the two species was about the same with both models, P_c of the species also differed only slightly. In the CA mixture, the difference between the models with respect to light partitioning was reflected in P_c, however to a lesser extent (about 3 vs. 6%). This could be attributed to light saturation in the top layers of the mixture where clover leaves were dominating. For grass, there was a close agreement between the relative differences in absorbed *PAR* and P_c predicted with the two models. This might be ascribed to the low light levels deeper in the canopy where grass had its highest *LAD* (Figure 2) and gross leaf CO_2 assimilation rate is mainly light-dependent.

Sensitivity analysis

The dependency on canopy structure parameters of light absorption and partitioning between the species and their canopy gross CO_2 assimilation rate were evaluated with the two models. Species *LAI*, height of maximum *LAD* (h_m), and k were varied in the sensitivity analysis (Table 5). The main effect of difference in k is shown in columns a and b. Under these conditions, where the species have the same height and h_m occurs at 50% of canopy height, the triangle *LAD* function works similar to the parabolic one and the ratio of simulated absorbed *PAR* by the species with model 1 is identical to the ratio of their k-values.

The importance of the triangle function with variable h_m is clear in columns c and d with the same conditions as in columns a and b but with h_m of clover twice that of grass. This difference changed the profile of absorbed *PAR* within the canopy and gross CO_2 assimilation rate of the species markedly, especially with model 2 where the effect of leaf dispersion was also included. With the same height and h_m of species, halving of clover *LAI* resulted in still a greater light absorption by clover using model 2 ($Abs_{G/C}$ = 0.84; column f). In addition, when h_m of clover was then doubled, the ratio $Abs_{G/C}$ declined to 0.69 (column h). It can be concluded from Table 5 that in all situations the ratio of absorbed *PAR* between the two species was closely related to their ratio of gross canopy CO_2 assimilation. It is also clear from this sensitivity analysis that in all cases the fraction absorbed *PAR* and the CO_2 assimilation rate by clover is underestimated when using the default version of the INTERCOM model.

Discussion

The experimental results showed that in both mixtures maximum clover *LAI* occurred at higher canopy layers than that of grass. Different patterns of vertical distribution of leaf area in grass-clover mixtures under cutting management are also reported in literature by Johnson *et al.* (1989), Woledge *et al.* (1992a,b) and Nassiri *et al.* (1996a). This is in contrast with the assumption of homogeneity of leaf area within the canopy or a parabolic leaf area density function as in the default version of the INTERCOM model. Replacement of the parabolic function by a triangle *LAD* function permitting a variable height of maximum *LAD* improved the results to a great extent.

Table 5 Conditions used for the sensitivity analysis and corresponding results for fraction absorbed *PAR* and rate of gross CO_2 assimilation of species at an incoming radiation of 200 J m^{-2} s^{-1} *PAR*.

		a	b	c	d	e	f	g	h
LAI	Grass	3	3	3	3	3	3	3	3
	Clover	3	3	3	3	1.5	1.5	1.5	1.5
Species height (cm)	Grass	30	30	30	30	30	30	30	30
	Clover	30	30	30	30	30	30	30	30
H_m (cm)*	Grass	15	15	10	10	10	10	10	10
	Clover	15	15	20	20	10	10	20	20
k	Grass	0.70	0.52	0.70	0.52	0.70	0.52	0.70	0.52
	Clover	0.70	1.00	0.70	1.00	0.70	1.00	0.70	1.00
Fraction absorbed *PAR*									
Model 1	Grass	0.47	0.32	0.35	0.22	0.61	0.46	0.52	0.37
	Clover	0.47	0.62	0.58	0.72	0.30	0.44	0.39	0.53
	G/C**	1.00	0.52	0.61	0.30	2.00	1.04	1.33	0.69
Model 2***	Grass	0.40	0.28	0.31	0.19	0.52	0.40	0.45	0.32
	Clover	0.50	0.66	0.62	0.75	0.33	0.48	0.42	0.57
	G/C**	0.82	0.42	0.51	0.25	1.59	0.84	1.07	0.56
Gross CO_2 assimilation rate (kg CO_2 ha^{-1} h^{-1})									
Model 1	Grass	32.8	22.7	25.0	15.6	42.5	32.6	36.6	26.3
	Clover	32.8	42.9	40.4	49.4	21.5	31.3	27.4	37.3
	G/C**	1.00	0.53	0.62	0.32	1.97	1.04	1.33	0.70
Model 2***	Grass	28.5	19.6	22.2	13.9	36.8	28.1	31.9	22.9
	Clover	34.6	45.5	42.7	51.2	23.2	35.3	29.7	39.9
	G/C**	0.82	0.43	0.52	0.27	1.58	0.84	1.07	0.57

*height of maximum *LAD*

**Ratio between Grass and Clover

***Leaf dispersion factor for each species calculated from the overall relation shown in Figure 4 (*L* = total cumulative *LAI*):

clover: $\xi = 1.135 - 0.589 \times L$ ($r^2 = 0.72$)

grass: $\xi = 0.681 + 0.132 \times L$ ($r^2 = 0.85$)

The residual deviation between modelled and measured light profiles in the mixtures could be explained by variation in the light extinction coefficient of both species as a result of different types of leaf dispersion. In clover, the leaf dispersion factor ξ decreased linearly with cumulative *LAI* and for grass the inverse pattern was observed (Figure 3). In an alfalfa/tall fescue mixture, Inoue (1995) reported a variable *k*-value for species and explained this variation as the effect of leaf dispersion. Nilson (1971) suggested that leaf dispersion within the canopy layer has a stochastic nature and can be described as a Markov process, i.e. the probability density of dispersion in each canopy layer can be calculated from the probability in its previous layer. Using the same concept, Andrieu and Sinoquet (1993) defined a dispersion parameter as a fitting value for unexplained residuals in a random canopy model. Although the fitted apparent *k*-values in our study coincided with an r^2 of 0.99 the simulated total absorbed *PAR* lagged behind the measurements (Table 4). However, when running the model with estimated fixed dispersion-free *k*-values ($k_{d,f}$) for the two species in combination with a depth-dependent leaf dispersion parameter gave a nearly perfect fit of the light profile in the mixtures (Figures 4 and 5). Kroppf *et al.* (1994) used a clumping factor (C_f) to correct for the low measured *k*-values of rice seedlings for the effect of clumping. We extended this correction factor to a dispersion factor (ξ) to include other types of leaf dispersion. ξ is the ratio of measured *k* to the theoretical *k* under the assumption of random dispersion ($0.8\sqrt{(1-\sigma)}$, Goudriaan, 1977). In general, the clover leaves were oriented regularly in the topmost layers of the canopy and changed to a clumped distribution with downward cumulative *LAI* leading to a decrease in the effective *k*, i.e. the product $k_{d,f}.\xi$ (Table 2 and Figure 3). However, in grass this pattern was reversed. Vertical gras occurred in the lower layers of the canopy, where the clover leaves were clumped. These gaps were filled by grass through a higher *LAD* and a higher effective *k*-value (regular or random dispersion, Table 1). Woledge *et al.* (1992a) and Nassiri *et al.* (1996b) also reported a higher *LAD* of grass at lower parts of the canopy.

Introducing the parameter for leaf dispersion gave a better estimation of light extinction, which was underestimated when dispersion was excluded from the model, in particular in the mixture with the large-leaved clover (Table 4). The results of the models with and without the dispersion parameter were the same for the mixture with the small-leaved clover which indicates that overall dispersion (between species dispersion) was random in this mixture, but regular in the mixture with the large-leaved clover. Ignoring this regular dispersion also resulted in an underestimation of light extinction over *LAI* (Figure 4). Acock *et al.* (1970) showed a 10% increase in light interception in a model canopy with an *LAI* of 1 and regular dispersion. The importance of leaf dispersion on light absorption decreases with increasing *LAI* (Acock *et al.* 1970). The simulated profile of absorbed *PAR* density over height (Figure

5) also showed that the underestimation of absorbed *PAR* by clover and overestimation of it by grass with the random dispersion model occurred only in the top layers of the canopy up to a cumulative *LAI* of about 2. In the lower layers of the canopy, no further changes in the simulated profiles between the different dispersion models occurred. Higher density of large-leaved clover leaves in the top layers together with a regular leaf dispersion and thus a higher effective *k* resulted in a large increase in the density of absorbed *PAR* by clover, which reached its maximum at about 20 cm height, but sharply declined at lower heights (Figure 5). Faurie *et al.* (1996) also reported higher light absorption by clover than grass when no nitrogen was applied. In our study the maximum absorbed *PAR* density in grass occurred at the same height as in clover. However, it decreased only slowly with height (Figure 5). This slow reduction can be explained by a linear increase of the leaf dispersion factor with increasing cumulative downward *LAI* (Figure 3) together with an increase in leaf area density from the top of the canopy to a height of 5 cm (Figure 2).

Conclusions

In conclusion, heterogeneity in vertical leaf area distribution and variation in leaf dispersion are the basic characteristics of the canopy structure of grass-white clover mixtures observed in this study. The model presented in this paper gives a better understanding of the canopy structure and light partitioning in grass-clover mixtures by taking into account these sources of vertical heterogeneity of the canopy. When using apparent values of *k* for both species, obtained by fitting and assuming random leaf dispersion, total absorbed *PAR* by the mixture as well as the share of clover in the intercepted *PAR* were underestimated. The findings that nearly perfectly fits the measured light profile in the mixtures were obtained only after the inclusion of leaf dispersion. This indicates that leaf dispersion showed much more variation with canopy height than leaf-angle distribution of the species. In this study we have used the inclined point quadrat method developed in the late 1950s by Warren Wilson (1960) for measuring several components of canopy structure. Nowadays, more sophisticated techniques like plant canopy analyzers (Chen *et al.*, 1997) and digitizing techniques (Thanisawanyangkura *et al.*, 1997) are available. However, our experience showed that the inclined point quadrat method is still a successful way of offering an inexpensive, non-destructive method of measuring leaf dispersion and leaf area density in all height layers of mixed canopies. However, with digitizing techniques diurnal leaf orientation behaviour and sunlit leaf area distribution may be recorded. The inclined point quadrat method can in principle be used to calculate the total sunlit leaf area index in monocultures (Acock *et al.*,

1970) and mixtures, but it is not possible to distinguish between the different species within the mixture.

Chapter 6

Canopy development, light interception and radiation use efficiency of perennial ryegrass and white clover grown in mixture and pure stands

M. Nassiri, E. A. Lantinga and A. Elgersma

Abstract

Canopy development, light interception and radiation use efficiency were studied during three regrowth periods in a mixture of a large-leaved white clover with perennial ryegrass and in grass and clover monocultures without N fertilisation. In all regrowth periods grass in monoculture had a poor growth compared to growth of grass in mixture. As a result a closed canopy was not reached in pure grass and only 40-50% of the total incoming photosynthetically active radiation (*PAR*) was absorbed by the end of each regrowth. In mixture, grass had a higher leaf area index (*LAI*) than clover during spring, but in summer and autumn clover showed a higher growth rate of leaf area. In all regrowth periods clover absorbed a higher fraction of *PAR* than its contribution to the *LAI* of the mixed canopy. Simulation of the profile of absorbed *PAR* by the species in mixture showed the different patterns for grass and clover. In mixture, partial overtopping of clover by grass occurred only during spring regrowth. Simulated elimination of species showed that by removal of grass above 15 cm height, clover captured the same fraction of light as in the entire absence of grass. However, for grass this was achieved only after removal of clover above 5 cm height. In contrast to ryegrass, clover had a lower radiation use efficiency (*RUE*) in mixture than in pure stand, possibly due to N shortage. This was most pronounced in spring, where grass was a stronger competitor for N. The difference in dry matter yield of grass in mixture and in monoculture was due to the higher *RUE* of grass in the mixture. However, the difference in productivity of clover in mixture and in pure stand was due to combined effects of light absorption and *RUE*. In all regrowth periods mixtures had a yield advantage over pure stands (land equivalent ratio >1). This advantage was the result of trade offs between the ratio of absorbed *PAR* and ratio of *RUE* of species in mixture and monoculture.

Key words: Perennial ryegrass, white clover, modelling, canopy, light interception, light partitioning, radiation use efficiency, land equivalent ratio.

Introduction

Of all the major environmental factors that determine competition in mixed canopies, solar radiation has received the most attention (Keating and Carberry, 1993). Competition for light in mixed plant canopies involves both interception and photosynthetic utilisation of the intercepted light by the species. The simple model (Monteith, 1977) based on the linear relationships between cumulative dry matter production and time integral of absorbed light provides a useful framework for analysis of the efficiency of canopies in capture and conversion of solar energy to dry matter. The slope of this linear relation, radiation use efficiency (*RUE*), displays the net assimilation gain of the crop per quantity of intercepted light (Sinclair and Horie, 1989), while the light absorption is a completely geometrical issue and depends on size and structure of the canopy (Baldocchi and Collineau, 1994). In spite of application of the concept of *RUE* in the growth analysis of various annual crops (Belanger *et al.*, 1992; Gosse *et al.*, 1986 among others), its use is less frequent in perennial forage crops (Cruz and Sinoquet, 1994) and there are very few data about *RUE* in mixed swards.

Differences in the yield of species grown in mixture compared to their pure stands has been reported for many intercropping systems (Willey, 1979; Ahmed and Rao, 1982; Marshall and Willey, 1983), including grass and clover (Haynes, 1980). However, it is not fully understood if the reported yield differences are due to a change of the light interception by a species in a mixed canopy or to changes in the *RUE* of a species in a mixed canopy compared to a pure stand. Barnes *et al.* (1990), using a detailed multispecies canopy model for the competition between wheat and wild oats, showed that the differences in canopy structure are more important than the differences in assimilatory characteristics on net carbon gain of species. In a study on the mixture and pure stands of *Arachis pintoi* and *Digitaria decumbens* (Cruz and Sinoquet, 1994), intercropping had no effect on the *RUE* of species. The contribution of each species to the growth of the mixture was only dependent on their light interception. There are also reports on the difference in *RUE* of species when grown in mixture compared to that in pure stands (e. g. Keating and Carberry, 1993). Differences have also been reported for grass and clover. Sinoquet *et al.* (1990) showed that tall fescue had a higher *RUE* in an unfertilised mixture with white clover than in monoculture, but the reverse was observed for clover.

Mixed canopies are generally non-homogeneous (Thornton *et al.*, 1990). Heterogeneity of canopy structure in a grass-clover mixture is due to different patterns of vertical distribution of their leaf area (Woledge *et al.*, 1992a; Nassiri *et al.*, 1996a), while the species have a more or less similar height. However, this heterogeneity is usualy ignored in light absorption and partitioning models for grass-clover mixtures (Johnson *et al.*, 1989; Rimmington, 1984; Sinoquet *et al.*, 1990). The model described in Chapter 5 clearly demonstrated that different patterns of leaf area distribution have a drastic effect on light partitioning and the CO_2 assimilation rate of species, which may not be ignored.

The work reported here deals with the seasonal patterns of canopy development, light interception and *RUE* of ryegrass and white clover grown as pure stand and in mixture, without N fertilisation. The performance of species in mixture or in monoculture was compared on the basis of absorbed light and *RUE*. Light partitioning between species was studied using the simulation model from Chapter 5. The effect of N fertilisation and cultivar choice is reported in an accompanying paper (Chapter 7).

Materials and methods

Design and management of the experiment

The experiment was established in autumn 1995 on a sandy soil in Wageningen, The Netherlands (Chapter 3). White clover (*Trifolium repens*) cultivar Alice (large-leaved) and perennial ryegrass (*Lolium perenne*) cultivar Barlet (diploid, erect) were sown in mixture and in monoculture at 4 and 25 kg ha^{-1}, respectively. Plot size was 2.8 x 8.5 m. A complete randomised design with 2 blocks was used. No N fertiliser was applied. Sampling started in May and continued till October 1996. All plots were cut at an approximate target yield of 2000 kg DM ha^{-1} above cutting at a stubble height of 5 cm. The clover monoculture and the mixture were cut five times (10 May; 17 June; 22 July; 2 September and 7 October) during the growing season, but the grass monoculture with no N was not harvested in July owing to its poor growth. In this paper the data of 3 regrowth periods are presented: spring (17 June-22 July), summer (22 July-2 September and early autumn (2 September-7 October). The performance of the mixtures is compared with that of pure clover and grass.

Measurements

Daily solar radiation and temperature were obtained from the Haarweg meteorological station, located within 500 m from the experimental site. The daily *PAR* (photosynthetically active radiation) is assumed to be half of the daily global radiation. The absorbed *PAR* by species in mixed and pure stands was measured weekly using a linear ceptometer at successive 5-cm layers in the canopy. The vertical distribution of the leaf area and the height of species was measured in all treatments and during each regrowth period at weekly intervals, using an inclined point quadrat with the inclination angle 32.5° (Warren Wilson, 1965). In each plot all contacts with points in 35 to 40 descents were recorded for grass and clover laminae.

For each regrowth period the total aboveground biomass of species was sampled weekly by cutting a 10x10 cm area. The harvested material was separated into grass and clover; the weight of each component was determined after drying for 24 hours at 70° C. In the calculation of cumulative aboveground biomass the dry weight of the stubble, left after cutting, was not included.

Light interception and partitioning

Light interception by species was calculated using a multilayer model for heterogeneous canopies (Chapter 5). The daily amount of absorbed light within layer h in the canopy (I_h, J m^{-2}) is described by:

$$I_h = (1-\rho)I_0\,(1-\exp(-\Sigma k_{d,f,i} L_{h,i}\,\xi_{h,i})) \qquad (1)$$

where I_0 is the *PAR* entering the top of layer h (J m^{-2}), ρ the reflection coefficient of the canopy, $L_{h,i}$ the leaf area index (*LAI*) of species i within layer h for a given day, $k_{d,f,i}$ the dispersion-free extinction coefficient of species i and $\xi_{h,i}$ is a parameter which shows the effect of leaf dispersion of species i in layer h. A leaf area density function (*LAD*) is used for calculation of *LAI* at any height in the canopy (L_h). Absorbed *PAR* by leaves of species i within layer h in the canopy ($I_{h,i}$; J m^{-2}) is then:

$$I_{h,i} = I_h \cdot \frac{k_i\,\xi_{h,i}\,L_{h,i}}{\Sigma(k_i\,\xi_{h,i}\,L_{h,i})} \qquad (2)$$

Totals over all layers yield the daily intercepted *PAR* for each species. Two sources of heterogeneity are assumed in the model: variation in light extinction coefficient and differences in leaf area density (*LAD*) of the species. The first source is dealt with by a dispersion factor (ξ), which accounts for variations in k-values as a result of leaf dispersion (Chapter 5). This parameter is unity for random dispersion, and less or more than unity in case of clumping or regularity, respectively. The second source is dealt with by introducing a triangular leaf area density function for each species with a variable height for maximum *LAD* with total height of species (Chapter 5). In the monocultures k-values were estimated as the slope of the linear regression between log-transformed values of I/I_0 and cumulative downward *LAI*. For mixtures, k-values of 1 and 0.52 were used for clover and grass respectively, and ξ for each species was estimated as described in Chapter 5. Reflection of the canopy was assumed as 5% of the total incoming *PAR* (Jones, 1992; Goudriaan and van Laar, 1994).

Total *LAI*, total height of species and height of maximum *LAD* (h_m) are the model inputs. Daily values of *LAI* were estimated using a non-linear relationship between weekly measurements for each treatment and the corresponding temperature sum (Kropff and Lotz, 1993). Daily height (H)

of species was estimated by fitting a logistic function to the weekly measurements and temperature sum (T_{sum}; °C d) (Spitters, 1989):

$$H = \frac{H_m}{(1 + a \times exp(-b \times T_{sum}))} \tag{3}$$

where H is the height of a species (cm) at a given thermal time, H_m the maximum height of each species (cm), a and b (°C d)$^{-1}$ are parameters. Daily values of h_m for a given species were expressed as a logistic function of species height (H):

$$h_m = \frac{h_{m(m)}}{(1 + c \times exp(-d \times H))} \tag{4}$$

where $h_{m(m)}$ is the maximum h_m and c and d are parameters. For calculation of the temperature sum the base temperature was set at 4 °C for grass and 7°C for clover (Harris, 1987).

