THE USE OF MATHEMATICAL MODELS TO INVESTIGATE CARBON AND NITROGEN RELATIONS OF A MYCORRHIZAL GRASS / LEGUME MIXED STAND.

NEIL STUART ECCLES

Degree awarded with distinction on 19 April 1995.

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg in fulfilment of the requirements for the degree of Master of Science.

Johannesburg 1995

ABSTRACT

This project represents the development of a comprehensive description of the growth of two plant species (namely white clover and Italian ryegrass) in a mixed canopy. Particular attention has been paid to the impact of two symbiotic associations (legume root nodules and vesicular arbuscular mycorrhizae) on the growth of the two plant species. The development of the description was divided into two parts. The first of these was a plant growth trial in which the effects of establishment of symbioses on plant growth, nitrogen status and biomass distribution was assessed. In the case of ryegrass, neither of the two symbiotic associations had any significant impact on growth, nutrient status or resource allocation. However, both symbioses played a significant role in the enhancement of clover growth and this enhancement of growth was apparently due to improved nutrient status of plants in association with symbionts. The improved nutrient availability in clover plants which entered into symbiotic partnerships also resulted in a greater proportion of plant biomass being located in above ground structures.

The second part of the description process involved the development of a mathematical growth model of the system. Growth was set as function of two resources: substrate carbon and substrate nitrogen. Carbon substrate acquisition was calculated by means of a mixed canopy photosynthetic model where light attenuation (and therefore photosynthesis) through the canopy was determined on the basis of the leaf area distribution in the canopy. Nitrogen acquisition was calculated on the basis of root geometry, and a description of the roles of the two symbiotic associations was included. The distribution of acquired resources within the model plants was described using a flux-utilization model similar to that developed by Thornley (1972). Simulation results were generally similar to growth trial data in the clover, although the model was unable to simulate the impact of mycorrhizal formation. In the case of the ryegrass, while biomass simulations were similar to observed data, nitrogen contents differed noticeably. The shortcomings of the model act as indicators of where gaps in our understanding of the system exist.

DECLARATION

I declare that this dissertation is my own unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.

20 day of JAN, 199~~4~~5.

ACKNOWLEDGEMENTS

I would like to acknowledge my supervisor, Dr. V.M.Gray and co-supervisor, Dr. C.J.Straker, and thank the members of my post graduate committee. I would also like to thank I.M.Weiersbye for proof reading a draft copy of the dissertation and my parents for their support.

I am grateful to the Foundation for Research Development and the University of the Witwatersrand for funding me during this work.

A poster illustrating the main points of chapter 3 was presented at the South African Association of Botanists conference in Johannesburg (Eccles, Gray and Straker, 1994).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

The system to be described in this dissertation comprises of four biotic components: a legume (*Trifolium repens* L.), a grass (*Lolium multiflorum* L.), symbiotic nodule-forming bacteria and symbiotic vesicular-arbuscular mycorrhizal fungi. In terms of the nitrogen and carbon relations, this is a fairly complex system, consisting of at least 16 state variables (Fig. 1.1). One reason for interest in such a system is that these intercrop systems are increasingly being used in agriculture. It has often been reported that, given the correct management, they produce higher yield per area (overyielding) when compared with monocultures of the component species (Reeves, 1992). In addition to the agricultural potential of intercrop systems, these system offer several interesting possibilities for formal description and analysis and it is on these that the bulk of the dissertation focuses.

The first subjects dealt with in this chapter are, the use of models as a descriptive tool in biology in general terms, and literature dealing with intercropping systems. The rest of the review is structured in the same way that the modelling approach categorises the system. Firstly above-ground processes are discussed. This discussion is rather brief since the whole of chapter 3 is dedicated to this subject. Following this, literature pertaining to below-ground processes is discussed. Particular emphasis is placed on the biology and physiology of the symbiotic associations. The reason for this emphasis is that to date, the role of symbionts in plant growth has been largely ignored in plant growth models. This literature thus serves as the foundation for incorporating these into the model developed. Aspects of general nutrient acquisition are also briefly dealt with in this section and are again considered in chapter 4. The last section of this literature review deals with resource allocation with particular reference to modelling above versus below-ground allocation.

1.2 THE MODELLING APPROACH

The classical view of modelling is that it is a tool to allow the integration of ideas generated through the conceptualisation of complex systems into organisational levels (Loomis *et al.*, 1979). Penning de Vries (1983) clearly defined the benefits to be gained from the use of modelling:

i. to help define and categorise the state of knowledge,
ii. to help locate gaps in knowledge,
iii. to make hypotheses explicit and thus set priorities for research,
iv. to allow integrated information to be made operational,
v. to provide a means for disseminating knowledge.

Mathematical models are particularly useful (if not essential) since mathematics provides a symbolic logic which is capable of describing ideas and relationships of great complexity while still retaining a simplicity of statement (Jeffers, 1988). Implicit in the use of a mathematical equation to describe the relationship between two or more

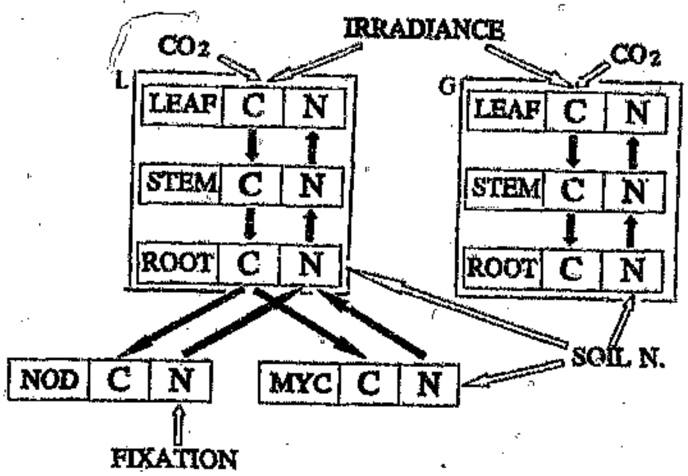

Fig. 1.1. Conceptualisation of the biotic components of the system including the legume (L), the grass (G), nodules (NOD) and the mycorrhiza (MYC).

quantities (variables) is the idea that a formal statement is being made about them, and by association, about the problem we are researching (Charles-Edwards, Doley, Rimmington, 1986).

1.3 INTERCROPPING

An intercropping system is defined as a system where two or more crop species are grown together on the same land, at the same time (Cordero and McCollum, 1979). It has been proposed that given appropriate species and management, these should be able to produce greater yields than the component monocultures, despite the occurrence of interspecific competition. In particular, a great deal of interest has been focused on the use of grass/legume mixtures which could produce benefits not only in terms of quantity, but quality as well (due to N fixing symbionts associated with the legume component) (Reeves, 1992).

Legume / non-legume intercrops are thought to be particularly productive combinations under conditions of limited soil nitrogen. It is believed that nitrogen fixation by the legume in some way (or ways) benefits not only itself, but the non-legume too. There are two possible mechanisms for this benefit:

1. It is possible that the nitrogen benefit is conferred as a result of reduced competitive exploitation of mineral nitrogen by the legume.
2. On the other hand, it is possible that there is direct transfer of nitrogen from the legume to the non-legume.

There are a number of possible mechanisms for such direct transfer, including decomposition or mineralisation, nodule or root exudates, or via a common mycorrhizal network.

There are a great number of reports on investigations of grass legume intercrops. Improved corn dry mass yield has been reported in intercropped corn and soybean fields (Herbert *et al.*, 1982), however, this was at the cost of decreased soybean yields. Improvements in corn grain yields have also been reported for the same mixture

(Crookston and Hill, 1979). Various other mixtures have also been investigated including: maize and winged bean which showed an increased protein content in the maize (Hikam *et al.*, 1992) and sorghum and pinto bean (Carr *et al.*, 1992). In addition, various forage crop mixtures have been investigated, including lucerne and various clover species as the legume component, with various species of grass such as perennial ryegrass, *Panicum virgatum* etc (Olsen, Kaiser and Al-Maskari, 1993). In the case of white clover and various ryegrass forage mixtures, it has been found that the clover is usually at a competitive disadvantage, despite having faster single leaf photosynthetic rates (Papadopoulos, 1992). This may be due to the grass having faster initial growth rates (Martin and Field, 1984), resulting in overtopping of the clover. This condition may be alleviated in a number of ways including careful selection of planting ratios (Papadopoulos, 1992). Another possibility is the use of symbiotic interactions to shift the competitive balance and this is investigated in chapter 2.

As with any agricultural system, intercrops are not without their problems. These include allelopathic effects such as those found between clover and rye (Badoux, 1993; Harkot and Warda, 1993; Snell and Quigley, 1993), finding the correct ratios of the component species so that the yields of neither are adversely affected (Crookston and Hill, 1979), as well as finding species that are tolerant of the same herbicides and pesticides (Cordero and McCollum, 1979).

1.4 ABOVE GROUND PROCESSES

Generally all above ground plant structures and activities are associated with the process of photosynthesis. This process can be considered at a number of levels ranging from molecular or biochemical to whole plant or canopy. A number of factors limit the rate of photosynthesis and these can be divided into intrinsic and external. Intrinsic factors include the amounts and catalytic activity of the photosynthetic enzymes such as ribulose-1,5-bisphosphate carboxylase-oxygenase (RUBISCO), and the rate at which substrates for these enzymes (eg. CO_2) diffuse to sites of photosynthesis. Formal descriptions of the activities of these enzymes are usually based on Michaelis-Menten kinetic formulations (eg Farquhar, von Caemmerer and Berry, 1980).

External factors influencing photosynthesis include light, atmospheric concentrations of CO_2 and O_2, and temperature (Marshall and Biscoe, 1980). In addition to these above ground external factors, below ground factors such as nutrient and water availability also play important roles: the amounts and activities of photosynthetic enzymes are strongly dependent on the supply of mineral nutrients such as nitrogen and phosphorus while the movement of CO_2 into the leaves is very tightly linked to transpiration (Pearcy *et al*, 1987).

