PRODUCTION AND PHYSIOLOGICAL RESPONSES OF ITALIAN RYEGRASS AND WHITE CLOVER GROWN IN MONO CULTURES AND MIXED STANDS

BY

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ABSTRACT

The aim of this study was to test the hypothesis that ryegrass and clover, when grown under optimal conditions in mixed stands, interact in response to available light energy. With other resources non-limiting, pasture yield is determined by the efficiency with which solar radiation is intercepted by canopies and converted into dry matter.

The clover-ryegrass interaction under varying light regimes, as experienced in the canopies of these pastures, was studied by investigating the growth and production of ryegrass and clover in relation to light harvesting abilities and photosynthetic utilization of intercepted light.

Pasture canopy structure and growth were studied under a four-weekly clipping treatment. The interception of photon flux density (PFD) in the pasture canopy was monitored diurnally and seasonally in mixed and mono cultures. Light use efficiency (CO_2 fixed/ unit absorbed PFD) as well as photochemical efficiency of photosystem II (F_v/F_m) were studied by monitoring CO_2 assimilation rates and chlorophyll fluorescence respectively.

The results obtained from this study indicated that interaction did occur between ryegrass and clover, cultivated in mixed pastures. The mixture was capable of more efficient light interception than the mono cultures, which resulted in higher productivity. Light interception abilities, as manifested in canopy architecture, and not physiological utilization of light energy, were found to govern the interaction between the two pasture components.

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LIST OF ABBREVIATIONS

A list of the most frequently used abbreviations include the following:

LAI - Leaf Area Index
N - Nitrogen
CO_2 - Carbon dioxide
PAR - Photosynthetically active radiation
PFD - Photon Flux Density
PI - Photon Irradiance
LI - Light Interception
IRGA - Infra-red Gas Analyser
P_{max} - Maximum photosynthetic rate
F_v - Variable fluorescence
F_0 - Minimum fluorescence
F_m - Maximum fluorescence
F_v/F_m - Quantum yield of photosystem II
q_N - Non-photochemical quenching
q_Q - Photochemical quenching
DM - Dry Matter

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CHAPTER 1: INTRODUCTION

Ryegrass (Lolium multiflorum cv. Midmar) pastures can be produced successfully only if high levels of nitrogen fertilizer are applied. Eckard (1989) recommended nitrogen fertilizer levels of 350 kg ha^{-1} to sustain ryegrass pastures for animal grazing. This high nitrogen application is associated with high costs. Therefore, in order to reduce costs and ensure a more economically viable solution, ryegrass is often grown in associated with legumes (e.g. white clover - Trifolium repens cv. Ladino). In addition to reducing costs, the quality of the pasture is also improved (Shelton 1990). Shelton (1990) stated that a greater total herbage yield might be obtained by growing a grass and a legume in association, rather than in separate swards.

The beneficial association of legumes and grasses is mainly related to the fixation of atmospheric nitrogen by rhizobia bacteria associated with the root nodules of legumes, allowing the clover to become independent of the soil nitrogen and in time this fixed nitrogen may become available to the grass component via the grazing animal (Haynes 1980). In this way herbage yield and productivity can be sustained. However, it is mainly the clover component in a mixed pasture which satisfies the energy requirement of grazing stock. But it is important to realize that too much clover in a grazing animal's diet may lead to a condition known as pasture bloat, which could be detrimental to the grazing animal (Howarth et al. 1978).

It thus becomes obvious that ryegrass (fibre-rich) and clover (protein-rich) complement one another in a mixed pasture to ensure a balanced diet for grazing animals. Improved digestibility, increased dry matter production and a more balanced mineral content have been reported for mixed pastures (Haynes 1980).

It is essential to manage these mixtures in order to maintain the different components. Competition between legumes and associated grasses in a mixed pasture is of great importance. Martin and Field (1984) and Donald (1963) stated that aerial resources are the main factors which result in competition between ryegrass and clover components in mixed stands. The dry matter production of actively growing pastures is limited by the interception and utilization of light energy (Ludlow 1978). The success and survival of individuals in these communities depend upon the way they intercept, compete for and respond to available light energy.

Competition for light in plant canopies consist of two important components: (1) the interception of photosynthetically active radiation (PAR) and (2) photosynthetic utilization of intercepted light. Light energy becomes attenuated as it penetrates a pasture canopy. Radiation is absorbed, transmitted and reflected by plant material and in this way becomes reduced in intensity as it passes through plant canopies. Caldwell (1987) stated that light interception depends mostly on canopy structure of the different components in a mixed pasture.

It is generally accepted that grasses normally have a competitive advantage over legumes with regards to light harvesting, due to differences in growth form and canopy height, and tend to dominate in mixed pastures. According to Thompson and Harper (1985), ryegrass has an upright growth form with closely packed tillers, and in a mixed pasture tends to outgrow the clover component which has a plagiotropic shoot system.

Most of the radiation intercepted by a field crop is absorbed by the leaves. Therefore, structure of the canopy is fundamental to light interception by plants. Haynes (1980) suggested that the most important feature of plants which determines their competitive ability for light interception is their height. Most often in mixed pastures the grasses are taller and more vigorously growing than the legumes and this leads to shading of the legumes. Charles-Edwards et al.(1986) postulated that the component in a mixture with the largest leaf area higher in the canopy is at a general advantage for light interception. The amount of leaf material present in a canopy is expressed as the Leaf Area Index (LAI), the ratio of total leaf to ground area beneath the canopy. Lang (1973a, cited in Frame and Newbould, 1986) has stated that many clovers have horizontally orientated leaves, they reach a critical LAI more quickly than grasses, and from this time onwards light within the canopy becomes a critical factor, limiting CO_2 fixation within the canopy. (Critical LAI can be defined as the LAI where maximum - not necessarily 100% - amount of light is being intercepted by a specific pasture canopy).

Leaf angle (inclination) is an important determinant of light interception. Legumes possess planophile or horizontally inclined leaves and are able to absorb light from only a few layers of leaves, while in the erectophile or vertically inclined leaves of grasses light is distributed more evenly. However, because of petiole elongation, clover leaves are held fairly high in the canopy (Dennis and Woledge 1982) and this enhances their competitive ability.

Light which has been intercepted by the leaves of pasture plants in the different layers of the canopy can be utilized during the process of photosynthesis for growth and production. Wilson and Ludlow (1983) stated that the dry matter production of plant canopies are dependent on the net photosynthetic contribution of their components. It is thus important to understand the behaviour of these components for predicting and understanding limitations to productivity.

Both Lolium multiflorum Midmar and Trifolium repens Ladino are C3 species, which suggests that the maximum photosynthetic rates are reached at relatively low light intensities. According to Wilson and Ludlow (1992) light is not a determinant of photosynthetic efficiency except at low PAR levels, as are experienced in the lower layers of the canopies.

Different seasonal growth patterns should also be considered when studying competitive interaction. According to Dennis and Woledge (1982), growth of white clover is greatest in midsummer when the growth of grass, whether in monoculture or in association with clover, is usually depressed. The poor performance of grass at this time, even when the pasture receives adequate water and minerals, is attributable to the low photosynthetic capacity of its canopy (Leafe 1972), resulting from a fall in the photosynthetic capacity of successive, newly-expanded leaves. This fall is in turn due to the increasingly poor light environment experiencing by the developing leaves as the sward gets denser.

It is important that management practices be applied correctly to mixed pastures in order to ensure a balanced pasture and to maintain both components in this pasture. Important management techniques to consider are: (1) clipping and (2) fertilization applications.

Defoliation (eg. by clipping or grazing) reduces leaf area and can alter canopy architecture. It may also change the photosynthetic characteristics of the sward by altering the age structure of leaves in the canopy and their light environment (Ludlow 1978 and Charles-Edwards et al. 1986). Dominance or suppression of species in a mixture can largely be illustrated by means of defoliation (Haynes 1980). Defoliation allows light to penetrate to levels where prostrate species display their leaf canopies and defoliation can restrict the ability of taller-growing species to elevate their leaves to shade prostrate species. Haynes (1980) cautioned that competitive relationships are altered by intensity, frequency and method of defoliation. Thus, frequency and intensity of defoliation must be planned in accordance with the growth habit and regrowth characteristics of individual components of a pasture.

The application of nitrogen fertilizer can have important impacts on the production, growth and ultimately competitive ability of the components of a mixed pasture. N-fertilizer stimulated ryegrass growth, but seemed to depress clover growth (Dennis and Woledge 1985) and Donald (1963). Dennis and Woledge (1985) noted that the negative nitrogen effect on clover growth was the product of competition. Increased grass growth resulted from increased nitrogen fertilization. This in turn caused shading of the clover component and reduced growth. Shading led to lower available light energy levels lower down in the canopy, where the clover component was situated (Chestnutt and Lowe 1970). Low nitrogen levels, in turn, benefitted the clover component, by reducing growth of ryegrass (Donald 1963). It thus becomes obvious that the application of nitrogen fertilizer plays a key role in production and balance of a ryegrass/clover mixed pasture. Nitrogen fertilizer has a direct effect on the competitive ability between species for light interception.

Presently there is little information on the behaviour of the individual components of mixed ryegrass/clover stands under Southern African conditions. This lack of information might lead to unsuitable management of these mixtures. The aim of this study was to investigate the physiological and growth responses of ryegrass and clover in a mixed pasture with seasonally varying light intensity. Ryegrass and clover were grown in monospecific stands and in mixtures. Two levels of N fertilizer were applied to the mixture. Grazing was stimulated by clipping. The development of the canopy and light interception ability in the interval between clippings were followed over the growing season when incident light levels vary naturally. Plants were also grown in pots and subjected to the same treatments, so that they could be removed to the laboratory for detailed physiological analysis. A better understanding of the interaction of the two pasture components in mixtures will enable improved future pasture management practices to be employed.

CHAPTER 2: LITERATURE REVIEW

The concept of planting legumes and non-legumes as pasture has been an important feature of agriculture from the early days (Nicol 1935, cited by Haynes 1980). The role of legumes in agriculture is increasing in importance. According to LaRue and Patterson (1982), legumes have been known to support livestock on poor soils. Haynes (1980) observed that grass-legume associations have been used in many countries of the world because a greater total herbage yield may be obtained by growing a grass and a legume in association, rather than in individual swards.

In terms of land use, growing crops in mixed stands is regarded as more productive than growing them separately (Andrew and Kassam 1976, cited by Ofori and Stern 1987). They stated that some of the established and presumed advantages of intercropping are; higher yields, greater land use efficiency and improved soil fertility through the addition of nitrogen by fixation and excretion from the legume component. Thus, pasture mixtures have the effect of intensifying crop production and exploiting more efficiently environments with limiting or potentially limiting growth resources (Trenbath 1982, cited by Ofori and Stern 1987).

Ofori and Stern (1987) observed that it would seem worthwhile to develop cropping systems that have the capacity to maximise crop yields per unit land area while keeping the fertilizer nitrogen requirement to a minimum. A mixed grass/legume pasture would fulfil this requirement as legumes have the ability to obtain nitrogen from the air, in symbiosis with bacteria from the genus Rhizobia (LaRue and Patterson 1981).

Shelton (1990) studied the use of legumes in Australian pastures and found that legumes have raised the fertility and productive output from often quite infertile land. The role of legumes in improving soil fertility can be related to improved soil nitrogen status and increased organic matter levels. Other consequences are: improved storage of other plant nutrients via an increased ion exchange capacity; improved soil physical condition and consequently water infiltration and storage capacity; improved biological activity of soils increasing the rate of nutrient cycling from the organic matter; increased vegetative cover on nitrogen deficient soils reducing soil erosion and leaching loss; and the indirect effect of fertilizers added to improve legume growth will improve soil fertility. Shelton (1990) thus concluded that there can be no doubt concerning the beneficial effects of using legumes in mixed pastures. Legumes are seldom grown in individual swards, as an animal grazing on pure legume stands could easily become bloated due to the high quality and content of proteins in legumes (Shelton 1990).

It is well known that a symbiotic relationship exists between legumes and soil bacteria of the genus Rhizobium which results in fixation of atmospheric nitrogen. The roots of the legumes become infected by the bacteria and form nodules. Frame and Newbould (1986) suggested that many factors influence this infection process. Rhizobia are abundant in soil only when associated with their host legume and they are strongly affected by adverse conditions such as heat, drought and acidity. Levels of nitrogen fertilizer applied to mixtures in which a legume is a component may also influence the symbiotic relationship between the host legume and soil bacterium. Haystead and Marriott (1979) (cited by Frame and Newbould 1986) stated that only a small supply of soil mineral nitrogen is needed by clover until nodules are formed and nitrogen fixation commences; thus the use of a "starter" fertilizer nitrogen can be beneficial. However, too much mineral nitrogen can depress nodule initiation and development (Sprent 1979, cited by Frame and Newbould 1986, and Davidson and Robson 1985). Young (1958) and Sprent (1983, cited by Frame and Newbould 1986), confirmed that application of nitrogen reduces nodulation and nitrogen fixation; the response varying with species, cultivar, Rhizobium strain, form of nitrogen, amount of nitrogen, time and site of nitrogen application, age and size of host plant, and prevailing environmental conditions. Van den Berg and Kruger (1988) stated that low levels of nitrogen fertilization (60 kg N ha^{-1}) on mixtures of Lolium multiflorum cv Midmar (Italian Ryegrass) and Trifolium vesiculosum cv. Amclo (Arrowleaf clover) gave the best results in terms of mixture production.

Shelton (1990) observed substantial amounts of nitrogen fixation by legumes in grazed pastures - yearly inputs of between 100 and 300 kg N ha^{-1} from good quality permanent legume-based pastures. Values for nitrogen fixation ranging between 100 and 200 kg N ha^{-1} year^{-1} were also reported (Barnes 1961, Strydom 1979, Evers 1980, cited by Frame and Newbould 1986). The amount of nitrogen fixed depends on photosynthates reaching the nodule, and thus on the amount of photosynthetically active leaf area in the sward (Frame and Newbould 1986). They also stated that a conflict exists between keeping sufficient clover leaf for nitrogen fixation and providing sufficient feed for animals. According to Stewart (1984, cited by Frame and Newbould 1986), an average of 30% of clover herbage over the season is a desirable target. Shelton (1990) stated that the amount of nitrogen fixed is directly proportional to the yield of the legume component of the pasture. Thus, good legume growth would ensure high levels of nitrogen fixation in the pasture.

Atmospheric nitrogen fixed by legumes is transferred to the companion grasses/species via the grazing animal (Davidson and Robson 1985) or by death, decomposition and mineralization of nodules, roots and other parts (Haystead 1983, cited by Frame and Newbould 1986). The consumption by livestock of clover shoots containing fixed nitrogen and the return of 80% of the nitrogen to the pasture as urine is the most rapid route for transfer, but it is also the route most subject to losses due to volatilization of ammonia and possibly due to leaching too. Thus, experiments conducted under a cutting regime should take into account that most nitrogen transfer occurs via the grazing animal.

In a study done by Brophy et al. (1987), where they used the isotope dilution method, they presented evidence that transfer of nitrogen from alfafa (Medicago sativa L.) and birdsfoot trefoil (Lotus corniculatus L.) to reed canarygrass (Phalaris arundinaceae L.) occurs and that the proportion of grass nitrogen (N) obtained from transfer was high. The grass derived 68% of its N from alfafa and 79% from trefoil. This N represented 13% of N_2 fixed by trefoil and 17% of that fixed by alfafa. They furthermore stated that N transfer occurred over a distance of 20 cm. Estimations of nitrogen transfer range from 26 to 154 kg N ha^{-1} depending on species composition (Simpson 1976, cited by Brophy et al., 1987). These authors also referred to Haystead and Marriott (1978), who showed that 6 to 12% of perennial ryegrass (Lolium perenne L.) nitrogen content was derived from neighbouring white clover in the field.

Smith (1987) accentuated the fact that the selection of companion legumes to be used in mixtures is very important. He observed that in intensively fertilized and irrigated pastures, the decline in pasture quality towards the end of the growing season is even more important than the cost of nitrogen. With the incorporation of a suitable legume into the grass sward, pasture quality is improved, nitrogen requirements are reduced and animal performance is enhanced. Smith (1987) tested seven legumes in combination with Lolium multiflorum cv. Midmar (Italian Ryegrass) and found that the perennial legumes (red and white clover) appeared to be the most suitable companion legumes. These species were superior to any of the annual legumes in dry matter yields, crude protein content and the ability to extend the growing season. He found that the growing season could be extended by at least six weeks. Furthermore, the saving in nitrogen fertilizer in such a grass/legume pasture may be considerable. High levels of nitrogen fertilizer are required for individual grass swards - in excess of 400 kg N ha^{-1} (Dannhauser 1980), while only "starter" nitrogen is required in mixtures.

Frame and Newbould (1986) stated that white clover (Trifolium repens cv. Ladino) is the most important pasture legume in temperate zones of the world. It is of value because of its wide climatic range, the high nutritional quality and digestibility of its herbage, and the significant contribution it makes to the economy of grass/white clover pastures by fixation of atmospheric nitrogen, especially in the absence of fertilizer nitrogen. Frame and Newbould (1986) further stated that white clover is not grown in monocultures because of the difficulties of keeping such swards weed free, low annual production, its short growing season, and concern about bloat and possible reproductive problems in grazing livestock.

Chestnutt and Lowe (1979, cited by Frame and Newbould 1986) stated that the choice of suitable companion grasses is important. It is well documented that grasses differ in their competitiveness to clover, but the adaptation to local growing conditions usually takes precedence over its suitability as a companion grass to clover.

Lolium multiflorum is considered the most important temperate grass species in South Africa, due to its superior winter yielding capacity (Van den Berg and Kruger 1989). However, to obtain acceptable dry matter production, very high nitrogen applications in excess of 400 kg N ha^{-1} (Dannhauser 1980) are necessary. The Midmar cultivar has an erect growth habit and reacts favourably to nitrogen fertilization. The yield of ryegrass in various parts of South Africa varies considerably, depending on the type of soil, fertilization, climate, irrigation and management. A production of 7 to 9 tons ha^{-1} under irrigation appears to be a good average, although a yield of 12 tons ha^{-1} is not impossible (Dannhauser 1980 and Morrison 1980). Dannhauser (1980) also stated that if irrigation is utilized, the farmer can successfully include ryegrass in his feed production program as it is high yielding and has both high nutritional value and carrying capacity.

Plants growing in mixtures compete above ground for space and light and below ground for space, water and nutrients. There is general agreement that white clover is at a competitive disadvantage when grown with most pasture grasses. The grasses are taller, have a greater mass of fine roots, and have less precise requirements of climate and soil nutrition for growth (Frame and Newbould 1986).

In order to obtain a well-balanced mixture of grass and clover; it is important to pay attention to relative seeding densities. According to Van den Berg and Kruger (1990) the ideal grass/clover seeding ratio should be approximately 70:30%. Chestnutt and Lowe (1970) concluded that a ratio of clover to grass seed of 3.5 to 22-25 kg ha^{-1} was adequate for obtaining a good clover:grass balance in a sward. Ludlow (1978) confirmed the short-lived effect of clover:grass seed ratio when he examined combinations of clover seeding density (1-9 kg ha^{-1}) and grass seeding density (3-14 kg ha^{-1}). He also found that a constant clover seeding density with varying grass seeding densities also had no significant effect on white clover performance.

Considering the many viewpoints on seeding densities in mixtures, it may be concluded that there is scope for considerable flexibility in the clover/grass seed ratio in seed mixtures. There has however, been a scarcity of work done on the critical seed density of white clover necessary to establish specific seedling populations.

Various authors have stressed the important effect that nitrogen has on the growth and production of grass/legume pastures. Ofori and Stern (1987) stated that nitrogen is one of the main factors influencing the production efficiency of grass/legume pastures. It was shown that the taller component suppresses the companion legume through shading, and this is accentuated by application of nitrogen fertilizer.

Donald (1963) stated that the balance between grass and clover in mixed swards is highly susceptible to environmental change. Studies have shown that a sward can become grass-dominated or clover-dominated according to the nutrient status or grazing management imposed on it.

The application of nitrogen fertilizers on grass/clover swards has an impact on herbage yield and productivity, increasing total yield of harvestable dry matter, although to a lesser extent than in a pure grass sward (Chestnutt and Lowe 1970). The yield increase is particularly valuable in the spring, but can be accompanied by negative effects on the clover component of the mixture. While the growth of the grass is greatly stimulated, that of the clover is depressed (Lowe 1966, cited by Chestnutt and Lowe 1970). The adverse effect of nitrogen on clover in a mixture appears to be an indirect one, acting through the grass plant (Dennis and Woledge 1985). Competition for light from the increased grass growth is thought to be the most likely mechanism (Donald 1963; Chestnutt and Lowe 1970), shading by the grass reduces the amount of carbon fixed by the clover. In an experiment conducted by Donald (1963), he found that low nitrogen gave almost pure clover whereas at a high nitrogen level, grass was markedly dominant. Thus, because of the progressive suppression of clover through shading when nitrogen is applied to mixed swards, it follows that fertilizer application and rhizobial activity cannot be used as additive sources of nitrogen. Applied nitrogen will continue to displace rhizobium nitrogen until displacement is complete. The effect of nitrogen on mixtures was also investigated by Van den Berg and Kruger (1988) where they found that it was more advantageous to apply low levels of nitrogen (60 kg N ha^{-1}) to ryegrass/clover mixtures in terms of dry matter production.

In contrast, high levels of fertilizer nitrogen are required where grass is grown in monocultures. Levels in excess of 400 kg N ha^{-1} was found necessary by both Dannhauser (1980) and Bartholomew and Chestnutt (1977). Eckard (1989) found the nitrogen requirement of Italian ryegrass to be 350 kg N ha^{-1}.

Eckard (1990) stated that too much fertilizer nitrogen applied, not only becomes economically wasteful, but nitrate-N could build up, which can lead to a toxic situation. April (autumn) and September (spring) appeared to be periods when nitrate-N in the plant could reach levels potentially toxic to ruminants.

The main function of pastures is to provide forage for grazing animals. Animals also deposit dung and urine and frequently sit, lie, scratch and paw on the pasture, as well as walking, running and jumping on it (Kemp 1984). Each of these activities has its own effect upon plant performance and needs to be considered when assessing the regulation of pasture productivity. As it is not always possible to standardize grazing effects in experiments, cutting techniques have frequently been used to simulate what seems to be the main effect of grazing, that is, herbage removal. Cutting regimes are defined as the frequency, intensity, uniformity and timing of the defoliation in relation to the development of plants or swards (Kemp 1984). However, according to Bryant and Blaser (1968), cutting does not simulate grazing exactly, as forage yields under cutting frequently exceed those under grazing, though the reverse can also be found.

Frame and Newbould (1986) observed that many cutting studies have shown that total herbage production from grass/white clover swards generally increases as the interval between defoliations is lengthened. White clover production was increased as the interval between defoliations was increased from 3 to 6 weeks, but the proportion of white clover in the total herbage decreased (Orr and Ludlow 1978, cited by Frame and Newbould 1986). Van Heerden (1986), in a study conducted in South Africa, investigated the production and quality patterns of a range of legumes and grasses under different defoliation frequencies, as he found a lack of information on defoliation programmes to assist in management. His results indicated that a 6 week cutting interval would be a reasonable compromise for the production of dry matter. However, he added that modifications might be necessary for different species to obtain a competitive balance in mixtures; for instance white clover and perennial ryegrass mixtures would be favoured by frequent cuttings (2 to 4 weekly).

This was confirmed by Smith, 1987 (also in a South African study), who tested the response of legume/ryegrass mixtures to 3 cutting intervals. He found that dry matter yields were significantly reduced when cutting interval was increased from 4 to 5 weeks. However, in ryegrass/white clover mixtures, cutting interval had no effect on dry matter yield. In the pure stands of ryegrass, dry matter yields were reduced, although not significantly, when interval was increased from 5 to 6 weeks.

Past work on grass/clover and grass swards generally showed that close cutting (25 to 50 mm from ground level) increased total herbage production considerably (up to 44%) compared with lax cutting (60 to 100mm), provided adequate recovery periods were allowed between defoliations (Frame and Newbould 1986). According to these authors white clover production and proportion were either little affected by closeness or else were enhanced by close cutting. Anslow (1967, cited by Frame and Newbould 1986), stated that the stimulus to total herbage production from close cutting has been attributed largely to more efficient light utilization, since a high proportion of a sward's production is in the lower layers. Furthermore, close defoliation will also stimulate grass tillering and utilization of regrowth by removing flowering shoots and permitting better light conditions at the base of the sward. The stimulus to the clover production will include better light utilization, while it may also benefit from reduced grass competition. Dennis and Woledge (1982) pointed out that better light conditions at the sward base will also encourage the number of clover growing points and promotion of photosynthetically efficient leaves.