Radiation use efficiency (RUE) and land equivalent ratio (LER)

Cumulative intercepted *PAR* for each species and for each regrowth period was calculated by summing the daily values obtained from the model. *RUE* (g DM MJ^{-1}) of each species was calculated as the slope of linear relationships, forced through the origin, between cumulative intercepted *PAR* and cumulative aboveground DM of that species during a given regrowth period. Land equivalent ratio of mixtures (that is the relative area required as two pure stands to produce an equivalent dry matter to a mixture (Willey, 1990)) was calculated on the basis of light interception and *RUE* of pure and mixed species. Total dry matter of mixtures (DM$_{mix}$) can be considered as:

$$\mathrm{DM_{mix}} = RUE_{\mathrm{g,mix}}\, PAR_{\mathrm{g,mix}} + RUE_{\mathrm{c,mix}}\, PAR_{\mathrm{c,mix}} \tag{5}$$

where subscripts g and c are used for grass and clover, respectively, and *PAR* is the cumulative absorbed *PAR* during each regrowth period. *LER* can then be formulated as:

$$LER = \frac{RUE_{g,mix}\ \ PAR_{g,mix}}{RUE_{g,pure}\ \ PAR_{g,pure}} + \frac{RUE_{c,mix}\ \ PAR_{c,mix}}{RUE_{c,pure}\ \ PAR_{c,pure}} \tag{6}$$

Results

Growth of leaf area

Growth rate of leaf area of the two species showed a significant variation during the season, both in mixture and monoculture. During spring, in mixtures grass showed a higher growth rate of leaf area than clover. At the end of the spring regrowth period, the grass *LAI* was about twice that of clover (Figure 1a). However, the increase of clover *LAI* in mixture was lower than in pure clover (compare Figures 1a and 1b). At the end of the summer regrowth period, clover *LAI* in mixture equalled grass *LAI* (Figure 1b) and was significantly higher than its value in spring ($P<0.05$). For a given temperature sum, clover *LAI* was lower in spring compared to summer, which indicates the higher growth rate of leaf area during the second regrowth period. Mixed grass had a significantly ($P<0.05$) lower growth rate of *LAI* in summer (Figure 1b) compared to spring (Figure 1a). More or less similar growth rates of leaf area during spring and summer were observed in clover monoculture (Figures 1d and e). In autumn, the growth rate of grass *LAI* in mixture (Figure 1c) was similar to that in spring (Figure 1a), while clover had a significantly ($P<0.05$) higher growth rate of leaf area both in mixture and monoculture compared to spring and summer (Figures 1c and f). Grass monoculture had a poor growth of leaf area compared to mixed grass in all periods (compare Figures 1a-c and d-f).

Light interception

Cumulative absorbed PAR

Linear regression of $\ln(I/I_0)$ on cumulative *LAI* gave *k*-values of 0.914 (±0.033) and 0.633 (±0.045), averaged over 3 regrowth periods for clover and grass monocultures, respectively (Table 1). In mixture, fixed *k*-values of 1 and 0.52 for clover and grass, respectively (obtained from an independent experiment described in Chapter 5), corrected by a leaf dispersion factor for each species, as will be shown below, gave a good approximation of the measured *PAR* within the mixed canopies.

The time course of total intercepted *PAR* by the mixed canopy and each species was simulated for monocultures and mixtures. The model was validated against measured total absorbed *PAR* in pure stands and entire mixed canopies (Figure 2), because direct measurement of light

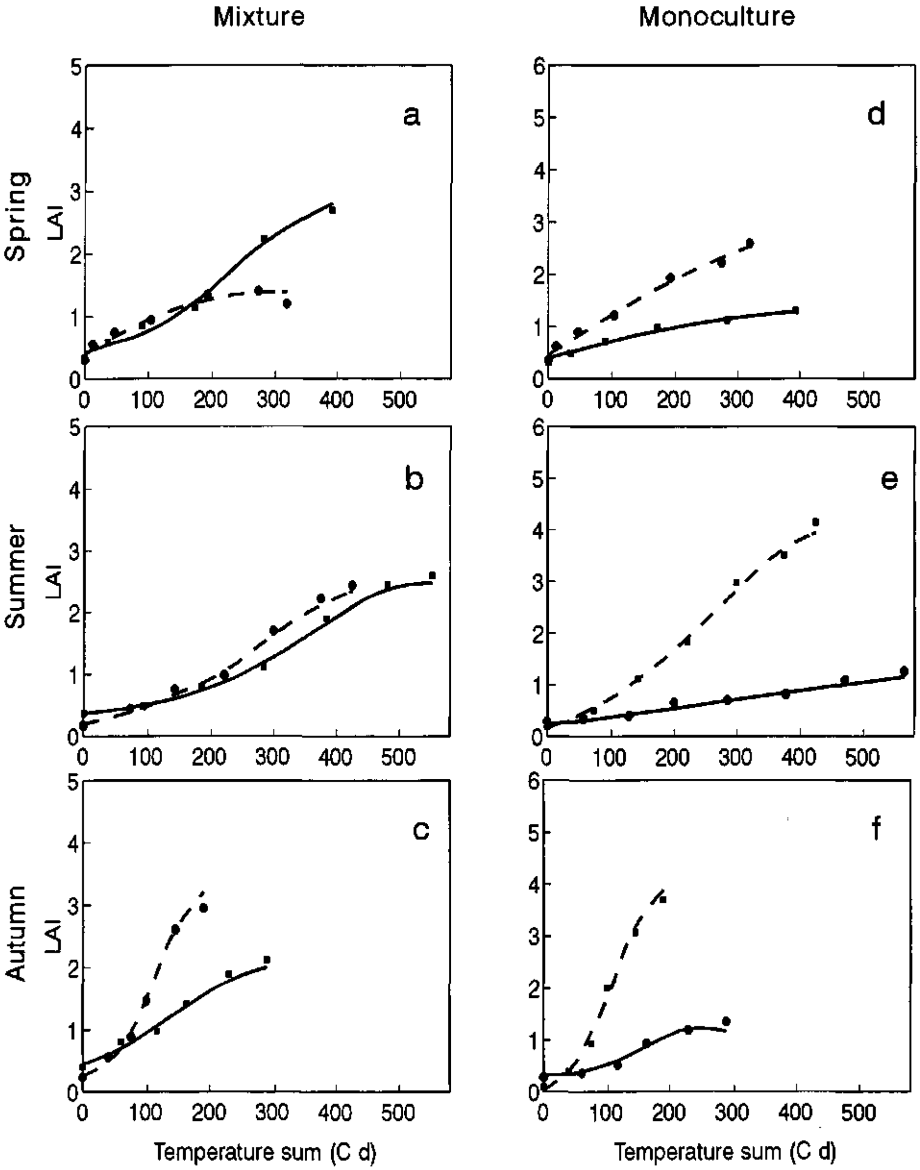

Figure 1. Leaf area index (*LAI*) of clover (----) and grass (—) in mixture and pure stands as a function of temperature sum in different regrowth periods; measured values for grass (■) and clover (●) are also shown. The base temperature for grass and clover was set at 4 and 7 °C, respectively.

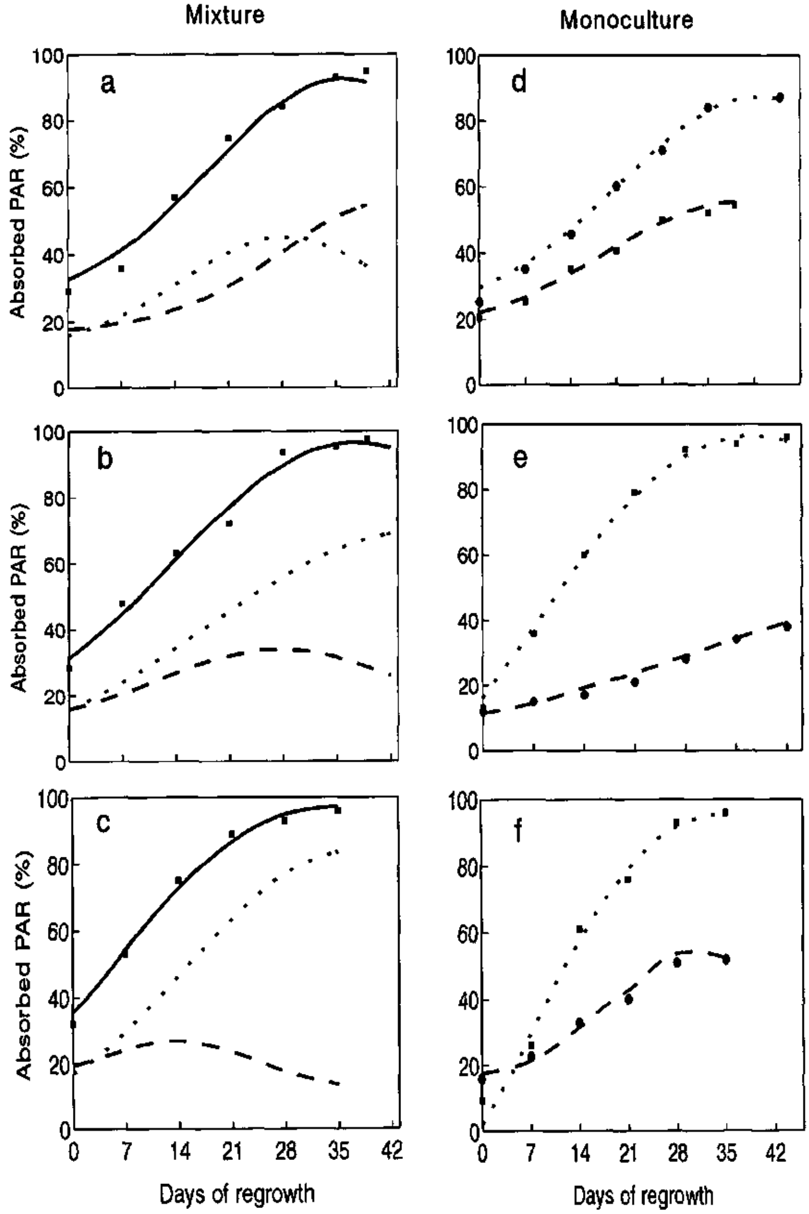

Figure 2 . Simulated time course of the percentage of total absorbed *PAR* (—) and fraction intercepted *PAR* by grass (—) and clover (.....) in mixture and pure stands during different regrowth cycles. Measured values for total fraction of absorbed *PAR* are also shown for the mixture (■) (Figures a-c), and clover (●) and grass monocultures (■) (Figures d-f).

Table 1. Apparent light extinction coefficient (*k*) of grass and clover in monoculture during 3 regrowth periods.

Species	Spring	Summer	Autumn	Mean
Grass	0.680	0.590	0.631	0.633 (±0.045)
Clover	0.879	0.943	0.922	0.914 (±0.033)

absorbed by the components of the mixed canopy was not possible. Good accordance between modelled and measured values indicated that the calculated *LAI* and *k*-values of species, corrected by a leaf dispersion factor, were able to explain the behaviour of the species in mixed canopy.

The daily fraction of absorbed *PAR* by monocultures followed the pattern of leaf area development. A closed canopy was not reached in clover monoculture in the first regrowth period and total absorbed *PAR* by the end of this period was 87% (Figure 2d). In summer and autumn, however, clover monoculture had a closed canopy (> 95% absorbed *PAR*) after 4 weeks (Figures 2e and f). Grass monoculture only absorbed about 40 to 50% of total incoming *PAR* by the end of each of the three regrowth periods (Figures 2 d, e and f).

In mixtures, in addition to the leaf area of species, light interception was also affected by canopy structure. In all regrowth periods, clover captured a significantly higher fraction of *PAR* than its contribution to *LAI* of the mixed canopy (compare Figures 1 a-c with Figures 2 a-c). At the end of the spring regrowth period, 41.3% of the total *PAR* was absorbed by clover (Figure 2a), while its *LAI* was half of the grass *LAI*. This advantage of clover in absorption of *PAR* was most pronounced in summer (Figure 2b) and autumn (Figure 2c).

Light profile within the canopy

The simulated profiles of absorbed *PAR* density (percentage of *PAR* absorbed per cm height) over height of mixed and pure canopies are shown in Figure 3. Profiles were different between species in mixture. In clover, going downwards in the canopy, a sharp decrease after a peak in the top layers was observed, but in grass *PAR* density decreased slowly with canopy depth. This pattern remained unchanged during the season (Figures 3 a-c). In spring, height difference resulted in the better light condition for grass in the top layers. During summer, both species had the same *LAI* in mixture, while total absorbed *PAR* by clover (area within the curves) was significantly ($P<0.05$) higher compared to that of grass. In addition, light interception in the clover canopy mainly occurred in the top layers. For example, at the end of the summer regrowth period more than 60%

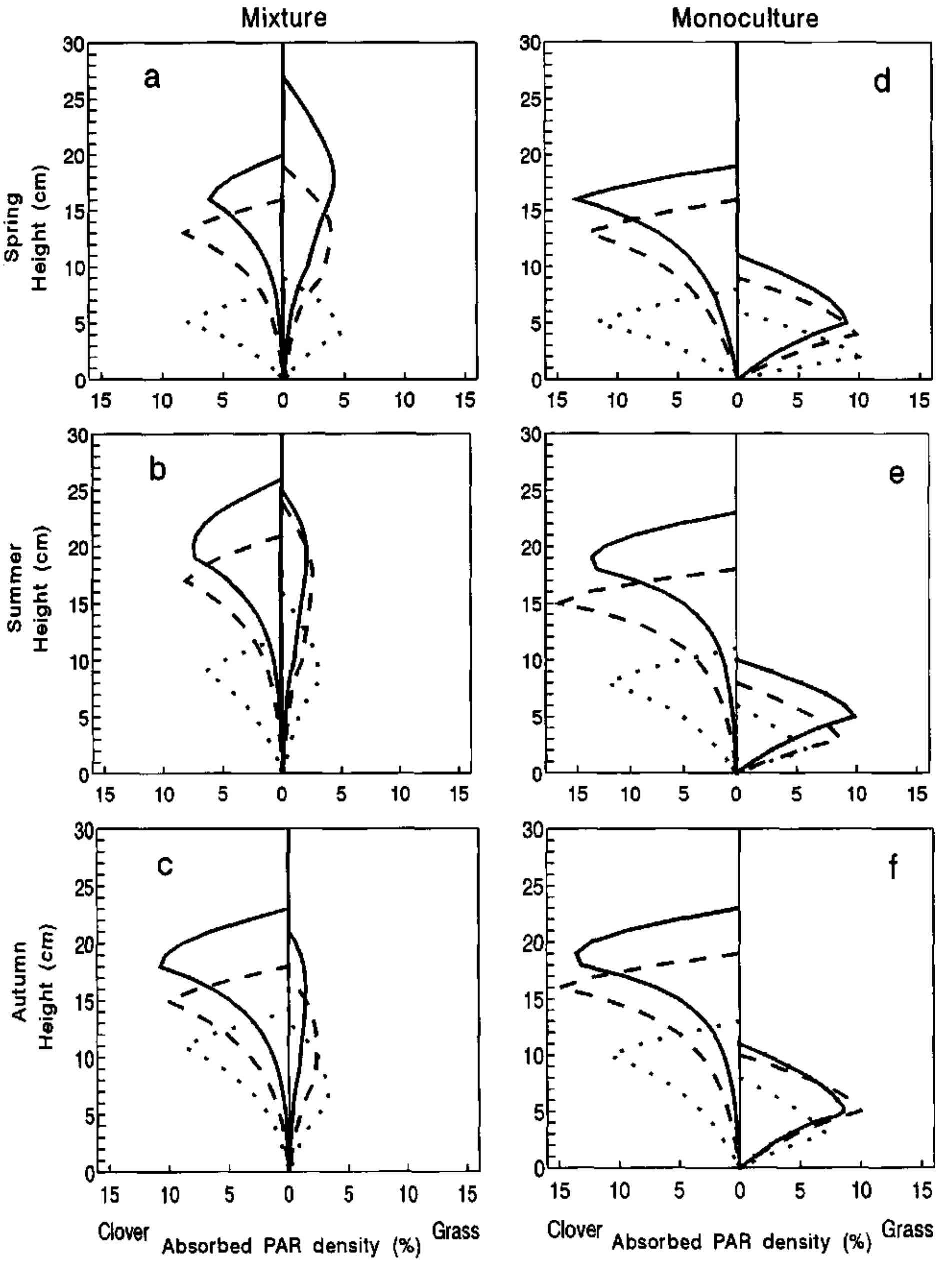

Figure 3. Simulated profile of *PAR* density for grass and clover grown in mixture and pure stand during different regrowth cycles. Development of profile is shown after 2 weeks (......), 4 weeks (-----) and by the end of regrowth (—).

of the total *PAR* was absorbed above 15 cm height (Figure 3b). This advantage of clover is due to its canopy structure with a higher proportion of its leaf area at a higher position in the canopy and its higher light extinction coefficient. In pure clover, the development of the *PAR* profile was the same as in mixture (Figures 3d-f). Poor growth of leaf area in grass monoculture led to an undeveloped *PAR* profile in this treatment compared to that of mixed grass, which was not affected by the season.

Zonation of the height of light competition in the mixture

The light demanded by grass or clover (that is the fraction of light that theoretically could be captured by one species in the absence of the other species) in the mixed canopy was simulated by eliminating the other species from the mixture assuming the canopy structure remains unchanged. The results were compared with the real amounts of captured light at the end of each regrowth period (Table 2). In spring, grass captured about 70% of its demand, but clover only 56%.

Table 2. Captured and demanded *PAR* (%) estimated for grass and clover grown in mixture. For simulation of the *PAR* demanded by one species, parameters of the other component were set at zero.

	PAR	Spring	Summer	Autumn
Ryegrass	captured	54.1	25.6	12.7
	demanded	76.6	68.9	61.5
	captured as % of demanded	70.6	37.2	20.7
White clover	captured	38.2	65.5	81.9
	demanded	67.7	81.0	88.0
	captured as % of demanded	56.4	80.9	93.1

In summer and autumn, when clover was in good light conditions, grass experienced a severe light deficit by absorbing only 37 and 20% of its demand, respectively. To find the critical height for light competition, each species was again eliminated from the mixture using a layer by layer simulated removal (Figures 4a and b). For clover, the most competitive zone of grass canopy was situated between 20 and 15 cm height. In summer and autumn, clover absorbed more than 90% of its demand by removal of grass above 20 cm. However, in spring removal above approximately 10 cm was needed to achieve this absorption level (Figure 4b). Clover had a wider competition zone than grass. In all regrowth periods grass was only able to absorb 90% of its demand, when clover was removed above approximately 12 cm (Figure 4a).

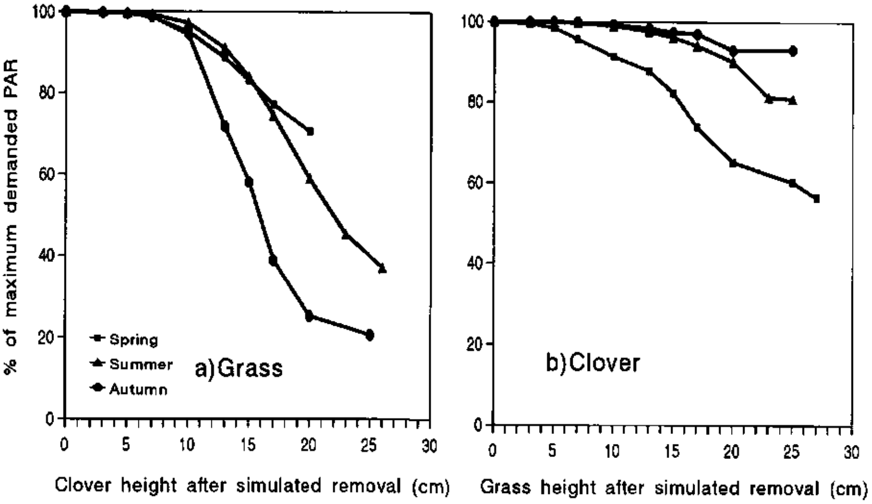

Figure 4 Simulated layer-wise removal of clover (a) and grass (b) and the resulting effect on captured *PAR* by grass (a) and clover (b) relative to its maximum demand.