Because of the obvious importance of photosynthesis in terms of plant growth and production (Osmond, 1987), numerous attempts have been made to describe the process formally (eg Farquhar, von Caemmerer and Berry, 1980; Marshall and Biscoe, 1980; Johnson and Thornley, 1983; Rimmington, 1984). Some models are based on detailed descriptions of the biochemical processes (eg Farquhar *et al.* 1980), however, these tend to be rather complicated and are difficult to extend to scales larger than leaf photosynthesis. Light is of obvious importance in driving photosynthesis (Marshall and Biscoe 1980). A very common approach to modelling leaf photosynthesis is therefore to amalgamate all intrinsic factors defining photosynthetic activity into two parameters: the light-saturated photosynthetic rate (AM or more commonly Amax), and the quantum yield efficiency (A or more commonly α), and then to describe photosynthetic rate as a function of irradiance. Since photosynthesis is dependent on a number of factors other than light, the rate is limited by the pace of the "slowest" process. Thus describing the rate of photosynthesis (of leaves) as a function of AM and A has often relied on the use of rectangular hyperbolic functions and similar analogues (eg Johnson and Thornley, 1983). These parameters (AM and A) need not be constant but can be defined as functions of various factors such as temperature (Johnson and Thornley, 1983), or leaf nitrogen content (Hirose and Werger, 1987).

The great advantage of this approach is that it is relatively easy to integrate such leaf photosynthetic descriptions to whole plant or even canopy descriptions (eg Johnson and Thornley, 1984). In order to do this it is necessary to incorporate some description of light attenuation through the canopy. It is also necessary to describe changes in the leaf area distributions through the canopy depth since this will define the light attenuation.

In general, photosynthesis models have been restricted to monoculture canopies although there are a few exceptions (Rimmington, 1984; Papadopoulos, 1992). The model developed by Rimmington (1984) is based on a very good description of light attenuation through mixed canopies, however, there are a number of oversimplifications in the scaling up from leaf photosynthesis to canopy photosynthesis. The model developed by Papadopoulos (1992) improved on the Rimmington model by incorporating the effects of environmental variables such as seasonal variability in temperature and irradiance. However, in the Papadopoulos model the description of canopy architecture and in particular leaf area distribution through the canopy was unrealistically static. The canopy photosynthesis model developed in chapter 3 represents an improvement on both of these previous models: Rimmington's description of light attenuation through a mixed canopy is combined with the integration developed by Johnson and Thornley (1984) for monocultures and the resulting equations are supported by a dynamic description of the distribution of leaf area within a mixed canopy.

1.5 BELOW GROUND PROCESSES

1.5.1 NUTRIENT ACQUISITION

It should be emphasized that the word acquisition infers that more is involved in getting inorganic nutrients into the plant than ion transport across cell membranes (Clarkson, 1985). There seems to be some uncertainty as to which factors are of greatest importance in nutrient uptake (Robinson, 1986). When plants are stressed by low nutrient availability, they commonly increase their root uptake capacity (equivalent to Davidson's (1969) root activity), and also increase their growth allocation to roots (Gutschick, 1993). These modifications allow plants to approach a balance between nutrient acquisition and photosynthate supply. From liquid culture experiments, the activity of uptake sites (often defined by the Michaelis-Menten constants K_M and V_{MAX}) is particularly important (Ingestad and Ågren 1988). This is because of rapid replenishment of the solution close to the root surface. However, in the case of plants in a soil medium, depletion sheaths/zones form around the root. In this situation changes in root growth appear to be more important than changes in the activity of uptake systems. Increased root growth allows exploration of greater volumes of undepleted soil.

From the perspective of the soil, fluxes of nutrients to replenish the depletion sheath is a salient feature (Gutschick, 1993). The mobility of the nutrients of interest in the soil may in fact blur the above generalisations regarding priority of allocation between activity or growth. For mobile nutrients (such as NH_4^+, NO_3^-, K^+), depletion zones may be rapidly replenished so that the situation resembles liquid culture experiments and the activity of uptake sites may be more important. On the other hand, for immobile nutrients (such as phosphorus), root growth is likely to be more important.

The above discussion applies to situations where nutrient supply is suboptimal to optimal. When nutrients are available in superoptimal levels, a key factor in the regulation of uptake is plant demand. Nutrient uptake will be limited by reducing both the uptake activity, and root growth rate relative to shoot growth. The most simple mechanistic explanation for this is that nutrient concentration in a tissue determines the photosynthate demand in that tissue, and because of the high levels of nutrients reaching the shoot, the shoot tissues will have a greater photosynthate demand. They will therefore retain a greater proportion of photosynthates, and a relatively smaller proportion will be allocated below ground (Ågren and Ingestad, 1987). However, this possibility is not considered in the nutrient acquisition model developed below since it is assumed that the system is experiencing suboptimal nitrogen levels. This is in line with the experimental work which was carried out (where the growth medium was river sand and the fertiliser applications were low to encourage establishment of symbionts). It is also in line with the fact that the great benefit of legume/grass intercropping is to be derived in nutrient-poor situations (see section 1.3)

1.5.2 NODULE-FORMING BACTERIA

Rhizobium-legume root nodules are highly organised hyperplastic, hypertrophic tissue masses derived from root cortical cells. There are three major groupings of nodules based on shape, meristematic activity and nitrogenous transport products: a. elongate-cylindrical nodules are formed with protracted apical meristematic development that assimilate fixed nitrogen into amides; b. spherical nodules with transient internal meristematic development that assimilate fixed N into ureides; c. collar type nodules that encircle the tap root (Vance *et al.,* 1988). The first of these three is found in *Trifolium* species. Following inoculation with specific rhizobial strains, a complex series of developmental alterations begins involving both partners, which culminates in the formation of nitrogen fixing root nodules. The importance of these symbioses stems from the ability of the bacterial symbiont to fix atmospheric nitrogen by means of nitrogenase under appropriate conditions. Globally, nodule mediated nitrogen fixation is responsible for fixing approximately 10^8 tonnes of nitrogen per annum (Downie and Johnston, 1988). This has obvious agronomic importance in terms of the potential to reduce applications of costly nitrogen fertiliser and has led to agricultural practices such as legume intercropping mentioned above (section 1.3).

The development of active nodules represents an intriguing example of joint differentiation in two different organisms, and it is apparent that the development involves specific expression of genes in both symbiotic partners (Downie and Johnston, 1988). The initial step in the development of the legume-*Rhizobium* interaction is believed to involve bacterial recognition of low molecular weight flavones and flavanones exuded from the legume roots (Downie and Johnston, 1988; Vance *et al.*, 1988). This is mediated, in part at least, by bacterial *nod D* genes. The interaction between these exudates and *nod D* genes results in the induction of several other bacterial *nod* genes (eg *nod ABC*, the gene products of which form a soluble factor that is required for root hair curling and possibly eliciting development of the nodule meristem) (Downie and Johnston, 1988). Specificity is an important feature, with particular legumes being infected only by a limited range of rhizobial strains or species. It has been suggested that this specificity is established at a very early stage and that this is achieved by combining plant-produced flavenoid signals with the bacterial signal to produce root hair curling factors (Downie and Johnston 1988).

The curling of the root hairs, initiated by signals from both symbiotic partners results in entrapment of bacteria within the curl and this is followed by active penetration, by the bacterium, through the plant cell wall. As this penetration occurs, root hair cell wall growth is re-orientated and appositional wall growth is initiated to form the so-called infection thread. Several hours prior to the penetration of the root cortex by the infection thread, signals from the rhizobial symbiont initiate a focus of cell division that later becomes the nodule meristem. This functions for an indeterminate period in the elongate-cylindrical nodules. The nodule meristem proliferates in advance of the infection thread, and nodule cells around this are invaded by the infection thread which releases bacteria into the cytoplasm of the host cells. As the bacteria enter they become enclosed by the peribacteroid membrane (PBM). Once inside the plant cells, differentiation of the bacteria into bacteroids occurs along with induction of nitrogenase activity.

1.5.2.1 FLUXES OF CARBON AND NITROGEN BETWEEN THE LEGUME AND NODULES

The growth and development processes described above, along with maintenance respiration, N_2 fixation and assimilation as well as transport of fixed nitrogen from the nodules represents a significant carbon sink to the host legume (for instance, nodule respiration may make up as much as 35% of the total root respiration according to Kouchi *et al.*, 1985). This is despite the fact that a certain proportion (10 - 14%) of these costs are accounted for by CO_2 fixation in the nodules through the activity of phosphoenolpyruvate carboxylase (Twary and Heichel, 1991). The primary carbohydrate transported to the nodules is sucrose, which is then cleaved in the plant fraction of the nodules by either alkaline invertase or sucrose synthase (Copeland, Vella and Hong, 1989). These enzymes have often been implicated in regulating sink strength by regulating the concentration of sucrose in some area of the plant. The mono- and disaccharides are processed in the plant cells and supplied to the bacteroids in the form of C4-dicarboxylic acids (Vance and Heichel, 1991).

In addition to the regulatory activity of sucrose synthase and alkaline invertase, the interface between the plant cell cytoplasm and the bacteroids represents a very important regulatory site for nodule activities (Aapels and Haaker, 1991; Rosendahl, Dilworth and Glenn, 1992). This interface comprises the peribacteroid membrane (PBM), the periplastic space and the bacteroid membrane. Fluxes of substances across this interface will regulate not only the exchange of carbon and nitrogen substrates between plant and bacteroids, but also the physical conditions (pO_2, pH and ionic environment) in the bacteroid (and thus the efficiency of N_2 fixation) (Rosendahl *et al.*, 1992).

There are a number of mechanisms that have been proposed to describe the exchange of carbon and nitrogen across the plant-bacteroid interface. One suggestion is that a malate/aspartate shuttle similar to that associated with mitochondria is involved (Aapels and Haaker, 1991). However, more recent evidence seems to indicate that this is unlikely (Rosendahl *et al.*, 1992). These latter authors suggest two alternatives. The first is that malate is converted to OAA[1] (by MDH[2]) and pyruvate (via malic enzyme). Transamination with amino donors such as glutamate would then produce aspartate and

---

[1] Oxaloacetic acid.

[2] Malate dehydrogenase.

alanine. How these amino acids might cross the PBM is not yet known although it has been suggested that nitrogen nutrition in symbiotic N_2 fixation may involve the plant providing amino acids in exchange for NH_4^+ (Vance and Heichel, 1991). The other alternative proposed by Rosendahl *et al.* (1992) is that transformations take place in the periplastic space through the activity of transaminases (although the presence of these has yet to be shown).