Van den berg and Kruger (1988) found that heavy defoliation

(50mm) tended to increase the Land Equivalent Ratio (an index to compare yields of mixtures relative to their respective pure stands) compared to light defoliation. They speculated that the reason for this could be that competition for light was more detrimental to both the grass and clover components in the light defoliation treatments.

Competition between grasses and legumes for light seem to be an important factor. The rate of dry matter production in crops depends on the efficiency of the interception of photosynthetically active radiation (PAR) (Monteith 1977 and Graham et al. 1988). Blackman and Black (1959, cited by Monteith 1977) stated that under conditions where growth is not restricted by temperature or by supplies of nutrients or water, maximum production of dry matter per unit area will be limited by leaf area index and the amount of solar radiation. According to Donald (1963), utilization of solar radiation is the factor governing the ultimate yield of any particular genotype or community. He went further to say that even where there is a shortage of water or nutrients, competition for light remains a factor of major importance.

Results from a study conducted by Graham et al. (1988) showed that competition for light is important in sorghum-pigweed mixtures. Even in well-watered, fertilized plots, drastic dry matter and yield reductions could be seen, which corresponded to similar reductions in the amount of light intercepted by sorghum.

Haynes (1980) stated that competition for light is unique. Incoming light energy is instantaneously available, it must be used or lost. According to Donald (1963) competition for light occurs whenever one plant casts a shadow on another or when one leaf shades another. Haynes (1980) pointed out that competition for light is between individual leaves, rather than between plants, because if leaves remain below compensation point for long periods, they are not supported by export of assimilates from other parts of the plants and die. In a pasture, foliage of each plant will be intermingled with that of several of its neighbours. Thus, the successful plant is not necessarily the plant with more foliage, but the plant which has its foliage in an advantageous position, relative to the foliage of its competitors for light interception. Thus, the physiological characteristics, canopy height and architecture and whole plant morphological characteristics determine peak photosynthetic rates and light competitive abilities of plants (Haynes 1980).

Rhodes and Stern (1978) also confirmed that competition for light occurs not only between different species but also between any leaves or photosynthetically active parts whenever one by its interposition reduces the light supply falling on another, thus reducing photosynthetic rate. These authors also stated that competition is regarded as having occurred when the yield of a plant in mixed culture is lower than that in monoculture.

Light becomes attenuated as it penetrates a foliage canopy and if sufficiently reduced, may impair the functioning and development of plants that become shaded in comparison with their performance in unshaded conditions (Rhodes and Stern 1978).

Brougham (1958) stated that growth rate is related to the amount of light interception and leaf area. The amount of light intercepted by the component species in a mixed pasture depends on foliage architecture and pasture geometry (Ofori and Stern 1987). Beyschlag et al. (1990) and Thompson and Harper (1988) also regarded canopy structure as playing an essential role in the balance of competition for light between species. Thompson and Harper (1988) stated that white clover grows as a plagiotropic shoot system (in contrast to stoloniferous, which is the incorrect term), rooting at the nodules. They stated further that this type of growth habit ensures that clover continuously "meanders" through the associated vegetation to ensure that favourable light environments are reached. The grass component has the advantage of possessing an upright, "phalanx" growth form, with closely packed tillers which enables light harvesting (Thompson and Harper 1988). Brougham (1958) considered orientation and shape of leaves as major factors governing light interception ability, although leaf thickness and density should also be taken into consideration. Shaw and Weber (1967) found that changes in plant growth habit affect light interception and distribution. They noted that most interception occurred in the outer portion of the canopy. They suggested that a more irregular plant canopy resulted in better light penetration of the canopy. Accordingly, they found that in soybean canopies yield was positively correlated with both the amount of leaf area and the volume of the canopy. According to Stern (1962) light interception depends on the following: angle of elevation of the sun, whether radiation is direct or diffuse, the density of the foliage canopy, the angle at which leaves are disposed, the reflection, absorption and transmission characteristics of the foliage and the extent to which gaps in the foliage or movement will allow sunflecks to penetrate. Monsi and Saeki (1953) have described light attenuation in quantitative terms ($I=I_0e^{-kd}$; where I = light intercepted by a canopy, I_0 = incoming light incident upon a canopy, k = extinction coefficient and d = canopy density) and determined the extinction coefficient for light energy (k) as it is attenuated throughout the sward. This varies between grass species and varieties (Rhodes and Stern 1978). Monteith (1973) found values of extinction coefficients for clover and ryegrass to be 1.10 and 0.43 simultaneously. Donald (1963) speculated that the growth potential of the clovers may be lower than the grasses due to the planophile nature of their canopies. However, for certain of the legumes this may be partly due to the delayed onset of growth in spring. Monsi and Saeki (1953) calculated the relative light interception by horizontal and erect foliage to be 1:0.44. Further, they showed that in 5 species with a leaf angle from the horizontal ranging from 0 to 75 degrees, the value of the extinction coefficient followed these theoretical expectations.

Duncan (1971) stated that leaf area and leaf angle should be considered together in any assessment of their effect on photosynthetic efficiency. According to him, the inefficiency of plant canopies with high LAI value which have layers of horizontal leaves at the top of the canopy suggests that the photosynthetic efficiency of grass-like plants might decrease rapidly as the upper ends of the leaves bent over. Barnes et al. (1990) and Rhodes and Stern (1978) also stated that plant attributes which are advantageous for increased production in pure stands may not necessarily be of advantage for competition in mixed stands. Increased LAI or more horizontally orientated leaves in upper canopy layers would be advantageous for a species competing in a mixture, but not necessarily of benefit for production in monocultures. Ludlow (1978) stressed that competition for light depended more on leaves gaining preferential access to limiting light than on the photosynthetic response.

Thompson and Harper (1988) Rhodes and Stern (1978) suggested that communities with erect foliage are the most productive under managements that maintain a high average leaf area index. Light penetration is greater into canopies of erect rather than horizontal leaves, clumped or random as opposed to regular foliage, and low rather than high leaf area density (Ludlow 1978). Red and blue wavelengths of PAR become relatively depleted within the canopy because they are absorbed more (Ludlow 1978).

Rhodes and Stern (1978) emphasized that competition for light cannot be considered in isolation, but should be seen through interaction with other factors. The major management variables, defoliation and nutrient status, have both been found to alter relative competitive abilities, although in many instances the precise role of competition for light has not been elucidated. Any factor that affects a plant's structure or ability to shade differentially in relation to its neighbours should have an effect on its relative competitive ability. Ross et al. (1972) formulated a model to describe the effects of nitrogen and light in grass-legume pastures. They described rates of production as functions of irradiance and leaf area index. Photosynthesis of the grass component was also seen as a function of the level of nitrogen available for growth. Different fertilizer or clipping treatments will have a marked effect on competitive abilities in mixtures (Rhodes and Stern 1978). They found that nitrogen application, by its stimulation of grass growth, increases the ability of the grass to shade the clover. Likewise, in some mixtures, more severe defoliation prevents shading of the legume and thus its relative competitive ability is increased.

Pearcy (1990) observed that leaves at the top of a plant canopy or in the most shaded understory sites may experience long periods of light intensities that approximate steady-state conditions. However, most leaves are subjected to rapid alternating periods of sun and shade because of sunflecks. Under these circumstances, a large fraction of carbon dioxide assimilation may occur under transient conditions. It has been shown that on clear days 30 to 60% of the daily carbon gain by understory plants can be attributed to utilisation of sunflecks (Pearcy 1987, cited by Pearcy 1988). Measurements of Chazdon (1984) showed that up to 80% of the total daily irradiance intercepted by understory leaves were in the form of sunflecks. Field gas-exchange measurements showed a similar percentage (20 to 80%) of the daily CO_2 exchange of leaves in the understory can be attributed to sunfleck utilization.

According to Pearcy (1990) much less attention has been given to the dynamics of light regimes in crop canopies than those of forest understories. Light regimes in soybean canopies were characterised by sunflecks shorter and brighter than those of forest understories (Pearcy 1990). The number of sunflecks varied from nearly 1800 per day in the upper part of the canopy to none for a few locations at the bottom. At most locations within the canopy, sunflecks contributed 40 to 90% of the daily PAR, and of this total approximately one third was contributed by sunflecks shorter than 10s.

Beyschlag et al. (1990) stated that success for competition for light between species is due to differences in either structural features or photosynthetic characteristics which allow one species to fix more carbon than its competitor. Wilson and Ludlow (1983) stated that the dry matter production by mixed plant canopies depends largely on the net photosynthetic contribution of its components. It is therefore important to understand the behaviour of these components, both for purposes of predicting and for identification of limitations to productivity. They further stated that leaf photosynthetic rates in a canopy are usually measured in one of two ways, both of which have drawbacks. Firstly $^{14}CO_2$ uptake or CO_2 exchange rates by individual leaves or parts of leaves enclosed in chambers are measured. However, in short and dense canopies, canopy structure was disturbed, resulting in disturbance of light distribution and photosynthetic rate. Furthermore, insufficient number of leaves can be measured to overcome variability among leaves. The above authors stated that photosynthetic rates can also be estimated from calculated light levels (based on a model of light distribution which takes account of canopy structure) and assumed photosynthetic capacities of leaves in each layer. It is assumed that all leaves in the canopy have the same capacity or that their capacities decline linearly or exponentially with depth. Wilson and Ludlow (1983) continued to say that these models are dependant on assumptions which are not always applicable. Leaf age may vary with depth and shaded leaves can acclimate and partially compensate for the lower light levels (Pearce and Lee, 1969).

Wilson and Ludlow (1983) estimated photosynthetic rates of grass (Setaria sphacelata var. sericea) and a legume (Desmodium intortum) leaves at different levels in a mixed canopy. They found that the light level decreased as leaf area index increased with depth in the canopy. The grass component had higher rates of photosynthesis in the upper strata where the relative illuminance was 60 to 100%. The superiority of the grass decreased with depth, and rates for the two species were comparable in the lower 2/3 of the canopy where most of the leaf area occurred. Thus for 75 to 85% of the canopy leaf area, rates for grasses and legumes were indistinguishable. This was due to the fact that photosynthesis is depressed more by shading in grasses than in legumes (Ludlow et al. 1974) and possibly because of the lower average illuminance at the surface of grass leaves which are more erect than legume leaves. They concluded that the difference in photosynthetic capacity is emphasized in the upper layers of the canopy where illumination is higher. These conclusions were supported by Ludlow (1978) who stated that preferential access to incident radiation is the most important determinant of competition between grasses and legumes.

Woledge et al (1989) investigated rates of photosynthesis of canopies of a mixture of ryegrass (Lolium perenne L.) and white clover (Trifolium repens L.) during winter months and the partitioning of total canopy photosynthesis between the two component species, using $^{14}CO_2$. They found that at a PAR of 552 µE m^{-2} s^{-1}, net photosynthesis for both components ranged between 3.51 and 18.31 µmol m^{-2} s^{-1}. Clover had a lower rate of photosynthesis than grass due to its canopy structure (leaves were nearer the base of the canopy than those of the grass) particularly in the second half of winter when photosynthesis per leaf area was least. Canopy LAI was small at this time (approximately 2) and it might be expected that there would be little mutual shading of leaves at small LAI. They found that the tendency of clover to have a lower rate of mean photosynthesis than its companion grass in winter to be reversed in summer, when clover leaves had a greater photosynthesis rate than grass. Clover leaves were predominantly near the top of high LAI canopies. They concluded that this seasonal difference in relative photosynthesis by grass and clover had a role in determining the growth pattern of mixed swards in which clover had a higher relative growth rate (RGR) than grass during summer.

Dennis and Woledge (1982) found the growth of white clover to be greatest in midsummer when the growth of grass is usually depressed. Leafe (1972) suggests that the poor performance of grass at this time is attributable to the low photosynthetic potential of its canopy, resulting from a decline in photosynthetic capacity of successive, newly-expanded leaves. This fall, in turn is due to the increasingly poor light environment experienced by the leaves as the sward gets denser. Dennis and Woledge (1982) found that petiole extension by the clover component played an important role in a mixed stand. The advantage of petiole extension to clover is twofold. The detrimental effect of shade on the development of the photosynthetic capacity of mature leaves is avoided and the leaves are well-placed in the canopy to receive light and thus have a high actual rate of photosynthesis. Thompson and Harper (1988) confirmed that clover has the ability for linear extension of the main axes whenever it became confined by neighbouring grasses. Brougham (1958) suggested phototropic movement of leaves in the canopy of white clover occurred, but later these movements were attributed more to petiole extension which positions the leaf in favourable positions for light interception. (Dennis and Woledge 1982; Boller and Nosberger 1985).

Dennis and Woledge (1982) stated that although the photosynthetic capacity of individual clover leaves is at least as high as that of perennial ryegrass, the published data on photosynthesis of communities suggests that grass swards have a higher photosynthetic rates. They continued by saying that sward photosynthesis depends not only on photosynthetic capacity of individual leaves, but also on the leaf area, how much light the leaves intercept, and how this light is distributed among the leaves. In an erect crop, light is more evenly distributed at a lower average intensity, and thus is more efficiently used than in a crop with horizontal leaves in which the upper leaves may be light saturated while the lower ones are in very dim light. They concluded by saying that the dry matter production are limited by the structure of the canopy rather than an intrinsically low photosynthetic capacity. This was previously also found by Sheehy et al. (1979) where they stated that light reception is mainly a function of canopy structure, which may determine photosynthetic productivity by limiting total light interception of young stands or by allowing light to penetrate easily into the dense foliage of older stands. In contrast, Boller and Nosberger (1985) found that the structure of the legume (white clover) canopy was less important in determining canopy photosynthesis compared with the effects of leaf age. Photosynthesis of older stands declined as a greater proportion of light was intercepted by senescing leaves. According to their studies the very young unfolding leaves occupied the lower levels of the canopy, while the recently unfolded leaves were in the top layers of the canopy (due to petiole extension). The older leaves were found in progressively lower layers. The light-photosynthesis (P-I) response curves of leaves of different age classes were found to be similar, indicating that leaf age had little influence on the P-I curves of individual leaves. Boller and Nosberger (1985) and Schwank et al. (1986) thus indicated the importance of petiole extension to young leaves which do not reach the top layers of the canopy, but are able to position in such a way that sunflecks in the lower parts of the canopy may be utilized. It was concluded that such behaviour would improve the species chances of survival even with tall-growing competitors.

Thomas and Davies (1978) conducted a study to investigate the effect of shading on the regrowth of Lolium perenne swards in the field. They found that the current herbage yields were only slightly affected when ambient light levels were reduced. Shading to 71%, which simulated light levels similar to conditions in autumn, caused no reduction in current herbage production. However, when the shaded leaves were defoliated and returned to full light, early regrowth from the depleted stubble was low compared with that from unshaded swards.

Woledge and Leafe (1975) found that in a ryegrass sward which has been cut, the first new leaves to be produced had high photosynthetic capacities. However, as the leaf area of the crop increased, leaves developed in lower light intensities, resulting in a reduction of more than 50% in their photosynthetic capacities. They calculated that this lead to a depression of about 30% in the photosynthesis of the whole canopy.

Bjorkman and Holmgren (1963) focused their investigation on the role of light intensity as a selective factor in the adaptation of the photosynthetic apparatus. They stated that light shows more variation than any other external factor which influences the photosynthetic apparatus of the plant. They studied reactions of the photosynthetic apparatus in plants taken from habitats with contrasting light environments but grown under controlled conditions of high and low light intensity. They observed that the photosynthetic response of plants is strongly modified by the light intensity of the growing conditions, but that the modifications are different for populations from exposed and shaded habitats. Clones from the exposed habitats had similar initial slopes of photosynthesis - irradiance curves (an expression of the efficiency of the photochemical process) in both light treatments, whereas the rates at light saturation were considerably higher for the plants grown in strong light. The clones from the shaded habitats showed considerably shallower initial slopes when grown in strong light than in weak light; whereas the rates at light saturation were about equal in both light treatments. They stated further that the populations from the shaded habitats had a higher quantum efficiency than the populations from the exposed habitats thus implying that the photosynthetic apparatus of the former is able to utilize weak light more efficiently than that of the latter. At light saturation, the populations from the exposed habitats had the higher photosynthetic, thus implying that the photosynthetic apparatus of the populations from the exposed habitats utilizes strong light more efficiently than that of the populations from the shaded habitats. Bjorkman and Holmgren (1963) also investigated the effect that strong light had upon mature leaves by transferring a plant grown in low light to high light intensity. Strong light treatment caused a depression of the photosynthetic activity of the leaf; both the photochemical efficiency and the rate at light saturation were affected. Their results also indicated that the chlorophyll content of leaves decreased when transferred from low to high light. However, when plants were once again subjected to weak light after a relative short time in high light, recovery took place - indicating that the inhibition of photosynthesis by high light is reversible. It was concluded that the photosynthetic behaviour of plants are consistent with the light intensities prevailing in the natural environments, indicating that they are a result of a genetic adaptation to habitat.

The quantum yield of various species exhibiting the C3 pathway of photosynthesis, have been studied by various authors. Ehleringer and Bjorkman (1977) found a value for quantum yield of 0.052 mol CO_2 E^{-1} to be representative. Ku and Edwards (1978) reported quantum yield of the C3 grass, <u>Triticum aestivum</u> to be 0.054 mol CO_2 E^{-1}. Ehleringer and Pearcy (1983) observed a value of 0.053 mol CO_2 E^{-1} for C3 grasses.

Beyschlag <u>et al</u>. (1990) investigated the photosynthetic characteristics of mixtures of wheat and wild oat grown in the field and found canopy photosynthesis to be most influenced by maximum photosynthetic capacity in the upper, light-saturated portions of the canopy and by quantum efficiency in the lower levels of the canopy. As leaves age the rate of photosynthesis at saturating irradiance (P_{max}) declines, but there is little change in the quantum efficiency (Johnson and Thornley 1984). They observed that P_{max} decreased down the length of a <u>Lolium multiflorum</u> leaf blade. In grass swards the leaves grow from the base of the sward and thus, during early stages of growth in a dense sward, their irradiance environment will be low (Johnson and Thornley 1984). However, the above authors cited Prioul <u>et al</u>. (1980) who have demonstrated that <u>Lolium</u> leaves can adapt when transferred from a low to a high irradiance environment.

Dennis and Woledge (1983) stated that the light conditions under which a plant grows can have a large effect on its photosynthetic capacity. Leaves which have developed in bright light have higher rates of light-saturated photosynthesis than those grown in dim light or in the shade of other leaves, which changes both the quality and intensity of light. Dennis and Woledge (1983) further stated that the reduction in a leaf's photosynthetic capacity as a result of development in dim light can have important effects on the photosynthesis and growth of crops.

In contrast to the above, Dennis and Woledge (1982) found that in white clover, in a white clover/perennial ryegrass mixture, there is little or no reduction in the photosynthetic capacity of successive leaves as the crop gets denser, even though new clover leaves are formed near the ground level. Even when nitrogenous fertilizer was applied and the grass component of the canopy became very dense, there was little reduction in the photosynthetic capacity of the clover leaves, although the proportion of clover in the sward was markedly reduced. Dennis and Woledge (1982) reasoned that the photosynthetic capacity of clover leaves might be less affected than grass leaves when growing in a dense sward, due to the fact that they are less sensitive to the light conditions in which they develop. Another reason might be that rapid petiole extension raises the developing laminae to the top of the sward while their photosynthetic capacity is still able to respond to the high irradiance they receive there.

Dennis and Woledge (1982) investigated the effects of the light conditions during development of a white clover leaf (Trifolium repens cv. Blanca) on its photosynthetic capacity. They found that newly expanded leaves which have developed in dim light or in the shade of other leaves, have lower photosynthetic capacities than leaves grown in bright light. They also found that leaves grown in dim light or shade had a higher specific leaf area. This has been interpreted as an adaptation to shade in that for each unit weight of leaf dry matter, a greater area of leaf is exposed to the available light.

Dennis and Woledge (1983) stated that plants detect shade by neighbouring leaves through a change in the light quality perceived by the phytochrome system. It is thus the intensity or quantity of light rather than its quality that is the factor determining photosynthetic capacity, as no difference could be detected between the photosynthesis of clover leaves grown in dim light or shade. An earlier study conducted by Dennis and Woledge (1982) showed that although clover leaves are formed at the base of a sward, in dim light, the petioles of successive leaves grow longer as sward height increases, so that each new laminae is raised to the top of the sward and into full sunlight where they reached similar photosynthetic capacities than those of leaves unshaded throughout their development. By contrast, a grass leaf has no petiole and grows from its base and by the time it is fully expanded, it is subject to a gradient of increasing shade from top to base, with the youngest part of the leaf remaining in dim light. According to authors such as Stern (1962) and Donald (1963) and Chestnutt and Lowe (1970), the difficulty of maintaining clover in a mixed clover/grass sward is a result of competition with grass for light, especially when nitrogenous fertilizer is added.

It was found by various authors that plant canopy architecture, as described elsewhere, played a much more important role in competition for light than single-leaf photosynthetic characteristics (Beyschlag et al. 1990, Ryel et al. 1990 and Kuppers, 1984).

The light environment within canopies and light interception in relation to growth and productivity has been the topic of numerous theoretical models. According to Johnson and Thornley (1984), models of crop growth and production can be divided into two component models: 1) light interception and canopy architecture; where the irradiance of a leaf is calculated at a given depth within the canopy, and 2) evaluation of the photosynthetic activity of a leaf as a function of its irradiance. Norman and Jarvis (1976) realised the usefulness of modelling as a tool in improving the understanding of interactions between radiation and vegetation by postulating a model describing radiation penetration in Sitka spruce (Picea sitchensis (Bong) catt.). Charles-Edwards (1986) modelled plant growth and developments in terms of the light environment in order to obtain a better understanding of the behaviour of the leaf canopy. He examined canopy structure and proposed a method of estimating the proportion on incident light energy intercepted by the canopy. He also defined light-use efficiency in terms of photosynthetic and respiratory activities. Ryel et al. (1990) stated that to describe the process of light competition in a mixed species canopy using direct measurements of light interception might prove to be difficult. Species could differ in height, inclination or orientation of leaves and might also overlap with other leaves. Canopy structure might also change through time. In addition, leaf age composition and photosynthetic capacity varies within canopies and differ among species. Therefore, simulation models could be implemented in estimating light interception and describing mechanisms of light competition. However, Caldwell (1987) observed that few models exist to examine light competition among mixed canopies. Therefore, Ryel et al. (1990) developed a detailed model to satisfy the above requirements. The model was tested for mixed wheat/wild oat and monospecific canopies in the field.

An intensive overview of the literature, as cited above, suggested the following:

As a consequence of competition, the productivity of each component is less in pasture mixtures than when each is grown in monoculture, but the combined productivity of a mixed stand is greater than either component in monoculture. Competition is predominantly at the level of light interception and so is very dependent on the architecture or structure of the canopy. This competition is influenced by factors such as nitrogen supply and grazing intensity. The effect of nitrogen fertilization appears to be an indirect one through canopy development, rather than direct physiological phenomena, in that high N gives rise to increased grass growth, which shades the clover out.

CHAPTER 3: MATERIALS AND METHODS

3.1. STUDY AREA

This study was conducted at the Roodeplaat Grassland Institute, situated approximately 20 km north-east of Pretoria (25°44'S, 28°11'E).

3.2. EXPERIMENTAL DESIGN

Italian Ryegrass (Lolium multiflorum cv Midmar), an annual species, and white clover (Trifolium repens cv Ladino), a perennial, were planted both in mixed and mono stands in field plots and in pots. The pots were placed in trenches in the field to ensure that all treatments were exposed to similar environmental conditions.

3.2.1. Field Trials

Plots of 12 m^2 (3 * 4 m) were layed out in the field, separated from one another by a 1m path (Figure 1). A total of 9 plots were placed in a randomized design:

1) Mono clover (3 plots)
2) Mono ryegrass (3 plots)
3) Mixtures (3 plots)

The mixed plots were divided into two sections to enable two different nitrogen applications to be administered to each mixture. (N1 and N2 - see below).