Radiation use efficiency

In Figure 5, the linear regression lines between cumulative absorbed *PAR* and accumulated dry matter of species, are plotted for each regrowth period. The slope is the radiation use efficiency (*RUE*). Overall, clover had a lower *RUE* than grass in the mixture. In contrast to ryegrass, clover always had a higher *RUE* when grown as a pure stand than in mixture. Clover had a very low *RUE* during spring in mixture and monoculture (0.53 and 1.02 g DM MJ^{-1} *PAR* respectively), which significantly ($P<0.05$) increased later in the season (Figures 5a-c). High *RUE* of mixed grass during spring (1.95 g DM MJ^{-1} *PAR*) significantly ($P<0.05$) declined to 1.3 g DM MJ^{-1} *PAR* in summer, followed by an increase in the last period (1.77 g DM MJ^{-1} *PAR*). Both in mixture and monoculture grass had the lowest *RUE* in summer (Figure 5e). The different performance of species in mixture and pure stand could be due to differences in absorbed light, as well as in *RUE*. These two factors are separated for clover using graphical analysis (Figure 5). For grass, the higher *RUE* during all three periods in mixtures was partly offset by less absorbed light in summer and autumn compared to monoculture (graphically not shown). Comparison of mixed and pure clover in spring and autumn (Figures 5a and c) showed that *RUE* and intercepted *PAR* contributed about equally to the higher DM production in clover monoculture. In summer, however, higher absorbed *PAR* in pure clover accounted for more than 70% of the observed differences in DM (Figure 5b).

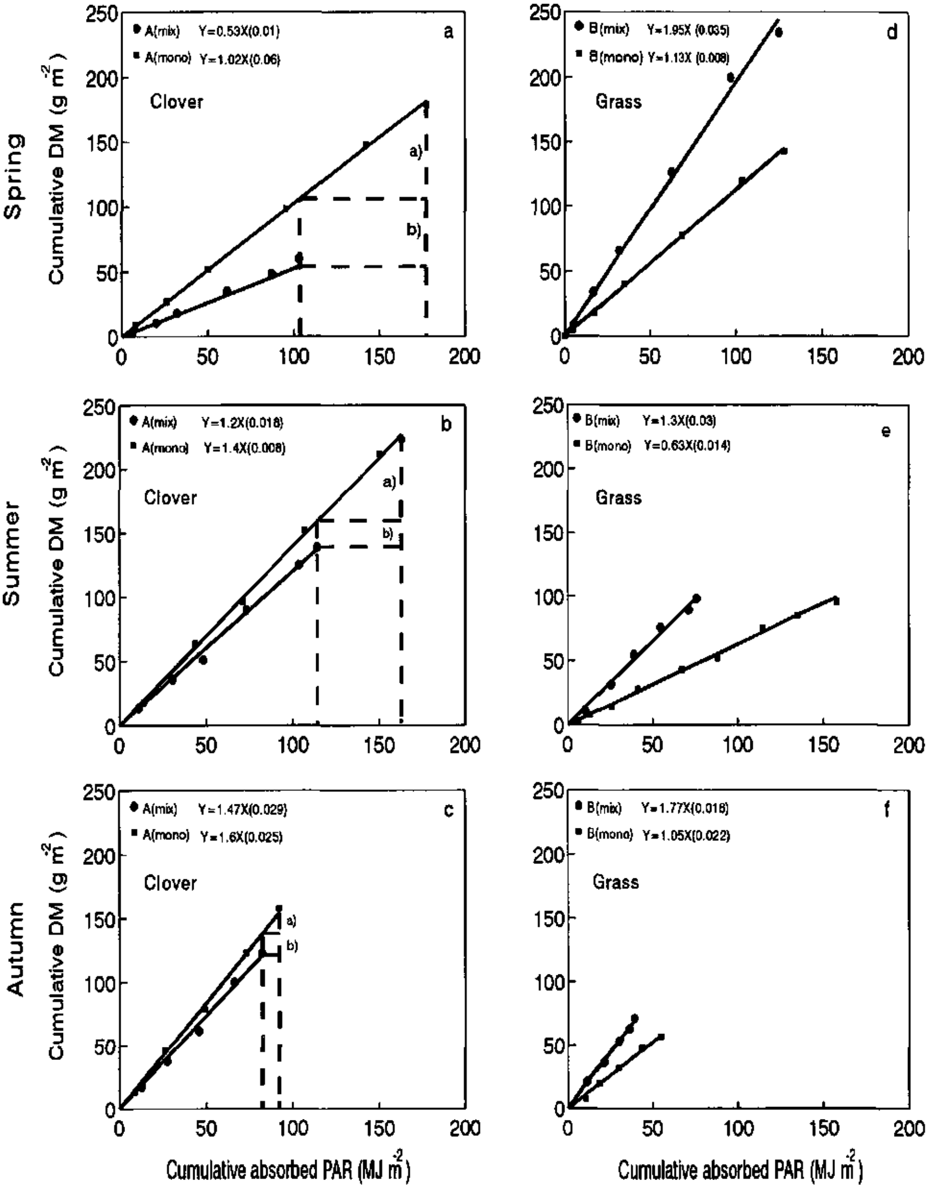

Figure 5 Linear relationships between cumulative DM and cumulative absorbed *PAR* (forced through the origin) for clover (a-c), grass (d-e) in mixed and pure stands. Figures between brackets are the standard errors of regression coefficient i.e. *RUE* (g DM MJ^{-1} *PAR*); a) and b) indicate the effects of *PAR* interception and *RUE*, respectively, on DM production. The arrow in Figure 5e indicates the moment of harvesting the mixture.

In summer, grass monoculture was allowed to grow for 4 more weeks. The results show that pure grass, without N, needed about 3 weeks more regrowth to compensate for its low *RUE* by absorption of more light (Figure 5d).

Land equivalent ratio

Over the summer and autumn regrowth cycles, mixtures had a distinct advantage over pure stands (*LER* = 2.30 and 2.01 for summer and autumn, respectively; Table 3). Mixture *LER* was influenced by both improvement of light interception in the mixed canopy and by changes in *RUE* of species in mixture compared to that of pure stands. The reduction in absorbed *PAR* and *RUE* of clover in mixture was compensated for by the enhancement observed in *RUE* of mixed grass compared to its pure stand. The trade-off between the ratio of absorbed *PAR* and the ratio of *RUE* of species in mixture and monoculture was reflected in the *LER* during the season.

Table 3. Land equivalent ratio (*LER*) calculated as the product of the ratio of absorbed *PAR* and radiation use efficiency (*RUE*) of species in mixtures and pure stands. *LER* of mixture is the sum of *LER* of both components.

	Summer		Autumn	
	Grass	Clover	Grass	Clover
RUE_{mix}/RUE_{pure}	2.06	0.86	1.68	0.92
PAR_{mix}/PAR_{pure}	0.83	0.69	0.71	0.89
Component *LER*	1.71	0.59	1.19	0.82
Mixture *LER*	2.30		2.01	

Discussion

Validity of the model

Simulation models for light competition between species in general assume a well-mixed canopy in which leaf elements are both horizontally and vertically homogeneous (Sinoquet *et al.*, 1990; Rimmington, 1984; Kropff, 1993). However, grass-clover mixtures are not vertically homogeneous under a cutting management (Johnson *et al.*, 1989; Woledge *et al.*, 1992a,b; Nassiri *et al.*,1996a; Chapter 4). Simulation results with grass-clover mixtures (Chapter 5) showed that the triangular *LAD* function of the model, used in the present study, gives a good estimation of leaf area distribution of species compared to other functions (e.g. the parabolic function in Kropff, 1993). In addition, the assumption of random leaf dispersion in the above mentioned models is not always valid for a mixed grass-clover canopy (Baldocchi and Collineau, 1994), where clover leaves have a regular dispersion and therefore a higher *k*-value in top layers of the canopy (Chapter 5). The results of this study showed a good agreement between simulated and measured values of the daily fraction of absorbed *PAR*, both in mixtures and in pure stands (Figure 2), which in turn validates the simulated pattern of light sharing between the species.

Canopy structure and light absorption by species

Grass had a low *LAI* and an undeveloped canopy when grown in a pure stand without N, while its *LAI* increased more than two times in mixture with clover. This beneficial effect of clover has been frequently reported in natural grasslands (Turkington and Harper, 1979) as well as artificially mixed swards (Harris, 1987). The growth rate of *LAI* of species in mixtures generally shows a seasonal pattern. The higher growth rate of grass in spring and its subsequent decline during summer (Harris, 1987), when clover experiences optimum growth conditions (Davies, 1992), was also observed in this study (Figure 1). After harvesting grass in the reproductive stage, the stubble containes a high amount of DM, i.e. part of the cut stems. In addition, regrowth is generally delayed compared to regrowth following the harvest of vegetative crop. In grass and clover monocultures the light absorption during each regrowth period followed the pattern of leaf area growth. However, in mixture in addition to *LAI*, leaf area distribution also had an important role in *PAR* interception and partitioning. The canopy layers containing most clover were towards the top of the canopy, which was due to a higher height of the maximum *LAD* (h_m) in clover than in grass, both in mixture and monocultures. The same pattern was observed by Woledge *et al.* (1992a and b). As a result, clover captured a significantly larger proportion of the light than its relative contribution to the *LAI* of the mixed canopy, leading to absorption of relatively more *PAR* per unit of leaf area by clover than by grass. This is in accordance with the results of Faurie *et al.* (1996). In their study, clover absorbed more light per unit leaf area while its proportion in total *LAI* was very low (less than 30%). This advantage can be explained partly by the higher *k*-value of clover (Frame and Newbould, 1986). However, its h_m has to be considered as well. The latter led to about 15% more light absorption by clover when the species had the same *LAI* and height, but the h_m of clover was twice that of grass (Chapter 5).

Critical zone of light competition

A strong relationship exists between plant height and competitive ability, and has been reported for many species (Berkowitz, 1988). This feature has, therefore, an important role in competition models. For example, in the model of Spitters and Aerts (1983) the light absorption by a species is weighted by the light transmitted to half the height of that species, which gives preference to taller species. However, it seems that this assumption looses its validity when the species have more or less the same height. In this situation, the height at which the *LAD* is maximal (h_m) has a crucial role (Chapter 5). Simulated removal of species in a mixture during summer, when both species had the same *LAI* and height, showed that clover was the stronger competitor, mainly because its h_m was about 10 cm above that of grass. Beyschlag *et al.* (1992) who studied asymmetric (one-sided) competition between two species with significant difference in height, showed that the negative effect of the tall species was removed only when it was cut to the height of the smaller species. However, in our simulation study, where species had a similar height (symmetric competition), each species will capture nearly all of its light demand by removing the other species above its h_m For clover with h_m at 20 cm, removal of the grass canopy above this height is enough to capture about 90% of its demand (Figure 4b; summer and autumn). However, for grass with h_m at 10 cm, clover has to be removed above about 12 cm (Figure 4b; all periods) to achieve this. The wider light competition zone of large-leaved clover cultivars over grass clearly explains the better performance of these cultivars under cutting, as reported by Swift *et al.* (1992) and Elgersma and Schlepers (1997). Besides, it also confirms their poor persistence under intensive grazing (Evans *et al.*, 1992), where top layers are continuously removed by animals.

Radiation use efficiency, species performance and land equivalent ratio

Grass had a higher *RUE* when grown in mixture compared to a pure stand, but the opposite was observed for clover (Figure 5), which is in accordance with Sinoquet *et al.* (1990). They found values of 1.63 and 1.1 (g DM MJ^{-1} *PAR*) for grass (tall fescue) and white clover, respectively, in a mixture. Gosse *et al.* (1986) obtained a potential *RUE* of 1.9 and 1.72 (g DM MJ^{-1} *PAR*) for grass and leguminous species, respectively. The variation in the values of *RUE* for mixed species found in the literature is partly due to errors in measurements (Gallo *et al.*, 1993), as well as in the assumptions made in the used simulation model (Thornton, 1990). Simulation is the only way to estimate light sharing in mixed canopies, where direct measurement for each component is not possible.

During spring, *RUE* of clover was significantly lower than during other regrowth cycles, both in mixture and in monoculture (Figure 5). This could partly be explained by its lower rate of CO_2 assimilation at low temperatures (Woledge, 1988). Seresinhe *et al.* (1994) showed that white clover in mixture is more dependent on symbiotic N fixation than when grown in pure stands, because of strong competitiveness of ryegrass with regard to the uptake of inorganic N. This effect, together with a decrease in clover N fixation at lower temperature (Nesheim and Boller,

1991) and a subsequent N shortage in mixed clover, probably resulted in a significantly lower *RUE* of mixed clover compared to pure clover during spring.

Long term simulation of clover variability (Schwinning and Parsons, 1996c) shows that it is hardly possible to give an optimal level for clover content in the mixture and in fact the balance between grass and clover is the main driving force of resource sharing and dynamics of species. Our results (Figure 5) showed that in spring clover had a lower performance in mixture mainly because of its lower cumulative absorbed light, while grass productivity in the mixture was promoted by a significant increase in its *RUE*. However, in spring and autumn *RUE* and absorbed *PAR* had equal contributions in different DM yield of clover in mixture and in monoculture.

The mixture *LER* obtained in this study (Table 3) showed advantage of mixtures over monocultures. However, it was not only determined by the clover content in the mixture, as was found by Menchaca and Connolly (1990), but also by the ratio of *RUE* of species in mixture and in pure stand. In fact the trade off between the two components of *LER* led to advantages of mixtures over the season compared to pure stands of grass and clover without N. However, LER decreased in autumn, where the clover content in DM was at its highest, which is in agreement with the conclusion of Evans *et al.* (1992) that successful grass-clover swards depend on the retention of clover, yet avoiding clover dominance.

Chapter 7

Effects of nitrogen on light competition, partitioning and radiation use efficiency in pure stands and mixtures of perennial ryegrass and white clover cultivars. Simulation and experiment

M. Nassiri, E. A. Lantinga and A. Elgersma

Abstract

The effect of repetitive nitrogen (N) applications (+N; 150 and 300 kg N ha^{-1} $year^{-1}$) compared to zero N (-N) on light absorption, partitioning and radiation use efficiency in mixtures of perennial ryegrass with a large and a small-leaved clover as well as their monocultures were studied during spring and summer. Light competition in mixtures was quantified using a demand-supply analysis.

Clover *LAI* was significantly decreased in +N compared to -N mixtures and monocultures. Grass *LAI* in fertilised mixtures was significantly higher than in -N mixtures and was close to that in the N150 monoculture. In -N mixtures partial overtopping of clover was only observed in spring. In +N mixtures, the large-leaved clover was overtopped only in spring, but strong overtopping of the small-leaved cultivar was observed during both spring and summer regrowth periods. In large-leaved clover, petiole lengths increased in +N mixtures but such a response was not obtained in small-leaved clover. Using this advantage, large-leaved clover raised its leaves to the top layers of the canopy which resulted in different light profiles of the two clover cultivars. Analysis of light competition showed that in spring, both in +N and -N mixtures, grass was the stronger light competitor. However, during summer large-leaved clover was the stronger competitor in -N mixture and had the same competitive ability as grass in +N mixture. Grass in N150 and N300 monocultures absorbed the same amount of light and the observed dry matter (DM) yield differences between these treatments resulted from the effect of N on radiation use efficiency (*RUE*). However, the difference in DM yield of grass between +N and -N mixtures was due to the combined effect of increase in intercepted light and *RUE*. Clover had a lower *RUE* than grass in monoculture as well as in mixtures with no difference between cultivars. The yield difference between clover cultivars in +N mixtures was the result of a difference in the amount of intercepted light.

Key words: Perennial ryegrass, white clover, light interception, light partitioning, radiation use efficiency, mixture, monoculture, nitrogen, light competition, simulation.

Introduction

Balance between grass and clover content is the main driving force of resource sharing and dynamics of these species in a mixed sward. This balance will be disturbed by application of N because clover persistence is incompatible with N fertilisation (Frame *et al.*, 1983; Laidlaw, 1984; Fisher and Wilman, 1995; Soussana and Arregui, 1995). Stern and Donald (1962) showed that the negative effect of N fertiliser on clover (*Trifolium subterraneum*) was due to its overtopping by the companion grass (*Lolium rigidum*). However, it has also been found that white clover leaves were not overshadowed by perennial ryegrass, neither in -N (Woledge *et al.*, 1992a) nor also in +N (Woledge, 1988) swards. Since light sharing between species was not studied in these experiments, Faurie *et al.* (1996), using a light partitioning model, re-analysed the data of Woledge (1988) and showed that the vertical dominance of clover is not present in swards with high N fertility.

In a previous experiment with -N ryegrass-white clover mixtures (Chapter 6), partial overtopping of clover was observed only in spring regrowth. In Woledge's experiment with large and small-leaved clover cultivars (Woledge *et al.*, 1992a) no N was applied, whereas the data of the +N experiment (Woledge, 1988, Faurie *et al.*, 1996) were obtained from a mixture with a large-leaved clover cultivar (Blanca) with a single N application in spring. However, it is evident that clover is more sensitive to repetitive N application. The intensity of damage depends on clover cultivar and is higher in mixtures with small-leaved clovers (Wilman and Asiegbu, 1982; Frame and Boyd, 1987). Unfortunately, light competition ability of small and large-leaved clover cultivars under repetitive N application has not been studied in detail.

The different performance of grass and clover in +N or -N mixtures could result from the change in the light intercepted by species and/or its use efficiency (*RUE*). It has been shown that *RUE* remains relatively constant among species with the same metabolic pathways (Russell *et al.*, 1989). Nevertheless, grass and clover have a different *RUE* when grown in mixture or in pure stand (Sinoquet *et al.*, 1990). However, the effect of N on the contribution of *RUE* and light interception to dry matter yield of species is not fully understood.

In this paper the response of large and small-leaved white clover cultivars in mixture with perennial ryegrass was studied under repetitive nitrogen applications during the growing season and the results were compared with -N swards and pure grass and clover stands. The objectives were quantification of the effect of clover cultivar and nitrogen on canopy development, light competition, light interception and *RUE* in mixed and pure canopies.

Materials and Methods

Experimental site, design and management

In 1996, a series of monocultures and mixtures ranging from extreme clover dominance to extreme grass dominance was established by application of varying levels of N to grass-clover mixtures sown in autumn 1995 on an actually nitrogen-deficit sandy soil at Wageningen, The Netherlands. The white clover (*Trifolium repens*) cultivars Alice and Gwenda (large and small-leaved, respectively) and two perennial ryegrass (*Lolium perenne*) cultivars Barlet (diploid, erect) and Heraut (diploid, prostrate) were used to make four different mixtures (mixtures hereafter referred to by the first letter of their component cultivar names). Monocultures of the clover cultivars were also sown. No fertiliser N was applied on clover monocultures. However, the mixtures were grown under two N levels, 0 (-N) and 150 kg N ha^{-1} (+N) during the growing season. The seeding rate was 4 and 25 kg ha^{-1} for clover and grass, respectively. Plot sizes were 2.8x8.5 m. In a second trial monocultures of both ryegrass cultivars were established in 2.8x7 m plots. The seeding rate was the same as in the mixtures (25 kg ha^{-1}). During the growing season, the monocultures received 3 levels of N (0 (N0); 150 (N150) and 300 kg N ha^{-1} (N300)). In both trials a complete randomised design with two replicates was used. Sampling started in May and continued till October 1996. In both trials, all plots were cut at an approximate target yield of 2000 kg DM ha^{-1} (which took 5 to 7 weeks depending on growth conditions) at a stubble height of 5 cm. This allowed for 5 cuts over the whole growing season with exception of N0 grass monocultures where poor growth allowed only 3 cuts on these plots. Increasing N doses in pure grass were applied during the growing season (Chapter 3, Table 1) to simulate the expected increasing amount of clover-derived N in mixtures (Elgersma and Schlepers, 1997). Analysing DM yield and growth of leaf area during the season (Chapter 3) showed no significant differences between grass cultivars. Therefore, in this paper the results are presented only for one grass cultivar (Barlet) during spring (10 May-17 June) and summer (22 July-2 September) regrowth periods.