It has been argued by a number of authors that because of the strong sink strength of the nodules, that the vast majority of carbon used is recently assimilated carbon as opposed to storage forms (Gordon *et al.*, 1985). Cralle and Heichel (1985) observed an 80% decrease in nodule fixation in alfalfa within 24 hours of shoot removal. Dabas, Swaraj and Sheorar (1988) reported the same findings; however, they also found that the levels of carbohydrate in the nodules were not decreased at the same rate as N_2 fixation suggesting that there was some other short term regulation mechanism. More convincing evidence for this "recent assimilate" theory comes from work using carbon isotope labelling, which showed that within 70 minutes, a greater proportion of labelled carbon was to be found in the nodules than in the rest of the plant (Gordon *et al.*, 1985). These authors argued that other studies may not have had sufficient resolution of early events since the turn[illegible]e-export and respiration) of labelled carbon in the nodules was very rapid. Kouchi *et al* (1985) also interpret their carbon labelling work as suggesting the importance of recently assimilated carbon; however, they also note that at night the primary source of carbon used by the nodules originated from starch stored in the leaves.

Other authors, however, have argued the opposite, ie that storage forms of carbohydrates are favoured by nodules since nodules appear not to be strongly perturbed by short term interruptions in photosynthesis (Boller and Heichel, 1983). In addition to this, Finn and Brun (1982) interpreted the lag of only six hours between decreasing photosynthesis and decreasing N_2 fixation (due to application of water stress) as evidence for the use of storage carbohydrates. The evidence for this theory is far weaker than the alternative. In the final analysis, it does not really matter whether nodules use recently fixed carbon or storage forms, and in fact there is evidence that nodules will in fact use whichever is available at the time (Vance and Heichel, 1991). The general conclusion that can be drawn is that there is strong coupling between carbon assimilation and nitrogen contents , and thus N_2 fixation (Boller and Heichel, 1983; Ryle, Powell and Davidson, 1992). Increases in the nitrogen supply to the plant may increase the photosynthetic ability (Hirose, 1988). This is in line with the idea of a "balance" (Minchin *et al.*, 1981) between the processes of C assimilation and N_2 fixation. If such a functional balance can be assumed then Davidson's (1969) formulation can be applied to the system in question. According to this formulation, nodule mass and activity should tend towards a balance with other plant activities such as photosynthesis. Thus plants with increased nodule mass should exhibit increased mass and/or activity of the rest of the plant, as was found by Cralle, Heichel and Barnes (1987). It is apparent from the literature that nodules respond to fluctuations in photosynthate supply by changes in mass, rather than changes in activity (Vance and Heichel, 1991; Ryle, Powell and Davidson, 1992). Thus responses to changes in photosynthate supply would be relatively long term.

Factors other than photosynthesis seem to be more important in short term regulation of N_2 fixation (mediated by regulation of nitrogenase activity). In particular the oxygen concentration in the nodule seems to be important (Dabas, Swaraj and Sheoran, 1988). The enzyme nitrogenase poses something of a paradox in that while its functioning is dependent on large quantities of ATP (which would usually be supplied by aerobic respiration), the enzyme is irreversibly denatured by O_2. It is for this reason that the root nodule symbiosis exhibits several adaptations for fine regulation of oxygen levels. These are: (a) an oxygen diffusion barrier in the nodule cortex; (b) synthesis of the O_2-binding protein leghaemoglobin (Lb) within the nodules which facilitates oxygen flux to the bacteroids within the infected zone; (c) plant redirection of glycolysis to malate with subsequent reductive formation of succinate under microaerobic conditions; (d) bacteroid utilization of C4-dicarboxylic acids rather than mono- and disaccharides to fuel nitrogenase activity; and (e) bacterial ATP formation coupled to a high-affinity terminal oxidase (Vance and Heichel, 1991).

From the above discussion, it can be seen that the legume-rhizobial system represents a fully integrated symbiosis in terms of hormonal, genetic and nutritional responses (Minchin *et al.*, 1981). The activity and biomass of nodules appear to approach functional balance with other plant processes being closely regulated both in the short term (perhaps by O_2 content), and in the long term (by way of photosynthate supply). Aspects of the carbon and nitrogen budgets of this component of the system will be dealt with formally in chapter 4.

1.5.3 VESICULAR-ARBUSCULAR MYCORRHIZAE

Vesicular arbuscular mycorrhizae (VAM) are symbiotic associations formed between vascular plants and fungal species of the order Glomales (Morton, 1993). The colonization of plant roots by VAM fungi involves the formation of intercellular hyphae and highly branched intracellular arbuscules and vesicles scattered throughout the root. The establishment of intracellular phases is believed to be achieved through wall degradation by cell wall-hydrolysing enzymes (Garcia-Garrido, Garcia-Romera and Ocampo, 1992). VAM symbioses are widespread, occurring in 80% of vascular plant species (Smith and Gianinazzi-Pearson, 1988), and within a single host species the possibility of common mycorrhizal networks is high. Also, many of the fungi that form mycorrhizas have low host specificity, and this has led to the suggestion that these fungi may link plants of different species (Newman, 1988). It has thus been suggested that VAM fungi may be able to bridge different plants, thereby breaking down some physiological barriers (Newman, 1988). This may shift the competitive balance in the direction of some members of a community (St-John and Coleman, 1983).

The infection process develops from an encounter between a hypha and a root. The dynamics of mycorrhizal infection are by nature complex because both fungal infection dynamics and root growth dynamics must be considered as well as feedback between these processes. Mycorrhizal infection can be modelled mathematically in a similar way to the spread of pathogens. Buwaldo *et al* (1982) have reviewed a number of modelling approaches based on permutations of a logistic type of growth model. However, this approach is purely empirical and does not take into account the effects of root carbon allocation (Thompson, Robson and Abbott, 1990; Andersen and Rygiewicz, 1991) or soil nutrient contents (Sylvia and Neal, 1990) on infection dynamics. As an alternative to this logistic approach, the mycorrhizal fungi may be considered, not as a different organism, but rather as an additional organ of the plant. The infection process is therefore regulated by the plants allocation regime which is in turn dictated by carbon and nutrient availability. Clearly this approach is only appropriate for non-parasitic symbioses.

VAM symbiosis appears to play an important role in uptake of nutrients and possibly water in vascular plants (Veenendaal *et al.*, 1992) by increasing the absorptive surface as well as the volume of soil explored. This can be very simply demonstrated with reference to cylinder geometry. By assuming that both the mycorrhizal fungal hyphae and the roots are cylindrical, and that the volume of soil explored is a function of the length of the cylinder, then by rearranging the following equations:

$$V_{ROOT}=\pi R^2_{ROOT}*L_{ROOT} \tag{1.1}$$

$$V_{MYC}=\pi R^2_{MYC}*L_{MYC} \tag{1.2}$$

(where V_{ROOT} and V_{MYC} are the volumes of the root and of the root and the mycorrhizal fungal hyphae; R_{ROOT} and R_{MYC} are the radii of the root and hyphae respectively; and L_{ROOT} and L_{MYC} are the lengths of the root and hyphae) we get expressions for the lengths of the respective structures:

$$L_{ROOT}=V_{ROOT}/\pi R_{ROOT}^2 \tag{1.3}$$

$$L_{MYC}=V_{MYC}/\pi R_{MYC}^2 \tag{1.4}$$

If it is then assumed that the mean radius of the root is of the order of 20 times that of the hyphae (Gutschick, 1993), then, for any given tissue volume the length of the hyphae will be 400 times (20^2) than the root.

When considering the surface areas of the root (SA_{ROOT}) and hyphae (SA_{MYC}):

$$SA_{ROOT}=2*\pi*R_{ROOT}*L_{ROOT} \tag{1.5}$$

$$SA_{MYC}=2*\pi*R_{MYC}*L_{MYC} \tag{1.6}$$

Then, by substituting for length and simplifying:

$$SA_{ROOT}=2*V_{ROOT}/R_{ROOT} \tag{1.7}$$

$$SA_{MYC}=2*V_{MYC}/R_{MYC} \tag{1.8}$$

Then if it is again assumed that the mean root radius is 20 times that of the hyphae, then it can be seen that for any particular volume of tissue, the root will have a 20 times smaller surface area.

In order to relate these volumes to biomass, it is necessary to introduce tissue densities ($g.m^{-3}$) for root (P_{ROOT}) and for the fungus (P_{MYC}):

$$V_{ROOT}=M_{ROOT}/P_{ROOT} \tag{1.9}$$

$$V_{MYC}=M_{MYC}/P_{MYC} \quad (1.10)$$

It is reasonable to assume that the values for P are more or less the same so that for any given volume the masses will be the same, and if all other things are equal (which they are not), then the costs (in terms of biomass allocated) of increasing the length and surface area of the absorptive structures will be far less in the case of the fungal hyphae.

The bulk of nutrient exchange between the fungus and the plant appears to occur at the intracellular interface. This interface comprises the membranes of the plant and fungal symbionts as well as the interfacial apoplasm. Although several modifications to the interfacial membranes have been hypothesised (eg increased permeability of the membranes), the only feature that has really been demonstrated experimentally is the localisation of H^+-ATPases on the membranes of both symbiotic partners (Gianinazzi-Pearson *et al.*, 1991). This is believed to play a very important role in the active transport of several substances into both of the symbionts. In addition to the H^+-ATPase linked transport mechanisms, a number of other mechanisms are thought to be involved including passive fluxes, and linking of metabolic processes (as in the case of nitrogen) (Smith and Smith, 1990). The polarities of these fluxes are generally not fixed and seem to depend largely on source-sink relations in the fungus and the host.

1.5.3.1 CARBON PHYSIOLOGY OF MYCORRHIZAE

It is widely accepted that mycorrhizal fungi are dependent on their host plant for carbon supply. Anderson and Rygiewicz (1991) noted that the extent of infection is at least in part regulated by the availability of carbohydrate from the shoot of the host plant. Also, the vigour of established mycorrhizal associations is affected by availability of carbon for maintenance. Because of this dependence, the general concept of carbon flux is that the process involves loss of carbon by the plant cell into the interfacial apoplast from where it can be taken up by the fungal partner. One suggestion is that plant-supplied carbon enters the apoplast by passive fluxes of sucrose or hexose sugars (Smith and Smith, 1990). This obviously necessitates a downward concentration gradient which has led to the postulated role of invertase in the apoplast. Whether or not there is any modification of the plant plasma membrane to increase its permeability is unclear (although Smith and Smith [1990] argue strongly against such a proposal). Carbon supply to the fungal symbiont may also be in the form of plant synthesised wall precursors (Smith and Smith, 1990). This mode of carbon supply is dependent on the modification of plant wall deposition at the interface by fungal mediated inactivation of the polymerisation process (Smith and Gianinazzi-Pearson, 1988).