Inoculated clover was already established at the start of the trial, it thus entering the second season of growth. The clover had been planted (broadcasted) the previous season (April 1991) at a seeding density of 10 kg ha^{-1} (both in mono and mixed stands). All clover seeds were inoculated with Rhizobium bacteria (Rhizobium leguminosarum (biovar trifolii) SR4). Inoculated seeds were covered with a layer of bentonite, to

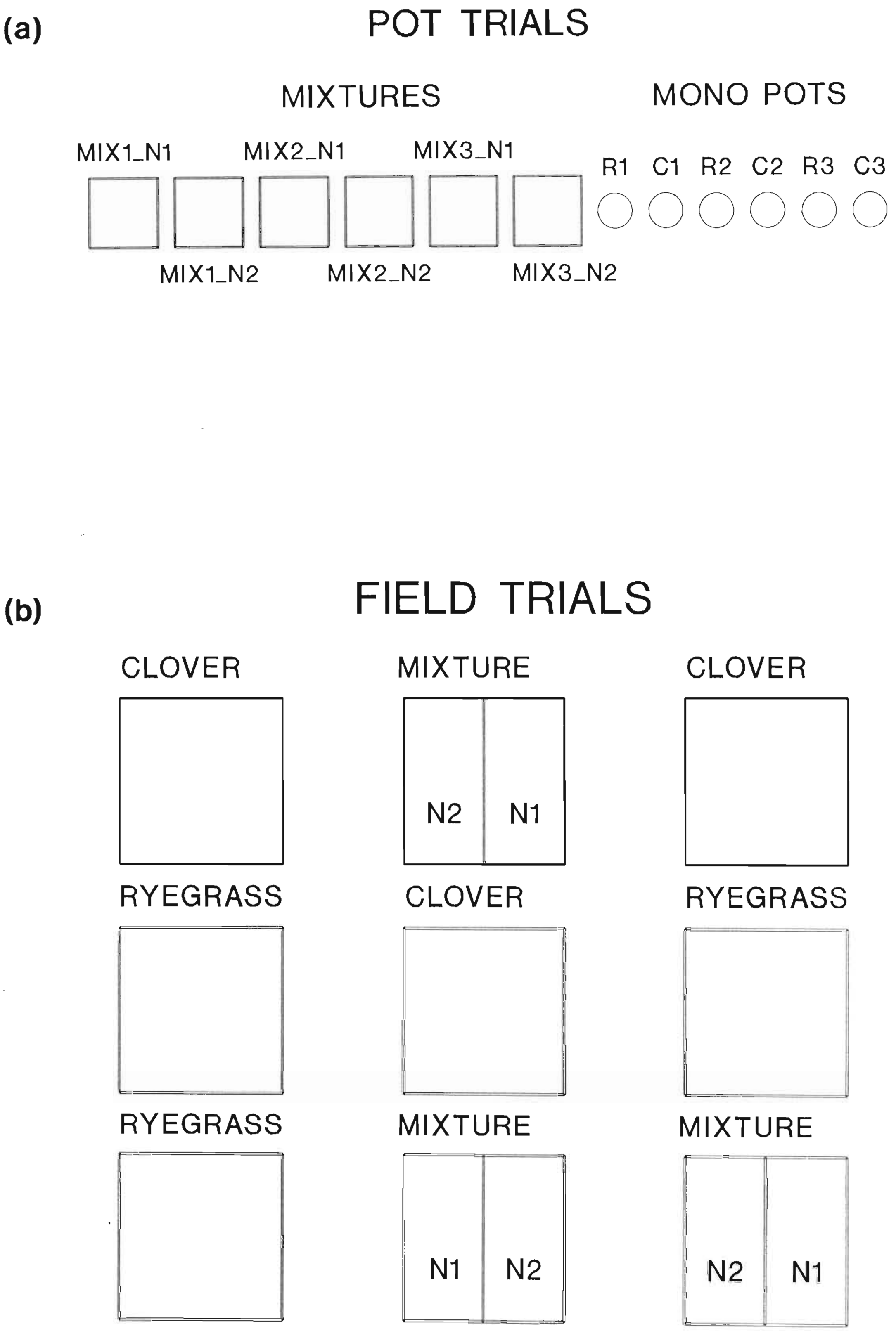


Figure 1. Schematic representation of (a) pot trials and (b) field trials

ensure that an alkaline pH, ideal for germination, was obtained. Ryegrass was sown into the clover plots in rows, at a seeding density of 25kg ha^{-1} (mono and mixed) on 16 April 1992. The ryegrass to clover seeding ratio were therefore 70% to 30% (Lehman and Meister 1985 cited Van den Berg and Kruger 1990). The row spacing in all treatments was 30cm.

All plots received nitrogen fertilizer (KAN/LAN, 28% N) and super phosphate (10.5%). To enable comparisons of growth and physiological parameters, it was decided to apply fertilizers in identical amounts to all treatments; although it should be kept in mind that clover usually receive less nitrogen (approximately 60-100 kg N ha^{-1}, Van den Berg and Kruger 1988) to ensure nodulation for nitrogen fixation. (The processes of nitrogen fixation and transfer were not investigated during this study and were thus not accounted for). Nitrogen fertilizer was applied in the concentration of 200 kg N ha^{-1} to all plots. A second nitrogen level (400 kg N ha^{-1}) was administered to half of each mixture plot. Thus, mixtures (split plots) received N1 (200 kg N ha^{-1}) and N2 (400 kg N ha^{-1}). All other plots received 200 kg N ha^{-1}. The above amounts was divided into 6 aliquots which were spread across 6 harvests (a growing period of 180 days). Super Phosphate (100 kg ha^{-1}) (Dannhauser 1980), was applied to all plots. This amount was also applied in aliquots over the growing season. Fertilizer was applied after each harvest, simultaneously with irrigation.

All plots were irrigated twice weekly and thus received 25 mm water per week (Eckard 1990).

3.2.2. Pot Trials

Identical treatments to the field plots were planted in pots in the same seeding densities as the field trials. The pots were kept in the field. Pot trials were used, as pots could be transported to the laboratory for certain studies such as gas exchange studies and chlorophyll fluorescence - see sections 3.6

and 3.7). Pots containing mono cultures had a diameter of 30cm and were 35cm deep. The mixtures were planted in square pots with dimensions of 60cm * 60cm * 30cm. All pots were placed in trenches in the field which were filled with vermiculite for insulation against temperature gradients. Soil from the field trials were used as growth medium for all cultures in pots. Pots received nitrogen (KAN/LAN) in the same concentrations as the field trials. Fertilization applications were applied on a more regular basis to compensate for leaching. In addition, a nutrient solution (excluding nitrogen) with the following contents, was applied once a week (constituents were made up to 1000ml with water):

1. 120.37 g l^{-1} $MgSO_4.7H_2O$ (5ml)
2. 136.09 g l^{-1} KH_2PO_4 (2.5ml)
3. 10 g l^{-1} FeEDTA (2.5ml)
4. 110.98 g l^{-1} $CaCl_2$ (10ml)
5. 74.55 g l^{-1} KCl (10ml)
6. Micronutrient solution (2.5ml):
6.1. 2.860 g l^{-1} H_3BO_3
6.2. 0.220 g l^{-1} $ZnSO_4.7H_2O$
6.3. 0.079 g l^{-1} $CuSO_4.5H_2O$
6.4. 1.015 g l^{-1} $MnSO_4$
6.5. 0.090 g l^{-1} $H_2MoO_4.H_2O$

(Due to differences in pot sizes (see above) monocultures received only 250ml, while mixtures received 1000ml nutrient solution per week).

3.2.3. Soil Analysis

A soil analysis, conducted by the National Institute for Soil and Irrigation (Pretoria), was performed on the experimental site, prior to commencement of this study. A summary of the results is given in Appendix 1.

3.3. GROWTH MEASUREMENTS

3.3.1. Dry matter production

All treatments were clipped at intervals of approximately four weeks. Plant material was cut to a level of 5 cm above ground level. The canopies of all treatments were divided into 3 sections, according to height from ground level: Top (15-24cm), Middle (10-15cm) and Bottom (5-10cm). These levels were kept constant in order to enable comparisons between different treatments for the whole of the growing season. Six harvests were performed throughout the experimental period, as follows:

6 June 1992 - all material was clipped to a level of 5cm above ground level (starting point)................day 0
9 July 1992 - harvest 1.............day 28
10 August 1992 - harvest 2.............day 60
4 September 1992 - harvest 3.............day 85
30 September 1992 - harvest 4.............day 111
29 October 1992 - harvest 5.............day 140
8 December 1992 - harvest 6 ....day 180

(Note: The lengthy interval (40 days) between harvests 5 and 6 was due to a severe hail storm on 8 November 1992 which heavily damaged all plant material).

During each harvest, a 1m^2 quadrat was placed randomly (rectangular to rows) in each plot. This quadrat was vertically adjustable to the three , previously defined, layers of the canopy. Samples were taken from all treatments in the three different layers of the canopy. Harvested plant material was separated into the different above-ground plant components (stems and leaves). Separated material was dried to a constant mass in an oven at 70°C. Thereafter, all plant material was weighed to obtain dry matter mass.

3.3.2. Leaf Area Index

Leaf area index (LAI) is the leaf area per ground area. LAI was determined according to the stratified clip method (Coombs et al. 1985). This method was used to study the distribution of leaves with canopy height. LAI was determined by harvesting the leaf matter in each layer of the canopy, as described above. Leaf area of clover and ryegrass were obtained by setting up regression curves as follows:

Leaf material was sampled from ryegrass and clover in both mixed and mono stands. Leaf area of various numbers of leaves was determined by using a Licor planimeter (Li3100). These leaves were dried and weighed. Leaf area was then plotted against leaf dry mass and linear regression curves were fitted to each curve. After each harvest, the dry mass of harvested leaf material could thus be used to obtain values for leaf area.

LAI was determined at each harvest for all treatments during the season and also between harvests on 2 occasions to get an estimate of LAI development between 2 successive harvests:
1) 20/7; 24/7; 31/7 - between harvests 1 and 2

2) 14/9; 21/9 - between harvests 3 and 4

3.4. MEASUREMENT OF PHOTOSYNTHETICALLY ACTIVE RADIATION (PAR)

3.4.1. Measurement of photon flux density (PFD)

Photon flux density (PFD) was measured at different layers of the canopies of ryegrass, clover and mixture (N1) in the field plots. (Mixtures with nitrogen level N1 were used for measurement of all physiological parameters and compared with mono cultures. Thus mixtures with nitrogen level N2 were incorporated into this study solely for purposes of establishing the influence of nitrogen on growth and production.) For measurements of PFD, the various canopies were divided into the three vertical layers, as described above. Measurements were taken prior to each harvest and also for the periods of LAI development, between harvests. Diurnal (daily between 09h00 and 15h00) as well as seasonal (September to December) measurements were made.

Measurements were taken with a sunfleck ceptometer (Model SF-80, Decagon Devices Inc.). The ceptometer has 80 light sensors placed at one-centimetre intervals along a 1m probe. This probe is inserted into the canopy, and a microprocessor scans the 80 sensors. In order to make reliable measurements and reduce errors due to spatial variation in the canopy, measurements were taken at ten different locations in each canopy layer and these readings were averaged by the ceptometer. The values of PFD obtained by means of the ceptometer were compared to those found by a quantum sensor (Licor Li-190SA). Values were not significantly different.

Variation in PFD was measured:
a) above the canopy (S)
b) in the different layers of the canopy - Top
   (for each treatment) - Middle
   - Bottom

Quadratic regression curves ($y=a+bt+ct^2$, where y=PFD and t=time (hours)) were fitted to each daily light measurement curve and the area underneath the curve was integrated to obtain a daily irradiance value. It was decided to fit quadratic regression curves to the measured daily light intensity curves (above canopy level as well as in the canopy layers) in this study as these equations described the data most accurately. It should be kept in mind that equations already exist for describing daily light variation which is applied mostly for modelling purposes; e.g. N. Papadopoulos (1992) modified an existing equation by Johnson and Thornley (1983) in order to describe daily irradiance; and Monteith (1965) described diurnal variation in PFD by a sine function.

3.4.2. Light interception by canopy leaves

Direct measurements

Radiation intercepted by the foliage in the canopy of each treatment was also measured by the ceptometer. Calculations were made as follows (Decagon Sunfleck Ceptometer User's Manual 1989):

$f = 1 - t$

where,

f = fractional interception
t = fractional transmission

fractional transmission of each canopy was in turn determined as follow:

$t = T/S$

where,

T = up-facing ceptometer reading at a certain depth in the canopy (Top, Middle and Bottom)
S = up-facing ceptometer reading above the canopy

As, described in the previous section, ten readings at different locations within each canopy layer was taken and averaged in order to obtain a reliable reading. The ceptometer was placed perpendicular to rows within each plot. To obtain comparable values, the ceptometer was placed in the same manner in all the plots which were measured.

Values of light interception were obtained in conjunction with measurements of PFD. Thus, diurnal and seasonal light interception changes between the different layers of the canopy could be established.

Indirect measurement of light interception

Daily light interception was also obtained by calculation, using the daily integrated light intensity values (obtained as described above in section 3.4.1.). For indirect light interception measurements, the daily integrated values obtained by calculation (integration of the areas beneath the light curves for changes in daily light intensity) was used for PFD values above the canopy (S) and at various depths within the canopies (T). These values were then used for calculation of fractional transmission and interception ($t = T/S$ and $f = 1-t$, respectively).

3.5. CHEMICAL ANALYSIS

3.5.1. Chlorophyll content

Chlorophyll content was determined from leaf material harvested in pots. These determinations were done prior to each harvest. (Pot trials were used for chlorophyll content determinations as parameters such as gas exchange and chlorophyll fluorescence were also determined on pot material). To obtain uniformity, material was collected before 10h00. For each treatment (mono ryegrass, mono clover, mix rye (N1) and mix clover (N1)), the canopy was divided into top, middle and bottom layers. Three replicates from each canopy layer were collected. The samples were transported to the laboratory on ice, where they were stored in the refrigerator in moisture-proof bags for approximately 30 minutes to ensure turgidity of the leaves.

A modified method of Arnon (1949) was used for chlorophyll content determination. 0.5g of fresh material was weighed out and cut into small pieces. 5 ml 80% acetone was added to material in a test tube. This material was mashed in a blendor (Janke and Kunkel ultra-turrox T25, fitted with a rotor S25N), for 3 minutes at 11000 rev min^{-1}. The blendor was washed with 5 ml acetone in order to obtain material entangled in the blendor. The suspension was then centrifuged for 10 minutes in a Beckman

GP Centrifuge at 3000 rpm. After centrifugation, the supernatant was decanted through filter paper into an erhlenmeyer flask. The pellet was resuspended in 10 ml acetone by means of a mixer (Janke and Kunkel IKA-Vibro-Fix-VF2) and centrifuged as described above. The above extraction procedure was repeated 3 times.

Absorbance was measured at 645nm and 663nm for chlorophyll b and a respectively. The slit width was 2 nm. Acetone was used to zero the spectrophotometer and absorbance was read against an acetone blank.

Total chlorophyll content was determined as follows:

$$C = (20.2(A645) + 8.02(A663))/1000$$

where,

C = total chlorophyll content (mg chl ml^{-1} chl solution)
A645 = absorbance at 645 nm
A663 = absorbance at 663 nm

Chlorophyll content was expressed on a fresh mass (FM) basis,

$$C' = C \text{ (mg chl ml}^{-1}\text{)} * \text{volume acetone/g fresh mass}$$

where,

C' = total chlorophyll content (mg chl g FM^{-1})

Chlorophyll content was determined on a fresh mass basis. Although much variation occurs in fresh material due to differences in moisture content, it has been shown by other authors that chlorophyll determination on such a basis produces realistic results (Bruinsma 1963). Chlorophyll content was eventually expressed on a leaf surface area basis (g chl m^{-2}) by using linear regression curves.

3.5.2. Nitrogen content

Total nitrogen content of leaf material was determined for all field treatments. Each canopy was divided into top, middle and bottom layers. Three replicates of each canopy layer were used for nitrogen determination. These determinations coincided with each harvest. Subsamples were taken from dried leaf-material , used for dry matter production determination. Samples were ground to a powder in a mill (Janke and Kunkel Mikro-Feinmuhle-Culatti MFC); using a 1.5mm mesh. Nitrogen was then determined according to the micro-Kjeldahl method, described by Coombs et al. (1985).

Digestion

0.5g of dry matter was weighed onto rizzla paper and put into Buchi digestion tubes, together with two Kjeltabs, containing $CuSO_4$, which acted as catalyst for the digestion process. 20ml H_2SO_4 (98%) was added to each tube. Tubes were placed onto a digestion block (Buchi 430 digestor). A manifold was secured on tubes (1 per 4 tubes) which was connected to a scrubber (Buchi 412 scrubber) on one end. The scrubber absorbed H_2SO_4 fumes which escaped as a result of the digestion process. During this process nitrogen was converted to NH_3 which reacted with H_2SO_4 to form $(NH_4)_2SO_4$. (In order to prevent volatilization of nitrate components, salicylic acid and zinc were added during the digestion process. This reduced all nitrate components to ammonium sulphate). The digestion process took approximately 30 minutes to completion, until content of the tubes was a clear green color. Tubes were then left to cool.

Distillation and titration

This was performed by the Buchi 322 distillation unit, which in turn was controlled by the Buchi 343 control unit. The contents of each tube were diluted with water. NaOH was added to the digestion chamber and steam was passed through the mixture. Ammonia liberated from the ammonium sulphate under the alkaline conditions distilled over into the boric acid. The ammonia was then titrated with HCl to an end point determined from a blank digestion containing all the reagents except the plant material.

3.6. PHOTOSYNTHESIS MEASUREMENTS

3.6.1. Determination of net CO_2 assimilation

Measurements of net CO_2 assimilation rate were conducted on pot trials. Measurements were made using ryegrass mono, clover mono, ryegrass mix (N1) and clover mix (N1) pots. Measurements were taken at the three different layers of the canopy (six replicates each). Pots used for measurements were transported to the laboratory and allowed to equilibrate for a period of 24 hours.

Measurements were taken using an Infra-red Gas analyzer (IRGA) (ADC 225 MK3) in the differential mode. The ADC 225 Mk3 CO_2-IRGA was calibrated regularly with 369.5 ppm special CO_2 gas and CO_2-free air from the purged air outlet from the CO_2 analyzer. Air was pumped from outside the laboratory with an ADC WA-197B pump. This air intake was passed through a buffer drum to minimize fluctuations. Part of this air was stripped of CO_2 and water vapour before entering the purged air inlet of the CO_2-IRGA by employing soda lime and "Drierite" ($CaSO_4$) respectively. The remainder of the air was split into two paths, one passing through the reference cell of the CO_2-IRGA (after passing through "Drierite" containers), and the other - the analysis stream - passed via the leaf cuvette, through "Drierite" and then through the analysis cell of the CO_2-IRGA. Calibrated rotameters were used to monitor flow rates and care was taken to prevent pressure fluctuations in the cuvette. Volume flow rate were kept constant at $400cm^3$ min^{-1} depending on CO_2 depletion rate in the cuvette.

A cuvette was constructed to enable single leaf measurements of treatments. This cuvette was constructed from aluminium (100mm * 100mm and 45mm thick). The two halves of the cuvette were placed together and tightened with wing nuts. Air flow through the cuvette was generated by means of an ADC pump. The plant material (leaf) were sealed inside the cuvette by means of O rings, which clamped over the leaf blade but were sufficiently soft to prevent damage. The ambient air temperature within the cuvette was regulated by means of a flow-through water jacket built into the cuvette. Water was circulated constantly through the cuvette. The upper part of the cuvette was fitted with a glass window (35mm in diameter) to enable actinic light to be shown onto the leaf within the cuvette. An actinic light source (Schott KL1500, fitted with a halogen lamp, 15V/150W) to which bifircated fibre-optics were attached (to ensure even distribution of light) provided adequate light intensity (in excess of 1000 µE m^{-2} s^{-1}), to ensure saturation of the leaf photosynthetic system. The light intensity of the light source was measured with a Licor Li190SA qauntum sensor. A thermocouple (Type K), which was attached to a datalogger (Licor Li-1000) was inserted into the cuvette to enable the measurement of air temperature (°C).

Measurements of CO_2 assimilation rate were taken only after CO_2 depletion inside the cuvette stabilized, to allow for maximum photosynthetic rate (Pmax) to be obtained. Following the measurement of gas exchange, the leaf being measured were excised and leaf area determined by means of a planimeter (Licor Li3100).

CO_2 assimilation rate was calculated as follows (Long and Hallgren 1985):

$$F = Vol.FR/1000 * 1/22.4 * 273.15/(273.15 + temp.) * ATM/101.3 * 1/60$$

where,

F = mole flow of air (mol s^{-1})

FR = flow rate (ml min^{-1}) of air

22.4 = volume in dm^3 of one mole of air at S.T.P.
temp.= air temperature (degrees Celsius)
ATM = atmospheric pressure (For Pretoria: 883mbar at 1212m above sealevel)

thus,

$A = (F/area) * delta[CO_2]$

where,

A = assimilation rate ($\mu molCO_2\ m^{-2}\ s^{-1}$)
F = mole flow of air (mol s^{-1})
area = leaf area (m^2)
$[CO_2]$ = difference of CO_2 concentration (ppm) between reference and analysis air lines

3.6.2. Responses of assimilation to irradiance

The photosynthetic rate responses to varying light intensities (PFD) (P versus I curves), were determined for the following treatments in pots:

mono ryegrass
mono clover
mix ryegrass (N1)
mix clover (N1)

An ADC 225 Mk3 IRGA, operating in the differential mode was used to determine photosynthetic rates (see section 3.6.1.). Single leaves were placed in the cuvette, as described above, and illuminated with a KL1500 light source (halogen lamp). Light intensity emitted was measured with a Licor Li190SA quantum sensor, placed inside the cuvette. Plant material inside the cuvette was exposed to light of decreasing intensities - from approximately 2500μmol m^{-2} s^{-1} to 2.5 μmol m^{-2} s^{-1} (controlled by settings on lamp).

Photosynthetic rates were determined from leaves exposed to decreasing light intensities.

The P versus I curve was described according to the equation by Potvin and Lechowicz (1990):

$$A = A_0[1-e^{-k(PFD-Lc)}]$$

where,

A = rate of CO_2 assimilation (μmol CO_2 m^{-2} s^{-1})

A_0 = predicted saturated rate of CO_2 uptake (μmol CO_2 m^{-2} s^{-1})

k = slope of the linearized relationship (light use efficiency) (CO_2 fixed per unit PAR)

PFD = light intensity (μmol m^{-2} s^{-1})

Lc = light compensation point

3.7. CHLOROPHYLL FLUORESCENCE

Chlorophyll fluorescence signals give information on the carbon reduction cycle and thus the process of photosynthesis. Although chlorophyll fluorescence has mostly to do with the photochemical conversion of light energy, the end-products (ATP and NADPH) are consumed during the Calvin cycle and the resulting effect of the trans-thylakoid gradient and the redox state of the primary acceptor of photosystem II (and thus fluorescence yield), is an indication of photosynthetic processes (Ogren 1990).

Weiss and Berry (1987) investigated the photochemical efficiency of photosystem II. They found that measurements of chlorophyll fluorescence during steady state photosynthesis could be used to obtain estimates of the apparent quantum yield for net electron transport. According to these authors, proper functioning of the photosynthetic apparatus is apparent the higher the value of variable fluorescence (F_v) relative to minimum fluorescence (F_o) and maximum fluorescence (F_m). The ratio F_v/F_m is the quantum efficiency of photosystem II. This ratio tends to be constant among many species and ecotypes. Bjorkman and Demmig (1987) reported values of 0.832 ± 0.004 for C3 plants. Baker et al. (1989) concluded that the quantum efficiency of photosystem II seems to be the factor that limits CO_2 assimilation at limiting levels.

Chlorophyll fluorescence of pot treatments was determined (ryegrass mono, clover mono, ryegrass mix (N1) and clover mix (N1)). Measurements (3 reps each) were taken at the top and the bottom of each canopy. Pots were transported to the laboratory and (as for gas exchange studies) allowed to equilibrate for 24 hours before measurements were taken.

1 cm^2 leaf discs were excised and placed on moist filter paper in petri dishes (Ogren and Baker 1985; Adams et al. 1990). (Intact leaves in a cuvette were also used, but this method was abandoned as it was found that the available cuvette scattered too much light, making accurate recording of chlorophyll fluorescence difficult).