Measurements

The *PAR* (photosynthetically active radiation) absorbed by mixed and pure canopies was measured weekly using a linear ceptometer at successive 5-cm layers from the top. Vertical distribution of leaf area and height of species was measured weekly in mixtures and monocultures during each regrowth period, using an inclined point quadrat with the inclination angle 32.5° (Warren Wilson, 1965) (see Chapter 5 for details). For each regrowth period the total aboveground biomass was sampled weekly by cutting a 10x10 cm area. The harvested materials were separated into grass and clover; the weight of each component was determined after drying for 24 hours at 70° C. In the calculation of cumulative aboveground biomass the dry weight of the stubble was not included, because stubble is usually left after harvest. At the end of the summer regrowth, the length of the clover petioles was measured in 50 randomly selected plants for each replication. The daily solar radiation and temperature were obtained from the meteorological station, within 500 m from the experimental site. The average daily temperature and daily total of incoming radiation during the season are presented in Table 1. The daily *PAR* is assumed to be half of the daily total radiation (Goudriaan and van Laar, 1994).

Table 1. Average minimum, maximum and mean daily temperature and daily totals of incoming global radiation during the growing season.

	Temperature (°C)			Global radiation
	Mean	Maximum	Minimum	(MJ m^{-2} d^{-1})
May	10.6	15.5	4.7	15.52
June	15.6	20.9	9.3	18.61
July	16.2	21.3	10.5	17.43
August	17.3	22.5	11.8	13.47
September	12.2	17.2	6.7	11.18

Light interception and partitioning

Light interception by species was simulated using a multi-layer model for heterogeneous canopies. The structure of the model is described in Chapter 5 and validated with an independent data set. The model calculates the daily amount of absorbed light by species *i* within layer *h* in the canopy ($I_{h,i}$ J m^{-2}). Summation over all layers gives the daily intercepted *PAR* for each species.

Total *LAI* and total height of species are the model inputs. The daily values of both *LAI* and height were estimated using non-linear relationships between weekly measurements for each treatment and the corresponding temperature sum (Kropff and Lotz, 1993). For the calculation of the temperature sum the base temperature was set at 4 °C for grass and 7°C for clover (Harris, 1987). In the monocultures, *k*-values were estimated as the slope of the linear regression between log-transformed values of the ratio of measured *PAR* within canopy depth to the top of the canopy (I/I_0), and the cumulative downward *LAI*. For mixtures, dispersion free *k*-values of 1.00 and 0.52 were used for clover and grass respectively, and a leaf dispersion parameter (ξ) for each species was estimated as described in Chapter 5. The reflection of the canopy was assumed to be 5% of the total incoming *PAR* (Goudriaan and van Laar, 1994).

Analysis of light competition

In mixtures, the moment at and the canopy layer in which light competition occurs were analysed by calculating the amount of light subjected to competition following the approach used in the CropSys model (Caldwell and Hansen, 1993) and described by Sinoquet and Caldwell (1995).

In the mixed canopy the distinguished light fractions are the light fraction actually captured by grass (G), actually captured by clover (C), actually captured by both grass and clover (GC), and the fraction captured neither by grass nor by clover (G^0C^0). The values for the fraction that theoretically could have been captured by grass if all clover leaves had been removed (fraction G^+) or the fraction that theoretically could have been captured by clover if all grass leaves had been removed (fraction C^+), were estimated by means of simulation. G^+ and C^+ are termed the light "demanded" by grass and by clover, respectively. The fraction $(G^+ - G) + (C^+ - C)$ is thus the light fraction (G^+C^+) subjected to competition. If $(G^+ - G) < (C^+ - C)$, then grass is the stronger light competitor and if $(G^+ - G) > (C^+ - C)$ clover is the better competitor. When the two fractions are identical, light is equally shared between the two species.

Using this approach the time course of light "captured" and "demanded" by the species as well as light subjected to competition could be simulated for each regrowth period by running the model in the presence of both species and by removal of grass or clover from the model. It is assumed that removal of a species has no effect on the structure of the remaining species.

Radiation use efficiency (RUE)

The cumulative intercepted *PAR* for each species and for each regrowth period was calculated by summing the daily values obtained from the model. The *RUE* (g DM MJ^{-1}) of each species was calculated as the slope of linear relationships, forced through the origin, between the cumulative intercepted *PAR* and the cumulative aboveground DM of that species during a given regrowth period.

during the season (Figures 5 a, b). Grass with a lower rate of leaf growth in the N0 treatment only absorbed 40 - 50% of the total incoming *PAR* by the end of each regrowth period (not shown). A closed canopy was also not found in pure clover in the first regrowth period. In summer, however, both clover cultivars had a closed canopy after 4 weeks (Figures 5c, d). The effect of clover cultivar on light absorption in a pure stand was practically zero.

In mixtures, in addition to the leaf area of species, light interception was also affected by canopy structure, mainly through the height of maximum leaf area density (h_m). Despite a strong response to N in absorbed *PAR* by grass, clover captured a significantly higher fraction of the light than its contribution to the *LAI* of the mixed canopy (compare Figures 2 and 7a, b). This advantage of clover in absorption of a higher *PAR* per unit leaf area was most pronounced in the -N mixture and in the summer growth cycles (Figure 7b). In addition, a substantial difference was observed between clover cultivars, in particular in -N swards, which was due to a higher h_m in the large-leaved clover compared to the small-leaved cultivar.

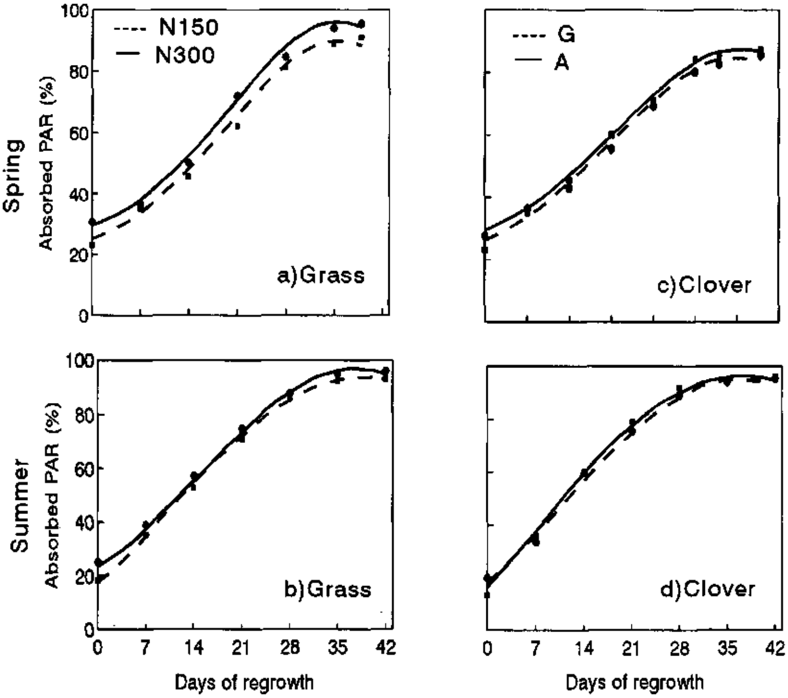

Figure 5. Simulated time course of total absorbed *PAR* (%) of grass and clover in pure stands. Measured values for pure grass in N150 (■) and N300 (●) and monocultures of Alice (■) and Gwenda (●) are also shown.

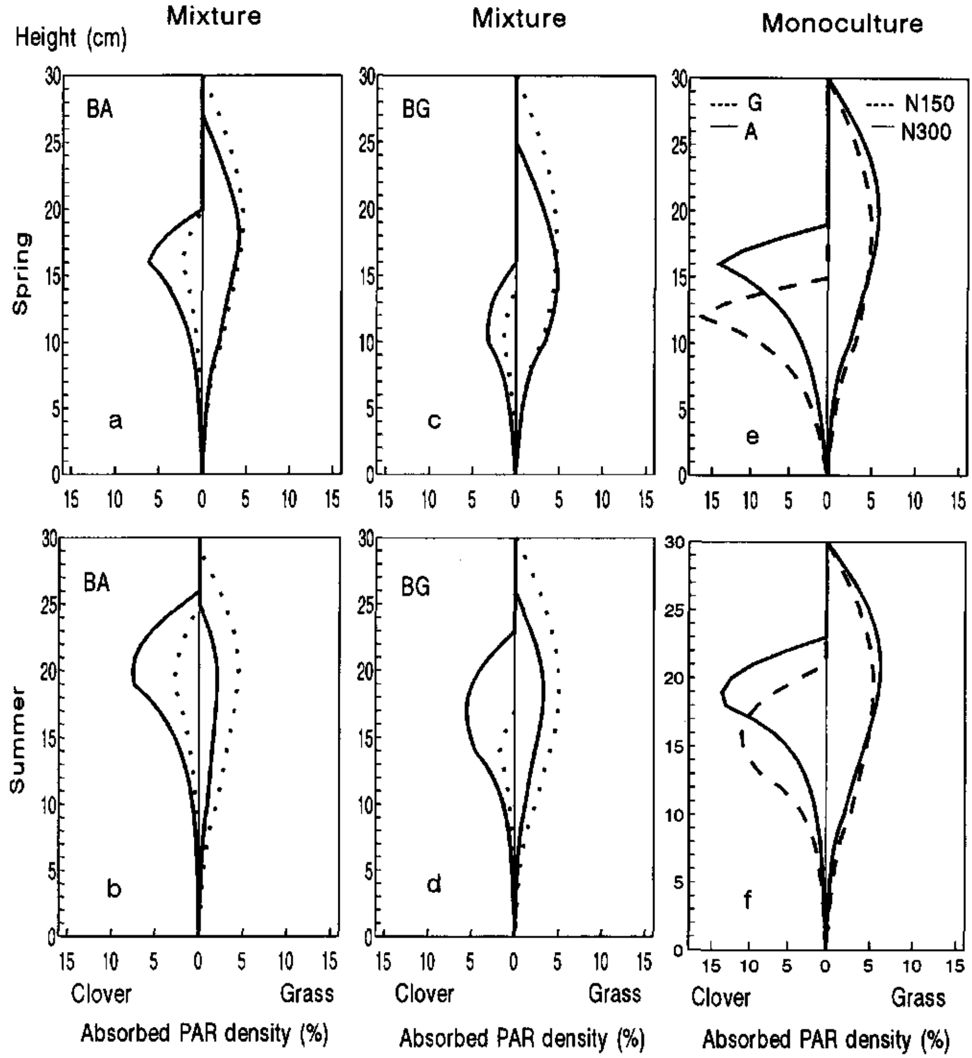

Figure 6. Simulated profile of *PAR* density for grass and clover grown in mixture and in pure stands at the end of spring and summer regrowth periods. In mixtures: -N (—) and +N (····); in monocultures: Gwenda (----), Alice(—), N150 (----) and N300 (—).

Vertical profile of light in mixed and pure stands

The model was used to study the vertical sharing of light between components of mixtures and to compare absorbed *PAR* of each component with that of their pure stands.

Monocultures: In grass monocultures the density of absorbed *PAR* over height was the same in the N150 and the N300 treatments and had an identical pattern during both regrowth periods (Figures 6e and f). In clover monocultures a significant difference ($P<0.05$) in the height of the maximum absorbed *PAR* density was observed between cultivars. The difference appeared from the second week of regrowth (not shown) and became larger by the end of the cycles. In contrast to grass, the profile of light density in clover decreased sharply downwards after a rapid increase in the top layers, which was in accordance with the leaf area distribution of species. In the N0 grass stand the low *LAI* and corresponding low *PAR* absorption resulted in an undeveloped *PAR* profile within the canopy (not shown).

Mixtures: The simulated profiles of *PAR* density over canopy height of mixtures by the end of the spring and summer regrowth periods are shown in Figures 6 a-d. In spring, clover was overtopped both in +N and -N treatments. However, the large-leaved clover had a greater maximum absorbed *PAR* density the in -N sward than the small-leaved cultivar (Figures 6a and b). However, in summer the grass was overshaded by clover in -N mixtures, in particular with the large-leaved cultivar Alice (Figures 6b, c and d). During summer, both species had the same *LAI* in the -N mixtures (Figure 2). However, both clover cultivars had a higher absorbed *PAR* density above 15 cm canopy height than grass. In addition, the total absorbed *PAR* by clover (area enclosed by the curves) was significantly higher ($P<0.05$) compared to that of grass. This advantage of clover is due to its canopy structure, with a higher proportion of its leaf area at a higher position in the canopy, and its higher light extinction coefficient. In the +N swards overtopping of clover was obvious in both growth cycles. While shading by grass was avoided in the large-leaved clover by increasing its h_m, no such avoidance strategy was observed in the small-leaved clover (not shown). Therefore, the height where the maximum absorbed *PAR* density occured was maintained in Alice at +N (Figures 6a and b), but lowered in Gwenda (Figures 6c and d).

Quantification of light competition in the mixed canopy, a supply-demand analysis

Light competition between species was quantified as the sum of differences between the supply and the demand for species in mixture (e.g. the light subjected to competition).

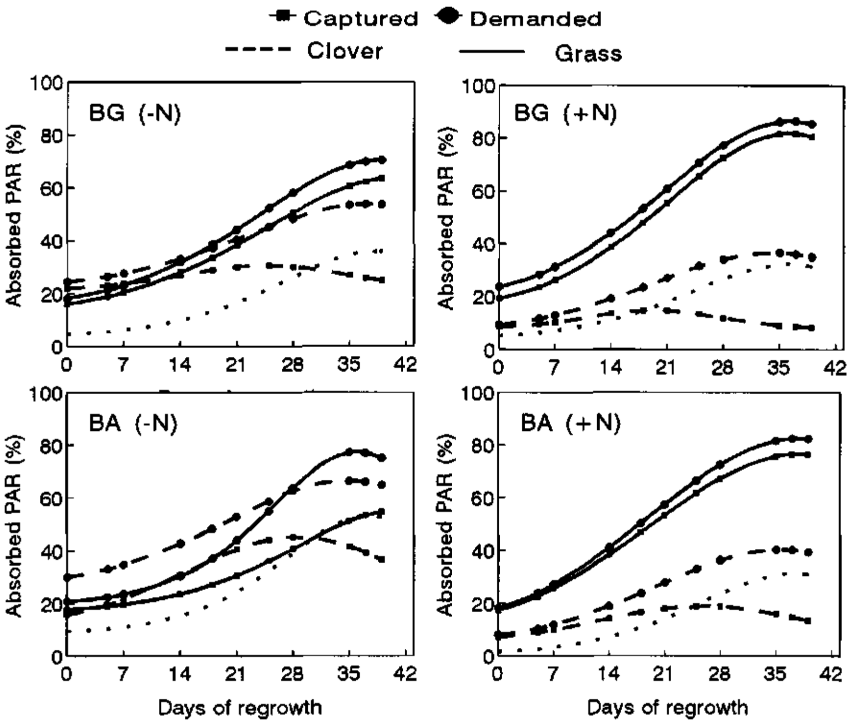

Figure 7a. Simulated daily fraction of captured and demanded *PAR* by grass and clover in different mixtures and the daily fraction of light subjected to competition (dotted line) during spring, see text for details.

Spring regrowth: In the +N mixtures the light captured by grass was very close to its demand and comparable to the N150 grass monoculture during the same period. In contrast, for clover a significant difference was found between the light supply and the demand, which was not affected by clover cultivar (Figure 7a). Since the fraction (G^+-G) was also lower than (C^+-C) the light subjected to competition was mainly intercepted by grass. This one sided competition in the +N mixtures increased slowly during the first 2 weeks of regrowth, but with a higher rate onwards. This reflects the higher grass *LAI* and overtopping of clover which became especially visible after 2 weeks (Figure 3a). In the -N mixtures, clover demanded more light than in the +N mixtures. In BG(-N), the difference between supply and demand was greater in clover than in grass and the amount of light subjected to competition was identical to that in BG(+N) (Figure 7a). Grass and clover had the same *LAI* in BG(-N) and BA(-N) (the same demand of grass and clover), but the large-leaved clover captured more light than the small-leaved cultivar. As a result, a higher fraction of light was subjected to competition.

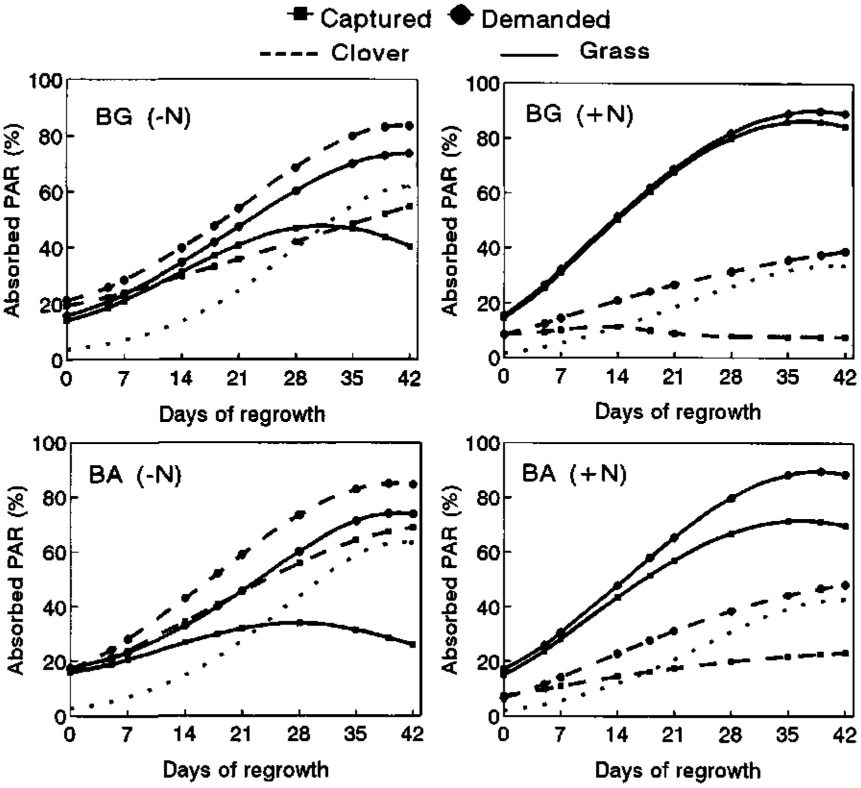

Figure 7b. Simulated daily fraction of captured and demanded *PAR* by grass and clover in different mixtures and the daily fraction of light subjected to competition (dotted line) during summer, see text for details.

Summer regrowth: During summer, grass demanded less light in the +N mixtures than in the monocultures, but it was significantly higher compared to its demand in -N mixtures (Figure 7b). While both clover cultivars had the same demand, both in the +N and the -N mixtures, the light really captured by clover was higher in the large-leaved cultivar (Figure 7b). In BG(+N), where clover was intensively overtopped, grass captured nearly all of its demand. In BA(+N), however, the difference between supply and demand in grass became significant after 3 weeks of regrowth. In the -N mixtures the light subjected to competition was higher than in the +N mixtures. In BG(-N) the fraction (G^+-G) was the same as (C^+-C) and therefore the light subjected to competition was equally shared between species. In BA(-N), however, clover captured a higher proportion of the light subjected to competition [(G^+-G) > (C^+-C)].