Processes involved with the uptake of this carbon by the fungal symbiont are also not particularly clear. One possibility is that this uptake is active and facilitated by way of an H^+-hexose sugar symport system where the H^+ gradient is generated by the H^+-ATPases which have been reported to occur in association with the fungal membrane (Gianinazzi-Pearson *et al.*, 1991). Another possibility is that the transfer may be passive, and therefore dependent on the maintenance of a gradient. Low sugar concentrations in the fungal symbiont may be maintained by conversion of sugars to "non-reusable" lipids and glycogen. In the ericoid mycorrhizal fungi, ^{14}C studies have suggested that host carbohydrates (eg. sucrose) are absorbed by the fungus and converted into mannitol and trehalose thus maintaining the concentration gradient (Stribley and Read, 1974). A similar situation has been reported in the ectomycorrhizae where host sucrose is converted to trehalose, glycogen and mannitol (France and Read, 1983). The involvement of trehaloses in carbon transport has also been suggested in the vesicular arbuscular mycorrhyzal (VAM) fungi. Schubert *et al* (1992) reported the occurrence of trehalose in the spores of VAM fungi as well as in the mycorrhizal roots.

In addition to this general concept of carbon transfer, France and Read (1983) note that the [illegible] an intricate relationship between the carbon and nitrogen physiology at the host-fungus interface. It is suggested that mycorrhyzal fungi may act as centres of nitrogen metabolism (Smith and Smith, 1990) and that the host plant may take up organic nitrogen in the form of amino acids or amides across the plant fungus interface in exchange for carbon based precursors. Irrespective of the mechanisms involved, the transport of these amino acids or amides provides a route for bidirectional movement of carbon over and above the net sugar flux needed for growth of the fungus (Smith and Smith, 1990).

It has also long been suggested that common mycorrhizal fungi may facilitate fluxes of carbon between different plants within a population or community (Stribley and Read, 1974). Hirrel and Gerdemann (1979) investigated the possibility of ^{14}C fluxes between onions infected with VAM fungi and found strong suggestive evidence for the flux of carbon between individual plants. More recently, Martins (1993) has also investigated this possibility and puts forward strong evidence for direct transfer (root-symbiont-root) of labelled carbon. In order for such fluxes to occur, interfacial transfer processes would have to be completely reversible. It is also possible that in plants sharing a common fungal symbiont one of the plants may support a greater proportion of the fungal "costs", while the other may derive as much benefit from the association in terms of nutrient absorption. There could thus be an indirect net carbon benefit to one of the host plants. Whether such fluxes (direct or indirect) would be sufficiently large to have any significant physiological impact is a matter for debate.

1.5.3.2 NITROGEN PHYSIOLOGY OF MYCORRHIZAE

It is widely believed that mycorrhizal fungi are involved in the uptake and assimilation of inorganic nitrogen and that some of this nitrogen is transferred to the host plant (Johansen, Jakobsen and Jensen, 1992). In the case of ammonium, uptake by mycorrhizae can be described by Michaelis-Menten kinetics (Jongebloed, Clements and Borst-Pauwels, 1991). The assimilation of ammonium in VAM involves the glutamine synthetase/glutamate synthase (GS/GOGAT) biochemical pathway rather than the glutamate dehydrogenase path (Smith and Smith, 1990). It has been suggested that VAM fungi may act as centres for ammonium assimilation. This would then involve all assimilation taking place in the fungus, with fungal products such as glutamine and glutamate being exchanged for sugars from the plant across the interface. This is however not the only possibility and the distribution of GS and GOGAT at the fungus-plant interface has not been determined and may have implications for the transport of nitrogen and carbon at this interface (see figure 2. in Smith and Smith, 1990).

Whether VAM fungi play a role in nitrate uptake is less clear and to date there is no evidence for nitrate reductase (NR) activity in VAM fungal hyphae. This is in agreement with the general microbial preference for ammonium over nitrate (Johansen *et al.*, 1992) and also with the fact that nitrate is relatively mobile in the soil and thus accessible to the root directly. NR activity has however been reported in VAM fungal spores (Ho and Trappe, 1975). It has also been demonstrated that VAM associations may enhance NR (and GS) activity in the roots of the host plant (Cliquet and Stewart, 1993). Recently, George *et al* (1992) have presented some evidence for the direct involvement of VAM fungi in the uptake of nitrate (although this evidence is inconclusive).

As was the case in carbon, it has been suggested that VAM fungi may be involved in interplant nitrogen transfer. This is a particularly interesting possibility when considering legume intercrop systems (as in this dissertation). Again, whether this transfer is facilitated by direct hyphal bridges, or whether it occurs through some more indirect route is still unclear. This issue has been investigated quite extensively using a number of methods generally involving nitrogen isotopes and root separation techniques (some of which are fairly complex [eg. George *et al.*, 1992]). Haystead *et al* (1988) presented some evidence for direct transfer based on experiments to investigate the possibility of ^{15}N exchange between *Trifolium repens* and *Lolium perenne*. Similar evidence was presented in Hamel *et al* (1991b) for a maize and soybean mixture. However, both sets of evidence are only suggestive due to the possibility of ^{15}N cycling from dead root matter. It is also unclear whether the magnitude of these apparently direct fluxes would be large enough to have any significant impact (Hamel *et al.*, 1991a; Hamel and Smith, 1991; Reeves, 1992). It has however, been suggested that the distinction between direct fluxes and indirect fluxes may in fact be artificial and that the root and rhizosphere should be considered as a unit; the "nutrisphere" (Bethlenfalvay *et al.*, 1990). The implication of this is that the most important issues are whether any flux is facilitated by mycorrhizal infection, and the magnitude of such fluxes, rather than the exact route which is followed. It is in fact likely that both direct and indirect transfer are involved.

1.5.3.3 PHOSPHORUS PHYSIOLOGY OF MYCORRHIZAE

Although not investigated in this study, phosphorus nutrition is a very important aspect of VA mycorrhizas, and has therefore been included in this review. Numerous studies have demonstrated a significant phosphorus benefit in plants grown in association with VAM fungi (Newman, 1988). Because phosphate has a very low diffusion coefficient in soil, absorption is enhanced by high "root" length density (cm.cm^{-3}) (Koide, 1991). The considerable hyphal contact with the soil is believed to extend the potential absorbing surface ("root") and the increased length may exploit a greater volume of soil outside the nutrient "deficiency zone" (Harley, 1989). In order for this to be of benefit to the host plant there must be some mechanism for the transfer of phosphorus. A physiological mechanism for this has been described by Smith and Smith (1990): phosphorus is actively absorbed from the soil by the fungal hyphae and then transported to the fungal-plant interfaces. At the interface loss of phosphorus by the fungal symbiont appears to be passive although some modification of the interface is possible to facilitate greater fluxes (Smith and Smith, 1990). Active uptake of phosphorus by the plant from the interfacial apoplast has been inferred due to the presence of H^{+}-ATPase's on the plant interfacial membranes (Gianinazzi-Pearson *et al.*, 1991).

Interplant transport of phosphorus has also been suggested and some evidence is available to support this from a number of radioactive P isotope studies. Intraspecific fluxes were investigated by Whittingham and Read (1982) in *Plantago lanceolata* and *Festuca ovina* respectively, while interspecific fluxes were investigated by Francis, Finlay and Read (1986). In both of these investigations the results confirmed that the presence of VAM infection could provide channels for direct interplant phosphorus transfer and that this may in some cases be significant in terms of growth.

However, other investigators have questioned whether fluxes are of any significance in terms of plant growth. Newman and Ritz (1986) used two mathematical models to test hypotheses regarding whether it was necessary to infer direct phosphorus transfer to explain the rates of transfer observed. This work suggested that the classical soil pool pathway was sufficient to predict observed fluxes. It is possible that any previously observed benefits of common mycorrhizal infection could be due to preferential cycling of P from dying roots of one plant to another of the same type of mycorrhizal infection (Eason, Newman and Chuba, 1991). Newman (1988) notes that movement of an isotope does not by itself prove net movement of an element. As is probably the case with nitrogen, it is likely that effects of mycorrhizal infection on interplant fluxes will depend on the physiological status of the two plants.

1.6 RESOURCE ALLOCATION MODELS

Plant resource allocation has very important implications in terms of yield of particular components of crop species. For this reason, the subject has been the focus of numerous modelling exercises (see Wilson, 1988 for review). From an evolutionary point of view a plant has a limited amount of resources available for expenditure, and natural selection should operate on the allocation of these in such a way that maximizes the contribution of the genotype to following generations (Wilson, 1988). This sentiment was repeated by Perrin (1992) who states that allocation patterns directly affect an organisms contribution to population dynamics and so they will be under strong selective pressure (and this evolutionary argument forms the basis for the so called teleonomic models described later).

As with any other modelling exercise, attempts to model resource allocation can be broadly divided into empirical or mechanistic (with many shades of grey in between). Empirical models can be defined as models based on statistical formulations that best correlate a set of observed variables with a response variable of interest (Reynolds, Hilbert and Kemp, 1993). Because of problems frequently experienced in constructing mechanistic models (eg oversimplifications and discontinuity in the performance) empirical models of plant growth will always remain useful to experimenters and modellers. They may also be a necessary precursor to the construction of mechanistic models for the simple reason that they provide accurate descriptive information that may be necessary for assessment of the mechanistic approach. The particular form of the function used is of no special physiological significance, but the accuracy in the fit achieved becomes the primary aim. The purpose of this empirical modelling is to describe reality in a convenient, simple way (Hunt, 1981).

A number of empirical models have been proposed to describe allocation of resources. In the most simple of these, the relative sizes of resource pools are described using simple allometric equations. These allometric relations are not the allocation, but rather the result of the allocation process (Gray[3]). This allometric approach to description of resource allocation is rather static and in view of the fact that there is some evidence that plants may exhibit adaptive allocation "strategies" (Iwasa and Roughgarden, 1984), several other empirical approaches have been developed.

The teleonomic approach mentioned above is based on the premise that plants allocate resources in such a way as to allow them to achieve some goal. The "goal" may be maximising growth of the plant (eg Johnson and Thornley, 1987; Hilbert, 1990; Hilbert and Reynolds, 1991; Gleeson, 1993) or the maximising of the contribution to subsequent generations (Cohen, 1971; Leòn, 1976; King and Roughgarden, 1982; Chiariello and Roughgarden, 1984; Iwasa and Roughgarden, 1984; Perrin, 1992). The use of these teleonomic models has been the subject of much debate: while some authors have suggested that certain teleonomic assumptions be adopted as paradigms (Hilbert and Reynolds, 1991), other authors have argued that there is no compelling evidence that plants are sentient creatures capable of forethought or planning (Charles-Edwards, Doley and Rimmington, 1986).