Chlorophyll fluorescence of leaves was determined with a pulse-amplitude modulated fluorometer (PAM 101, H.Walz, Effeltrich, F.R.G.) attached to a strip chart recorder (Pederson) as follows (Pammenter et al. 1992):

All leaves were dark-incubated for a period of 15 minutes before measurements commenced, to enable the oxidation of photosystem II (PSII) primary acceptor Q_A (Krause and Weiss 1984). The measuring beam (pulse) of PAM 101 was switched on and a low-intensity modulated light beam (<10 µmol m^{-2} s^{-1}) at a frequency of 1.6 kHz, not sufficient to excite chlorophyll, was shone on the leaf by means of fibre-optics (101F). This was done in order to determine dark fluorescence level (F_o). A saturated pulse (3000 µmol m^{-2} s^{-1}) was then administered from the actinic light source (Schott KL1500), through the fibre-optics. This pulse lasted approximately 1 s. After this pulse was allowed to recover, a second saturated pulse (5000 µmol m^{-2} s^{-1}) was administered after the first. These saturated pulses were given in order to get an indication of the maximum fluorescence level (F_{max}). In order to obtain the value for the actual F_m value, F_m was plotted against 1/I to determine peak height at infinite flash intensity (Markgraf and Berry 1990). An actinic light (Schott KL1500 lamp) source was then switched on and with the aid of bifircated optics, an even light beam of approximately 1000 µmol m^{-2} s^{-1}) was shone onto the leaf. When the steady state (F_s) has been reached, 2 saturated pulses (as described above) were given in the presence of the white actinic light, to get a measure of F_m'. This parameter is required in the calculation of quenching parameters. In order to get a value for actual dark fluorescence (F_o'), the actinic light was switched off and a far-red light pulse (>720nm) of three second duration was given with the help of a far-red light filter (RG9 filter).

The quantum efficiency of photosystem II (fluorescence yield) was determined as follows:

quantum yield = F_v/F_m
where,
F_v (variable fluorescence) = $F_m - F_o$

Quantum yield is an indication of the optimal photochemical efficiency of photosystem II.
Chlorophyll fluorescence quenching parameters were then calculated according to Weiss and Berry (1987) as follows:

$q_p = 1 - (F_s-F_o')/(F_o')$
where,
q_p = photochemical quenching
F_s = fluorescence at steady state
F_o' = actual dark fluorescence

$q_n = 1 - (F_m' - F_o')$
where,
q_n = non-photochemical quenching
F_m' = actual max. fluorescence

3.8. STATISTICAL ANALYSIS

Data was analyzed by means of the statistical software package, Genstat 5 Release 2.2; unless stated otherwise. Multiple comparisons of two factor analysis of variance were done by using the statistical test of Tukey. The Tukey test considers the Null hypothesis $Ho:\mu B=\mu A$ versus $HA:\mu B\neq\mu A$ (Zar 1984). It should be noted that the Tukey test is considered to be a very strict test of variance beyond a sample size of twenty. However, to obtain uniformity, this test was used for analysis of data throughout this thesis, unless stated otherwise.

The following abbreviations are used for describing the treatments:

Ryegrass mono	Rmono
Ryegrass mixture, nitrogen level of 200 kg N ha^{-1}	RmixN1
Ryegrass mixture, nitrogen level of 400 kg N ha^{-1}	RmixN2
Clover mono	Cmono
Clover mixture, nitrogen level of 200 kg N ha^{-1}	CmixN1
Clover mixture, nitrogen level of 400 kg N ha^{-1}	CmixN2
Total mixture, nitrogen level of 200 kg N ha^{-1}	MixN1
Total mixture, nitrogen level of 400 kg N ha^{-1}	MixN2

CHAPTER 4: RESULTS

4.1. DRY MATTER PRODUCTION

4.1.1. Total DM production (leaves and stems)

Dry matter (DM) production (g m^{-2}) was calculated for leaves, stems and the total of above. Dry matter production (DM production) was determined for all treatments during the growing period of 180 days. The starting date (day 0) was considered to be 6 June 1992. Table 1 gives the dry matter production at the end of the growing season for leaves, stems and the total of these two parameters. (Data was tested for homogeneity by means of Bartlett's test, after which a multiple comparison of means was performed by using the test of Bonferroni for homogenous data).

Table 1: Cumulative dry matter production (g m^{-2}) for the growing season ($p<0.05$)

TREATMENTS	TOTAL DM	LEAF DM	STEM DM
Rmono	1123	848	259
Cmono	1359	831	524
RmixN1	986	681	298
RmixN2	1149	860	312
CmixN1	557	380	198
CmixN2	535	349	180
MixN1	1543	1060	496
MixN2	1684	1209	492
	LSD: 296.42 CV : 8.69%	LSD: 190.73 CV : 8.04%	LSD: 108.26 CV : 10.28%

From the results in Table 1, it is clear that no significant differences exist in DM production of Mixtures N1 and N2. However, Ryegrass in mixture N1 and mixture N2 had a higher DM production than Clover in mixture N1 and mixture N2 respectively. Both mixtures (N1 and N2) exhibited significantly higher DM productions than all other treatments, with the exception of Cmono which did not differ significantly from Mixture N1.

The DM production of ryegrass was similar in mono and mixed treatments, while clover had a higher DM production in mono than mixed treatments. Both ryegrass and clover had similar DM production values with nitrogen levels of 200 and 400 kg ha^{-1}. It should also be noted that ryegrass in both mixtures (N1 and N2) had higher DM productions than the corresponding clover in mixtures.

4.1.2. **Total leaf DM**

DM of ryegrass leaves was similar in mono and mixed cultures; while that of clover was higher in mono than mixed cultures.

DM of both ryegrass and clover leaves was similar in mixtures with nitrogen levels of N1 and N2. The ryegrass in both mixtures produced more leaf dry matter than the corresponding clover component.

Mixtures N1 and N2 exhibited the highest accumulation of leaf DM than all the treatments.

4.1.3. Total stem DM

DM of ryegrass stems was similar in mono and mixed cultures while clover accumulated more stem material in mono than mixed plots.

It should also be noted that clover (mono) produced more stems than ryegrass mono and mixtures.

Ryegrass produced similar amounts of stems in both mixtures (N1 and N2). Clover followed the same pattern.

4.2. SEASONAL CHANGES IN DRY MATTER (DM) PRODUCTION (GROWTH CURVES)

Seasonal changes in DM production for the whole experimental period (180 days) were calculated for all treatments. The relationship of dry mass versus time is illustrated in the form of growth curves. Hunt and Parsons (1974) devised a computer program for analysing growth functions. This program fits the polynomial relationship (linear, quadratic or cubic) to the data which describes the growth pattern the most accurately i.e. log $y=a+bt+ct^2+dt^3$, where y = dry matter production (g m^{-2}) and t = time (days) from planting date.

Logistic curves were fitted to all data and the resultant growth curves of total DM production (leaves and stems) are illustrated (see below). Growth curves for leaf DM and stem DM will only be discussed and not illustrated graphically. For each growth curve, the upper and lower confidence limits (95% level) are illustrated. The area between the upper and lower confidence limits are shaded. Growth curves are significantly different where no overlapping of confidence limits (shaded areas) occur.

4.2.1. Seasonal changes in total DM production

Table 2 gives the growth curves describing the change in total dry matter for all treatments over time.

Table 2: Summary of seasonal variation in DM production over time for all treatments ($p<0.05$), where y = dry matter production (g m^{-2}) and t = time (days) from the planting date

Treatment	Curve Description
Rmono	$\log y=0.2365+0.1620t-0.0014t^2+0.000004t^3$ (R^2=98.2%)
Cmono	$\log y=0.1340+0.1459t-0.0011t^2+0.0000029t^3$ (R^2=99.2%)
RmixN1	$\log y=0.2693+0.0973t-0.00035t^2$ (R^2=96.8%)
RmixN2	$\log y=-0.1108+0.1395t-0.00095t^2+0.000002t^3$ (R^2=99.1%)
CmixN1	$\log y=0.1293+0.1390t-0.0011t^2+0.0000031t^3$ (R^2=99.0%)
CmixN2	$\log y=0.0078+0.1372t-0.0011t^2+0.000003t^3$ (R^2=99.2%)
MixN1	$\log y=0.0714+0.1588t-0.001231t^2+0.0000032t^3$ (R^2=99.5%)
MixN2	$\log y=0.018+0.1615t-0.00125t^2+0.0000032t^3$ (R^2=99.4%)

The growth curves for all treatments, as shown in Table 2, were all compared with one another. Only the growth curves of treatments which showed significant differences in growth rates over time are illustrated as figures (see Fig.2). Comparisons between growth curves of treatments not displayed were similar.

Details of comparisons between the growth curves of the various treatments, illustrated in Fig.2, are displayed in Table 5 below (see section 4.2.3).

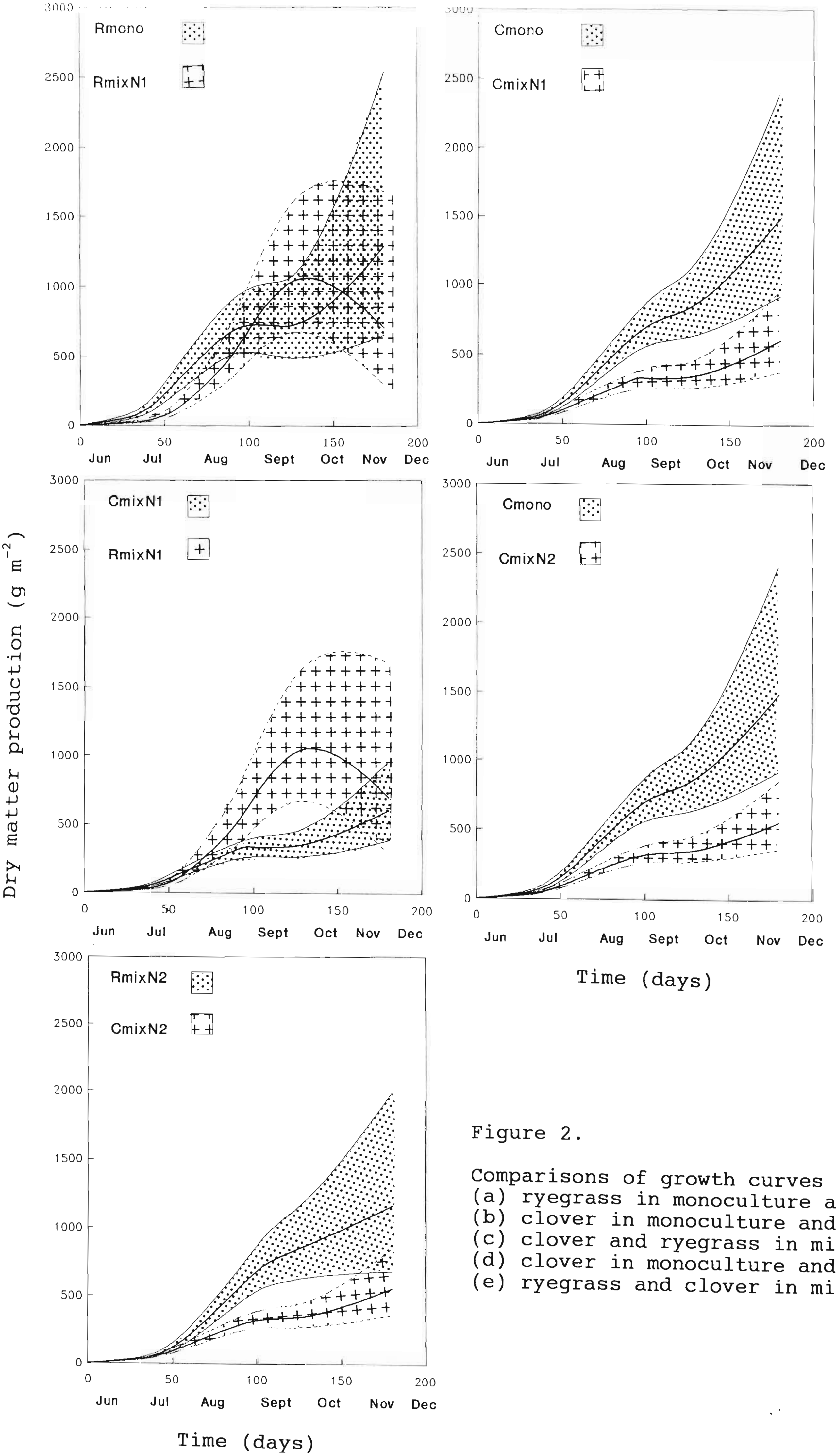


Figure 2.

Comparisons of growth curves between
(a) ryegrass in monoculture and mixN1,
(b) clover in monoculture and mixN1,
(c) clover and ryegrass in mixN1,
(d) clover in monoculture and mixN2 anc
(e) ryegrass and clover in mixN2

4.2.2. Seasonal changes in leaf DM

Descriptions of fitted curves depicting seasonal changes in leaf DM production in all treatments are shown in Table 3.

Table 3: Summary of growth curves describing seasonal changes in leaf DM production over time ($p<0.05$), where y = dry matter production (g m^{-2}) and t = time (days)

Treatment	Curve Description
Rmono	$\log y=0.2170+0.1620t-0.0014t^2+0.0000037t^3$ ($R^2=98.4\%$)
Cmono	$\log y=0.1011+0.1418t-0.0011t^2+0.0000029t^3$ ($R^2=99.3\%$)
RmixN1	$\log y=0.2815+0.0955t-0.0035t^2$ ($R^2=96.2\%$)
RmixN2	$\log y=-0.1274+0.1391t-0.00096t^2+0.0000022t^3$ ($R^2=98.9\%$)
CmixN1	$\log y=0.0950+0.1406t-0.0012t^2+0.0000033t^3$ ($R^2=99.2\%$)
CmixN2	$\log y=0.0041+0.1334t-0.0011t^2+0.0000028t^3$ ($R^2=99.2\%$)
MixN1	$\log y=0.0434+0.1594t-0.0013t^2+0.0000033t^3$ ($R^2=99.5\%$)
MixN2	$\log y=0.0054+0.1595t-0.0012t^2+0.0000032t^3$ ($R^2=99.5\%$)

As described in section 4.2.1., leaf dry matter growth curves of all treatments were compared and the details of curves which were found to differ significantly from one another, are summarized in tabular format in Table 5 (see section 4.2.3). (Leaf DM growth curves are not shown graphically; since leaf DM production will be thoroughly discussed in the section on Leaf Area Index - see section 4.3).

4.2.3. Seasonal changes in stem DM production

Seasonal changes in stem DM production is also included for the sake of completeness (Table 4). Growth curves are not shown for stem DM production, but differences in production between treatments are summarized below in tabular format in Table 5.

Table 4: Summary of curves describing seasonal stem DM production over time ($p<0.05$), where y = dry matter production (g m^{-2}) and t = time (days)

Treatment	Curve Description
Rmono	$\log y=-0.1082+0.0517t-0.000106t^2$ ($R^2=98.6\%$)
Cmono	$\log y=0.0718+0.0756t-0.00023t^2$ ($R^2=99.5\%$)
RmixN1	$\log y=0.1043+0.0385t$ ($R^2=86.2\%$)
RmixN2	$\log y=-0.734+0.0738t-0.000206t^2$ ($R^2=96\%$)
CmixN1	$\log y=0.0319+0.0610t-0.000178t^2$ ($R^2=98.8\%$)
CmixN2	$\log y=-0.281+0.064t-0.0002t^2$ ($R^2=96.0\%$)
MixN1	$\log y=-0.0489+0.0748t-0.000224t^2$ ($R^2=99.3\%$)
MixN2	$\log y=-0.2410+0.0786t-0.00024t^2$ ($R^2=98.2\%$)

From Table 4 it can be seen that the growth curve for treatment RmixN1 produced a lower correlation coefficient ($R^2=86.2\%$) than for the other treatments, which signifies a poorer fitting. This could be due to experimental error during data collection or large variation in stem DM production between the three replicate RmixN1 plots.

Table 5. Comparisons between growth curves of treatments which were found to differ significantly at the 95% confidence limits

Treatments compared	Period (days) of difference	Growth rates (g m^{-2} day^{-1}) for periods
Curves describing total dry matter production		
Rmono and RMixN1	22 to 68	24.10 - 465.55 9.42 - 196.97
Cmono and CMixN1	55 to 169	200.61 - 1211.30 129.34 - 451.46
CMono and CMixN2	39 to 180	75.04 - 1500.06 48.56 - 576.11
RMixN1 and CMixN1	93 to 149	555.33 - 1173.09 310.36 - 353.73
RMixN2 and CMixN2	65 to 159	255.18 - 1012.92 170.48 - 382.61
Curves describing leaf dry matter production		
Rmono and Cmono	39 to 74	107.4 - 507.9 62.38 - 314.36
Rmono and RMixN1	16 to 74	11.87 - 507.91 5.58 - 229.39
Cmono and CMixN1	65 to 174	237.89 - 852.33 166.69 - 345.84
Cmono and CMixN2	39 to 180	62.38 - 900.27 42.46 - 364.4
RMixN1 and CMixN1	92 to 152	450.84 - 837.27 251.78 - 250.49
RMixN2 and CMixN2	50 to 171	110.16 - 817.75 77.89 - 301.07
MixN1 and Cmono	39 to 110	49 - 775.69 62.38 - 517.5
MixN2 and Cmono	39 to 127	92.75 - 896.8 62.38 - 549
Curves describing stem dry matter production		
Cmono and CMixN1	28 to 180	7.44 - 499.80 4.96 - 26.50
Cmono and CMixN2	28 to 180	7.44 - 499.80 3.92 - 171.36
MixN1 and Rmono	28 to 156	3.511 - 216.05 6.49 - 477.78

MixN2 and Rmono	28 to 148	5.88 - 462.34 3.51 - 184.9
MixN1 and CMixN2	39 to 160	12.53 - 490.38 6.9 - 173.16
MixN2 and Rmono	28 to 148	5.88 - 462.34 3.511 - 184.9
MixN1 and CMixN2	43 to 156	14.81 - 483.72 8.38 - 167.23
Rmono and Cmono	28 to 161	3.51 - 236.5 7.44 - 531.87

4.3. LEAF AREA INDEX (LAI)

4.3.1. Leaf area versus DM regressions

Leaf area of all treatments (ryegrass and clover both in the mono and mixed plots) was determined by using leaf area versus dry mass regression curves, as described in chapter 3.
Linear regression curves (y=mx+c) were fitted for all treatments (where y = dry mass (g), x = leaf area (cm^2) and m = slope of linear regression):

Ryegrass mono : $y=x*(0.00388)+0.0312$ (R^2=99.5%, $p<0.001$)
Clover mono : $y=x*(0.003127)-0.0101$ (R^2=97.5%, $p<0.001$)
Ryegrass mix N1: $y=x*(0.00471)-0.00150$ (R^2=97.9%, $p<0.001$)
Clover mix N1 : $y=x*(0.00425)+0.00762$ (R^2=98.8%, $p<0.001$)

4.3.2. Seasonal change in canopy LAI for all treatments

Figure 3 illustrates the seasonal change of LAI in the various canopy layers for all treatments. It should be noted that the coefficient of variation is large (41.7%) which might lead to certain changes in LAI being non-significant.

(It should be kept in mind that LAI was determined at each harvest date, subjected to a cutting regime and thus indicate the amount of leaf material which was present after growth of approximately four weeks. The terms increase and decrease in LAI (as used below) thus indicate the rate at which leaf area is produced in the period between harvests).

Top of the canopy (Figure 3)

LAI of Rmono and RmixN1 increased from day 28 reaching a peak at day 111, after which LAI decreased again towards the end of the experimental period (day 180). The seasonal pattern of LAI for RmixN2 is similar to Rmono and RmixN1 except that the peak of LAI is reached at day 85, decreasing towards the end of the season.

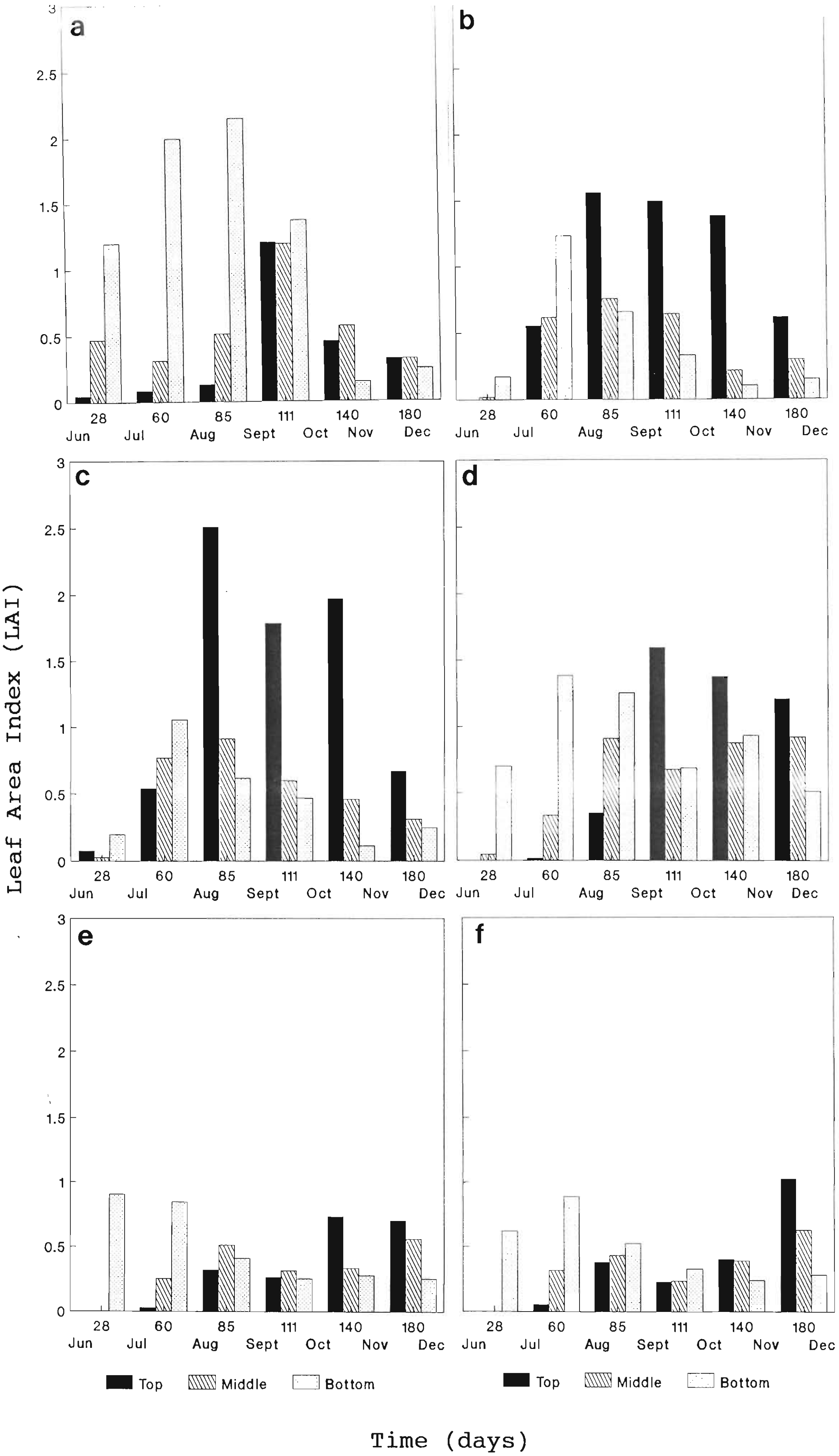


Figure 3. Seasonal variation of Leaf Area Index (LAI) in the canopy layers of (a) ryegrass mono, (b) ryegrass mixture N1, (c) ryegrass mixture N2, (d) clover mono, (e) clover mixture N1 and (f) clover mixture N2 ($LSD_T=0.871$, $p<0.001$).

LAI of CmixN2 increased towards the end of the growing period. CmixN1 also increased towards day 180, although this increase was found to be non-significant. (This might be attributed a large coefficient of variation of 41.7%). LAI of Cmono increased towards day 111 and stayed constant towards the end of the season.

Middle of the canopy (Figure 3)

LAI of Rmono increased significantly from day 28 to day 111 after which it decreased to day 180. This decrease was however not significant. LAI of RmixN2 reached a maximum at day 85, decreasing again to day 180, the decrease also being non-significant. LAI of RmixN1 also showed a (non significant) decrease towards the end of the season.