Critical zone of light competition

The light competition region in the mixed canopies were studied by the simulated removal of each species from the top downwards, at the end of both regrowth periods.

Simulated removal of grass: In spring, when clover was overtopped by grass, in all mixtures grass had a wider competition zone over canopy depth and clover reached 90% of its demanded light after moving all grass leaves above about 10 cm height. Besides, the rate of increase in the fraction captured by clover per cm removal of grass canopy was higher in the BG mixtures compared to that in the BA mixtures (Figure 8a). During summer, clover cultivar and nitrogen had a significant effect on the light competition zone (Figure 8c). In this period the response of clover in the BG(+N) mixture to grass removal was the same as in spring, but in BG(-N) clover achieved 90% of its demand when grass was removed above 15 cm. The response of the large-leaved clover in BA(+N) was similar to that in BG(-N), while in BA (-N) clover reached 90% of its demand already by removing grass above 20 cm.

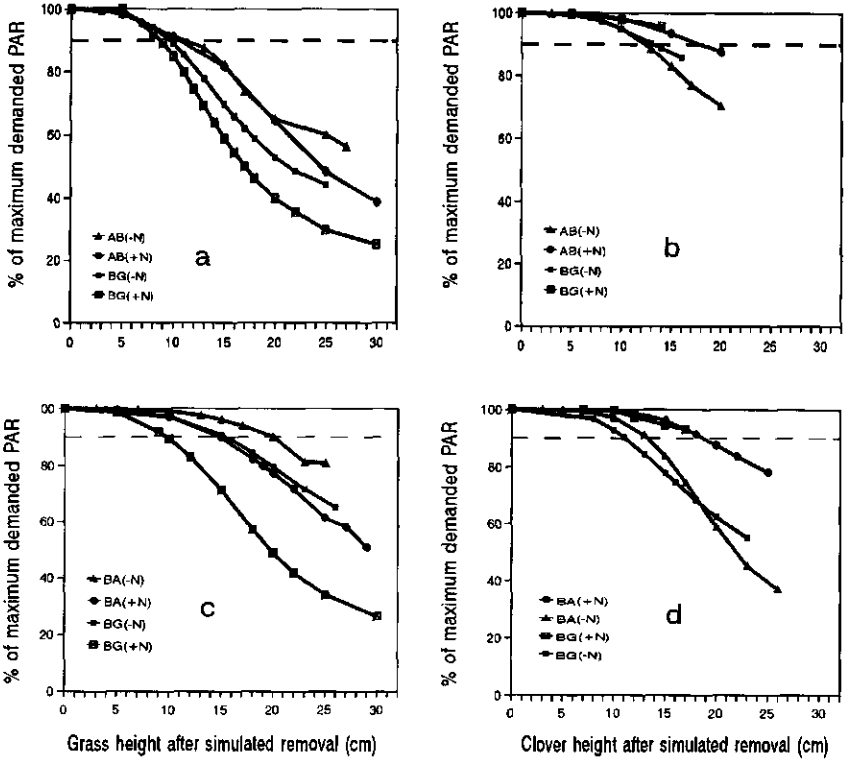

Figure 8. Increase in light captured by grass and clover during layerwise simulated removal of species from the top of the canopy at the end of spring (a and b) and summer (c and d) regrowth.

Simulated removal of clover: Since in spring the light captured by grass was close to its maximum demand, grass showed a small response to removal of clover, but in the -N mixtures removal of clover above 12 cm was needed for grass to reach 90% of its maximum demand (Figure 8b). The response of grass to clover removal was more pronounced in summer, particularly in the -N mixtures (Figure 8d). The rate of increase in light captured by grass per cm removal of clover was higher in BA(-N) compared to that in BG(-N). Therefore, grass reached 90% of its demand when the large-leaved clover was removed above 13 cm, compared to 11 cm for the small-leaved clover (Figure 8d).

Radiation use efficiency

Table 3 gives the final biomass and accumulated radiation by grass and clover at the end of the two regrowth periods, together with the calculated radiation use efficiency (*RUE*) of each species in mixtures and pure stands.

Perennial ryegrass: Grass had a higher *RUE* in mixtures than in monocultures (Table 3). The low *RUE* of pure N0 grass (1.13 and 0.63 g DM MJ^{-1} *PAR* in spring and summer, respectively) increased signicantly in the -N mixtures. The application of N led to an increase in the *RUE* of the grass monoculture. However, the difference between the N150 and the N300 monocultures was only significant in summer. Despite this increase, the *RUE* of the mixed -N grass was higher than that in N150 and N300 in spring, and the same as that in the fertilised monocultures in the summer. The grass in the +N mixtures had a higher *RUE* than in the -N mixtures, but the difference was only significant in summer (Table 3).

In both regrowth periods the grass DM in fertilised monocultures was significantly higher than in N0 pure grass. This DM yield difference was due to the combined effects of a higher *RUE* and a greater amount of cumulative absorbed *PAR* by the fertilised grass (Table 3). However, the observed yield difference between N150 and N300 grass in summer resulted mainly from the higher *RUE* in the N300 treatment. Grass had a higher DM yield in the +N than in the -N mixtures. In spring this difference was due to an improvement in absorbed *PAR* by grass in the +N mixtures, but in summer both absorbed *PAR* and *RUE* contributed to the increased grass DM yield (Table 3).

White clover: In contrast to ryegrass, clover had a higher *RUE* when grown as a pure stand than in mixture. No significant difference in *RUE* was obtained between clover cultivars, both in mixtures and in pure stands (Table 3). Both cultivars had a very low *RUE* during spring, which increased later in the season. In the -N mixtures and in the monocultures the same *RUE* and the same amount of cumulative absorbed light led to identical DM yields of cvs. Alice and Gwenda (Table 3). The application of N to the mixtures had no effect on clover *RUE*, but led to a significant reduction in absorbed *PAR* by clover. However, in the +N mixtures the reduction in cumulative absorbed *PAR* was significantly higher in small than in large-leaved clover. This can explain the observed difference in DM yield between clover cultivars in fertilised mixtures during spring and summer (Table 3).

Table 3. Total dry matter and cumulative absorbed *PAR* by grass and clover grown in mixed or pure stands together with radiation use efficiency (*RUE*) of species in different regrowth periods (*RUE* is the slope of the linear relationship between cumulative absorbed *PAR* and dry matter, forced through the origin).

	DM (g m^{-2})		Cumulative absorbed *PAR* (MJ m^{-2})		*RUE* (g DM MJ^{-1})	
	Grass	Clover	Grass	Clover	Grass	Clover
Spring regrowth (10 May-17 June)						
Mixtures						
BA(-N)	234.2b	68.5a	124.9b	128.8a	1.95ab	0.53a
BA(+N)	400.4a	31.2b	194.7a	54.1d	2.14a	0.55a
BG(-N)	276.8b	52.7a	149.8b	95.3b	1.86b	0.54a
BG(+N)	396.7a	13.3c	206.4a	36.0d	2.02ab	0.41a
Monocultures						
B(N0)	142.4b	-	127.8b	-	1.13b	-
B(N150)	403.2a	-	237.1a	-	1.68a	-
B(N300)	434.1a	-	255.6a	-	1.68a	-
Alice	-	179.2a	-	177.5a	-	1.02a
Gwenda	-	162.3a	-	171.1a	-	0.96a
Summer regrowth (22 July-2 September)						
Mixtures						
BA(-N)	98.2b	138.7a	75.4b	113.6a	1.30b	1.20a
BA(+N)	213.9a	68.4b	134.9a	56.7b	1.53a	1.21a
BG(-N)	123.1b	125.7a	95.1b	102.8a	1.26b	1.22a
BG(+N)	234.7a	36.9c	157.9a	25.4c	1.50a	1.31a
Monocultures						
B(N0)	55.1c	-	87.9b	-	0.63c	-
B(N150)	190.0b	-	160.6a	-	1.19b	-
B(N300)	231.7a	-	177.3a	-	1.32a	-
Alice	-	223.1a	-	163.2a	-	1.40a
Gwenda	-	218.2a	-	159.1a	-	1.38a

Figures accompanied by different letters are significanly different ($P < 0.05$). Comparison should be made within mixtures or within monocultures for each regrowth period separately.

Discussion

Overtopping of clover in mixed canopies

Woledge *et al.* (1992a) observed in an experiment with a large and a small-leaved clover cultivar in mixtures with ryegrass and tall fescue, without N fertiliser, that both clover cultivars were only slightly overtopped during spring. We observed the same results in -N mixtures in spring and in summer (Figures 3a,b and 6a,c). In +N mixtures, both clovers were overtopped in spring, with a higher intensity in the small-leaved clover, while during summer overtopping was only found in the BG(+N) mixture. It seems that the conclusion that clover will not be overtopped even in +N mixtures (Woledge, 1988) is valid only when large-leaved clover is considered, with a single spring N application. Small-leaved clovers under repetitive applications of N will be severely overtopped by grass. Wilman and Asiegbu (1982) showed that the length of clover petioles will increase in response to N application. In the present study this response was observed only in large-leaved clover, especially in summer. Gautier *et al.* (1995) studied the effect of light quality (red : far-red ratio) on the outcome of competition in grass-clover mixtures. They hypothesised that a low red : far-red ratio after cutting will stimulate the growth of clover petioles, giving clover the ability to put its leaves in the upper part of the canopy, but when N is applied this response is not sufficient to overcome the greater growth of grass. Our results showed that large-leaved clover, using this genotype-related response (Gautier *et al.*, 1995), avoided overtopping by grass to some extent in spring and completely during summer in +N mixtures (Figure 4a). However, such a response was not observed at all in the small-leaved cultivar when N was applied (Figure 4b).

Light competition in space and time

Light competition starts when one species captures a lower fraction than its demand in absence of the other species (Sinoquet and Caldwell, 1995). The amount of light subjected to competition is then determined by the degree of association between the foliage of the mixed canopy both in space and in time. During spring, the application of N resulted in more space above the clover canopy for grass compared to the unfertilised mixtures, especially above the small-leaved clover (Figure 3a). In this period the canopy was closed after 35 days of regrowth and the intensity of competition (sum of the difference between light demand and supply of species) remained unchanged or showed a slight increase after canopy closure (Figure 7a). In spring, both in +N and -N mixtures, grass was the stronger light competitor ($(G^{+}-G) < (C^{+}-C)$). In summer, this pattern was only found in the +N mixtures. During this regrowth period the canopy was closed after 30 days in the -N mixtures. Simulation results showed however, that the light subjected to competition increased after canopy closure in the -N mixtures, which was due to a reduction in the fraction captured by grass (Figure 7b). In summer, the large-leaved clover cultivar had a higher competitive ability in the -N mixture $((G^{+}-G) > (C^{+}-C))$. However, in BG(-N) and BA (+N), where the fraction $(G^{+}-G)$ was identical to $(C^{+}-C)$, both species had the same competitive ability and the light subjected to competition was equally shared. Davidson and Robson (1986) suggested that the balance between species remains unchanged after canopy closure and the competitive ability of clover depends on the events preceded before this time. Our results similarly showed that if clover would be able to avoid overtopping by grass during the first weeks of regrowth, then it can compete strongly with grass after canopy closure. This happened in the -N mixtures, particularly in summer and especially in mixtures with large-leaved clover (Figures 3b and 6b).

Simulated downward removal of clover showed that in spring grass, with its higher light competitive ability and wide competition zone, obtained the main part of its light demand above clover height. The significant height difference between species during this period resulted in a low level of light competition (a lower fraction of the light subjected to competition). This is in accordance with the conclusion of Thornton *et al.* (1990), that the intensity of competition is low when the leaves of each species are situated in separate zones. This pattern was changed in summer when the height differences were small.

Interestingly, grass and clover showed the same response to simulated removal in BA(+N) and BG(-N) mixtures (Figures 8c and d), in which an identical light sharing pattern between species was observed (Figure 7b). However, in BA(-N) clover had a wider competition zone than grass. Keddy (1989) suggested that competition between pairs of species is symmetric when they are similar in size. Our results show that asymmetric light competition can be observed between species with a similar height when one species has a greater height of maximum leaf area density. This is in accordance with Chapter 5, where it is shown that the stronger competitor is not the plant with most leaves, but the plant which has its leaves in an advantageous position in the canopy.

Light interception raiation use efficiency and dry matter yield in mixtures and pure stands

The *LAI* was the main determinant of light interception by monocultures. Similar *LAI* and the same *RUE* in clover monocultures led to an identical *PAR* interception and DM yield in the large and small-leaved cultivar (Table 3). The availability of N had a substantial effect on the

RUE of pure stands of grass. The increased *RUE* with higher levels of N supply was in accordance with findings of Sinclair and Horie (1989) and Belanger *et al.* (1992). The difference in DM yield of N0 grass compared to fertilised grass was due to the combined effect of lower *PAR* interception and lower *RUE* in unfertilised grass (Table 3). In spite of different *LAI*s, N150 and N300 grass absorbed the same amount of *PAR* in all regrowth periods. Therefore, the observed differences in DM yield between fertilised grass was only due to *RUE*. Belanger *et al.* (1992), in a study of tall fescue with different N levels, also showed that above a given *LAI*, when the canopy is closed and light interception is complete, N will only affect *RUE*.

In accordance with Sinoquet *et al.* (1990) grass had a higher *RUE* when grown in mixture compared to a pure stand, but the inverse was observed in clover (Table 3). Higher DM yield of grass in +N compared to -N mixtures was due to both an increase in light interception and *RUE*, but the effect on absorbed *PAR* was dominating. On average, the *PAR* interception by grass in the +N compared to the -N mixture increased by 47 and 71% in spring and summer, respectively, while the increase in *RUE* was only 9 and 18%, respectively (Table 3).

The high values of *RUE* of mixed grass obtained during the first cycle, corresponding to its reproductive development, were due to a low application rate of N in spring as well as a higher proportion of DM allocated to aboveground material during this stage (Parsons and Robson, 1982). Davidson *et al.* (1986) and Lantinga *et al.* (1996) showed that N has a strong effect in retention of DM in grass shoots at the expense of investment in roots. In addition, grass has a high rate of CO_2 assimilation during spring, which decreases towards summer (Woledge and Pearse, 1985). This explains the observed reduction in *RUE* of grass in summer regrowth.

Clover growth, however, has been found to be more depressed by low temperature than that of grass (Davidson *et al.*, 1986; Arnott and Ryle, 1982), probably as a result of its lower rate of CO_2 assimilation under these conditions (Woledge, 1988). This accounts for the low *RUE* of clover obtained in this study, both in mixtures and in pure stands during the first cycle (Table 3). A further increase of clover *RUE* during summer was due to the promotion of its assimilatory capacity with temperature (Dennis and Woledge, 1982). In spite of the higher rate of CO_2 assimilation of clover than that of grass during summer (Dennis and Woledge, 1982; Chapter 8), clover still had a lower *RUE* than grass during summer. This might have been the result of a higher proportion of *PAR* absorbed per unit of leaf area of clover than grass, in particular in -N mixtures where the vertical dominance of clover was significant (Faurie *et al.*, 1996) (Figure 6) since photosynthetically light-saturated leaves of clover are less efficient than shaded leaves of grass (Sinclair and Horie, 1989).

Faurie *et al.* (1996) observed in controlled environment studies an increase in clover *RUE* in +N mixtures. However, under field conditions the *RUE* of mixed clover was not affected by N (Table 3). This could be partly due to an increase in non-photosynthetic supporting organs (petioles) of clover in +N mixtures (Wilman and Asiegbu, 1982) and a lower leaf weight ratio (*LWR*) (Chapter 3).

Introduction

N affects plant growth through leaf expansion and canopy development, and through leaf CO_2 assimilation. Leaf CO_2 assimilation depends on the activity of photosynthetic enzymes in the chloroplasts (mainly Rubisco) (Field, 1991). In C_3 species the chloroplasts, where the CO_2 is fixed, contain about 75% of the N in leaf tissues (Chapin *et al.*, 1987; Evans, 1989a). Generally, the light saturated CO_2 assimilation rate (A_{max}) of single leaves is highly correlated with the N concentration in leaf tissues (Field, 1983; Field and Mooney, 1986; Evans, 1989a). However, Lantinga *et al.* (1996) showed a "down-regulation" in the leaf N-CO_2 assimilation relationship with increasing N input levels. Field and Mooney (1986) argued that the correlation between the concentration of leaf N and A_{max} is a general case, which applies to plants across a wide range of plant communities.

Since leaf A_{max} responds strongly to N, it is evident that canopies with a low avarage N concentration will maximise CO_2 assimilation when upper leaves, which are more frequently exposed to high light, have a greater leaf N concentration than leaves lower in the canopy (Hirose and Werger, 1987a, b). Numerous studies have revealed that leaf N concentration declines with depth in closed canopies in many plant species (e.g. Field, 1983; Walters and Field, 1987; Lemaire *et al.*, 1991; Ellsworth and Reich, 1993; Anten, 1995). This phenomenon is generally believed to be related to the changing light profile within the canopy. It has been suggested that, given a fixed amount of N available to the leaves, plants re-allocate N in order to optimise total canopy CO_2 assimilation (Hirose and Werger, 1987a; Hirose *et al.*, 1988). Goudriaan (1995), using a mathematical analysis, showed that the maximum canopy CO_2 assimilation rate is reached when the N distribution over the leaf canopy follows the light profile. This leads to the conclusion that canopy CO_2 assimilation will depend not only on the amount, but also on the vertical distribution of N within the canopy (Pons *et al.*, 1993; Wright and Hammer 1994; Connor *et al.*, 1995; Hikosaka and Terashima, 1996).

Studies of N distribution in canopies are mainly limited to single species. Lemaire *et al.* (1991) studied the N distribution within a lucerne canopy and suggested that the results obtained in pure stands could be extrapolated to mixtures. Anten and Werger (1996) showed in mixtures of two species differing in height that the pattern of leaf N distribution depends on the position of the species in the canopy. Experimental results (Chapter 4) showed that in grass and clover the pattern of leaf area and light distribution over canopy height was different in mixtures and monocultures. This can affect the leaf N distribution as well. However, very little is known about the importance of the N distribution profile for daily CO_2 assimilation in multispecies canopies, such as grass-clover mixtures.

This papers deals with the vertical distribution of leaf N within canopies of grass and clover in pure stands and in mixtures as influenced by the level of fertiliser N, and the consequences for canopy CO_2 assimilation. The objectives were (1) to show the effects of light competition between species on the development of leaf N profiles in mixture compared to monoculture and (2) to compare the effects of different patterns of leaf N distribution on canopy CO_2 assimilation in monoculture and mixture.

Materials and methods

Plant material and experimental design

Mixtures and monocultures of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) were used in this study. The swards were sown in autumn 1995 on a sandy soil in Wageningen, The Netherlands (Chapter 3). A large-leaved white clover cultivar (Alice) was sown in mixture with a diploid perennial ryegrass cultivar (Barlet) in 2.8 x 8.5 m plots in two replications. The seeding rate was 4 and 25 kg ha^{-1} for clover and grass, respectively. In the grass and clover monocultures plot size and seeding rates were similar to those used in mixture. During the growing season the mixtures received two levels of N (0 and 150 kg ha^{-1}), referred to as -N and +N hereafter. No N was applied to the clover monoculture. Grass monocultures received 3 levels of N (0, 150 and 300 kg $ha^{-1}y^{-1}$). The swards were cut at a target yield of 2000 kg DM ha^{-1}, which took 5-7 weeks, depending on weather conditions. There were 5 regrowth periods during the growing season. At the end of each regrowth period whole plots were harvested at 5 cm above ground level. The results presented in this paper are taken from one regrowth period (22 July-2 September) in 1996.