---

[3] Personal communication

Despite criticisms levelled at teleonomic approaches, there are two lines of supporting evidence: Firstly there is a strong logical argument that plants capable of responses tending towards optimality will be selected for during evolution (Charles-Edwards, Doley and Rimmington, 1986). Secondly, models based on teleonomic assumptions have often been shown to correspond well with observed patterns (Reynolds and Thornley, 1982; Johnson and Thornley, 1987; Hilbert and Reynolds, 1991).

Although the logic of this evolutionary argument seems to be reasonable, the actual mechanisms whereby plants achieve optimal allocation are somewhat less biologically understood (Lèon, 1976; Chen *et al.*, 1993). In order to gain an insight into the mechanisms, a number of so called mechanistic models have been developed. As is noted in Reynolds, Hilbert and Kemp (1993) one person's mechanism is another person's empiricism. So called mechanistic models are however, characterised by being made up of empirical and phenomenological descriptions of the component parts of the system, an approach which is dependent on a hierarchical view of plant processes (Charles-Edwards, Doley and Rimmington, 1986). The mechanisms proposed to date can be broadly divided into two categories (Wilson, 1988): source-sink based mechanisms, and hormone based mechanisms, although the distinction is often blurred. In the case of the former, allocation of resources occurs as a result of flux of resources from source organs to supply the demands of sink organs. The magnitude of these fluxes is dependent on relative source and sink strengths. Source strength is assumed to be dependent on the rate of acquisition of the resource, which is in turn related to the size and activity of the source organ. Sink strength is dependent on the rate of utilization of the resource, which is again dependent on the size and activity of the sink organ.

This proposed mechanism has been formalised into what has been dubbed the "Thornley approach" (Wilson, 1988) after Thornley (1972 a and b) and applied successfully to several systems (eg forest growth in Thornley 1991). The model is based on fluxes of substrates (such as carbon which is supplied by the shoot compartment or nitrogen which is supplied by the root), and their utilization for growth. The phenomenological equations describing the fluxes are based on concentration gradients and resistance to flux, while the utilisation of substrate is described by two-substrate enzyme kinetics. Growth in the shoot and root are dependent on the concentrations of carbon and nitrogen substrates in those organs (or meristematic tissue associated with those organs).

Although the vast majority of observations on the plastic responses of plants are compatible with the "Thornley type" of approach, a number of authors have suggested that the simple translocation / sink models are not adequate to explain allocation in certain experimental observations. These anomalous situations often arise when the plant has specialised structures associated with storage eg. as in carrots (Benjamin and When, 1980). It is for this reason that hormones have been suggested as integrating agents between above and below ground processes.

Rather than being a totally conflicting theory, the hormone based mechanisms are an extension of the source-sink approach (and it should be noted that the possibility of hormonal signals is never explicitly excluded in the "Thornley type" models). According to the hormone approach, source and sink strength are not only dependent on supply and demand but also on levels of various plant hormones. From studies based on exogenous applications of plant growth factors and their synthetic analogues, it has been shown that these substances may markedly affect plant growth rates and morphogenesis (Guern, 1987). Since growth and development is only possible if necessary resources are available, it has been extrapolated that hormones must exert some controlling effect over the partitioning of these. From recent literature there are a great number of papers that describe the effects of growth factors in partitioning, or events which are linked to partitioning. Such effects include the regulation of source and sink strength by regulation of key enzymes (eg. the enhancement of nitrate reductase by cytokinins [Lu, Ertl and Chen, 1992], or the induction of sucrose synthase by gibberellic acid [Sowokinos and Varns, 1992] to mention just two). Fluxes may also be regulated by modifications of membrane transport characteristics (Peters and Felle, 1991) or even by regulation of the development of tissues associated with transport (xylem and phloem).

Because of the diversity of possible roles of plant growth factors in regulating partitioning, hormones have to date, not been incorporated into formal mathematical models of partitioning. However, recently Johnson *et al.*, 1991 incorporated hormonal control into a model of water flow through plants thereby opening the way for a formal description based on hormonal signals.

Wilson (1988) reviewed both the hormone and the Thornley models and has given a detailed account of the behaviour of Thornley's model in response to various factors, including ontogeny, inorganic nutrients and so on. As a conclusion to this review, Wilson (1988) suggest that the "Thornley model" should be the mechanistic paradigm for shoot to root partitioning. This conclusion is based on an Occam's razor (parsimony) argument.

1.7 REVIEW SUMMARY AND PROJECT OBJECTIVES

To conclude, the discussion presented in this chapter focuses on several key features of the intercrop system comprising of a grass and a legume. As mentioned above (1.3), the general rationale behind legume intercrop systems is that the nodulated legume component may improve the nitrogen status of the whole system. This review therefore lays the foundation for the experimental and modelling exercises that are discussed in the following chapters. These were carried out with the primary objective of investigating this improvement of nitrogen status. Particular emphasis has been placed on the physiological role of the two microbial symbioses which occur in the system, namely *Rhizobium* nodules and vesicular arbuscular mycorrhizae. The significance of the legume nodules is obvious as this association is responsible for the fixation of nitrogen, however, the role of VA mycorrhizas is slightly less obvious. VA mycorrhizas are usually associated with improved phosphorous acquisition (1.5.3.3). However, recent findings have suggested that VAM fungi may be important in terms of interplant fluxes of nitrogen (1.5.3.2) and it was this possibility that was the rationale for incorporating these fungi into the experimental design.

The approach followed in investigating the primary objective was thus three fold:

1. To monitor the nitrogen and carbon status in a controlled intercrop system as affected by both nodules and VA mycorrhizas.

2. To develop a mathematical description of the carbon and nitrogen dynamics of the

system, which could be calibrated using the data generated in 1 above.

3. It was initially hoped that this mathematical budget could be further used to investigate the significance of carbon and nitrogen fluxes from the legume to the grass by way of some form of VAM hyphal connection as discussed in 1.5.3.1 and 1.5.3.2. However, as is discussed later (chapter 2), the grass failed to enter into a VAM symbiosis so that the concept of hyphal bridging was not appropriate for this particular system.

CHAPTER 2: ALLOCATION OF BIOMASS AND NITROGEN IN A GRASS/LEGUME MIXED STAND AS AFFECTED BY NODULES AND MYCORRHIZAS.

2.1 RATIONALE

There were two primary objectives to the experimental work that was carried out: Firstly to gain some insights into the actual carbon and nitrogen dynamics in a real, grass/legume mixture as affected by the presence of either nodules on the legume component or mycorrhizal infection; and secondly to allow calibration of the model described in chapter 4 against real data.

2.2 MATERIALS AND METHODS

2.2.1 PLANT GROWTH CONDITIONS

The growth period started in mid-November with the planting of 5 seeds of both white clover and Italian rye in each of 60 - 15cm diameter (area equivalent to $0.0177m^2$) pots. The potting medium used was twice-autoclaved river sand. Once the seeds had germinated the pots were thinned to two plants per species per pot (ie 4 plants per pot) which translates to a density of approximately 113 seeds/m^2 ground for both clover and rye. A very simple factorial experimental design was used, with two factors (MYC and NOD) each at 2 levels: +MYC and -MYC and +NOD and -NOD as defined below. The +MYC treatments were inoculated at planting with 10g of fresh grass root material collected from Melville Koppies nature reserve, while the -MYC treatments were inoculated with 10g autoclaved root material from the same site. The inoculum used was thus not a pure inoculum but rather a mixture of rhizosphere components. Fundamental to this is the observation that nodule-forming bacteria tend to be highly specific (Downie and Johnston, 1988) whereas VAM fungi tend to be rather promiscuous (Newman, 1988). The +NOD treatments were inoculated with 50 ml inoculum isolated as described below (2.2.2) at 1 week after planting, while the -NOD treatments received 50 ml of the same inoculum which had been autoclaved. Pots were watered daily, and once a week 100ml of fertilizer solution (3 ppm phosphorus and 46 ppm nitrogen)(appendix 2) was applied to each pot. No phosphorus compensation was carried out.

The initial growing period was four weeks whereafter five harvests were carried out, one every two weeks. At each harvest three replicates were taken at random for each treatment. The plants harvested were separated into their components (roots*, stems, leaves and nodules), oven dried at 60°C for three days, and then weighed on an analytical balance. The only exception to this procedure for harvesting was the root harvests (*) from which a fresh sample was taken prior to drying. This was used for the estimation of intraradical mycorrhizal infection. The dry mass of this root subsample was calculated as the product of the fresh mass of the subsample and a dry : fresh mass ratio. This was then added onto the dry mass of the remaining sample to give the total component biomass. The dry : fresh mass ratio was calculated by dividing the dry mass of the sample remaining after removal of the subsample, by the fresh mass of the sample remaining after removal of the subsample for each sample. In clover the stems were taken to be both petioles and stolons. A subsample (or in some cases the entire sample) of the dry plant material was then used for the determination of the nitrogen contents as described below (2.2.6).

2.2.2 ISOLATION OF RHIZOBIUM

Active nodules from white clover plants growing at the university were ground up using a mortar and pestle in a few drops of the liquid growth medium (Appendix 2). This was then plated out onto solid selection medium (Appendix 2) on a lamina flow bench and allowed to grow up for a day in the dark at 25 °C. Colonies that did not stain red in response to congo red were then selected and plated onto a second selection plate. This selection procedure was repeated five times in order to get as pure a culture as possible before transfer to the liquid medium. Once in liquid medium the *Rhizobium sp.* was allowed to grow up for one week before being applied to the plants in liquid form.

2.2.3 STAINING OF ROOTS FOR VAM INFECTION

Roots were stained according to the method described in Koske and Gemma (1989). This method is an improvement on several of the earlier methods employed from the point of view that potentially harmful chemicals (particularly phenol and lactic acid in staining and destaining procedures) are eliminated where possible. In addition, the number of ingredients is kept to a minimum. The fresh root subsample taken from each plant in each pot was fixed and stored (overnight usually) in 10ml 50% ethanol. (This step was not necessary if the roots were to be used immediately for staining.)

The actual clearing and staining were carried out as follows:

1. Tissue clearing:

In order to disrupt the cell membranes, so that cell contents are lost, the root material was placed in 20ml of 2,5% potassium hydroxide (w/v) and heated at 90°C for 45 minutes. This treatment was found to be the most effective in clearing the tissue, without causing too much damage to the cortex. The more rapid approach of autoclaving the samples for three minutes was found to be very damaging to the cortex.