LAI of Cmono increased towards day 180, while LAI of CmixN1 and CmixN2 stayed the same throughout the season, although slight increases can be seen from day 28 to day 85.

Bottom of the canopy (Figure 3)

LAI of Rmono exhibited the same pattern in the bottom layers as in the top and middle layers of the canopy. LAI of RmixN1 and RmixN2 decreased from day 60 towards day 180, with no significant decrease in LAI of RmixN2.

LAI of CmixN1 and CmixN2 did not differ throughout the season, but LAI of Cmono showed a slight decrease from day 60 towards the day 180.

4.3.3. Distribution of LAI in the canopy layers of all treatments

It was found, after statistical analysis of the data, that canopy layer played an important role in LAI distribution. Figure 4 depicts the relationship between canopy layers for the different treatments. (Seasonal changes, see above, are not taken into

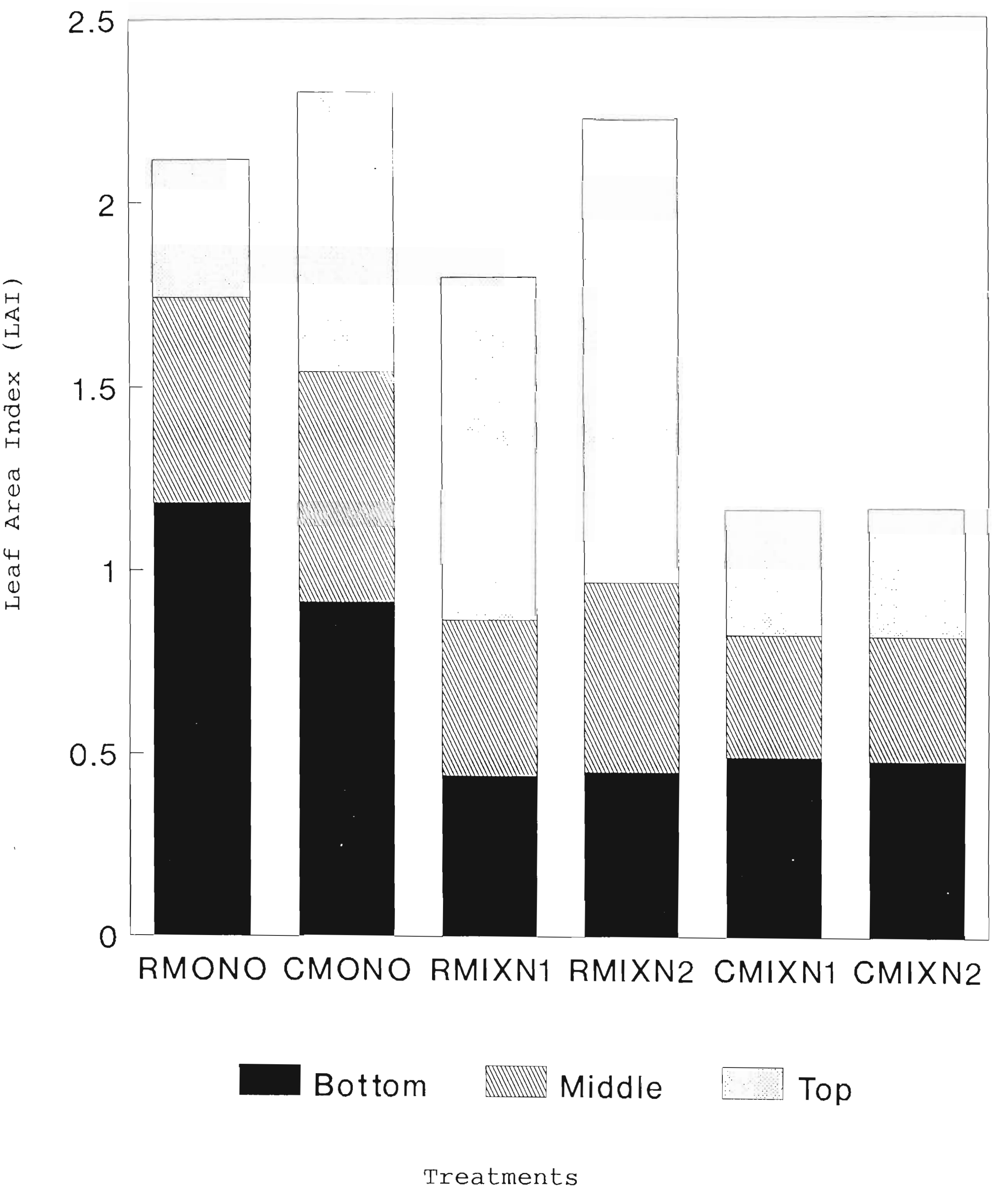


Figure 4. Distribution of Leaf Area Index (LAI) in the canopy layers (top, middle and bottom) of all treatments (LSD_T=0.308, $p<0.001$)

account when comparing canopy layer-treatment interaction. Values of LAI in the various canopy layers throughout the season were averaged and compared).

Most of the LAI in Rmono was situated in the bottom layers of the canopy, while LAI of RmixN1 and RmixN2 was significantly higher in the top layers. No differences could be found in canopy distribution of LAI for Cmono, CmixN1 and CmixN2, although total LAI for Cmono was higher than in the mixtures.

LAI in the top layers was the highest in RmixN2, followed by RmixN1 and Cmono. CmixN1 and CmixN2 exhibited the lowest LAI of all treatments in the top layers of the canopy. No differences between treatments was seen in the middle layers of the canopy. LAI in the bottom of the canopy was highest in Rmono and Cmono with RmixN1 and RmixN2 showing the lowest LAI.

4.3.4. Seasonal distribution of total LAI for all treatments

For determination of total LAI, the canopy layers of each treatment were totalled. Figure 5 depicts the seasonal variation of total LAI.

It can be seen that LAI of Rmono increases up till day 111 after which LAI decreases towards day 180. The LAI of RmixN1 and RmixN2 exhibits a similar pattern, obtaining a maximum in LAI at day 85.

The LAI of Cmono increased towards the end of the season. CmixN1 and CmixN2 also seemed to increase, but analysis of the data showed that the increase in LAI was non-significant.

Comparisons between LAI of treatments showed that ryegrass had the highest LAI from day 28 till day 111. The LAI of Cmono was the highest near to the end of the season (day 140 and 180).

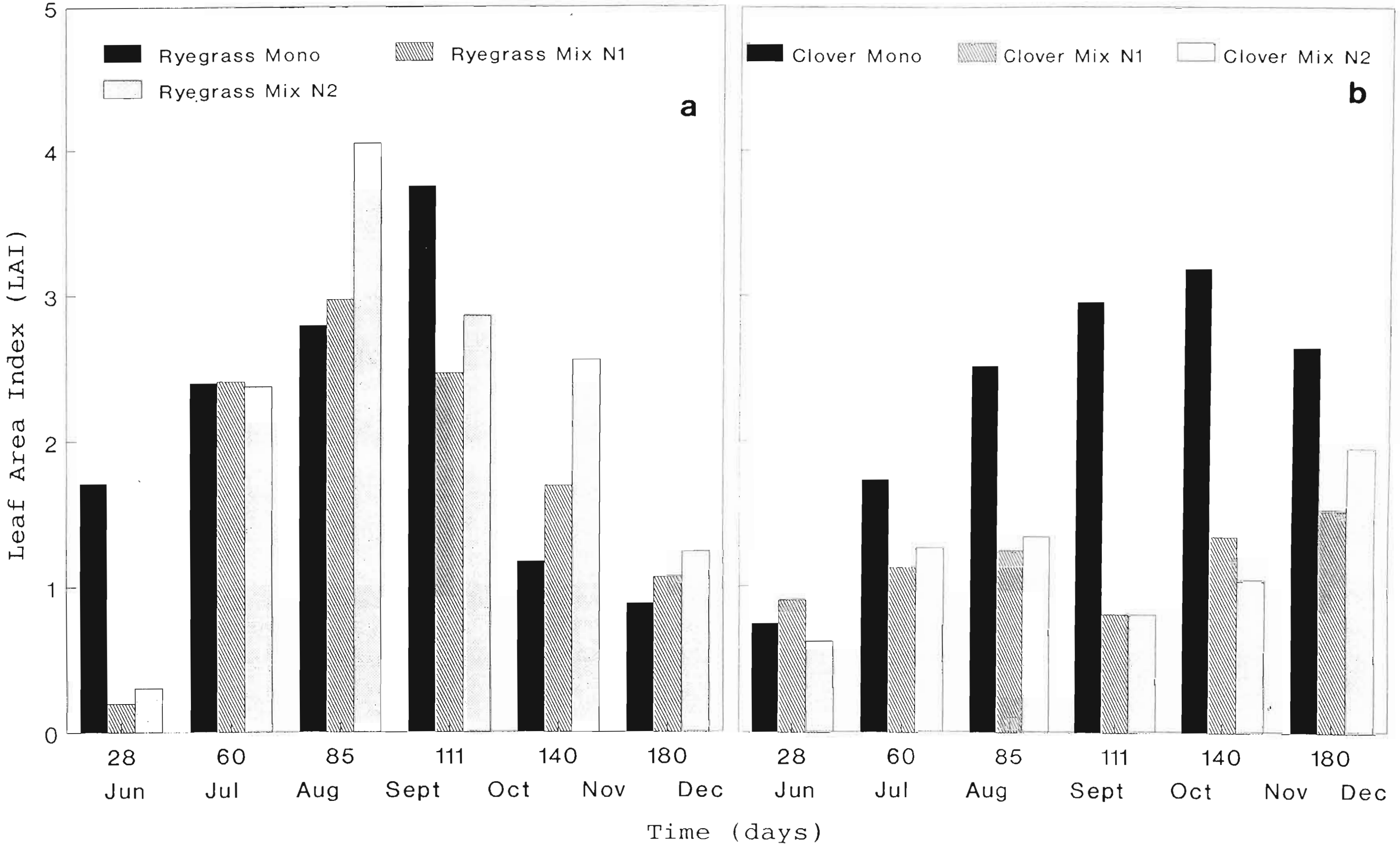


Figure 5. Seasonal variation in total canopy Leaf Area Index (LAI) for (a) ryegrass and (b) clover treatments (LSD_T=1.348, $p<0.001$)

4.3.5. The development of LAI between harvests

LAI development between days 28 and 60 (harvests 1 and 2)

Since the canopies of all treatments had not been well developed this early in the experimental season, the development of total LAI were considered, as is depicted in Figure 6(a) and (b).

During the development of LAI (for a period of 32 days), no differences could be distinguished between the various treatments. (LAI was the highest at day 60 in all ryegrass treatments). As could be expected, LAI increased significantly over this period. Although the LAI of CmixN1 and CmixN2 did not show significant increases, it can be accepted that the amount of leaf area present did increase between the periods of clippings.

LAI development between days 85 and 111 (harvests 3 and 4)

Figure 6(c) and (d) illustrate the total LAI development for the above period (26 days). According to statistical analysis of the data there are no significant increases in LAI from 10 to 26 days after harvesting. This might be due to a fairly high coefficient of variation (31%), as LAI of all treatments does increase towards day 111. Alternatively, it might be stated that the statistical test used was unsuitable for the above data.

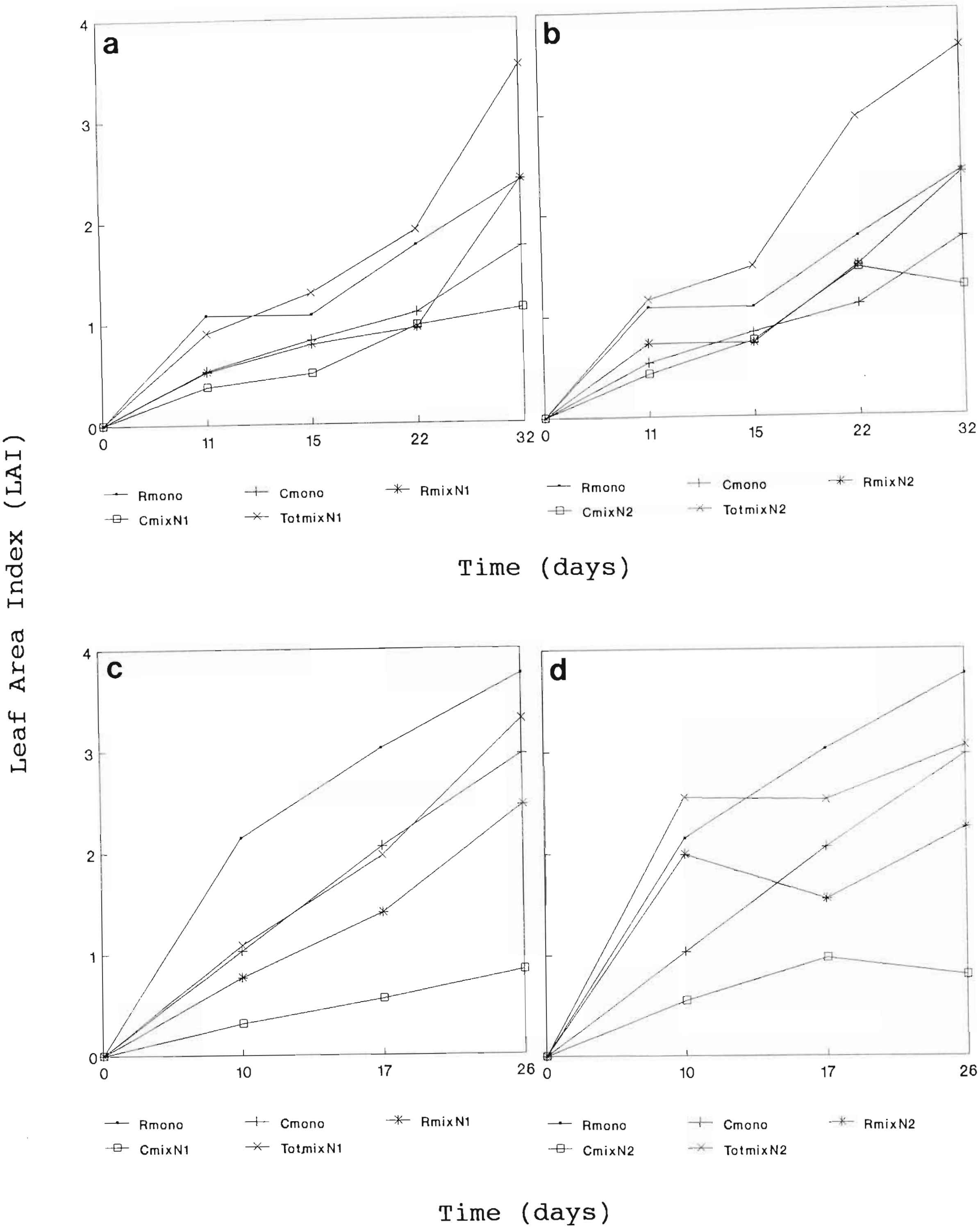


Figure 6. The developement of LAI between harvests 1 (day 28) and 2 (day 60) for a period of 32 days, with day 28 as starting point (a and b); and between harvests 3 (day 85) and 4 (day 111) for a period of 26 days, with day 85 as starting point (c and d)
(a and b: $LSD_T=1.209$, $p<0.001$)
(c and d: $LDS_T=2.221$, $p<0.001$)

4.4. THE LIGHT REGIME

4.4.1. Measurement of daily light intensity above the canopy

Changes in daily light intensity was determined throughout the season, prior to each of the canopy harvests, in order to obtain a measurement of available (non-intercepted) light energy for utilization by the pasture canopy. Daily measurements were taken between 09h00 and 15h00. Quadratic regression curves were fitted to each of the daily light intensity curves ($y=a+bt+ct^2$), which are depicted in Figure 7. Table 6 gives a description of the fitted curves illustrated in Figure 7, for the six harvests throughout the season.

Table 6: A description of the light intensity curves obtained from measurements made above the canopy, where y = light intensity (μmol m^{-2} s^{-1}) and t = time (hours)

Time (days)	Curve description	R^2 (%)	p
28 (9 Jul)	$y=-7806+1521t-63.46t^2$	99.9	<0.001
53 (4 Aug)	$y=-7745+1503.5t-62.12t^2$	99.6	<0.001
82 (1 Sept)	$y=-11043+2130t-88.56t^2$	94.7	0.001
109 (28 Sept)	$y=-7382+1564.8t-64.10t^2$	99.3	0.048
137 (26 Oct)	$y=-5626.3+1297.6t-53.329t^2$	100.0	0.01
172 (1 Dec)	$y=-9188+1895.2t-76.31t^2$	99.6	0.002

The area under each of the six quadratic curves, depicted in Figure 7, were integrated between times 06h00 and 18h00 in order to obtain a value for total daily incoming radiation incident on the pasture. Integration proceeded as follows:

$$\int_{6}^{18}(a+bt+ct^2)dt = [at+bt^2/2+ct^3/3]_{6}^{18}$$

$$= 18(a+18b/2+18^2c/3)-6(a+6b/2+6^2c/3)$$
$$= 18\ (a+9b+108c)-6(a+3b+12c)$$
$$= 12a+144b+1872c$$

The values of a,b and c, constants obtained after integration of each the curves displayed in Figure 7, were calculated by using Genstat 5 Release 2.2. and are shown in Appendix 2.
Daily photon irradiance values were given as moles m^{-2} day^{-1}.

4.4.2. Seasonal variation in radiation incident on the canopy

Seasonal variation in light intensity was obtained by calculating integrated daily light intensity values throughout the season, by using the integration method discussed above. The seasonal variation in radiation were described by fitting a logistic growth curve ($y=24.76+46.27/(1+e^{-0.1188(x-98.97)})$) to the data, where y = daily light intensity (mol m^{-2} day^{-1}) and x = time (days) (Figure 8).

4.4.3. Measurement of photon irradiance in the canopy of ryegrass mono

Measurements of photon irradiance were taken in the top, middle and bottom of the canopy. Daily measurements, similar to those described above, were taken throughout the season. Quadratic curves were also fitted to all daily data. Data for days 82, 109, 137 and 172 (corresponding to dates prior to harvests 3,4,5 and 6) are depicted in Figure 9 for the top (a), middle (b) and bottom (c) of the canopy.

Daily light intensity (photon flux density) values for each canopy layer were obtained by means of integration of the areas beneath the curves shown in Figure 9. Seasonal variation of photon irradiance could thus be obtained for each canopy layer (Table 7). The integration equation ($y=12a+144b+1872c$),

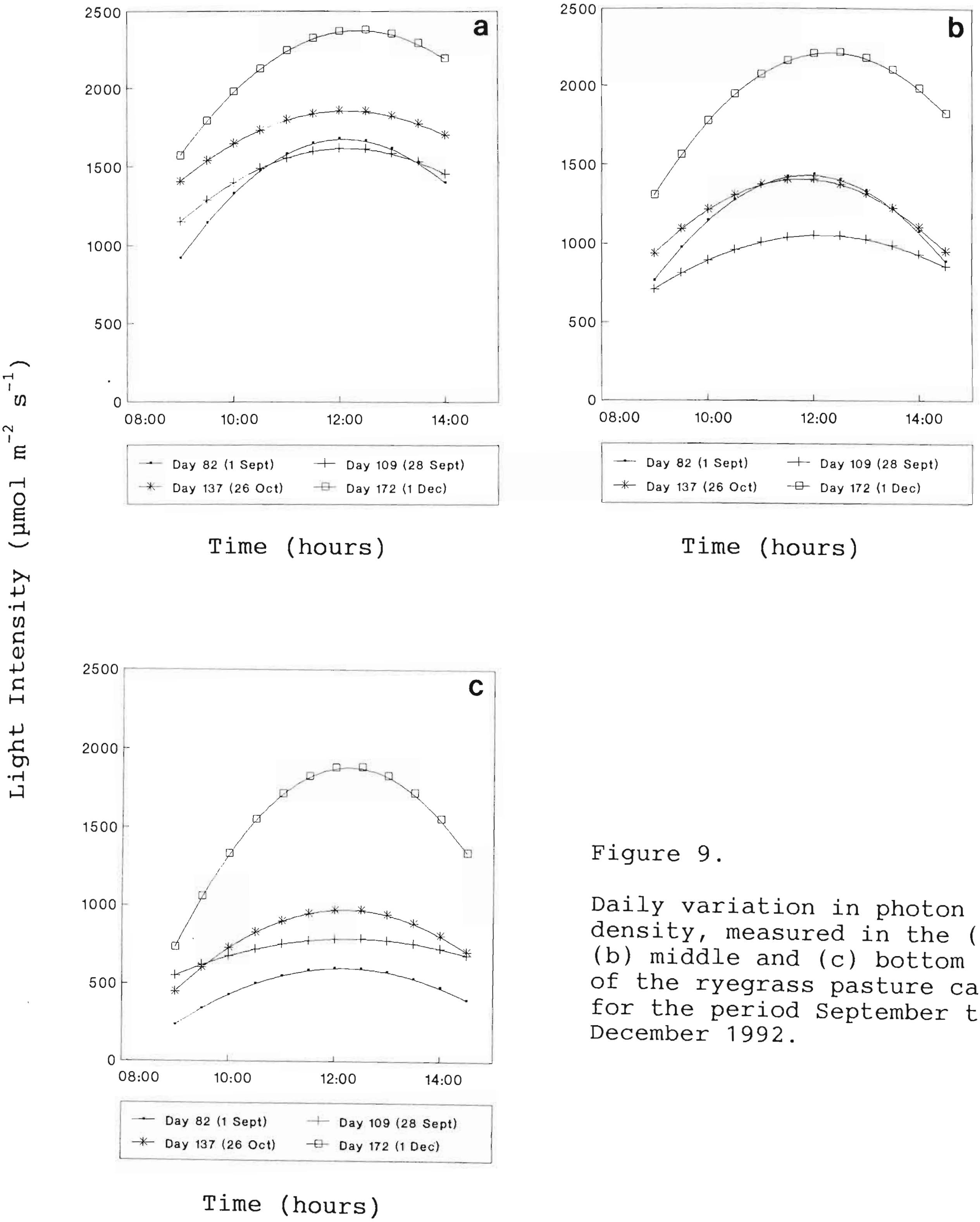


Figure 9.

Daily variation in photon flux density, measured in the (a) top, (b) middle and (c) bottom layers of the ryegrass pasture canopy for the period September to December 1992.

described above, was used to obtain values for total daily PFD. (Values of a,b and c were obtained by means of statistical analysis using Genstat and are shown in Appendix 2).

Table 7: Seasonal photon irradiance (PI) values for the different canopy layers of ryegrass grown in monoculture

Time(days)	Integrated PI (moles m^{-2} day^{-1})		
	Top	Middle	Bottom
82	31.89	20.31	7.05
109	45.26	27.23	22.74
137	56.69	28.94	15.51
172	65.87	52.61	24.75

(It should be noted that the above data is not the contributions of each canopy layer to light measured in the total canopy, but rather a manifestation of the cumulative light regime as found in these specific locations).

4.4.4. Measurement of photon irradiance in the canopy of clover mono

Daily measurements of PI were also taken in the various canopy layers throughout the season. Figures 10 depicts the daily variation in light intensity for the top, middle and bottom layers of the canopy. Daily PFD (photon flux density) trends were described by fitting quadratic curves.

The areas under the curves of daily PI variation was also integrated, as described previously in order to obtain values for seasonal variation in PI in the different canopy layers. (Table 8). Integration parameters (a, b and c) are given in Appendix 2.