Measurements

The vertical distribution of photosynthetically active radiation (*PAR*, 400-700 nm) was measured weekly with a linear ceptometer in 5-cm canopy layers.

At weekly intervals, all plant material in a 10 x 10 cm area was cut at ground level. The samples were taken to the laboratory and cut in 5-cm layers with a paper cutter, keeping the leaf inclination as natural as possible. The material of each layer was separated into grass leaf, grass (pseudo-) stems plus leaf sheaths, clover leaf blades and clover petioles. The leaf area of both species in each layer was measured with an electronic leaf area meter (Li-Cor 3100®, Li-Cor Inc., Lincoln, NE, USA). Leaf dry matter (DM) was measured after drying at 70 °C for 24 hours. Dried leaves were ground and their N concentration (g N g^{-1} leaf DM) was measured by Kjeldahl analysis, using an autoanalyser with a continuous-flow analysing system. For each species, the leaf N concentration was multiplied by the specific leaf weight (SLW, g m^{-2} leaf) obtained for each layer, to calculate the leaf N concentration on a leaf area basis (g N m^{-2} leaf) for each layer.

The leaf CO_2 assimilation measurements were conducted on 8, 16, 23 and 30 August in grass and clover monocultures. The light saturated CO_2 assimilation rate of grass and clover leaves was measured at 210 J m^{-2} s^{-1} *PAR* using an open gas exchange system (ADC, UK) with different leaf chambers for grass and clover. At each date, measurements were conducted on ten clover leaves and six grass leaves from each N treatment. During the measurements the temperature and relative humidity of the leaf chamber were in the range of 17-23 °C and 70-90%, respectively. After these measurements the area of the leaves was determined with an electronic leaf area meter. Their N concentration was measured after drying at 70 °C for 24 hours.

Calculations

The leaf N distribution and canopy CO_2 assimilation were studied in the -N and +N mixtures, clover monoculture and the N150 grass monoculture.

N profile: The leaf N concentration of each layer within the canopy (N_h, g N m^{-2} leaf) was fitted to the Hirose and Werger (1987a) equation:

$$N_h = N_0 \exp(-k_N L_h / L) \qquad (1)$$

where N_0 is the leaf N (g N m^{-2} leaf) at the top of the canopy, k_N the extinction coefficient of N, L the total leaf area index (*LAI*) and L_h the *LAI* at depth h within the canopy. A value of 0 for k_N indicates a uniform profile of leaf N per unit area, in which all leaves have the same N concentration equal to the mean. The value of k_N increases with increasing non-uniformity in N distribution (Hirose and Werger, 1987a).

N_0 and k_N were estimated from the log-transformed form of equation 1:

$$\ln N_h = \ln N_0 + k_N (L_h / L) \qquad (2)$$

Light profile: Light absorption by species was calculated using a multi-layer canopy model for grass-clover canopies (Chapter 5). In the monocultures, the apparent *k*-values were estimated as the slope of the linear regression between log-transformed values of I/I_0 and the cumulative downward *LAI*. This gave apparent *k*-values of 0.60 and 0.94 for grass and clover, respectively (Chapter 7). In mixtures dispersion-free *k*-values of 0.52 and 1.00 were used for grass and clover, respectively (Chapter 5). Reflection of the canopy was assumed to be 5% of the total incoming *PAR* (Goudriaan and van Laar, 1994). The daily total of incoming radiation ($J\ m^{-2}\ day^{-1}$) was recorded at a meteorological station situated about 500 m from the experimental site. Daily *PAR* was assumed to be half of the daily total radiation. The daily course of *PAR* above the canopy (I_0) was calculated based on daylength, latitude, day of the year and the sine of solar height, as described by Goudriaan and van Laar (1994).

Canopy CO_2 assimilation: The gross CO_2 assimilation light response for leaves can be approximated by: (Goudriaan and van Laar, 1994)

$$A_h = A_m\,(1\text{-}\exp\,(-\,\varepsilon\, I_a\,/\,A_m)) \qquad (3)$$

where A_h is the gross CO_2 assimilation rate ($kg\ CO_2\ ha^{-1}\ h^{-1}$), A_m is the maximum CO_2 assimilation rate ($kg\ CO_2\ ha^{-1}\ h^{-1}$), ε the initial light use efficiency ($kg\ CO_2\ ha^{-1}\ h^{-1}/(J\ m^{-2}\ s^{-1}$) and I_a is the absorbed *PAR* ($J\ m^{-2}\ s^{-1}$). The gross canopy CO_2 assimilation rate (P_c) of each species was calculated based on the absorbed *PAR* by that species. For each species the instantaneous A_h of each canopy layer can be calculated from the estimated absorbed *PAR* in that layer.

The effect of leaf N profile on canopy CO_2 assimilation was compared with a uniform N distribution. For a non-uniform N profile, A_m of each canopy layer was calculated based on the leaf N concentration in that layer (N_h, equation 1) and the established relationship between A_m and leaf N. For a uniform N profile, k_N was set at zero and the N concentration of each layer equal to the mean. For each species the mean N concentration of leaves (N_m) was calculated as the total leaf N concentration of that species (N_t) divided by total *LAI* (L):

$$N_m = 1/L \int N_h\, dL \qquad (4)$$

Woledge and Dennis (1982) reported that leaves of perennial ryegrass and white clover have the same assimilatory characteristics.

Therefore, the value of $\varepsilon = 0.36$ ($kg\ CO_2\ ha^{-1}\ h^{-1}/(J\ m^{-2}\ s^{-1})$), reported for ryegrass by Wilson (1975), was used for both species, assuming ε is independent of leaf N concentration (Wilson, 1975; Ehleringer and Pearcy, 1983). The daily total gross canopy CO_2 assimilation (P_{day}, $kg\ CO_2\ ha^{-1}\ day^{-1}$) of grass and clover was calculated by integration of instantaneous rates over canopy height and daylength.

To isolate the effect of leaf N distribution on daily canopy CO_2 assimilation (Hirose and Werger, 1987a) temperature was assumed to be constant throughout a day.

Results

Distribution of leaf N

Both in mixture and in monoculture the leaf N concentration (N_h) of grass and clover decreased from the top to the base of the canopy. The estimated values of N_0 (leaf N concentration at the top of the canopy) and k_N (extinction coefficient of N) for different days from the start of regrowth are shown in Table 1. In grass, N_0 tended to decrease towards the end of the regrowth period, particularly in the -N mixture, but such a tendency was not observed in clover.

Table 1. Estimated values of N_0 (g N m^{-2}) and k_N based on equation 1 for grass and clover in mixture and monoculture. The values are shown for four successive weeks during summer regrowth (22 July-2 September)

Days of regrowth	Treatment	Grass			Clover		
		N_0	k_N	r^2	N_0	k_n	r^2
21 days	Mixture (-N)	1.925	0.489	0.972**	2.068	0.492	0.931**
	Mixture (+N)	1.922	0.450	0.934**	1.915	0.531	0.961**
	Monoculture*	1.873	0.497	0.966**	2.296	0.343	0.958**
28 days	Mixture (-N)	1.644	0.506	0.876**	2.076	0.602	0.941**
	Mixture (+N)	2.030	0.549	0.947**	2.169	0.679	0.932**
	Monoculture	2.109	0.633	0.911**	2.303	0.486	0.988**
35 days	Mixture (-N)	1.680	0.607	0.895**	2.217	0.650	0.946**
	Mixture (+N)	1.761	0.646	0.981**	2.228	0.741	0.942**
	Monoculture	1.963	0.689	0.886**	2.223	0.519	0.875**
42 days	Mixture (-N)	1.582	0.622	0.937**	2.012	0.689	0.947**
	Mixture (+N)	1.750	0.702	0.903**	2.145	0.845	0.928**
	Monoculture	1.732	0.761	0.938**	2.158	0.605	0.860**

* the grass monoculture received 150 kg N ha^{-1} y^{-1}

** significant at P <0.01

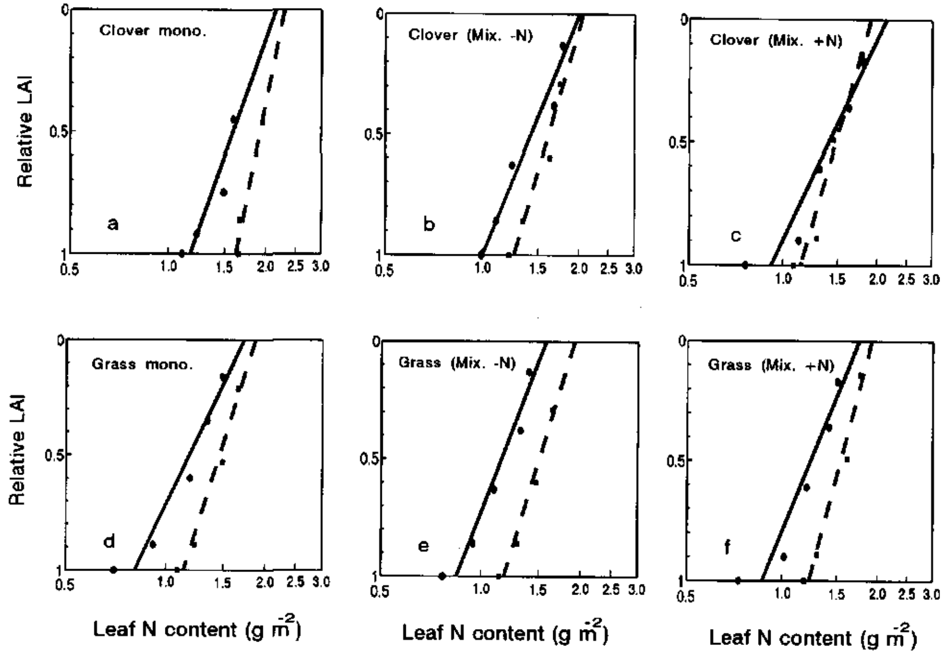

Figure 1. Profile of leaf N (N_h) as a function of relative LAI accumulated from the top of the canopy for grass and clover. Data are shown for 21 (■, ----) and 42 (●, —) days from the start of regrowth (22 July). For regression coefficients see Table 1.

In both species, k_N increased with increasing canopy *LAI* during time (Figure 1). Grass had more or less the same k_N in the +N and the -N mixtures. For grass, k_N in monoculture was close to that in the +N mixture, and significantly higher than in the -N mixture after 28 and 42 days of regrowth (Table 1, Figure 1). When the sward was relatively open (21 and 28 days from the start of regrowth), clover had a lower k_N in monoculture than in mixtures. However, in closed swards (after 28 days) k_N was about the same in the pure stand and in the -N mixture. In the +N mixture, clover had a significantly higher k_N than in other treatments, which was most pronounced after 6 weeks of regrowth (Table 1, Figure 1).

Relation between relative leaf N and relative *PAR*

To test whether the observed differences in vertical distribution of N_h measured at 4 successive harvests were correlated with the light profile within the canopy, relative leaf N (N_h/N_0) was plotted against measured relative light at each canopy position. (Figure 2). A curvilinear decline in relative leaf N with relative light (I/I_0) within the canopy was obtained.

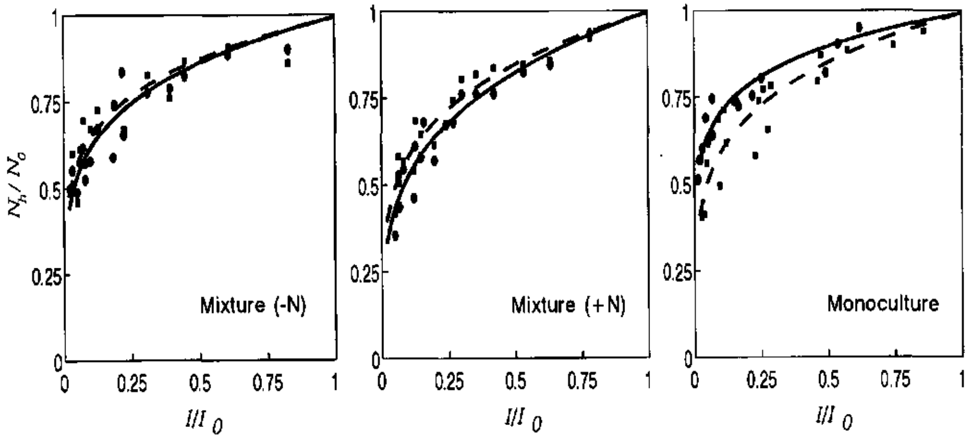

Figure 2. The relation between the relative leaf N concentration (N_h/N_0) and relative *PAR* (I/I_0) measured at different layers within the canopy. Data points of four successive measurements at 21, 28, 35 and 42 days after regrowth for grass (■) and clover (●) are combined. Clover (—); grass (----); for equations see Table 2.

Table 2. Curvilinear equation fitted to the values of N_h/N_0 and relative light at different positions within the canopy (see Figure 2). Data of 4 successive harvests for grass and clover are pooled.

Treatment	Grass	r^2	Clover	r^2
Mixture (-N)	$N_h/N_0 = (I/I_0)^{0.185}$	0.897**	$N_h/N_0 = (I/I_0)^{0.206}$	0.931**
Mixture (+N)	$N_h/N_0 = (I/I_0)^{0.232}$	0.945**	$N_h/N_0 = (I/I_0)^{0.277}$	0.944**
Monoculture*	$N_h/N_0 = (I/I_0)^{0.224}$	0.932**	$N_h/N_0 = (I/I_0)^{0.145}$	0.959**

* the grass monoculture received 150 kg N ha^{-1} y^{-1}
** significant at $P < 0.01$

A more rapid decline in N_h/N_0 was achieved as the relative light decreased (i.e. at the bottom layers of the canopy). Fitted equations ($N_h/N_0 = a\ (I/I_0)^b$; Hirose and Werger, 1988) for grass and clover are given in Table 2. Irrespective of treatment, parameter *a* was set to one. This means that the N concentration was maximal in leaves exposed to maximum light (Figure 2). In grass, parameter *b* in monoculture was close to that of grass in the +N mixture and higher than in the -N mixture. In clover, parameter *b* was lowest in monoculture and highest in the +N mixture (Table 2).

Effect of leaf N on leaf CO_2 assimilation

Both in grass and clover, A_{max} was linearly related to leaf N with a positive slope. The slope of the regression lines was the same for the four measuring dates. Therefore, the data were pooled (Figures 3a and d). The slope of the response in clover (1.086) was significantly higher than that of grass (0.647).

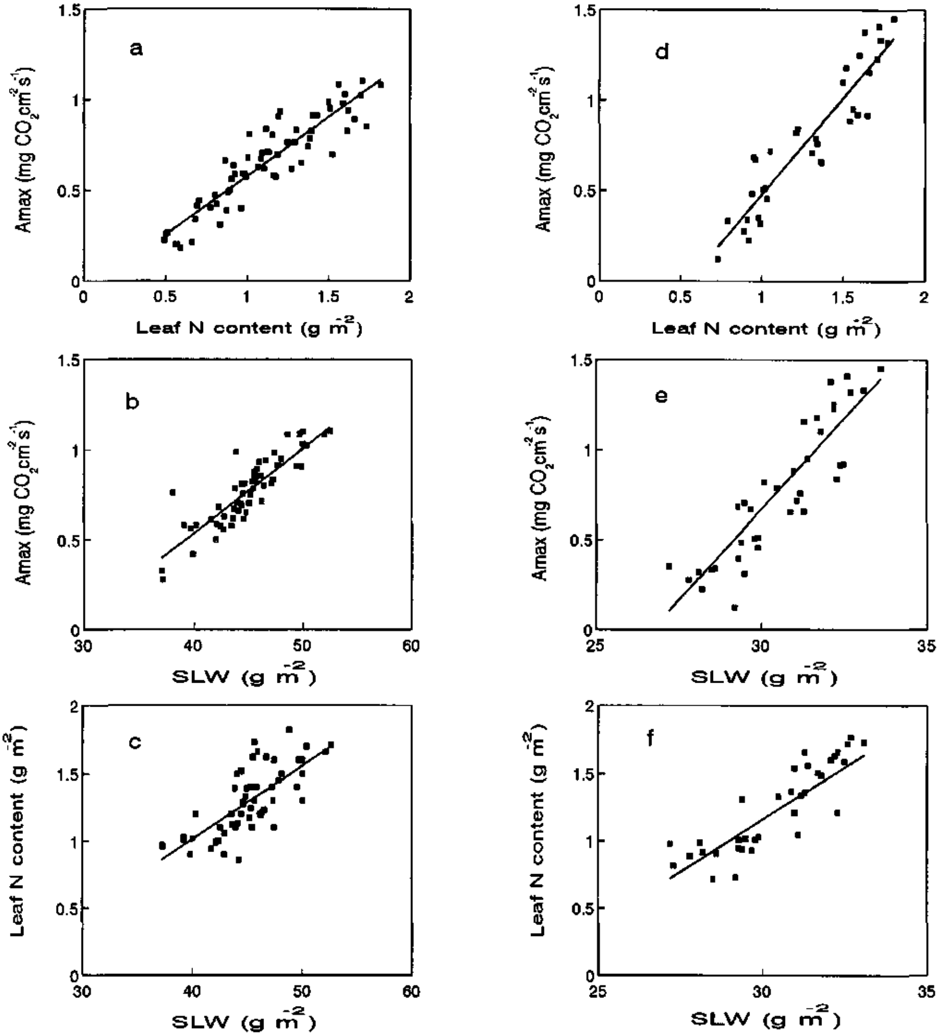

Figure 3. Linear relationships between A_{max} and leaf N concentration, A_{max} and SLW and leaf N concentration and SLW in grass (a-c) and clover (d-f). Data of four measurements during August were pooled. For regression coefficients see Table 3.

Based on established relations (Table 1 and Figures 3 a and b), $A_{max} = 0$ occurred at a leaf N concentration of 0.53 and 0.14 (g m^{-2}) for clover and grass, respectively. The A_{max} of both species was also positively related to specific leaf weight (SLW) (Figures 3 b and e) and again there was a higher slope in clover (Table 3). The regressions (Table 3) showed that A_{max} decreased with declining SLW. A significant and positive relation was found between the leaf N concentration and the SLW for both species (Table 3 and Figures 3 c and f), indicating that thicker leaves had a higher N concentration. To test these relations, the vertical distribution of leaf N and SLW of grass and clover are shown in Figure 4. In both species a gradient of SLW and leaf N concentration was observed over canopy height. Thicker leaves, with a higher SLW (Figures 4 a and b) and a higher N concentration (Figures 4 c and d), were located at the top layers of the canopy. Lower canopy layers contained thinner leaves, with a lower N concentration. Based on the results obtained from single leaf measurements (Figure 3) this pattern will lead to a gradient of A_{max} within the canopy, with different slopes, depending on species and treatment.

Table 3. Regression analysis of maximum rate of leaf CO_2 assimilation (A_{max}, mg CO_2 m^{-2} s^{-1}) on leaf N concentration (g m^{-2}), of A_{max} on specific leaf weight (SLW, g m^{-2}) and of leaf N concentration on SLW for grass and clover. The data of four measuring dates were pooled.

	Intercept	Slope	n	r^2
A_{max} on leaf N				
Grass	-0.066 (0.102)	0.647 (0.036)	64	0.832**
Clover	-0.637 (0.146)	1.086 (0.072)	38	0.864**
A_{max} on SLW				
Grass	-1.467 (0.087)	0.049 (0.003)	57	0.787**
Clover	-5.594 (0.172)	0.207 (0.017)	38	0.812**
Leaf N on SLW				
Grass	-1.167 (0.168)	0.054 (0.006)	57	0.550**
Clover	-3.999 (0.159)	0.172 (0.015)	38	0.778**

** significant at $P < 0.01$

Leaf N distribution and canopy CO_2 assimilation

The importance of observed (actual) distribution of leaf N compared to a uniform profile of leaf N was studied by simulation of the daily canopy CO_2 assimilation in monoculture and mixture (Table 4). The overall benefit of the actual over the uniform leaf N profile for the daily canopy CO_2 assimilation was low (in all cases less than 10%). The assimilatory gain of the actual profile increased during time. For all treatments it was highest after 42 days of regrowth when the canopy was closed and the leaf N profiles were fully developed (Figure 1 and Table 1). Figure 5 shows the simulated daily course of absorbed *PAR* and the gross canopy CO_2 assimilation under actual and uniform leaf N profiles of grass and clover after 42 days of regrowth.