2. Rinsing and bleaching:

Following the tissue clearing step, the roots were rinsed several times with distilled water and bleached with alkaline hydrogen peroxide (3ml 20% NH_4OH in 30ml 3% H_2O_2) for 45 minutes. This was followed by a thorough rinsing in distilled water. Although this step to lighten dark roots is optional (Koske and Gemma, 1989) it was carried out routinely on all the samples in this study as it was found to give enhanced clarity when viewing under a microscope.

3. Acidification:

Inadequate acidification is reported to result in poor binding of the trypan blue to the VAM structures (Koske and Gemma, 1989). It was therefore necessary to acidify the samples which were left highly alkaline by the preceding two stages. In order to achieve this acidification, the root samples were transferred to 20ml of 1% hydrochloric acid and left overnight.

4. Staining:

The material was stained in trypan blue (0.05% trypan blue in acid glycerol) at 90°C for 45 minutes. Because of the absence of phenol this stain has the advantage of having relatively low toxicity. It also stains the fungus blue which has greater contrast than certain of the alternatives such as acid fuscin. The roots were then destained in acid glycerol (500ml glycerol, 450ml water, 50ml 1%HCl).

2.2.4 ESTIMATION OF INTRARADICAL VAM COLONIZATION

There are essentially three methods for assessing levels of infection based on microscopy (as opposed to chemical estimation of chitin). The most elementary (and least accurate) approach is a simple subjective estimation of infection levels. Another approach is to base the estimation on the ratio of microscope fields of view with infection to the total number of fields considered. Although less subjective than the previous approach it can easily be demonstrated (McGonigle *et al.*, 1990) that this approach often leads to overestimation of infection. The last group of methods is based on permutations of a grid or line intersect method, where the infection level is determined from the ratio of infected intersections to uninfected intersections. The approach followed in this work was described in McGonigle *et al* (1990) and represents, perhaps, the least subjective approach available. Stained roots were cut into 1cm long pieces and 10 of these root sections were mounted parallel to the short axis of microscope slides in glycerine. These were observed at a magnification of x160. The field of view of the microscope was moved using the stage graticule to make 10 complete passes across each root segment. There were thus a total of 100 intersections counted per plant and therefore a total of 200 intersections per species per replicate.

Except where the cortex was missing, all intersections between roots and the eyepiece crosshair were scored. Rotation of the crosshair ensured that each intersection was at right angles to the long axis of the root. At each intersection, the roots were scored into one of six categories:

1. No fungal material in the root,
2. Arbuscules (Plate 1),
3. Vesicles (Plate 1)
4. Arbuscules and vesicles
5. Hyphae but no arbuscules and vesicles
6. Hyphae not seen to be connected to arbuscules or vesicles.

Arbuscular colonisation (AC) and vesicular colonisation (VC) were calculated by dividing the count for categories 2+4 and 3+4 above respectively by the total number of intersections examined. Hyphal colonisation (HC) was calculated as the ratio of infected intersections to total intersections.

2.2.5 NITROGEN ASSAY

These were carried out using the Dorich and Nelson (1983) method which is a

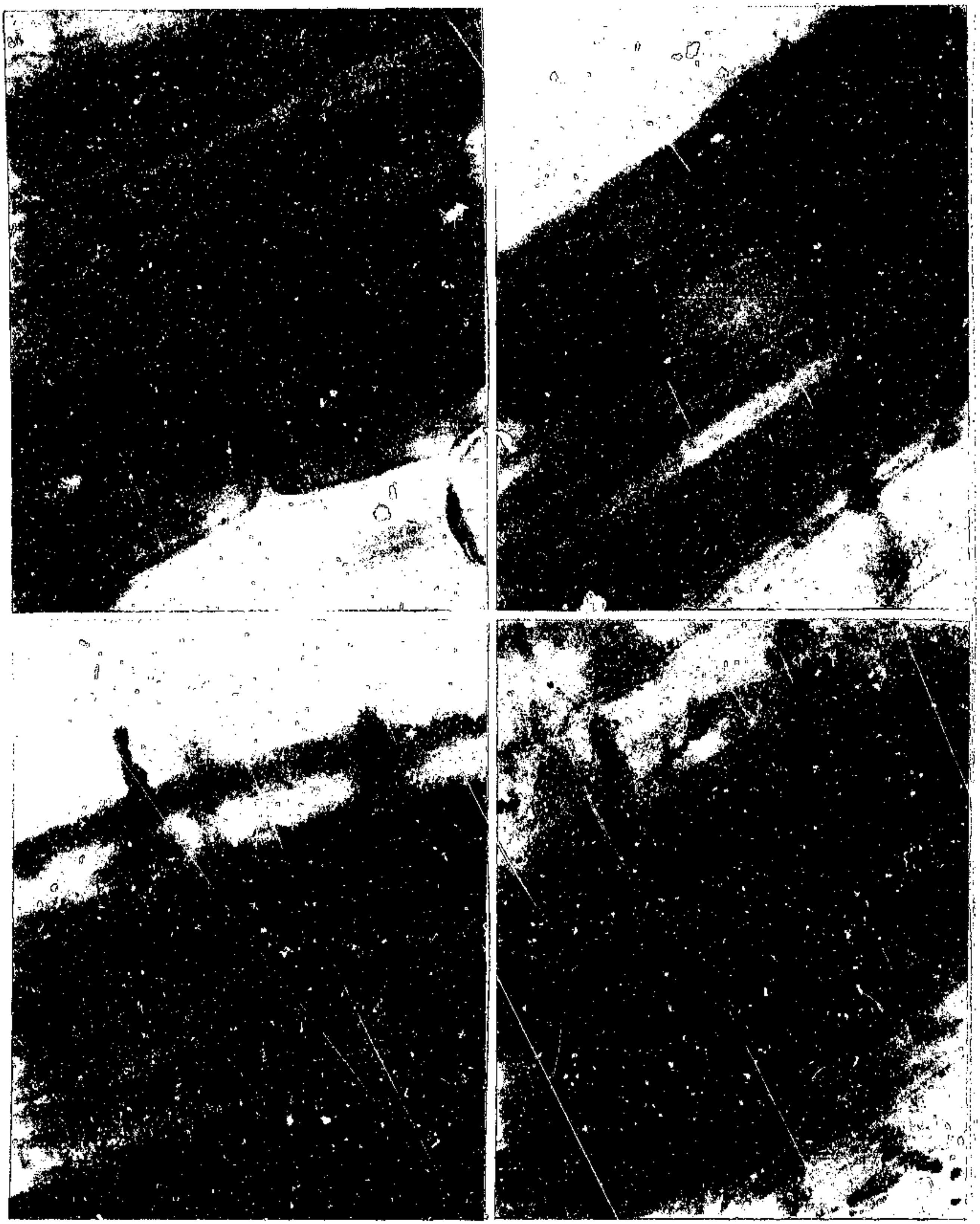

Plate 1. Intraradical VAM fungal structures in the roots of the white clover at harvest 5. v=vesicles, a=arbuscules and h=hyphae. All scale bars = 1.4 µm.

colorimetric method, using a hydrogen peroxide-sulphuric acid digestion step and an indophenol blue colour development.

The first step in the determination was to digest the plant material. For this, approximately 0,1 g of plant material was added to a small amount of selenium catalyst and 4.4 ml digestion mixture (Appendix 2iii), in a digestion tube. These samples were then digested at 360°C in a fume cupboard until the solutions became clear. They were then allowed to cool and 25ml of de-ionized water was added. The solutions were allowed to cool again and were then made up to 50ml with de-ionized water. Blanks were also prepared for use as standards.

For the preparation of the standard curve, an initial ammonium sulphate stock solution (0.25 M) was diluted in two steps. In the first dilution, 5ml was made up volumetrically to 250 ml. Following this; 0, 5, 10, 15, 20 and 25 ml of this intermediate stock were added to 4.4 ml of digested blanks and then diluted to 50 ml. This gave a series of working standards with NH_4^+ concentrations ranging from 0 M to $5*10^{-6}$ M. Three replicates were made up for each concentration.

For the colour development, 1ml from each of the working standards and each of the plant digests was pipetted into 25 ml volumetric flasks. 1 ml of solution A (appendix 2iii) was then added to each of these. 2N NaOH was then added dropwise until the solution changed colour from pink to yellow. This ensured that the solutions were in the correct pH range. 2ml of solution B (appendix 2iii) followed by 4ml solution C (appendix 2iii) were then added and the flasks were made up to volume with de-ionized water. They were then left at room temperature for 60 minutes to allow colour development.

The absorbances were read at 620 nm on a Varian DMS 90 UV visible spectrophotometer. For each harvest, a separate standard curve was produced to account for differences in the reagent solutions (which were made up fresh for each harvest). These were constructed by plotting absorbance versus concentration of ammonium nitrogen and the concentrations of the plant digests were determined from the standard curve. These were then used to calculate the concentration of nitrogen ($gN.gDM^{-1}$) in the component.

2.2.6 STATISTICAL ANALYSES

Because of the factorial nature of the treatment structure the statistical convention generally applied to all measured variates in this dissertation was to partition the variances at each harvest by way of analysis of variance. This is based on the assumption that the necessary conditions for ANOVA's were satisfied. The exceptions to this convention were the comparisons between mycorrhizal infection levels in the +NOD and -NOD treatments and the comparison between nodule biomasses in the +MYC and -MYC treatments. In the case of the former, the data were first arcsine transformed before running simple two factor ANOVA's. The arcsine transformation was used because of the binomial nature of the data and because they were expressed as percentages. In the case of the latter comparison, simple two factor ANOVA's were used. Some caution needs to be expressed regarding the reliability of these two tests because of the low degrees of freedom upon which they are based. However, as will be discussed later, the general lack of significance of the MYC*NOD interactions shown in the standard factorial based ANOVA's serves as confirmation of the lack of significance detected by these two tests. It was decided that the 5% p-level would be taken as the significance cut-off. All significant differences are recorded in the text since these allow parallels to be drawn between onset of infection and impact on plant growth and nitrogen status.

2.3 RESULTS

2.3.1 LEVELS OF MYCORRHIZAL FUNGAL INFECTION

Whereas the clover components in the +MYC treatments generally showed reasonable levels of intraradical infection (particularly in the later harvests) (table 2.1), no infection could be detected at any stage during the experiment in the ryegrass. In addition, no infection was detected in either species in the -MYC treatments, confirming the effectiveness of autoclaving the inoculum to prevent infection. The high variability in the levels of infection (reflected by the standard errors of the means in table 2.1) in all but the last harvest was due to some of the +MYC replicates not being infected.

When comparing the levels of mycorrhizal infection between the +NOD and -NOD treatments, the levels were consistently slightly higher in the +NOD treatment (although these differences are not statistically significant).