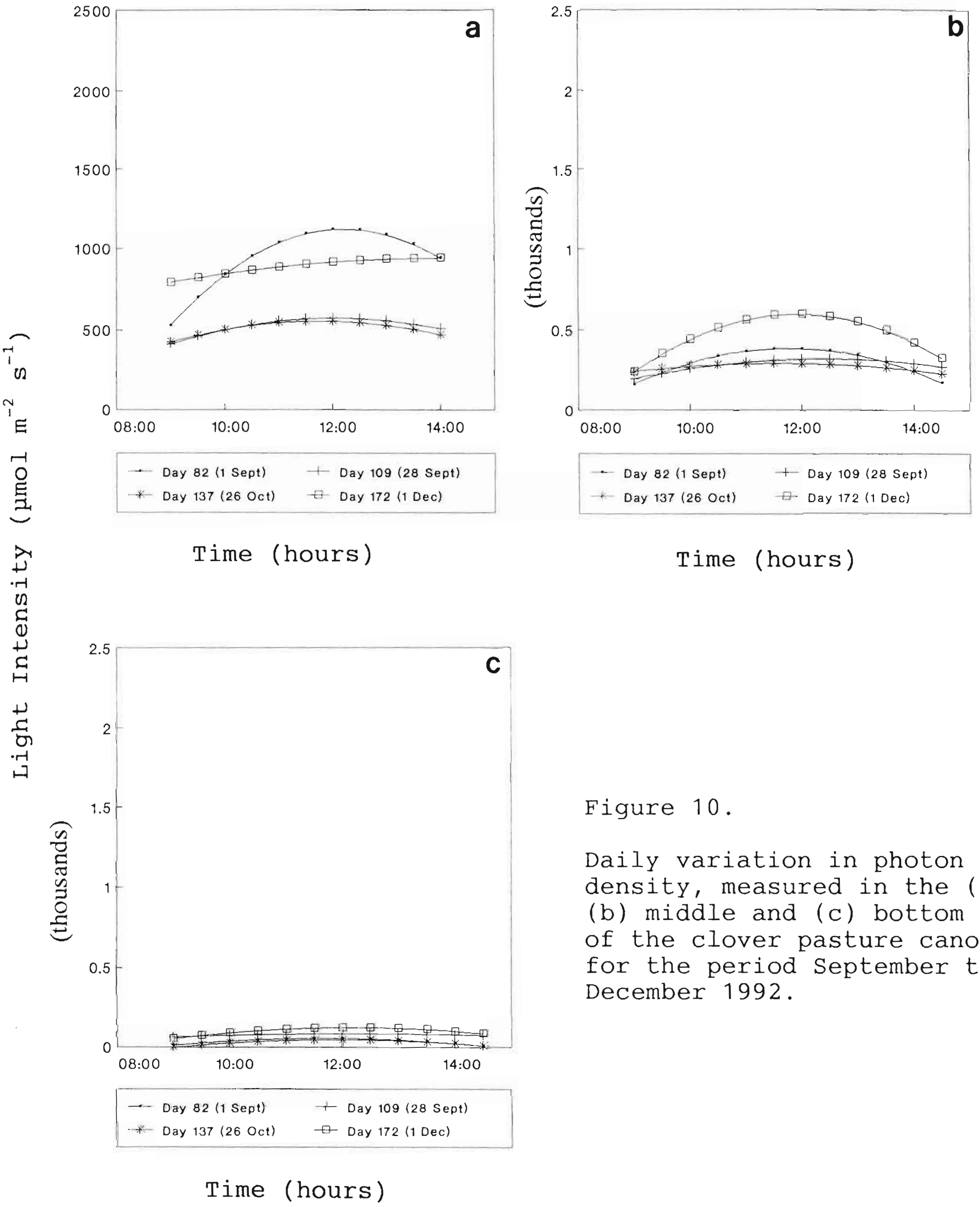


Figure 10.

Daily variation in photon flux density, measured in the (a) top, (b) middle and (c) bottom layers of the clover pasture canopy for the period September to December 1992.

Table 8: Seasonal PI values for the canopy layers of clover grown in monoculture

Time(days)	Integrated PI values (moles m^{-2} day^{-1})		
	Top	Middle	Bottom
82	18.62	1.69	-0.68 = 0
109	15.93	8.20	2.70
137	15.32	8.65	-0.91 = 0
172	36.58	4.32	1.80

4.4.5. Measurement of PI in the canopy layers of the mixture (N1)

Figure 11 illustrates the daily variation in PI in the top, middle and bottom layers of the canopy of the mixed pasture. Quadratic curves were also fitted to each of these data sets. These curves were integrated (see above) in order to obtain values for seasonal variation in PI in the various canopy layers of the mixture (Table 9).

Table 9: Summary of seasonal PI variation in the canopy layers of the mixed pasture

Time(days)	Integrated PI values (moles m^{-2} day^{-1})		
	Top	Middle	Bottom
82	2.03	0.60	-0.04 = 0
109	10.34	1.95	0.07
137	10.65	-0.83 = 0	0.02
172	60.62	56.21	13.12

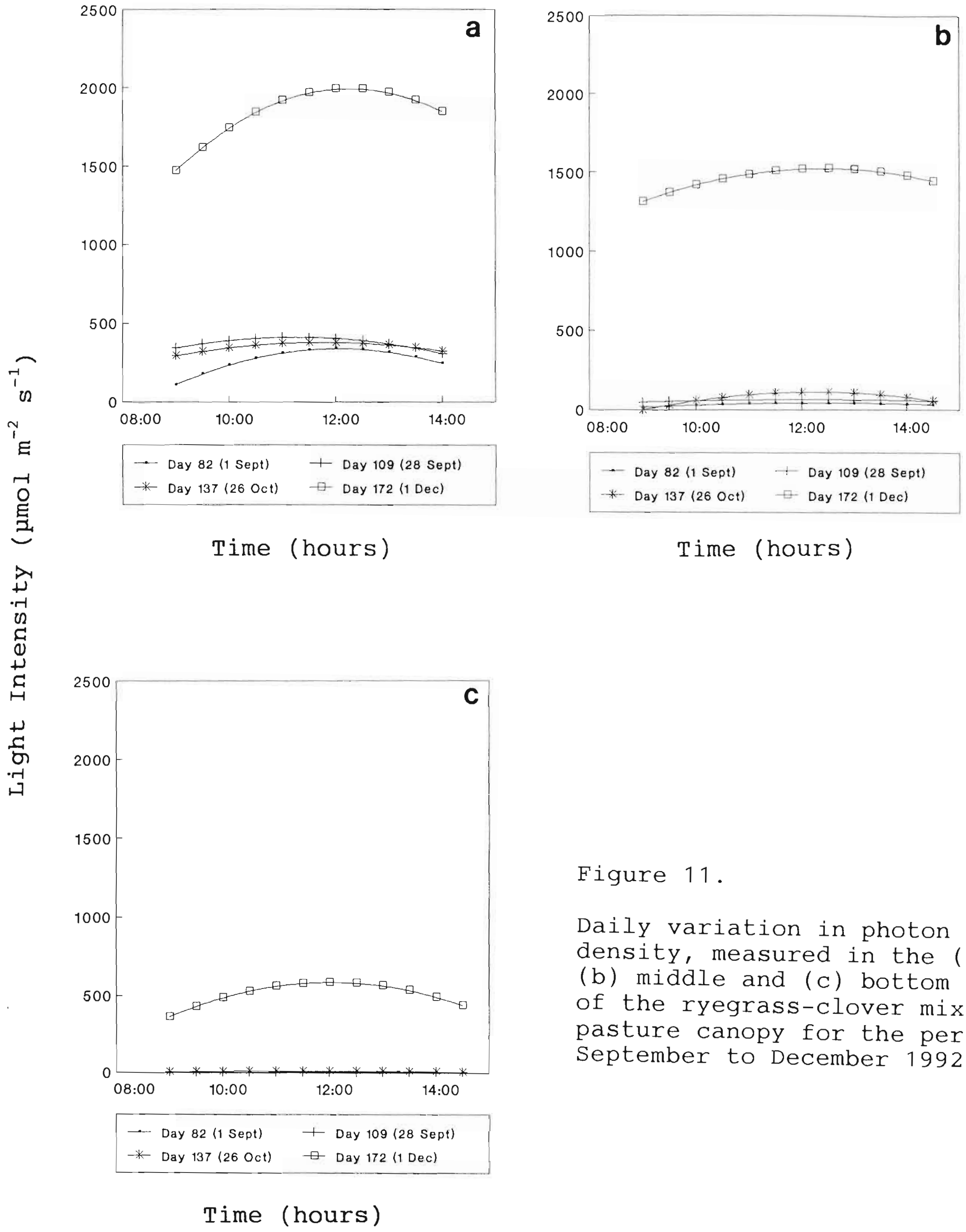


Figure 11.

Daily variation in photon flux density, measured in the (a) top, (b) middle and (c) bottom layers of the ryegrass-clover mixed (N1) pasture canopy for the period September to December 1992.

4.4.6. Comparison of photon irradiance measured in the bottom layers of all treatments

Integrated photon irradiance values in the bottom layers of ryegrass, clover and mixture (N1) were compared throughout the season (harvest 1 to harvest 6) in order to establish differences (Table 10). The photon irradiance values in the bottom layers are used for comparative purposes, as these values are representative of the total light intensity penetrating through each canopy as a whole (i.e. radiation reaching the bottom of each canopy).

Table 10: Comparison between Daily PI reaching the bottom layers of ryegrass, clover and mixture(N1)

Time(days)	Integrated PI Values (moles m^{-2} day^{-1})			
	ryegrass	clover	mixture	non-intercepted PI (above the canopy)
28	0	0	0.10	23.62
53	5.008	0.30	0.12	26.21
82	7.05	0	0	30.07
109	22.74	2.7	0.07	60.31
137	15.51	0	0.02	70.25
172	24.75	1.80	13.12	71.27

4.5. CANOPY LIGHT INTERCEPTION

As described in chapter 3, percentage light intercepted by each canopy layer of all treatments was measured using a ceptometer. Measurements were made throughout the experimental period. Daily measurements were made from 08h00 to 15h00. Analysis of the data showed that there were no distinguishable patterns for daily light interception in all canopy treatments. Thus no mathematical curves could be fitted to the data. The aim was to integrate the area under each curve to obtain a daily value for light interception. In order to compare seasonal trends, it is necessary to obtain a daily value for light interception. Daily values can also be obtained by calculating the average daily light interception. However, it was decided to calculate the percentage light interception by using the photon flux density values described above in section 4.3. Daily integrated values for PFD were obtained for each canopy layer throughout the experimental period. Percentage light interception could thus be calculated by using the following formula:

$$\% \text{ Light interception } (\%LI) = (1-T/S)*100$$

where,

T = daily integrated PI in the canopy
S = Daily integrated PI above the canopy

The integrated % LI values were fairly similar to average values obtained with the ceptometer. Table 11 gives an indication of integrated %LI values for days 82 (harvest 3), 109 (harvest 4), 137 (harvest 5) and 172 (harvest 6).

(Data from harvests 1 and 2 were not used, as the canopy layers of the treatments were inadequately developed at this time, therefore data from harvests 3,4,5 and 6 will be compared).

Table 11: Contribution of each canopy layer to total integrated canopy light interception (%) for all treatments

TREATMENT	CANOPY	DAY 82 (1 Sept)	DAY 109 (28 Sept)	DAY 137 (26 Oct)	DAY 172 (1 Dec)
Ryegrass	Top	0.00	24.95	19.3	7.58
	Middle	32.46	29.90	39.50	18.60
	Bottom	44.10	7.44	19.12	39.09
	Total	76.56	62.29	77.92	65.27
Clover	Top	38.08	73.59	78.19	48.67
	Middle	56.30	12.81	9.50	45.27
	Bottom	5.62	9.13	12.31	3.53
	Total	100.00	95.53	100.00	97.47
Mixture	Top	93.25	82.86	84.84	14.94
	Middle	4.75	13.91	15.16	6.19
	Bottom	2.00	3.11	0.00	60.46
	Total	100.00	99.88	99.97	81.59

When comparing total % light interception (LI), it can be seen that the ryegrass canopy intercepted less light (approximately 70%) than the canopies of clover (98%) and the mixture (100%), which were very similar in light interception abilities.

(It should be noted however, that the light intercepted by the canopy of the mixture exhibited an abnormal pattern on day 172. Most of the incoming radiation was intercepted in the bottom layers of the canopy (and not in the top layers) and total %LI was much reduced - 81.59%. The mixture canopy was thus not well-developed which could be attributed to damage caused by the hail storm (mentioned in section 3.2), or another factor like ryegrass senescence or a nutrient deficiency.

From Table 11, it can also be seen, that light interception patterns in each canopy layer changed throughout the season. Ryegrass intercepted most of the incoming radiation in the bottom layers of the canopy on days 82 and 172, while most light was intercepted in the middle layers on days 109 and 137 (September and October).

Most of the incoming radiation was intercepted in the middle layers of the clover canopy on day 82. On days 109 and 137, the top layers intercepted most of the radiation, while on day 172 most of the interception took place in both the top and middle canopy layers.

The mixture interception most radiation in the top layers of the canopy, except on day 172 when most light interception occurred in the bottom layers.

4.5.1. Relationship between light interception and leaf area index

The amount of photon irradiance being intercepted in the canopy of each treatment is closely related to the amount of foliage present in each canopy i.e. canopy density. Therefore, the relationship between light interception and the LAI of each treatment was investigated. Figure 12 illustrates the relationship between % light interception and LAI for ryegrass, clover and the mixture.

Ryegrass (Figure 12a) intercepted 72.73% of incoming radiation at a LAI of 3.0. When LAI was increased beyond 3.0, more light was not intercepted. Clover (Figure 12b) showed maximum light interception of 93.51% at a LAI of 2.5. The ryegrass-clover mixed canopy intercepted all of the incoming radiation (100%) at a LAI of 3.5.

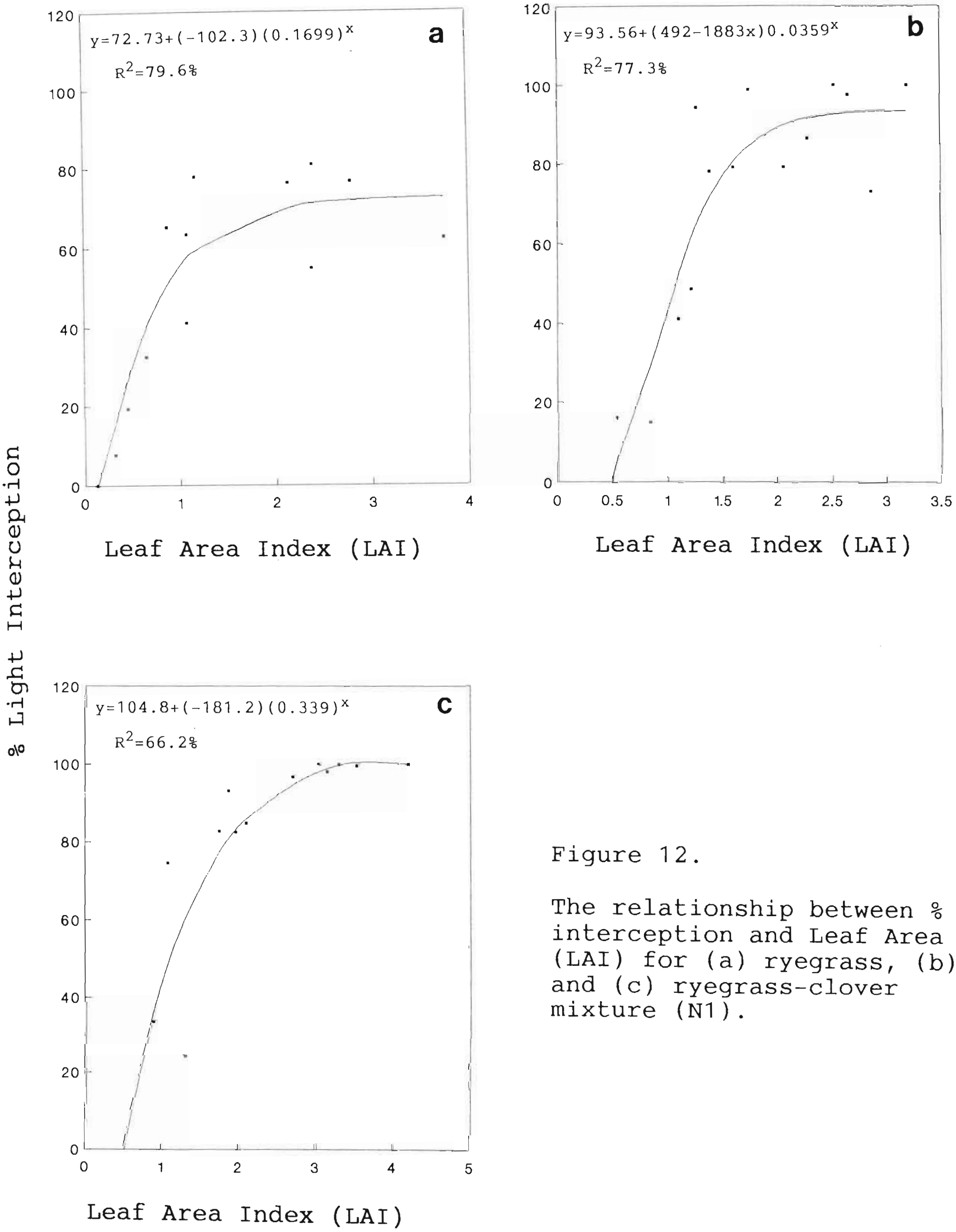


Figure 12.

The relationship between % light interception and Leaf Area Index (LAI) for (a) ryegrass, (b) clover and (c) ryegrass-clover mixture (N1).

4.6. CHLOROPHYLL CONTENT

Chlorophyll content (g chl m^{-2} leaf surface) was determined in the canopy layers of all treatments throughout the experimental period. Chlorophyll content determined on a fresh mass basis was converted to a leaf area basis by using linear regression curves.

Figure 13 illustrates the seasonal changes in chlorophyll content.

No statistically significant differences in chlorophyll content were evident between canopy layers of each treatment. Therefore, an average chlorophyll value for the canopy as a whole was determined for each treatment.

Seasonal changes in chlorophyll content could be seen for ryegrass in the mono cultures, where the chlorophyll content increased significantly towards the end of the season (0.371 to 0.731 g chl m^{-2} leaf area). Chlorophyll content of ryegrass in the mixture and clover in mono stands increased towards the end of the season, but this increase were found to be non-significant. The chlorophyll content of clover in the mixture showed no significant seasonal pattern.

Comparisons between treatments showed that no difference in chlorophyll content could be distinguished, except towards the end of the season where ryegrass in both the mono and mixed stands had significantly higher chlorophyll contents that clover in mono and mixed stands (0.731 and 0.739 g chl m^{-2} leaf area in contrast to 0.297 and 0.423 g chl m^{-2} leaf area).

(Statistical analysis of variance was done according to the Bonferroni method for homogenous data).

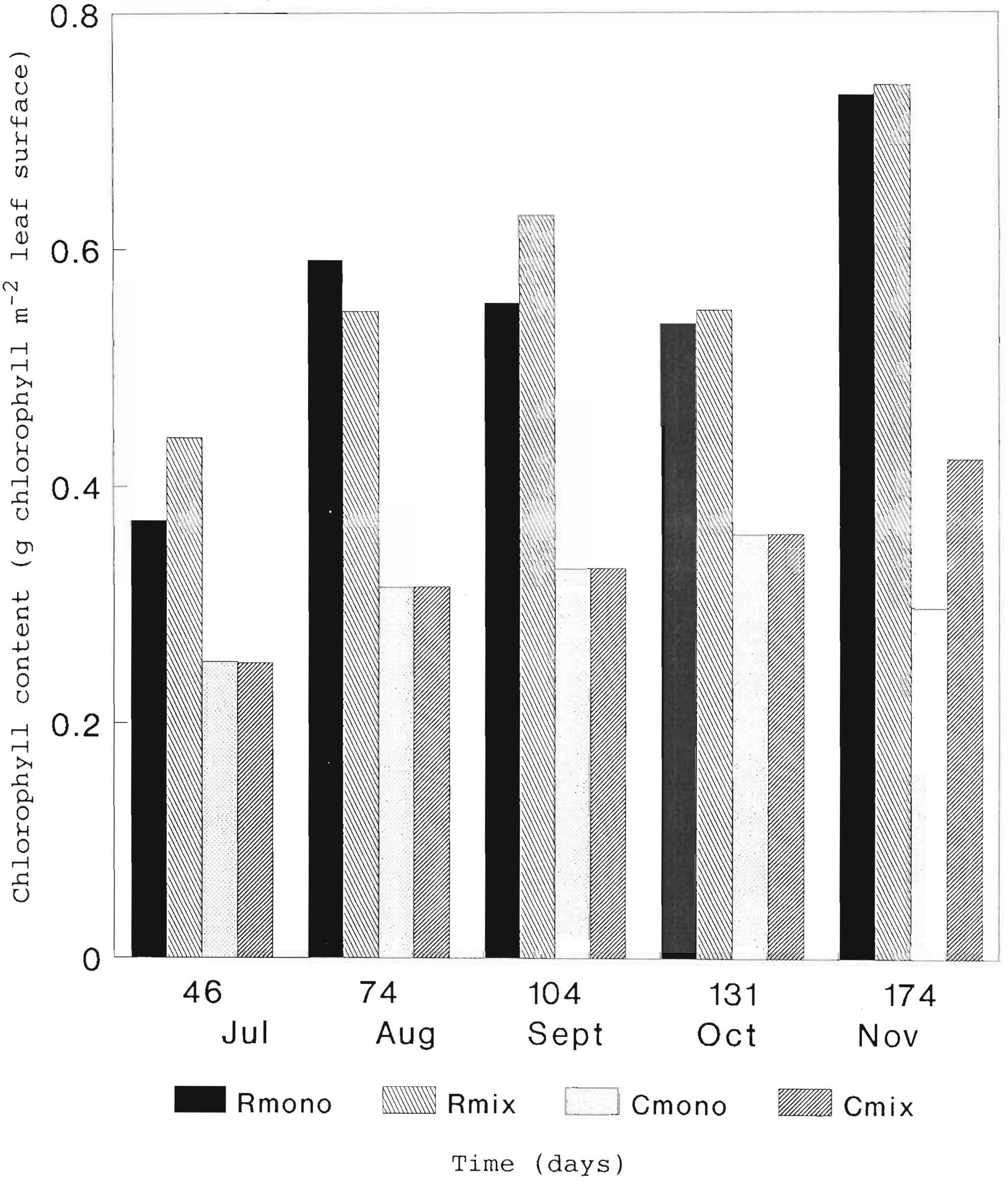


Figure 13. Seasonal variation in chlorophyll content for ryegrass and clover in both mono and mixed (N1) stands (LSD=0.303, $p<0.001$)

4.7. DETERMINATION OF NITROGEN CONTENT

4.7.1. Leaf nitrogen concentration

Leaf nitrogen concentration (%) of the different canopy layers of each treatment (averaged throughout the season) is illustrated in Figure 14. (A single statistical analysis was performed on the data set, including all treatments and all canopy layers). The nitrogen concentration in canopy layers exhibited statistical differences with concentration decreasing significantly from the top of the canopy downwards towards the bottom in all treatments.

In the top layers clover mono and clover in Mixture N2 had the highest nitrogen concentration (6.0 and 6.2 respectively). Ryegrass mono had the lowest nitrogen content of 2.9%.

Clover in mixtures N1 and N2 had the highest nitrogen content in both the middle and the bottom layers with ryegrass mono containing the least nitrogen in both middle and bottom layers.

4.7.2. Nitrogen content (g N m^{-2})

The nitrogen content of each canopy layer, expressed per unit ground area (g N m^{-2}), was calculated from the nitrogen concentration and LAI of each canopy layer, and the data are presented in Figure 15 (values of all harvests were averaged). This permits the contribution of each canopy layer to the total leaf nitrogen pool to be assessed.