In monoculture, the hourly absorbed *PAR* was the same for grass and clover except around midday. However, in mixtures the light partitioning between species was determined by their *LAI* and canopy structure. In both mixtures, clover absorbed more light with regard to its contribution to the total *LAI* (Figures 5 a and b, Table 4). In both species, the daily canopy CO_2 assimilation followed the pattern of light absorption (Figures 5 d-f).

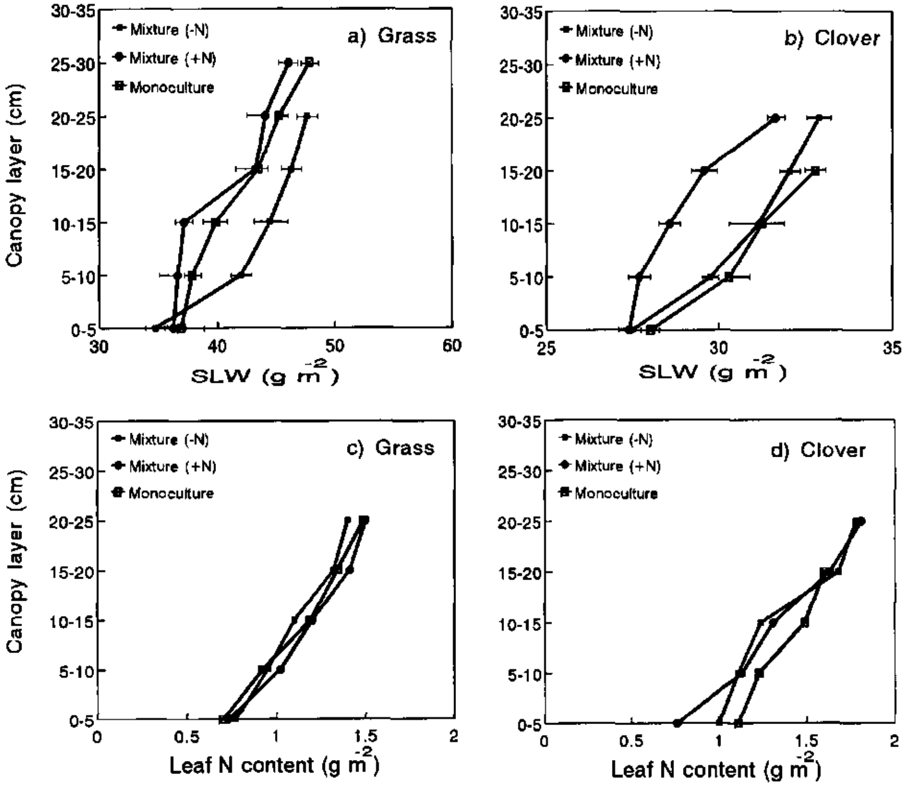

Figure 4. Vertical distribution of specific leaf weight (SLW, g m^{-2}) and leaf N concentration (N_h, g m^{-2}) for grass and clover in mixture and monoculture. Data points show the profiles 42 days from the start of regrowth. Error bars in a) and b) represent the standard errors of SLW.

Table 4. Simulated daily gross canopy CO_2 assimilation (P_{day}, kg ha^{-1} day^{-1}) of grass and clover in mixture and monoculture after 21, 28, 35 and 42 days from the start of regrowth (22 July). For each date, total daily incoming *PAR* (I_0, MJ m^{-2} day^{-1}), LAI and simulated fraction of *PAR* (f_a) absorbed by each species are shown. P_{day} was simulated for the actual leaf N profile, based on data shown in Table 1, and for a uniform leaf N profile (k_N = 0 and leaf N equal to N_m).

			Grass				Clover			
					P_{day}				P_{day}	
Days of regrowth	Treatment	I_0	LAI	f_a	actual	uniform	LAI	f_a	actual	uniform
	Mixture (-N)		1.19	0.33	102.4	101.6	1.10	0.49	137.4	135.2
21 days	Mixture (+N)	4.2	2.06	0.59	184.2	180.6	0.45	0.20	43.1	42.6
	Monoculture		2.20	0.73	228.8	224.5	1.72	0.80	240.9	237.0
	Mixture (-N)		1.83	0.34	264.0	262.2	1.63	0.56	405.9	401.6
28 days	Mixture (+N)	10.4	3.10	0.68	497.8	486.2	0.59	0.21	143.4	139.3
	Monoculture		3.20	0.85	630.9	618.3	2.60	0.91	641.5	619.1
	Mixture (-N)		2.37	0.31	118.9	117.4	2.12	0.66	238.2	231.2
35 days	Mixture (+N)	5.4	3.98	0.71	271.1	268.0	0.72	0.24	80.1	78.2
	Monoculture		4.15	0.92	350.1	345.7	3.20	0.95	350.7	345.9
	Mixture (-N)		2.49	0.29	145.6	139.1	2.34	0.68	417.5	385.1
42 days	Mixture (+N)	8.3	4.30	0.69	398.8	372.2	0.80	0.26	150.1	137.9
	Monoculture		4.51	0.93	541.6	503.7	3.91	0.97	541.2	517.0
	Mixture (-N)		2.49	0.29	227.2	215.3	2.34	0.68	671.6	618.8
42 days	Mixture (+N)	13.2*	4.30	0.69	622.4	578.2	0.80	0.26	236.8	216.5
	Monoculture		4.51	0.93	828.9	766.4	3.91	0.97	825.6	792.3

* I_0 set equal to the observed maximum daily incoming radiation during the 42 days of regrowth.

In monocultures, grass got a higher benefit from the actual N profile (7.5%) than clover (4.7%). In mixtures, the increase in canopy CO_2 assimilation of clover, using actual compared to uniform profiles, was higher than in monoculture (8.4 and 8.8% in -N and +N mixtures, respectively). However, for grass the benefit of a heterogeneous leaf N profile was lowest in the -N mixture, but it was the same in the +N mixture and in monoculture (Table 4 and Figure 5). The effect of radiation level on assimilatory gain of the actual N profile was studied by simulation of daily CO_2 assimilation of closed canopies (42 days of regrowth) under a clear sky (I_0 = 13.2 MJ m^{-2} day^{-1}), Table 4). The canopy assimilation increased under a high radiation level, but the benefits of the actual N profile remained unchanged.

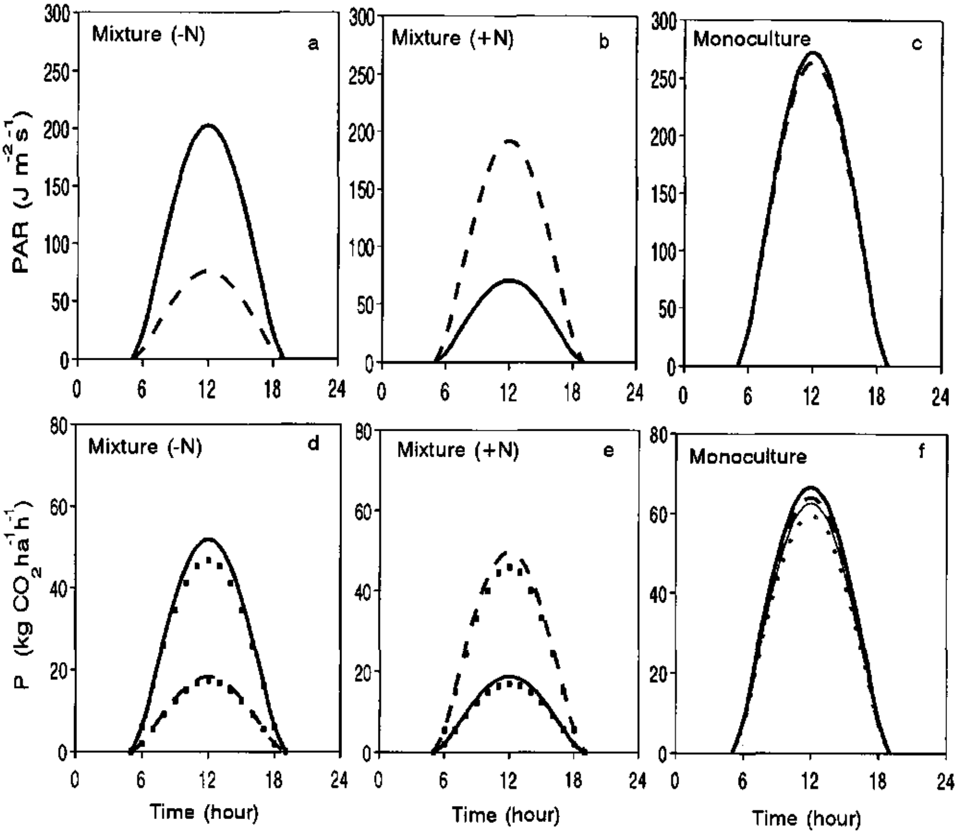

Figure 5. Simulated daily course of absorbed *PAR* (a-c) and simulated daily course of gross canopy CO_2 assimilation, based on actual and uniform leaf N distribution within the canopy (d-f) for grass and clover in mixture and in monoculture, 42 days after the start of regrowth. Clover (—); grass (---). CO_2 assimilation based on a uniform N profile is also shown, (▪▪▪▪) in d and e; (.....) for clover and (—) for grass in f.

The vertical distribution of the total daily canopy CO_2 assimilation (Figure 6) showed different patterns for grass and clover in monoculture compared to mixtures, which reflects the patterns of leaf area and light distribution. In clover the top canopy layers in monoculture had a larger contribution to the daily CO_2 assimilation. However, grass showed a more uniform pattern. Figure 6 also shows that the differences in CO_2 assimilation rate between actual and uniform leaf N profiles were highest at top layers, where the light absorption was also high. Deeper in the canopy, where CO_2 assimilation was limited by light, both profiles gave the same result.

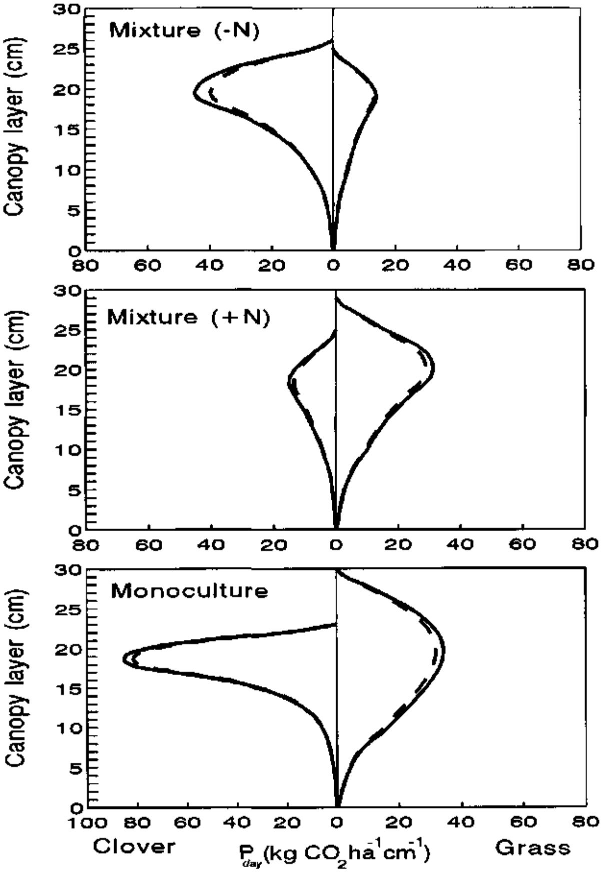

Figure 6. Simulated profile of daily total gross canopy CO_2 assimilation for grass and clover 42 days after the start of regrowth, based on actual (—) and uniform (----) leaf N distribution. The area under each curve gives the total daily canopy CO_2 assimilation; values are shown in Table 4.

Discussion

Leaf N concentration is considered to be strongly correlated with assimilatory capacity, both within and among C_3 species (Field and Mooney, 1986). Van Keulen *et al.* (1989) collected many examples from the literature, covering a wide range of species, and showed that the relation between A_{max} and leaf N concentration on an area basis can be approximated by a straight line. The same was found for the perennial herb *Solidago altissima* (Hirose and Werger, 1987 a,b), potato (Marshall and Vos, 1991) and rice (Peng *et al.*, 1995).

A linear relation was also reported in perennial ryegrass (Woledge and Pearse, 1985) but no data were available for clover. Lantinga *et al.* (1996) showed that at high N levels, where a significant part of leaf N is not present in the active form of Rubisco, the leaf CO_2 assimilation response to leaf N concentration is lower than at low N levels. However, in the present study where lower N input level were used, this "down regulation" effect was less clear and as a result a linear relation was obtained for grass and clover (Figure 3). The photosynthetic response of clover to leaf N showed a higher slope and intercept than for grass (Table 3). This indicates that the photosynthetic capacity was not equally sensitive to leaf N in both species. Differences in the utilisation of N in CO_2 assimilation might be the cause of the differences in canopy response. In accordance with the results reported for alfalfa (Delaney and Dobrenz, 1974) and soybean (Hesketh *et al.*, 1981), A_{max} in grass and clover was positively correlated with SLW, but with a lower slope in grass (Figure 3 and Table 3). Field and Mooney (1986) predicted that the intercept of the A_{max}-N relationship should be negative, since theoretically a minimum investment in leaf N is required for CO_2 assimilation to occur. We also obtained a negative Y-intercept in the response of A_{max} to leaf N and SLW for both species. However, the threshold of leaf N, below which assimilatory activity is zero, was higher in clover than in grass.

Leaf N concentration decreased exponentially with depth in many canopies (Hirose and Werger, 1987 a,b; Aerts and Caluwe, 1993; Anten, 1996), but reported values of the extinction coefficient of N, k_N, differ considerably. Hirose and Werger (1987a) obtained k_N values ranging between 0.67 and 0.86 for dense stands of *Solidago altissima*, and between 0.35 and 0.89 for open stands. Schieving *et al.* (1992) reported values of 0.45 and 2.8 for open and dense stands of *Carex acutiformis*, respectively, and a value 0.68 was found for sunflower (Sadras *et al.*, 1993). Our results showed an increase in k_N with increasing total *LAI* during regrowth (Figure 1). In the +N mixture, where grass had a higher contribution to the total *LAI*, its k_N was the same after 6 weeks of regrowth as in monoculture receiving the same amount of N, but lower than in the -N mixture (Table 1). In the -N mixture, clover had the same k_N as in monoculture, being lower than k_N in the +N mixture (Table 1). The same differences were observed for the distribution of N_h and SLW over canopy height (Figure 4).

Leaf age (Field, 1983) and light gradient (Hirose *et al.*, 1988) are the two mechanisms which have been proposed for regulation of the leaf N distribution within the canopy. Experimental results with *Carex* (Pons *et al.*, 1993), sunflower (Evans, 1993) and lucerne (Lemaire *et al.*, 1991) showed that the partitioning of leaf N concentration responds to changing light, rather than to leaf ageing. Reviewing this issue, Werger and Hirose (1991) also concluded that the leaf N partitioning is mainly influenced by the light distribution in the canopy. Our results also show that the light environment appears to be the main regulator of the N allocation within the canopy, as evidenced by the significant relationship between leaf N and light extinction throughout the canopy (Figure 2). Thus, the profile of N_h varied between the measurements (Table 1 and Figure 1), but when expressed on the basis of the light in each layer, a common response was observed (Figure 2). The light profile in turn depends on the canopy structure (i. e. the distribution of LAI and light extinction coefficient, k). Clover leaves, with a higher k-value, were positioned at the top layers, but grass with a lower k-value had a higher proportion of its *LAI* down in the canopy (Chapter 4). This led to different light profiles, and therefore different leaf N distribution patterns between species in monoculture (Figure 1). The results in Table 2 and Figure 2 show that parameter b was lower in the clover monoculture (0.145) than in the grass monoculture (0.224), resulting in a higher k_N for grass compared to clover. Lemaire *et al.* (1991), using the same analysis on a lucerne canopy, showed a gradual decrease in relative leaf N up to a relative light extinction (I_h/I_0) of 0.15, followed by a sharp decrease beyond this point. They suggested that this inflection point corresponds to the light compensation point below which net daily gain of canopy CO_2 assimilation is negative. Our results in monoculture support this conclusion, although grass and clover had different patterns of leaf N distribution in response to the light profile. The higher k_N of grass resulted in a lower relative N (N_h/N_0) at the light compensation point compared to clover (Figure 2). Mooney *et al.* (1981) also concluded that the difference in N profiles in response to light level might be species-dependent. In the -N mixture, where clover leaves were dominating at the top layers (Chapter 4), the pattern of N distribution for clover with light was much closer to that in monoculture. However, for grass it was similar in the grass-dominated +N mixture and the monoculture (Figure 2). In the +N mixture, clover had a significantly higher k_N than in the -N mixture and monoculture (Table 1). This resulted in a significantly lower N concentration at the lower parts of the canopy (Figure 3 and 4d), which was close to the threshold value (0.56 g m^{-2}) for leaf N concentration obtained for photosynthetic activity (Table 3). Sadras *et al.* (1993), combining the results from different experiments, concluded that *LAI* is the main determinant of k_N and differences between species are negligible. This could be valid for single species canopies. However, the present results show that in mixed canopies the distribution of *LAI* also plays an important role. Therefore, the results obtained from monocultures could not be directly extrapolated to mixtures, as was suggested by Lemaire *et al.* (1991). Anten and Werger (1996) studied the relation between the leaf N concentration and the light profile in a mixture of two species differing in height and showed that the different pattern of leaf N distribution in the dominant and the subordinate species depended on the position of the leaves in the light gradient within the canopy. Evans (1989b, 1993) observed interaspecific variation for leaf N distribution in response to low light and concluded that, within the same species, strategies of leaf N partitioning may change in response to N nutrition. A similar adaptive response of grass and clover to the light climate within the canopy was also observed in the profiles of SLW (Figure 4 a and b). These results support the conclusion that shaded leaves have a lower SLW than leaves in high light (Hirose *et al.*, 1988), because alteration of SLW is an important mode of acclimation to shade in many species (Björkman, 1981).

Considering the relation between A_{max} and leaf N concentration (Figure 3) the decreasing profile of N_h will lead to a gradient of A_{max}. In this study, ε (initial light use efficiency) was set constant and independent to leaf N for both species, so the leaf CO_2 assimilation response to the different N profiles depends only on the response of A_{max} to the leaf N concentration. Hirose and Werger (1987a), using a model for the distribution of leaf N in a dense canopy of *Solidago altissima*, found that the heterogeneous canopy realised over 20% more canopy CO_2 assimilation than the canopy with a uniform N distribution. However, in the present study the benefit of actual versus uniform leaf N profiles was low. In nearly-closed canopies (28 days of regrowth) no significant difference in canopy CO_2 assimilation was obtained between the two profiles (Table 4). Although the canopies in monoculture and mixtures were closed after 35 days of regrowth, the difference between both profiles in terms of daily canopy CO_2 assimilation was only found in the last week (Table 4). This might be explained by the increase in k_N in the last week of regrowth (Table 1) and the higher radiation level at day 42 than day 35. However, simultion results showed that the effect of radiation level was less important than k_N (Table 4). The gains achieved by actual versus uniform N distribution show considerable variation in different studies. They range from 1% for *Lepechinia calycina* (Field, 1983), 4.5-8.6% for *Eucalyptus grandis* (Leuning *et al.*, 1991), 9.9% for *Medicago sativa* (Evans, 1993), ≈10% for *Helianthus annus* (Gimenez *et al.*, 1994), 13% for *Acer saccharum* (Ellsworth and Reich, 1993) and 21% for *Solidago altissima* (Hirose and Werger, 1987a) to about 36% for *Lysimachia vulgaris* (Pons *et al.*, 1989).