2.3.2 COMPONENT BIOMASSES

The time series data for the component biomasses of clover and rye are presented in figures 2.1 - 2.4. The biomass values were calculated by dividing the dry masses in each pot by 0.0177 m^2 to convert them to $gDM.m^{-2}$ground. In addition to these values, nodule biomasses and shoot:root ratios are also presented below.

Table 2.1. Levels of intraradical VAM fungal infection of white clover in +MYC treatments (expressed as a percentage). HC = hyphal colonisation; AC = arbuscular colonisation; VC = vesicular colonisation. Data are means of 3 replicates ± SEM.

Harvest#		HC	VC	AC
1	+NOD	0±0.00	0±0.00	0±0.00
	-NOD	0±0.00	0±0.00	0±0.00
2	+NOD	12.5±86.55	2.41±1.03	4.53±1.86
	-NOD	10.87±5.01	1.33±0.63	6.01±3.16
3	+NOD	25.22±10.32	3.63±1.63	6.10±2.54
	-NOD	14.81±4.38	5.47±1.83	6.67±0.47
4	+NOD	33.33±16.88	6.13±2.87	6.61±2.87
	-NOD	13.77±1.50	4.53±1.15	7.10±1.32
5	+NOD	77.33±6.21	11.03±0.59	51.77±3.98
	-NOD	69.13±5.24	10.00±1.78	42.50±4.91

2.3.2.1 TOTAL BIOMASS

In the case of the ryegrass, there was an almost linear increase in the biomass over the

84 day growth period (fig. 2.1b). No significant ($p=0.05$) factor or interaction differences were found at any of the harvests. In contrast, in the clover, the biomass curves differed markedly for the different treatments (fig 2.1a). This divergence was reflected in the fact that there were significant differences between the NOD treatments in the last three harvest ($p=0.026$, 0.001 and 0.001 respectively) as well as significant differences between the MYC treatments in the final harvest ($p=0.042$). In all cases where significant differences were detected, the means for plants with symbionts were greater than the -MYC-NOD treatment, confirming the beneficial nature of these relationships. No significant combinational effects were shown at any of the harvests. This may be related to the fact that there were no apparent differences between the levels of infection of either NOD or MYC as a result of the presence or absence of the other microbial symbiotic partner (see sections 2.3.1.1 and 2.3.2.6). Differences may have been detected with more replicates or a longer growth period. While the curve for the -MYC-NOD treatment rapidly reached a ceiling value at just over 1 $gDM.m^{-2}$ground, the other three treatment curves were all more or less exponential in form, with the +MYC+NOD having the highest final biomass followed by the -MYC+NOD and then the +MYC-NOD.

2.3.2.2 LEAF BIOMASS

Leaf biomasses followed much the same trends as total biomasses in both clover and rye. Again, there was an almost linear increase in the leaf biomasses in the rye over the experimental period (fig. 2.2b) while in the clover, the -MYC-NOD curve rapidly reached a ceiling which was in contrast to the other treatments curves which had an almost exponential form (fig. 2.2a).

Statistically, no significant factor or interaction differences were found in the ryegrass at any of the harvests. In the clover, differences were detected between the NOD treatments in the last two harvests this time (p=0.001 in harvest 5 and 0.011 in harvest 4) while the p value for the third harvest was significant at the 10% level (p=0.087). A significant difference between the MYC treatments was detected in the final harvest (p=0.036). Again, no interaction differences were detected.

2.3.2.3 STEM BIOMASS

The stem biomass components for both clover and rye also follow the same general trend as described for total biomasses (fig 2.3 a and b). Significant differences between the NOD treatments were detected in the last two harvests only (p=0.001 in both harvests 4 and 5) and between the MYC treatments in the final harvest (p=0.036). Again no interaction differences were detectable.

2.3.2.4 ROOT BIOMASS

As would be expected, root biomasses of both clover and rye also follow the same general trends as the above components (fig 2.4 a and b). The significant differences between the NOD treatments detected in the last two harvests had p values of 0.001 in harvest 5 and 0.009 in harvest 4 while the difference between the MYC treatments in the final harvest had a p value of 0.014. No interaction differences were detectable.

2.3.2.5 SHOOT : ROOT RATIOS

Although many authors (Wilson 1988) have suggested that shoot : root ratios are fairly constant or at least developmentally constant, the results of this experimental work suggest that the ratio was in fact responsive to the treatments in the case of clover (fig 2.5a). Once again the differences were statistically significant in the case of the NOD treatments in the last two harvests ($p=0.001$ in harvest 5 and $p=0.009$ in harvest 4). In the case of the MYC treatments however, no significant differences were detected. While the shoot:root ratio of the control treatment decreased continuously over the growth period, the shoot:root ratio of all the experimental treatments increased in the last two harvests. This can be taken as evidence in support of models which have some sort of responsive shoot\root allocation mechanism (eg Gleeson and Tillman, 1992). In the case of rye, (fig 2.5b, there was a general decline in shoot:root ratio over the whole growth period.

2.3.2.6 NODULE BIOMASS

As was the case in VAM fungal colonization (2.3.1.1 above), infection of clover plants

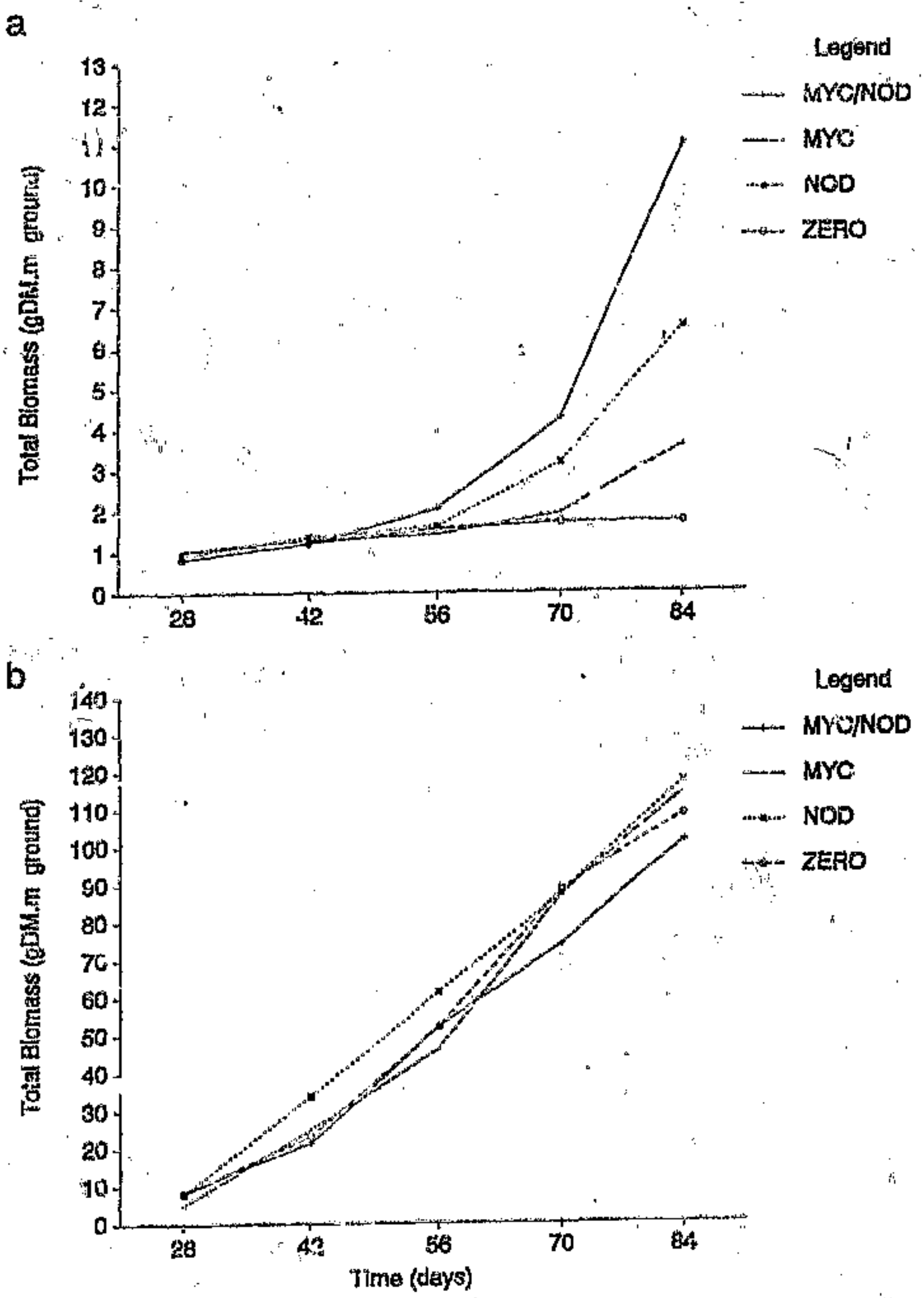

Fig 2.1. Total biomass (g dry matter.m-2 ground) of white clover (a) and Italian ryegrass (b), over time. Data are means of three replicates.