The largest nitrogen pool was located in the top of the canopies, except for ryegrass mono and the clover in mixtures where no significant difference between canopy layers could be found. Comparing between treatments, it can be seen that in the top layer of the canopy, clover mono and ryegrass in both mixtures had the highest amount of nitrogen. Clover mono had the largest nitrogen pool of all treatments in both the middle and bottom

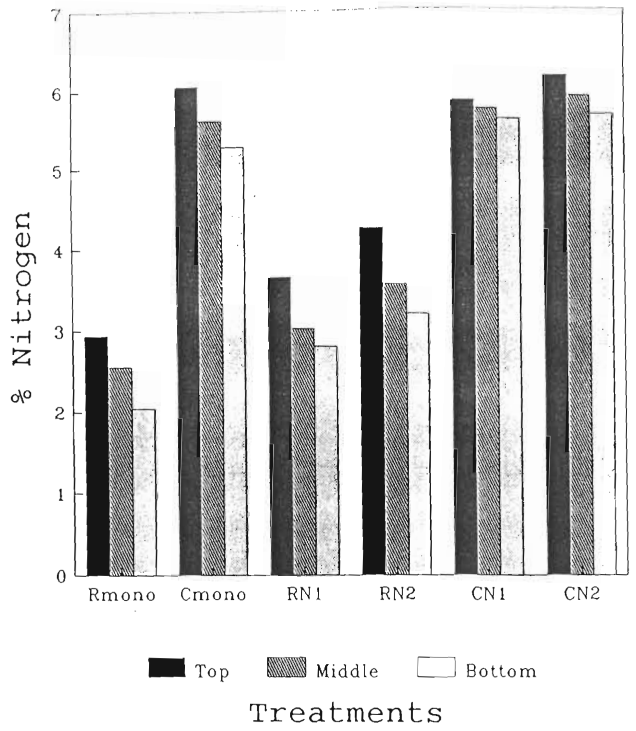


Figure 14. Distribution of nitrogen concentration between the top,middle and bottom layers of the canopies of ryegrass and clover in both mono and mixed (N1 and N2) stands (LSD_T=0.122, p<0.001)

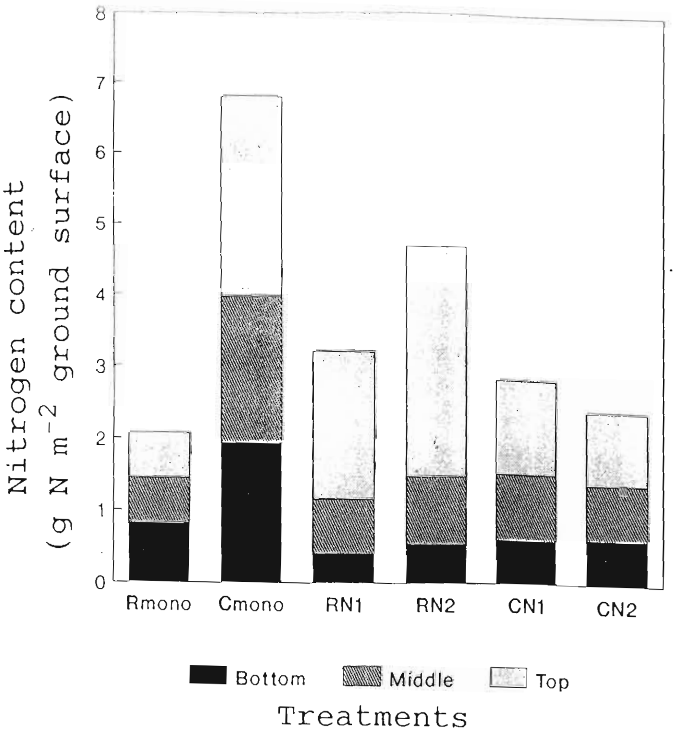


Figure 15. Proportional contribution of the different canopy layers of ryegrass and clover in both mono and mixed (N1 and N2) stands to total plant nitrogen (LSD_T=0.852, p<0.001)

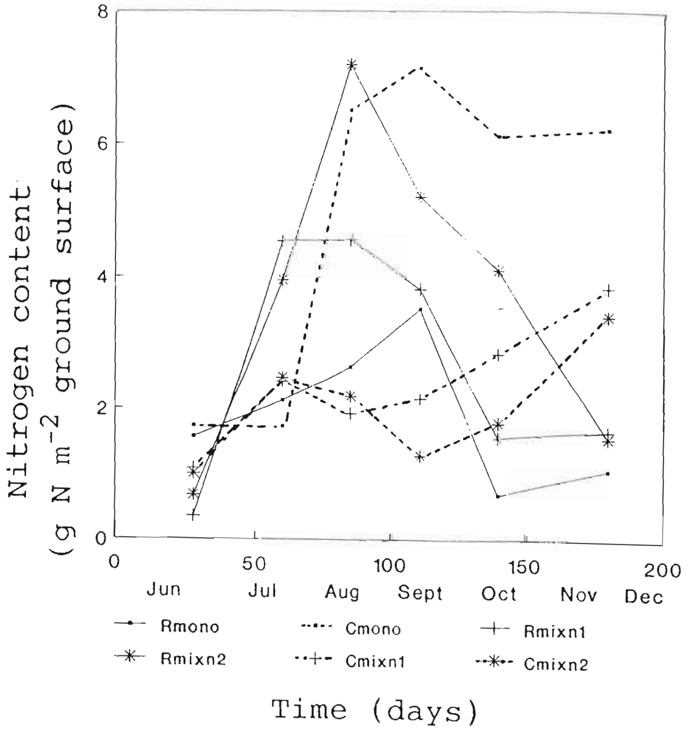


Figure 16. Seasonal variation in total nitrogen content for all treatments (LSD_T=2.523, p<0.001)

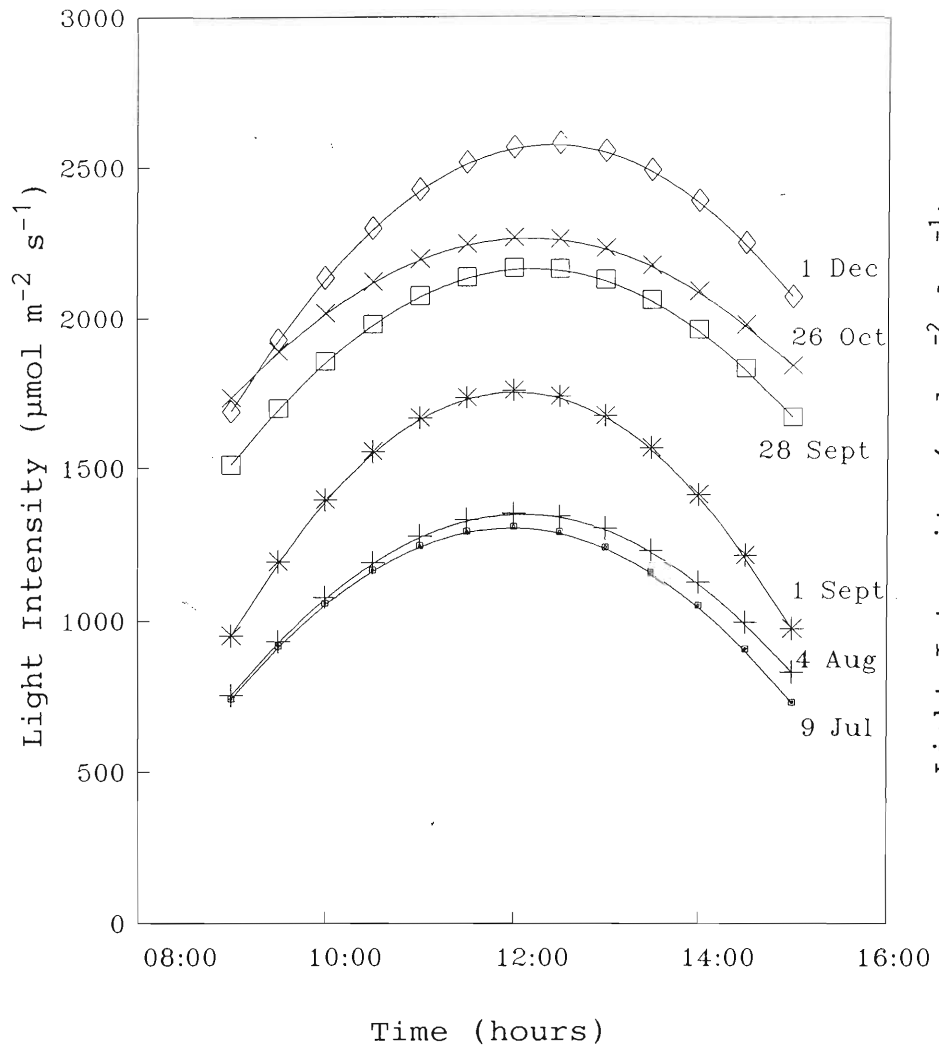
canopy layers.

Figure 16 shows the variation in total nitrogen (the whole canopy) between treatments for the experimental period (days 28 to 180). From this figure the following can be seen:

Ryegrass mono increased in nitrogen content to day 111 and then decreased again towards the end of the growing season. Ryegrass in both mixtures also showed this pattern of initial increase until midseason with a following decrease towards the end of the season. Clover mono and clover in mixture N1 increases significantly towards day 180. Clover in mixture N2 also appeared to increase, although not significantly.

Comparing between treatments, it was found that initially (day 28) all treatments had similar nitrogen contents. The nitrogen content of ryegrass in the mixtures increased significantly. At day 85 ryegrass mixture N2, clover mono and Ryegrass mixture N1 had significantly higher nitrogen values than ryegrass mono and clover in mixtures N1 and N2 (7.201, 6.512, 4.549 g N m^{-2} compared to 2.657, 1.933 and 2.202 g N m^{-2} respectively). Towards day 180; the nitrogen content of clover mono was significantly higher (6.226) than the other treatments.

It should be stressed once more that the above data was obtained from trials subjected to a clipping regime. Therefore, seasonal changes in nitrogen accumulation by the canopy represent changes in nitrogen pools during the growth between harvests. A decrease in canopy nitrogen content thus does not indicate a decrease in canopy nitrogen, but rather a decrease in the rate of nitrogen accumulation whilst growing between harvests. This could be due to either (or both) a lower leaf area produced or leaves with a lower nitrogen concentration.



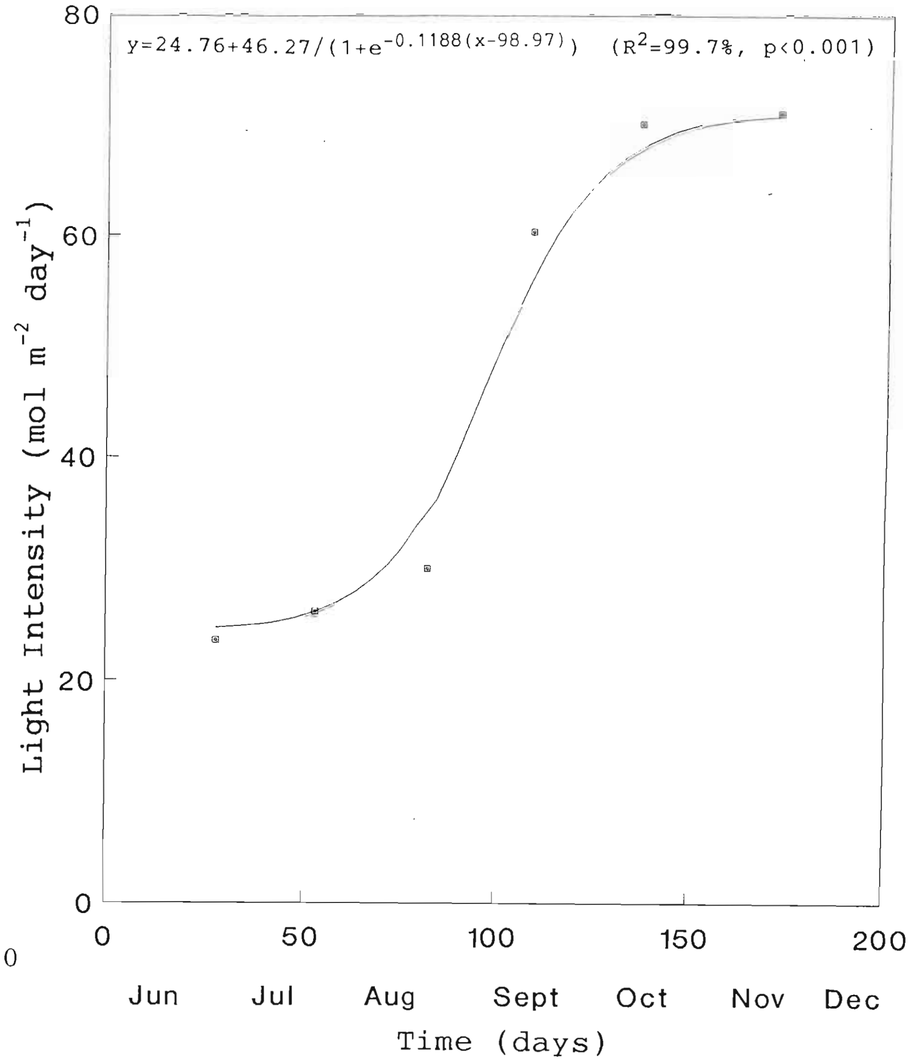


Figure 7. Daily variation in photon flux density, measured above the pasture canopy throughout the season

Figure 8. Seasonal variation in photon flux density, measured above the pasture canopy

4.8. CARBON DIOXIDE ASSIMILATION

4.8.1. Determination of maximum CO_2 assimilation rates

Net carbon dioxide assimilation rates of leaves of pot grown plants were measured at different leaf positions representative of the different canopy layers. Measurements were made on ryegrass mono, clover mono and mixture N1. Table 12 gives the maximum photosynthetic rates obtained for the above treatments.

Table 12. Maximum CO_2 assimilation rates ($\mu molCO_2\ m^{-2}\ s^{-1}$) measured in canopy layers

	Top	Middle	Bottom
Rmono	17.217	12.892	11.576
RmixN1	19.040	10.000	9.607
Cmono	18.997	14.910	13.354
CmixN1	18.770	11.947	11.017

LSD = 2.8150 (Bonferroni method)
CV = 6.336%

Maximum photosynthetic rates were higher in the top than the middle and bottom layers of all treatments. The middle and bottom layers did not differ from each other in terms of max. photosynthetic rates. There were however no significant differences in maximum assimilation rates between the various treatments - with the exception of clover mono in the middle layers, which had higher photosynthetic rates than the other treatments.

4.8.2. Determination of photosynthetic responses to varying irradiance levels (P versus I curves)

Photosynthetic responses to varying irradiance levels were investigated for all the treatments, using single leaves from the top and bottom layers of each canopy. Curves were fitted by non-linear regressions, using the regression function (Mitscherlich) described by Potvin and Lechowicz (1990) (see Chapter 3).

Photosynthetic light response curves of the top layers of ryegrass and clover in both mono and mixed stands are illustrated in Figure 17. A comparison between these curves showed that they were similar in both their linear slopes (α - light use efficiencies) and the maximum photosynthetic rates obtained. The photosynthetic apparatus of all treatments were shown to be saturated at PFD values in excess of 1000µmol m^{-2} s^{-1}.

Tables 13A and 13B give a comparison of the parameters obtained from curve fittings done of the P versus I curves for both leaves measured in the top and bottom layers of all treatments. For both the top and bottom layers, no significant differences between the various parameters could be found between the various treatments. When comparing between the top and bottom canopy layers, it can be seen that the P_{max} values in the bottom layers were significantly lower than in the top layers (as mentioned in section 4.8.1. above). The values for quantum efficiencies (α) were significantly higher for leaves measured in the bottom layers than in the top layers.

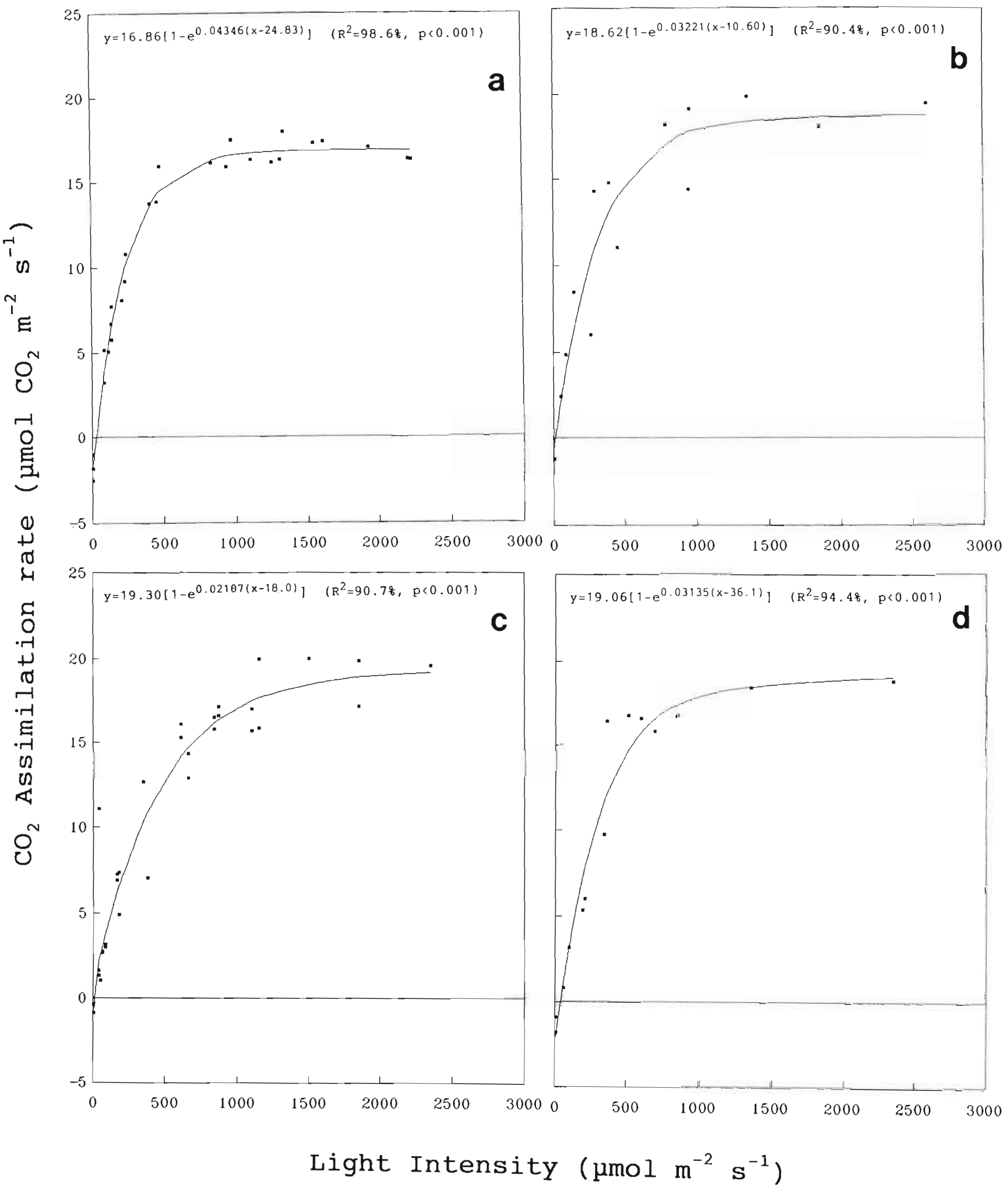


Figure 17. The relationship between photon flux density and CO_2 assimilation rate for (a) ryegrass mono, (b) ryegrass mixture N1, (c) clover mono and (d) clover mixture N1.

Table 13A. A comparison of the parametres obtained of P versus I curves for single leaves from the top layers of the canopy

	P_{max} μmol m^{-2} s^{-1}	α molCO$_2$ E^{-1}	light comp. point μE m^{-2} s^{-1}	R^2 (%)
Rmono	16.862	0.0470	24.83	98.6
RmixN1	18.620	0.0370	10.60	90.4
Cmono	19.300	0.0223	18.00	90.7
CmixN1	19.060	0.0360	36.10	94.4

Table 13B. A comparison between the parameters obtained of P versus I curves for single leaves from the bottom layers of the canopy

	P_{max} μmol m^{-2} s^{-1}	α molCO$_2$ E^{-1}	light comp. point μE m^{-2} s^{-1}	R_2 (%)
Rmono	12.026	0.0661	24.15	99.0
RmixN1	11.836	0.0713	13.58	98.1
Cmono	13.059	0.0528	9.18	99.1
CmixN1	11.619	0.0686	29.97	99.0

4.9. CHLOROPHYLL FLUORESCENCE

Chlorophyll fluorescence parameters were determined as described in Chapter 3. F_v/F_m, which is a measures of the quantum use efficiency of photosystem II, photochemical quenching (q_P) and non-photochemical quenching (q_N) were calculated for ryegrass and clover, in both mono and mixed stands (in the top and bottom layers of the canopy), and are presented in Table 14. (q_N and q_P were both measured at saturating light conditions).

Table 14. Chlorophyll fluorescence parameters

Treatments	F_v/F_m	q_N	q_P
Cmono (top)	0.788	0.783	0.356
Cmono (bot)	0.784	0.777	0.428
Rmono (top)	0.781	0.885	0.498
Rmono (bot)	0.747	0.873	0.373
CmixN1 (top)	0.756	0.755	0.505
CmixN1 (bot)	0.710	0.707	0.598
RmixN1 (top)	0.748	0.839	0.401
RmixN1 (bot)	0.715	0.831	0.412

LSD:	0.0329	0.0774	0.2308
CV :	1.428%	3.144%	16.934%

(The statistical analysis were done using the test of Bonferroni)

The top canopy layers had significantly higher values of F_v/F_m than the bottom layers, with the exception of Cmono, where no differences between canopy layers could be distinguished.

There were also differences between treatments. Ryegrass in mono stands had a higher quantum use efficiency than ryegrass in the mixtures. Similarly, clover in mono stands exhibited higher rates than clover in mixtures. Clover mono in the bottom canopy layers had a higher quantum use efficiency than ryegrass mono in the bottom canopy layers, while ryegrass and clover in the mixtures (in all canopy layers) had similar rates.

There were no significant differences in q_N between the canopy layers in all treatments. Ryegrass mono had a higher q_N value (0.879) than clover mono (0.780), while ryegrass in mixtures also had a higher value (0.835) than clover in mixtures (0.731).

No significant differences in q_P could be detected between canopy layers in each treatment and also between the various treatments. However, clover in mixture N1 had a significantly higher photochemical quenching (0.598) than clover in the monoculture (0.356).

CHAPTER 5: DISCUSSION AND CONCLUSIONS

Rhodes and Stern (1978) stated that competition has occurred between two components grown in a mixed culture when yield of a component in mixture is lower than that in monoculture. This statement, however, can be seen as an oversimplification, as the above might merely be a manifestation of interaction between pasture components rather than the occurrence of competition. Therefore, care should be taken when using the term "competition". It should first be established whether interaction between pasture components for a limiting resource does occur, before the nature of the interaction can be discussed. It was the main aim of this study to investigate the nature of interactive responses between ryegrass and white clover, grown in a mixed pasture, in relation to the prevailing light environment in the pasture canopy.

Differences in total dry matter production were found for the various treatments in this study. Measured values of total production were much higher in the mixtures than in the monocultures. Pasture yield values of 15 tons ha^{-1} yr^{-1} for mixture N1 and 16 tons ha^{-1} yr^{-1} for mixture N2 compared favourably with values cited in the literature (values obtained from the national trials in the United Kingdom, Frame and Newbould 1986). Ryegrass production in monostands of 11 tons ha^{-1} yr^{-1} seemed realistic when compared to values obtained by Smith (1987) in a clipping study conducted at Ermelo, where he found yield values of 15 tons ha^{-1} yr^{-1} for grass pastures receiving 400 kg N ha^{-1}. Davidson and Robson (1986) found values in the order of 12 tons ha^{-1} yr^{-1} for ryegrass production. Smith (1987) also found dry matter values for white clover, under a 4-weekly clipping regime, to be 13 tons ha^{-1} yr^{-1}. Clover monoculture yield in this study was 13 tons ha^{-1} yr^{-1}. There were no significant difference in the yields between ryegrass and clover grown in monocultures. For both ryegrass and clover, the component yield was decreased in mixtures relative to monocultures (Table 1).

Differences between yields obtained in mixtures and monocultures (yield of mixtures exceeded that of monocultures) is indicative of interactive responses between components in the mixtures. This interaction must be related to morphological and/or physiological characteristics.

Ludlow (1978) stated that pasture yield is limited by both light interception and utilization. He defined competitiveness for light as the ability of plants to capture available light. According to Beyschlag *et al.* (1990), competition for light is influenced more by differences in canopy structure than physiological characteristics of individual leaves. The results obtained from this study also indicate that light interception by the pasture canopy is the major factor determining growth and interactive responses.

Canopy structure and architecture affects the penetration and distribution of photon flux density (PFD) in pasture canopies. A study of the canopy structures of ryegrass and clover in mono stands and in a mixture showed major differences.