Several factors have been considered as explanation to the benefit of a non-uniform leaf N distribution to the daily CO_2 assimilation rate. Hirose and Werger (1987a) showed that the benefit is greater in canopies with a high *LAI* and a high N concentration. A mathematical analysis of Goudriaan (1995) showed that the benefit of a non-uniform leaf N distribution is not noticeable below a *LAI* of 2. The gain also depends on the A_{max}-leaf N relation (Leuning *et al.*, 1991).

Our results showed that in mixed canopies in addition to *LAI*, the position of the leaves of the species within the canopy may also be important for the benefit from a non-uniform N distribution. In the present study, the maximum benefit of a heterogeneous leaf N profile was higher in the grass (7.5%) than in the clover (4.6%) monoculture. However, in both the +N and the -N mixture the increase of the daily canopy CO_2 assimilation due to the N profile was higher for clover (Table 4 and Figure 5), which reflects the different patterns of leaf area distribution and therefore light absorption by species in a mixed canopy (Chapter 4), as well as the different profiles of leaf N (Figure 2) and the stronger assimilatory response of clover to leaf N compared to grass (Figure 3).

Chapter 9

General discussion

A key limiting factor in herbage production systems is often the supply of N. This limitation can be largely overcome by the addition of N fertiliser. The alternative, and environmentally desirable, approach is to utilise a legume and reduce reliance on N fertiliser. In temperate climates associations of perennial ryegrass and white clover form the basis of low input systems, where clover improves not only herbage quality, but also provides an input of fixed atmospheric N to enhance the supply of N available to the plant (Peel and Lloveras, 1994). While N fertiliser can be applied at any time, biological N fixation is highly variable depending on clover content, availability of soil N (Crush, 1987) and weather conditions. This may lead to the conclusion that success of a grass-clover mixture is mainly determined by the clover content. However, the relation between clover content in mixture and its productivity it not fully understood (Harris, 1987). Schwinning and Parsons (1996c) suggested that the uniformity of mixtures is not necessarily the measure of success and that short- and longer-term periodic fluctuations in clover content can be an indication that the system functions properly. Thus, it is hardly possible to define an optimal white clover content and in fact an important management goal is to achieve a proper balance between grass and clover. This balance controls sward productivity, feeding quality of herbage and the environmental safety.

A considerable amount of research has focused on how plants (and animals) interact to determine the species composition of grass-clover mixtures (Rhodes and Harris, 1979; Haynes, 1980; Parsons *et al.*, 1991b). White clover responds to many environmental and managerial variables. Combination of these variables results in a complex pattern of growth and interactions, which can be only explained through a modelling approach (Parsons *et al.*, 1991b).

Interactions in grass-clover mixtures

In mixed swards interactions may occur in time and space or both. Temporal interactions occur when the conditions favour one species during part of the season, but the other thereafter. Spatial interactions occur when neighbouring plants compete for a limited resource, either aboveground (e.g. light) or belowground (e.g. soil N). Both types of interaction will result in a variation in clover persistence, sward composition and productivity.

Temporal interactions

Schwinning and Parsons (1996c) described two sources of variation for clover content. An intrinsic source, which mainly explains long term (between years) variation, and external factors which lead to short term variations (within year). As appears from numerous investigations, seasonal variation of sward composition is associated with weather conditions (e.g. Haynes, 1980; Davies, 1992).

Swards are grass-dominated during spring and will change to clover dominance during summer, leading to seasonality in the growth pattern of species (Chapter 3). However, the amplitude of species growth strongly depends on management (e.g. cutting frequency, combination of cultivars and N fertilisation). Comparison of clover cultivars with different leaf sizes showed that, irrespective of cutting frequency, large-leaved clover cultivars had a higher yield than medium or small-leaved cultivars (Elgersma and Schlepers, 1997; Chapter 2). The results showed that cutting frequency may affect the aggressivity of clover over grass through changing the light environment and the duration of shading (Chapter 2).

The supply of N is considered one of the major factors influencing interactions between grass and legume plants in pastures (Thornley *et al.*, 1995). The uptake of N, symbiotic fixation and transfer of N can all be closely involved in such interactions and thus affect the grass-clover balance. This work (Chapters 3 and 4) showed that in unfertilised swards the reliance of grass on N fixed by clover resulted in reduced interspecific competition, increased possibility of coexistence and higher total yield through synchronised growth pattern of species. However, in N-fertilised swards clover growth was always limited by light. Irrespective of the companion grass, large-leaved clover cultivars are more persistent in N-fertilised swards (Frame and Boyd, 1987; Chapter 3). The present study showed that large and small-leaved clover cultivars followed different strategies in response to the applied N. These strategies, which were regulated by the patterns of allocation of DM to leaves or supporting tissues (petioles), had an important role in the different performance of clover cultivars in the +N and -N mixtures (Chapter 3).

Spatial interactions

The importance of spatial patterns for species interactions have been considered by many ecologists. To study the role of spatial patterns on plant interaction in grass-clover mixtures detailed descriptions of such patterns are needed. Under cutting, where the species are well mixed and the horizontal heterogeneity is rather low compared to grazed swards (Edwards *et* *al.*, 1996), vertical heterogeneity has a much more important role in spatial interactions. Analyses of inclined point quadrat data showed different patterns of leaf area distribution of grass and clover (Nassiri *et al.*, 1996a; Chapter 4). Clover had a higher proportion of its LAI at the top canopy layers, but the opposite was observed in grass. In addition, in both species a non-random leaf distribution was evident from relative variance data (Chapter 5). Heterogeneity of leaf area distribution and leaf dispersion can be considered as the main determinant of the spatial interactions, which in turn affect light absorption and partitioning between species in mixed grass-clover canopies (Chapter 5).

Modelling light absorption in grass-clover canopies

Plant canopy models have been used extensively to address numerous ecological questions. Few comprehensive models currently exist which are specifically designed to calculate light partitioning in grass-clover canopies (Ross *et al.*, 1972; Rimmington, 1984; Sinoquet *et al.*, 1990; Parsons *et al.*, 1991b). These models have the disadvantage that they do not take into account spatial heterogeneity, although the importance of such non-uniformity has been emphasised (Thornton *et al.*, 1990).

In this study spatial heterogeneities in the structure of the mixed grass-clover canopies were taken into account in a relatively simple way (Chapter 5). Different patterns of leaf area distribution of grass and clover were described by a triangular leaf area density (*LAD*) function with a variable height of maximum *LAD* (h_m). Analysis of inclined point quadrat data showed some departure from random leaf distribution as a result of leaf dispersion, which limits the use of a fixed light extinction coefficient (k). To overcome this source of heterogeneity, a fixed species-dependent apparent k-value (k_a), which shows the combined effects of leaf angle distribution and leaf dispersion, was replaced by a fixed dispersion-free k-value ($k_{d\text{-}f}$) corrected by a variable dispersion factor for each species.

Simulation results suggested that prediction of the canopy models for light partitioning between perennial ryegrass and white clover could be improved considerably by taking into account the vertical heterogeneity (Chapter 5).

For the specific situation discussed in Chapter 5, with overall random leaf distribution, light partitioning between grass and clover was more sensitive to the patterns of vertical distribution of leaf area that to the leaf dispersion.

Modelling light competition in grass-clover mixtures

Plants of similar height (such as grass and clover) can compete for light, and each plant exerts some shading effect on its neighbours, usually in proportion to its size and its contribution to canopy LAI. Such cases of symmetrical (two-sided) competition may involve mutual shading at several levels, which is quite different from asymmetric (one-sided) light competition, where one species simply overtops another. While the outcome of asymmetric light competition is determined by the height of species (Spitters and Aerts, 1983), results of the present study showed that in grass-clover mixtures the height of the maximum leaf area density of species is crucial (Chapters 5, 6 and 7), emphasising the importance of spatial interactions.

In this study grass was the strongest competitor for light in spring, irrespective of its companion clover cultivar or fertilisation treatment. However, in unfertilised swards this changed during the growing season, so that in summer both large and small-leaved clover cultivars had a competitive advantage (Chapters 6 and 7). The different pattern of leaf area distribution between clover cultivars resulted in a wider competition zone for the large-leaved clover and thus gave it a better competitive ability than the small-leaved cultivar (Chapter 6). Therefore, even in N-fertilised mixtures, where the small-leaved cultivar was strongly overtopped during the season (asymmetric competition), the large-leaved clover was still able to compete with grass (Chapter 7). Associated with the shift in competitive balance due to N fertilisation there were differential effects of N on the growth form of clover, such as a change in petiole lengths and in the height of the maximum leaf area density (Chapters 4 and 7).

Considering the results described in Chapter 5, a relatively small but differential change in the structural properties of the two competing species can lead to noticeable changes in light absorption and canopy CO_2 assimilation, which were sufficient to explain the observed shifts in the competitive balance (Chapter 7).

Modelling canopy CO_2 assimilation in grass-clover mixtures

CO_2 assimilation models for mixed canopies are usually structured based on the partitioning of light between species, calculation of CO_2 assimilation rates based on absorbed light by each species and its integration over canopy height and daylength. This necessitates a detailed description of canopy structure, as became evident from the spatial heterogeneities in vertical leaf area distribution and horizontal leaf dispersion (Chapter 5).

The result of this study showed that light saturated rates of leaf CO_2 assimilation in grass and clover were strongly related to leaf N concentration, which in turn followed the light profile within the canopy (Chapter 8). While the effect of leaf N on CO_2 assimilation of monospecific canopies has been studied in many species, information about leaf N distribution and its consequences for of a mixed canopy is scarce. Simulation of canopy CO_2 assimilation in grass-clover mixtures and their monocultures showed that the assimilatory benefit of the actual compared to a uniform leaf N gradient was low, but the maximum gain of species was different in mixture and in monoculture (Chapter 8).

It is therefore concluded that in grass-clover mixtures, canopy structure has a more important effect on production than the assimilatory characteristics of leaves (Chapters 5, 6, 7 and 8).

Radiation use efficiency

Dry matter production is almost proportional to the intercepted light with a slope considered as radiation use efficiency (RUE) (Monteith, 1977). Clearly, RUE involves both maintenance and growth respiration, which may not directly depend on light, and CO_2 assimilation , which is directly related to light interception.

In this study clover had a lower RUE than grass, both in mixture and monoculture, which could be explained by the higher absorption of light by clover relative to its contribution to the total LAI (Faurie *et al.*, 1996; Chapter 7) and by the higher shoot and root respiration in clover than in grass (Soussana *et al.*, 1995b), as well as the extra energy cost of N_2 reduction in clover root nodules. Under a high N nutrition the RUE of grass increased, both in mixture and in monoculture (Chapter 7). The yield difference of grass between +N and -N could be explained by the combined effects of RUE and light interception. While in +N mixtures the large-leaved clover captured more light than the small-leaved cultivar, no difference was observed in their DM yield, indicating that the beneficial effect of absorption of more light was compensated by the higher percentage of sunlit leaves and by the cost of raising leaves to the upper canopy layers (Chapter 7).

Competition ability and yielding ability

An important question about mixtures is whether they have any advantage over pure stands. Unfertilised grass-clover mixtures showed yield advantages (expressed as land equivalent ratio, LER) over unfertilised grass and clover monocultures (Chapter 6) indicating that the relative efficiency of resource utilisation was higher in mixtures. This was due mainly to an increase in the LER of the grass component. Ryegrass reacted to white clover as an enhancing factor in its environment, seemingly due to the transfer of clover N (Menchaca and Connolly, 1990). However, the magnitude of this enhancement, and therefore the yield advantage of the mixture, declines as soil fertility increases and species balance shifts to grass dominance.

A comparison of two clover cultivars with the same yield potential in monoculture, but a different competitive ability in mixture (Chapters 4 and 7), showed that monoculture performance is not necessarily a predictor for the behaviour in mixtures. Indeed, yielding ability is not necessarily the same as competitive ability (Hill, 1997). Despite the same yield of the two clover cultivars in fertilised mixtures, the large-leaved clover cultivar was a stronger competitor than the small-leaved cultivar, reflecting the structural difference between cultivars. In present study the young swards (only one year old) were compared. Preliminary results of the second year of growth of the same mixtures and monocultures (unpublished data of 1997) showed that both clover cultivars had the same productivity in monocultures. However, in fertilised swards the average clover content in total DM during the summer regrowth period was about 44 and 25% in the large and small-leaved cultivar, respectively.

Conclusion

This study quantified the complex interactions between perennial ryegrass and white clover, with their interspecific reactions changing both in space and time. The INTERCOM model (Kropff and van Laar, 1993) was used as a conceptual framework for modelling these interactions. The seasonality in growth, in response to environmental conditions, was the main source of temporal interactions. The canopy structure of species was the main source of spatial interactions. Light partitioning models in grass-clover mixtures can be improved by taking into account the vertical heterogeneity in leaf area distribution and leaf dispersion. The effect of canopy structure on canopy CO_2 assimilation and productivity of species appeared to be more important than the effect of the profile of leaf N over canopy height on CO_2 assimilation per species. Clover cultivars had a different competitive ability, which was independent from their yield potential in monoculture. Clover persistence under cutting may be improved by using cultivars with a higher competitive ability for light, based on their structural characteristics.

Summary

In temperate climates perennial ryegrass-white clover mixtures provide a low input alternative to fertilised grassland production systems through the N fixing ability and high nutritive value of clover. However, sustainable productivity of mixed swards depends mainly on clover persistence, which is highly responsive to environment and management.

The thesis includes the results of two years field experiments (1995, 1996) on growth characteristics of white clover and perennial ryegrass in mixture and monoculture. Experiments were carried out in Wageningen, The Netherlands.

The objective of the research presented in this thesis was to study the complex interspecific interactions in grass-clover mixtures under a cutting management, using a modelling approach on the basis of the INTERCOM model. Therefore, basic information about seasonal growth and structural characteristics of species in response to environment and management was necessary.

In Chapter 2, the dynamics of sward composition, light absorption and DM production in response to cutting frequency was studied in three white clover cultivars with a different leaf size (large-leaved cv. Alice, medium-leaved cv. Retor and small-leaved cv. Gwenda) in mixture with perennial ryegrass (cvs. Barlet and Condesa) on a clay soil. The patterns of light absorption by the whole canopy followed the increase of leaf area of species. There was no effect of cutting frequency or companion grass cultivar. Clover cultivars maintained their share in *LAI* and DM yield during regrowth, particularly in summer and autumn. However, significant differences were found between clover cultivars in terms of DM yield and *LAI*. DM yield and *LAI* were highest in the large-leaved cv. Alice and lowest in the medium-leaved cv. Retor, which was less persistent and winterhardy than the other two cultivars. As a result, grass production was influenced by the aggressivity of its companion clover cultivar.

In Chapter 3, the seasonality in growth of grass and clover in response to repetitive N applications was studied in mixture and monoculture. Large-leaved white clover cv. Alice and small-leaved cv. Gwenda and the diploid perennial ryegrass cultivars Heraut (prostrate) and Barlet (erect) were grown either as mixtures or monocultures on a sandy soil. The swards ranged from clover dominance to grass dominance, resulting from the application of varying levels of N fertiliser. In the unfertilised mixtures (-N), grass had the highest proportion of its annual yield in spring, and its yield reduced remarkably during summer. However, for clover the opposite pattern was observed. The application of N led to a significant increase in grass DM yield and *LAI*, both in mixture and monoculture, with no difference between grass cultivars. As a result of the extra growth of grass in response to applied N, the harmonic seasonality in species growth was disturbed in the +N mixtures. While the growth of both clovers was suppressed by N fertilisation. The large-leaved clover showed a significantly better competitive ability than the small-leaved cultivar and maintained its content in the +N mixtures during summer and autumn. The difference between cultivars was mainly due to the changes in the patterns of allocation of DM to the leaves and to the supporting tissues (petioles) in response to N supply.

In Chapter 4, the vertical distribution of leaf area, DM and light was studied in the same swards described in Chapter 3. Grass and clover showed a different leaf area and DM distribution, both in mixture and in monoculture. Clover had a higher proportion of its *LAI* and leaf DM in the top layers of the canopy, while the opposite was observed for grass. In clover, the height at which maximum *LAI* occurred was a function of total height and was higher in the large-leaved than in the small-leaved cultivar. In addition, it was affected by N application. In grass, the height of maximum *LAI* was much lower than in clover and was not influenced by cultivar or N. The vertical light profile in the -N mixtures, where the canopy was dominated by clover, was close to that of the clover monocultures. However, in the grass-dominated +N swards it was similar to that of the grass monocultures receiving the same amount of N.

The experimental results presented in Chapter 4 clearly showed the different patterns of leaf area distribution of species in mixed canopies. In Chapter 5, a multi-layer light absorption and partitioning model for a mixed grass-clover canopy is described. The model accounted for vertical heterogeneity by introducing a triangular leaf area density (*LAD*) function with a variable height of maximum *LAD* (h_m) for each species. The relative variance obtained from inclined point quadrat data showed the different types of leaf dispersion over canopy height for grass and clover. The effect of leaf dispersion on the light extinction coefficient (k) of species was also taken into account by introducing a dispersion-free k-value ($k_{d\text{-}f}$, reflecting only leaf angle distribution) corrected by a variable leaf dispersion factor. When compared with the default version of the model with a fixed apparent k-value, modelling light partitioning and absorption in the mixed canopy was improved by using a triangular *LAD* function and introducing a dispersion-corrected $k_{d\text{-}f}$-value for each species. Sensitivity analysis on the model parameters showed the crucial role of h_m on light partitioning between species.

In Chapters 6 and 7, the light partitioning model was used to simulate the seasonal patterns of light absorption and radiation use efficiency (*RUE*) in mixtures and monocultures of grass and clover and to quantify light competition. In the -N mixtures, partial overtopping of clover was only found during spring. In the +N mixtures the small-leaved clover was strongly overtopped, both in spring and summer. However, for the large-leaved clover this happened only in spring. Simulation results showed that in spring both in +N and -N swards grass was a stronger competitor for light than clover. However, in summer large-leaved Alice was a better competitor in the -N mixtures than Gwenda and had the same competitive ability as grass in the +N mixtures. Clover had a lower *RUE* than grass, both in mixture and in monoculture. The yield difference between clover cultivars was mainly related to the amount of absorbed light. However, the higher DM yield of grass in +N compared to -N mixtures was the combined effect of increased light interception and *RUE*.

In Chapter 8, the vertical distribution of leaf N and its relation with leaf and canopy CO_2 assimilation was studied in mixture and monoculture. In both species, a positive relation was found between the light saturated rate of leaf photosynthesis (A_{max}) and leaf N concentration, but with a stronger response in clover. The leaf N concentration in grass and clover exponentially decreased with cumulative *LAI*, leading to a N gradient in the canopy parallel to the light profile, with the same qualitative patterns in mixture and monoculture. The canopy CO_2 assimilation of species was simulated, based on the actual and on a uniform leaf N profile. While the overall photosynthetic gain of a non-uniform N profile was low, the maximum benefit for grass was obtained in monoculture and for clover in mixture.

In Chapter 9, patterns of temporal and spatial interactions in grass-clover swards and their role in species competition and productivity of mixtures are summarised. Temporal interactions resulted in seasonality in growth of species leading to a yield advantage of unfertilised mixtures over monocultures through a better utilisation of resources. However, spatial interactions played an important role in competitive ability. It was concluded that in terms of competitive ability and productivity, canopy structure characteristics are much more important than assimilatory characteristics at the leaf level.

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Curriculum vitae

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