The distribution and orientation of leaves in a canopy is of major significance in relation to light interception. Monsi and Saeki (1953) stated that light energy penetrating a canopy is a function of the incident light energy and leaf area index (an adaptation of Beer's law). Brougham (1958) and Graham *et al.* (1988) observed an exponential relationship between the amount of light intercepted and cumulative LAI for numerous crops. This relationship was confirmed in this study where a similar correlation was found for the treatments investigated. Here, the term "critical LAI", as first used by Brougham (1958), is of value. Critical LAI can be defined as the value for LAI where the maximum amount of incident light energy is being intercepted by a specific pasture canopy (maximum interception by a leaf canopy is not necessarily 100%). When LAI is increased beyond the critical LAI value, no further increase in light interception will be observed, due to factors such as canopy closure which results in shading of leaves found in the lower canopy levels. A value for LAI of 5 for a ryegrass-clover mixture (in Australia) was found by Brougham (1958) to intercept 95% of the incident radiation. Critical LAI values are strongly influenced by canopy structure and growth forms and will vary between mono and mixed stands. An important assumption is made in connection with the relationship of LAI versus light interception, namely that all the leaves of a species have uniform light intercepting capacities and also, no account is taken of changes in light quality distribution, vertically through the pasture.

Leaf orientation and shape are important considerations when studying canopy structure. Ryegrass in mono stands has a relatively open canopy structure, with erect leaves (long and slender), directing incoming radiation to lower levels of the canopy (light is being reflected from the surface of ryegrass leaves downwards to lower canopy layers) and also allowing for direct light penetration towards the bottom layers of the canopy. Most of the LAI in the ryegrass monoculture canopy seem to be distributed towards the bottom of the canopy (Figure 4), thus enabling the whole canopy to intercept most of the incoming radiation. However, seasonal changes in canopy growth (LAI distribution) could be observed towards the end of the growing season (Figure 3a). This phenomenon can be attributed to the annual nature of ryegrass, where the reproductive phase is entered near the end of the growing season. From Figure 12a, it can be seen that the critical LAI value for ryegrass in mono stands is 3, where maximum light interception is 72.73% of the incident radiation. Results on the seasonal variation in total canopy LAI (Figure 5), shows that the critical LAI is exceeded only between September and October (Day 111) when a value of 3.75 for LAI was obtained; implying that maximum light was intercepted (72.73%). The ryegrass canopy was well-developed at this time. The phenomenon of leaf flagging (Brougham 1958) was also observed during this period. This can be described as the tendency of the leaf to bend towards the horizontal as it elongates. This mechanism acts to increase leaf area exposed to light and would therefore also increase the amount of light being intercepted by the canopy.

Values obtained for light interception (maximum of 77.92% was measured in the field) exceeded that of the maximum potential light interception (72.73%) calculated from LAI versus light interception graphs. This might be attributed to the fact that the exponential curve describing the relationship between LAI and % light interception is an under-estimation of the real situation (R^2=79.6%), due to possible noise in the data set. However, a realistic indication is still provided of the light regime within the ryegrass canopy, which can be used as a guideline for the implementation of management strategies.

The clover monoculture canopy is very different from that of ryegrass monoculture. Clover leaves are horizontally disposed and are therefore more favourably orientated to intercept light energy during most of the day than an equal area of ryegrass. Various authors (Brougham 1958 and Ludlow 1978) have observed phototropic leaf movements for white clover, whereby the leaves orientate themselves facing the sun throughout the day. However, proof for this phenomenon is still lacking and doubtful. These movements are phytochrome dependent, and due to light quality changes within the canopy (Wilson and Ludlow 1992), it is unsure whether phytochrome could trigger these movements (Ludlow 1978). Suffice to say that clover leaves remain horizontal in the canopy to capture light most effectively. Maximum light is received at the top of the canopy by light saturated leaves. In this study, no differences in LAI distribution with depth through the canopy was observed. However, it was noted that bigger leaves were placed at the top of the canopy; while numerous smaller leaves could be found in the lower canopy layers.

Total canopy LAI, produced during inter-harvest periods, varied seasonally, but reached a peak value of 3.10 between October and

November (Figure 5). Values recorded for light interception ranged between 95.53% and 100%. Critical LAI of clover was found to be 2.5 (Figure 12) when interception is saturated at a maximum of 93.51%. Brougham 1958, found the critical LAI of white clover to be 3.5. Total light interception was greater in clover monoculture than ryegrass monoculture, due to the more closed canopy structure of clover. It should be noted that a high LAI was maintained in the instance of the clover canopy towards the end of the season; while the LAI of ryegrass declined due to annual canopy deterioration towards December.

The ryegrass-clover mixed canopy had a higher pasture yield than both the monocultures. This implied that the mixture was able to intercept (or utilize) light energy more effectively. The canopy structure consisted of erect ryegrass leaves as well as horizontal clover leaves. Ryegrass grown in the mixture formed more tillers that bore into the top layers of the canopy, resulting in most ryegrass LAI in the top layers, throughout the season. Clover exhibited a similar canopy structure as in monoculture. It was stated by Boller and Nosberger (1985) that white clover in mixed canopies can utilize sunflecks and therefore, although situated lower in the mixed canopy than ryegrass, are exposed to an adequate light environment. This ability was not investigated in this study, but might play a role in the ability of clover to interact successfully with ryegrass with regard to the light environment in the mixed canopy. Another characteristic attributed to clover growth form is the phenomenon of petiole extension. Thompson and Harper (1988) and Schwank *et al.* (1985) noted that clover, when in the vicinity of grasses, grows almost linearly upright by extension of the petiole to ensure that clover leaves are favourably placed for light interception in the top layers of the canopy. This phenomenon was not observed in this study. Clover in the mixed canopy produced less stem material (1978 kg ha^{-1} yr^{-1}) relative to clover in monoculture (5241 kg ha^{-1} yr^{-1}), having similar stem:leaf ratios. (0.631 for clover monoculture and 0.521 for clover in mixture N1). (The overall production of clover in mixtures was reduced relative to clover in monocultures). The mixture was found to have a higher total LAI than the respective monocultures, reaching values of 4.21. With a critical LAI of 3.5 where 100% of incident radiation was absorbed; it is not surprising that the mixture obtained the highest yield of all treatments. Critical LAI was exceeded in the mixture between July and October; implying that LAI was allowed to develop to such an extent that no resultant benefit in light interception was obtained due to shading effects caused by canopy closure. Light interception was complete at a LAI of 3.5. An increase in LAI to very high values might lead to a decrease of light being intercepted due to shading of lower canopy layers. The importance of correct management will be discussed later in this section. Ludlow (1978) stated that the erect structure of grass leaves and horizontal clover leaves, combined in a mixed pasture canopy, contributed to minimizing differences in "competitiveness" and leads to stability in mixtures.

Intercepted light is utilized by the process of photosynthesis. Both ryegrass and clover are C3 species, which utilize only the Calvin cycle for CO_2 fixation. The light level at which photosynthesis saturates generally is below that of full sunlight in C3 species (Bjorkman 1981). Results from this study indicated that all treatments had similar light requirements for light saturation of photosynthesis i.e. in excess of 1000 µmol m^{-2} s^{-1}. Due to the fact that light levels decrease with increasing depth through canopies, most photosynthesis takes place in the upper parts of plant canopies (Beyschlag *et al.* 1990) where leaves are light saturated. The results from this study confirmed these findings. In all treatments studied, maximum rates of CO_2 assimilation were highest in the top layers of the canopies (ranging from 10.040 to 17.217 µmol CO_2 m^{-2} s^{-1}); and decreased significantly towards the bottom layers of the canopy (in the range of 9.607 to 13.354 µmol CO_2 m^{-2} s^{-1}, Table 12). These results compared favourably with values cited by Woledge et al. (1989). There were however no differences in photosynthetic rates between treatments in each canopy layer, indicating similar capacities for CO_2 assimilation for all treatments. Decline in photosynthetic capacities from the top to the bottom of the canopy indicates adaptation to high light conditions, but might also be attributed to (1) the lower light environment, experienced in the bottom layers due to interception in top layers and thus shading effects, results in the photosynthetic apparatus not saturated in the bottom canopy layers) and/or (2) leaf age gradients in the pasture canopy (as pointed out by Wilson and Ludlow 1983) results in newly formed leaves and older senescing leaves being found in the lower canopy layers.

However, as shown by Wilson and Ludlow (1992), light becomes a determinant of photosynthetic rate only at low PFD levels, such as occur in the lower levels of canopy. An investigation into the light use efficiencies (quantum yield) of assimilation of CO_2, showed that no differences between any of the treatments occurred, in either the top or bottom layers (quantum yield for the top layers varied between 0.0223 and 0.0470 mol CO_2 E^{-1}, Table 13a). However, the quantum yield in the bottom canopy layers were found to be higher than for those measured in the top layers (ranging between 0.0528 and 0.0713 mol CO_2 E^{-1}, Table 13b), showing adaptation to low light conditions. There were no differences between the treatments in these responses.

Measurements of photochemical activity, and particularly emittance of chlorophyll fluorescence, were undertaken to complement measurements of gas exchange. In this way more information on carbon assimilation could be obtained. Baker _et al_. (1989) showed that the above parameters are valuable in obtaining a physiological and biochemical bases for understanding the possible differences in the ability of leaves to assimilate CO_2.

The quantum use efficiency of photosystem II (F_v/F_m, Table 15) in the chloroplasts shows that the top layers of all treatments had significantly higher F_v/F_m values than the bottom layers (also expressed in higher CO_2 assimilation rates). Both ryegrass and clover in monocultures had higher quantum use efficiency rates than in mixtures.

Absorbed energy not used in photochemistry is dissipated as either fluorescence or heat energy. Photochemical and non-photochemical fluorescence quenching can be used to distinguish between allocation of energy to photochemistry (electron transport sustaining CO_2 reduction) and to radiationless dissipation processes. Quenching is defined as any kind of decrease in chlorophyll fluorescence emission. Demmig and Winter (1988) showed that non-photochemical quenching is well correlated with changes in electron transport rate in response to light. Results from this study showed that clover in monoculture had a higher quantum use efficiency of photosystem II in the bottom canopy layers than ryegrass in monoculture. Ryegrass in monoculture exhibited higher non-photochemical quenching values (0.885) than clover in monoculture (0.783) which indicates that more radiationless decay occur in ryegrass. This is an indication that ryegrass is more efficient at dissipating excess energy (q_P and q_N was measured at saturating light intensities). Similarly, ryegrass in mixture also exhibited the same pattern as above in relation to the clover component in mixture in both the top and bottom canopy layers.

Determinations of chlorophyll-pigments were made in conjunction with chlorophyll fluorescence and gas exchange studies. No differences were found in chlorophyll content between the treatments, except towards the end of the growing season when ryegrass (mono and mixed) had much higher chlorophyll contents than clover (mono and mixed) (Figure 13). This increase in ryegrass chlorophyll content might be an adaptation to compensate for a decrease in leaf area, in order to maintain photosynthetic ability towards the end of the season. (Chlorophyll values of white clover compared favourably with values obtained by Howarth et al. (1978) for white clover.)

Results from this study are therefore in agreement with various authors (Ludlow 1978; Dennis and Woledge 1983; Beyschlag 1990) who found that dry matter production and pasture growth is determined more by light interception ability of the pasture canopy than photosynthetic utilization of intercepted light energy.

The interaction between components in a mixture for light energy cannot be seen in isolation but needs to be considered in relation to other variables. Canopy structure is greatly influenced by management practices like nutrient application and clipping (i.e. grazing) - both of which will be considered below.

Before discussing the importance of nutrient status on pasture canopy structure; it should be kept in mind that the present study focused on the interaction of above-ground components, but the importance of root competition and its effect on canopy architecture should not be excluded.

As discussed by numerous authors (e.g. Smith 1987 and Eckard 1990), the application of nitrogen fertilization is one of the financially costly requirements of ryegrass pastures. This fertilizer cost has been overcome to a large extent by the introduction of a nitrogen-fixing legume, like white clover, into mixtures. (The process of nitrogen fixation and transfer from the clover to the grass component was beyond the extent of this study: many studies have been conducted on this subject). Nitrogen transfer is difficult to quantify in a clipping study, where a major nitrogen source from decomposing leaves, is constantly removed and little transfer is expected in a relatively short experimental period as in this study. (Frame and Newbould, 1986, observed a slight increase in soil fertility only after the fourth harvest of the season). For these reasons, mineral nitrogen fertilizer was applied to all experimental plots, with an added nitrogen treatment to mixtures to monitor the effect of nitrogen on the interaction of pasture components. No significant differences were found between either ryegrass or clover in mixture N1 and mixture N2; although ryegrass increased slightly in mixture N2 while clover decreased to a small extent in mixture N2 in reaction to the increased nitrogen level. Various authors have reported on the detrimental effect nitrogen fertilizer has on the growth of the clover component in mixtures (Chapter 2). Clover growth is depressed mainly due to shading caused by increased ryegrass growth. This phenomenon could not be seen to such an extent in this study, possibly because the length of the inter-harvest interval did not permit growth to such an extent. Of significance in this study is the differences in nitrogen content found between the various treatments. Clover in both mixtures N1 and N2, as well as clover in monocultures had a much higher nitrogen concentration than ryegrass in both monocultures and mixtures (Figure 14). These results are similar to results obtained by Davidson and Robson (1985) in a glasshouse study, where they found that clover had a much higher nitrogen content than ryegrass (although a nitrogen content of 3% can be regarded as sufficient for clover). As this study did not focus on nitrogen metabolism, it can only be speculated as to why clover nitrogen content is so much higher than that of ryegrass. It might be that clover has a higher nitrogen requirement for growth, as it is restricted by its position of leaves in the canopy and does not have the ability to expand leaf area as fast as ryegrass. Secondly, it is important to take nitrogen availability (and not only content) into account when monitoring growth (here differences in uptake by the roots of both components might be important). By looking at the nitrogen pool present in each canopy treatment (i.e. nitrogen as a fraction of total above-ground dry matter content, Figure 15), it was noted that ryegrass in mixture had a higher nitrogen content than ryegrass in monoculture. This could imply that nitrogen transfer did occur from clover in mixture to the ryegrass component, although no increase in production was evident for ryegrass in mixture.

Another important factor to consider is that of clipping (grazing). Clipping alters canopy structure, as leaf material is removed and the light regime within the canopy is changed. Canopy structure development and architecture should therefore be taken into account when decisions on clipping strategies are being made. For maximum production of a pasture canopy, LAI should be allowed to develop in such a way that critical LAI is reached for maximum light interception. Pasture growth and production does not benefit if canopy LAI develops beyond critical LAI. Canopy closure would result with a decrease in light being intercepted by leaves in the lower canopy layers. Leaf death due to shading could also result. Results from this study indicate that the mixed canopy might benefit from improved management, if the clipping interval was reduced from 4 weeks to 3 weeks. A 4-weekly clipping interval was found to allow LAI to develop beyond the critical LAI value, with canopy closure resulting. The monocultures of ryegrass and clover, however, did not exceed the critical LAI values during the 4-weekly clipping period.

CONCLUSIONS

Ryegrass and white clover are cultivated extensively in mixtures in South Africa because of numerous reasons mentioned in Chapter 2, eg. cost savings of nitrogenous fertilizers, higher pasture quality and high dry matter production. This type of mixture is used mainly as winter feed for grazing animals (high animal production has been reported on these pastures, Goodenough, personal communication 1993, Cedara Agricultural College, Pietermaritzburg, RSA). Management strategies are formulated mainly on grounds of studies conducted on nutrient status and nutrient requirements of plants. Few studies have been initiated in South Africa which have concentrated on morphological and physiological characteristics of ryegrass and clover in mixed pastures. Much information can be obtained from studies conducted on mixtures in the United Kingdom, New-Zealand and Australia, although these findings are difficult to extrapolate to South African conditions as environmental conditions are different and should be taken into account. Management of cultivated pastures in South Africa itself also differs from region to region due to variability in environmental factors and should be adapted to ensure maximum yield.

It was the aim of this study to investigate morphological and physiological responses of components in a mixed pasture in reaction to the light environment experienced in the pasture canopy. It can be concluded that morphological parameters (canopy structure), rather than physiological responses, influenced the interactive responses between ryegrass and clover. Competitive ability is expressed as the ability of a species to place most of the leaf area in the upper canopy layers and to orientate leaves favourably for maximum light interception. Ryegrass and clover, due to their different canopy structures, minimizes the negative competitive effects in mixed canopies. (Negative competition is defined as competition to the detriment of one component). Both components are maintained successfully in the mixed canopy throughout the season to ensure a balanced grazing pasture for animals, and complement each other. (Yields obtained in mixtures are higher than for those cultivated in monocultures). If managed correctly, ryegrass-clover mixtures can be of great economical value to agriculture in South Africa as a winter/spring pasture to maintain high rates of animal production.

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APPENDIX 1

Analysis of soil obtained from study site.

Particle size distribution	REP 1	REP 2	REP3
c/g sand 2 - 0.5 mm	4.1	4.8	5.5
m sand 0.5 - 0.25 mm	13.7	15.4	7.8
f sand 0.25 - 0.1 mm	13.2	12.8	14.9
v/b f sand 0.1 - 0.05 mm	6.7	6.4	8.2
c/g silt 0.05 - 0.02 mm	8.8	8.8	9.9
f silt 0.02 - 0.002 mm	13.6	13.0	13.8
clay 0.002mm	37.2	36.2	36.9
P mg/kg Ambic	23.7	22.5	20.8
C %	1.17	1.17	1.16
N %	0.072	0.077	0.074
pH H_2O	7.06	7.08	7.03
Exchangeable/Extractable cations me/100g oven dry (100 °C) soil			
Na	0.56	0.41	0.39
K	0.29	0.33	0.33
Ca	11.91	10.69	10.94
Mg	6.04	5.89	5.91
S value	18.80	17.32	17.57
T value (CEC)	13.60	15.35	13.60
% Fe	1.99	2.19	1.97
% Al	0.139	0.150	0.142
% Mn	0.0829	0.0828	0.0828

APPENDIX 2

Table 1. Integration parametres (a, b and c) obtained from integration of daily light intensity data

Treatment	Time(days)	a	b	c
INTEGRATIONS FOR ABOVE THE PASTURE CANOPY				
	Day 28	-28.10	5.48	-0.228
	Day 53	-27.88	5.41	-0.224
	Day 82	-39.75	7.67	-0.319
	Day 109	-26.58	5.63	-0.231
	Day 137	-20.25	4.67	-0.192
	Day 172	-33.08	6.82	-0.275
TOP LAYERS OF THE CANOPY				
Ryegrass	Day 82	-9825.76	1900.03	-78.4388
	Day 109	-5398.07	1155.96	-47.6012
	Day 137	-4848	1103.75	-45.4157
	Day 172	-8433.41	1745.65	-70.4469
Clover	Day 82	-7461.70	1405.87	-57.5495
	Day 109	-1905.02	411.46	-17.0759
	Day 137	-1749.92	391.80	-16.6483
	Day 172	-197.20	161.54	-5.7340
Mixture	Day 82	-3195.9	585.68	-24.2646
	Day 109	-1326.61	308.80	-13.7159
	Day 137	-1134.91	256.69	-10.8900
	Day 172	-5366.14	1199.59	-48.8822
MIDDLE LAYERS OF THE CANOPY				
Ryegrass	Day 82	-9945.02	1914.96	-80.5408

	Day 109	-4157.55	860.33	-68.8260
	Day 137	-7108.30	1447.59	-35.4867
	Day 172	-10303.50	2033.33	-82.5555
Clover	Day 82	-3630.23	682.28	-28.9617
	Day 109	-1332.40	267.25	-10.7995
	Day 137	-736.18	177.70	-7.6667
	Day 172	-5324.18	992.41	-41.5680
Mixture	Day 82	-328.92	60.44	-2.4518
	Day 109	-172.41	39.39	-1.6360
	Day 137	-1530.36	268.26	-10.9488
	Day 172	-1237.34	445.55	-18.000
BOTTOM LAYERS OF THE CANOPY				
Ryegrass	Day 28	-7392.71	1289.01	-52.0779
	Day 53	-7070.59	1272.15	-51.7902
	Day 82	-4805.88	889.68	-36.5834
	Day 109	-2512.27	537.54	-21.8707
	Day 137	-6670.06	1253.87	-51.3935
	Day 172	-14527.41	2678.56	-109.2462
Clover	Day 28	-5266.43	953.35	-39.8325
	Day 53	-1271.47	234.06	-9.8092
	Day 82	-757.60	138.91	-5.9300
	Day 109	-199.90	46.59	-1.9018
	Day 137	-758.12	135.20	-5.6751
	Day 172	-908.14	169.49	-6.9494
Mixture	Day 28	4314.50	-549.79	19.0100
	Day 53	-581.27	97.91	-3.7869

	Day 82	-77.32	13.54	-0.5516
	Day 109	-25.82	5.21	-0.2244
	Day 137	-142.86	26.61	-1.1281
	Day 172	-2819.83	566.11	-23.5233

APPENDIX 3

Table 1. Equations describing variation in light intensity for the various treatments

Daily PFD above the canopy of all treatments (µmol m^{-2} s^{-1})			
Day number	equation	R^2 (%)	p
28	$y=-7806+1521t63.46t^2$	99.9	<0.001
53	$y=-7745+1503.5t-62.12t^2$	99.6	<0.001
82	$y=-11043+2130t-88.56t^2$	94.7	<0.001
109	$y=-7382+1564.8t-64.10t^2$	99.3	<0.001
137	$y=-5626.3+1297.6t-53.329t^2$	100	<0.001
172	$y=-9188+1895.2t-76.31t^2$	99.6	<0.001
Daily PFD (µmol m^{-2} s^{-1}) in the canopy layers of ryegrass			
Top layers of the canopy			
82	$y=-9826+900t-78.4t^2$	93.6	0.008
109	$y=-5398+1156t-47.60t^2$	99.2	0.053
137	$y=-4848+1103.7t-45.42t^2$	99.8	0.027
172	$y=-8433+1745.7t-70.45t^2$	99.7	0.002
Middle layers of the canopy			
82	$y=-9945+1915t-80.54t^2$	91.4	0.003
109	$y=-4158+860.3t-35.49t^2$	91.9	0.164
137	$y=-7108+1448t-61.49t^2$	98.4	0.073
172	$y=-10303+2033t-82.56t^2$	99.8	0.001
Bottom layers of the canopy			
82	$y=-4806+889.7t-36.58t^2$	88.7	0.006
109	$y=-2512+537.5t-21.87t^2$	44.7	0.429
137	$y=-6670+1254t-51.39t^2$	71.3	0.310
172	$y=-14527+2679t-109.2t^2$	97.4	0.013
Daily PFD (µmol m^{-2} s^{-1}) in the canopy layers of clover			
Top layers of the canopy			
82	$y=-7462+1406t-57.55t^2$	97.5	0.012
109	$y=-1905+411.5t-17.08t^2$	85.3	0.221
137	$y=-1750+391.8t-16.65t^2$	85.7	0.218

Table cont.

172	$y=-197.2+1610.5t-5.74t^2$	84.6	0.754
Middle layers of the canopy			
82	$y=-3630+682.3t-28.96t^2$	91.6	0.042
109	$y=-1332+267.3t-10.80t^2$	100	*
137	$y=-736.2+177.7t-7.667t^2$	63.6	0.349
172	$-5324+992.4t-41.57t^2$	56.2	0.219
Bottom layers of the canopy			
82	$y=-757.6+138.9t-5.930t^2$	89.3	0.054
109	$y=-199.9+46.59t-1.902t^2$	89.6	0.186
137	$y=-758.1+135.2t-5.675t^2$	79.2	0.263
172	$y=-908.1+169.5t-6.949t^2$	83.2	0.084
Daily PFD (μmol m^{-2} s^{-1}) in the canopy layers of the mixture			
Top layers of the canopy			
82	$y=-3196+585.7t-24.26t^2$	96.0	0.020
109	$y=-1327+308.8t-13.72t^2$	91.6	0.167
137	$y=-1135+256.7t-10.89t^2$	32.1	0.476
172	$y=-5366+1200t-48.88t^2$	96.8	0.016
Middle layers of the canopy			
82	$y=-328.9+60.44t-20.45t^2$	93.3	0.034
109	$y=-172.4+39t-1.636t^2$	95.1	0.127
137	$y=-1530+268.3t-10.95t^2$	91.4	0.170
172	$y=-1237+445.6t-18t^2$	84.0	0.080
Bottom layers of the canopy			
82	$y=-77.32+13.54t-0.552t^2$	73.6	0.132
109	$y=-25.82+5.205t-0.2244t^2$	84.7	0.132
137	$y=-142.9+26.61t-1.128t^2$	62.1	0.652
172	$y=-2820+566.1t-23.52t^2$	85.7	0.072