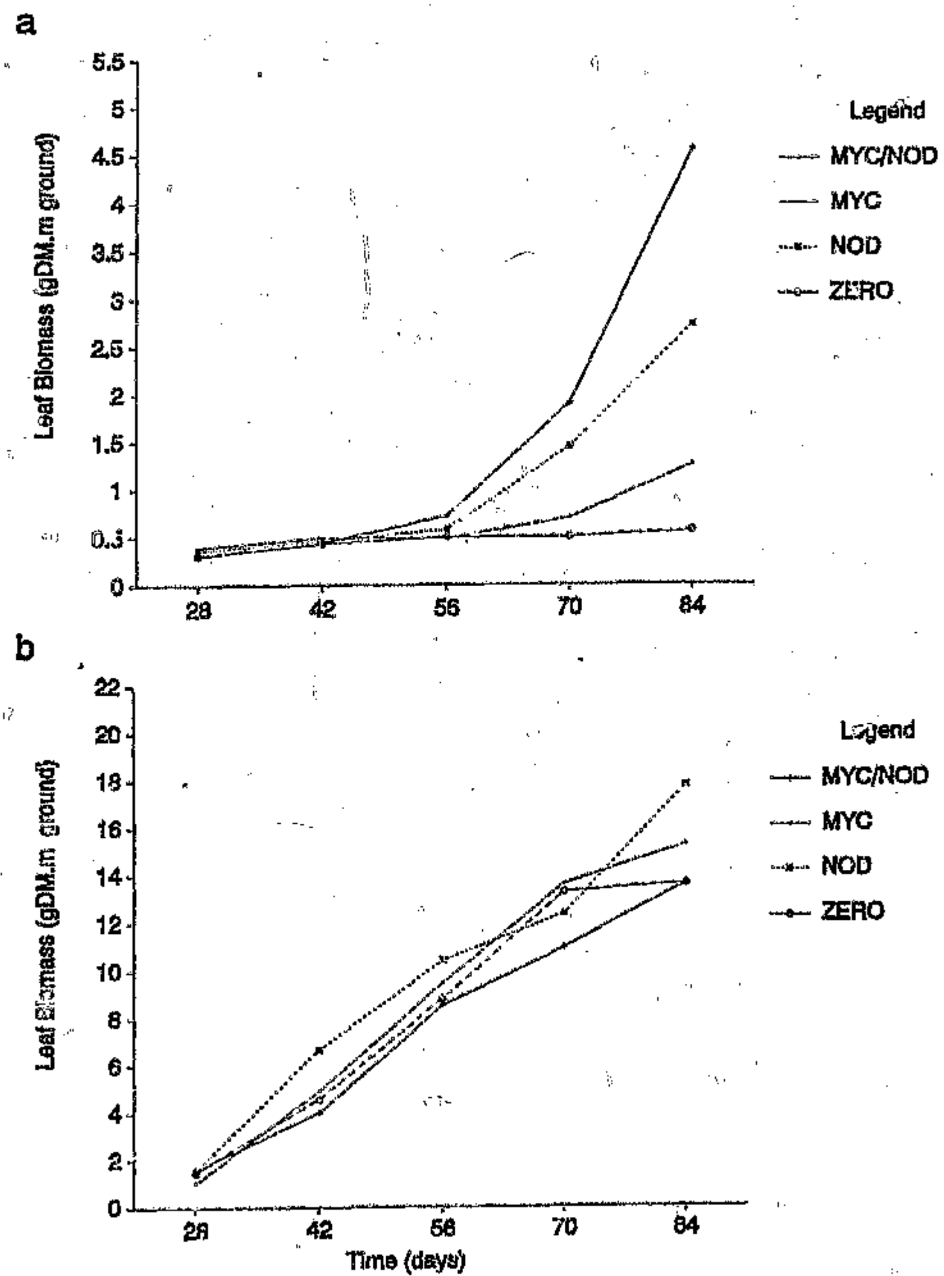

Fig 2.2. Leaf biomass (g dry matter.m-2 ground) of white clover (a) and Italian ryegrass (b) over time. Data are means of three replicates.

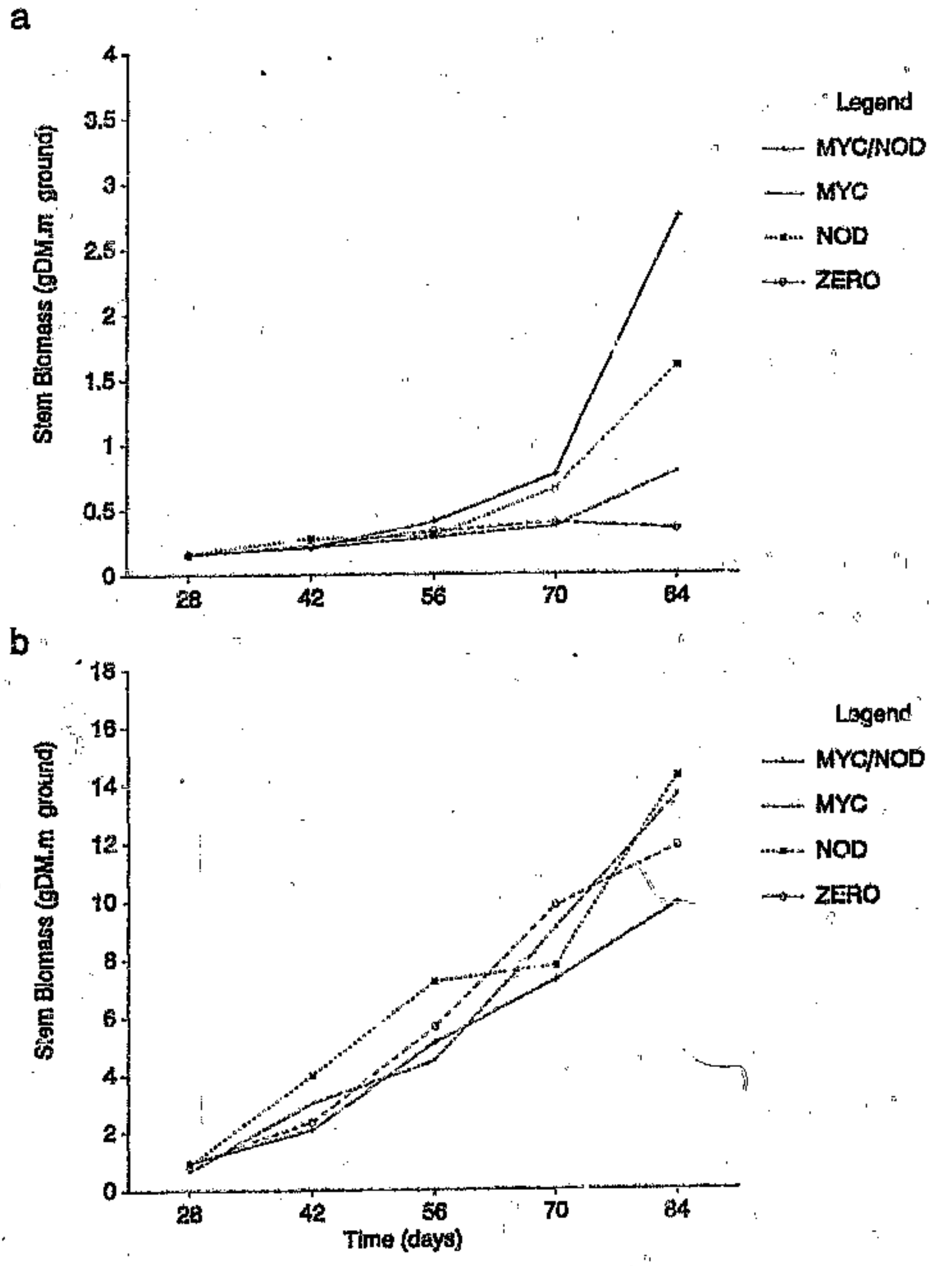

Fig 2.3. Stem biomass (g dry matter.m-2 ground) of white clover (a) and Italian ryegrass (b) over time. Data are means of three replicates.

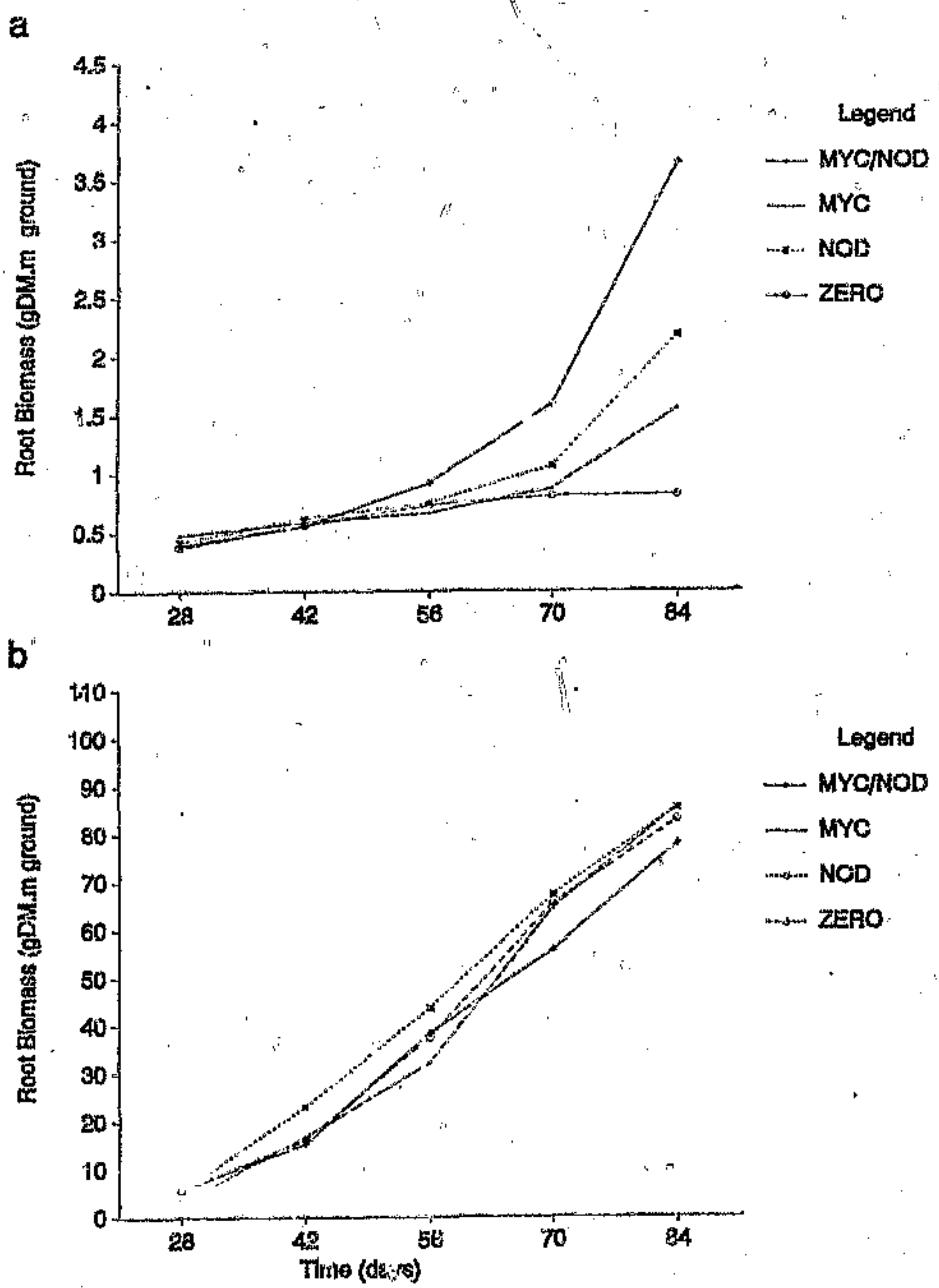

Fig 2.4. Root biomass (g dry matter.m-2 ground) of white clover (a) and Italian ryegrass (b) over time. Data are means of three replicates.

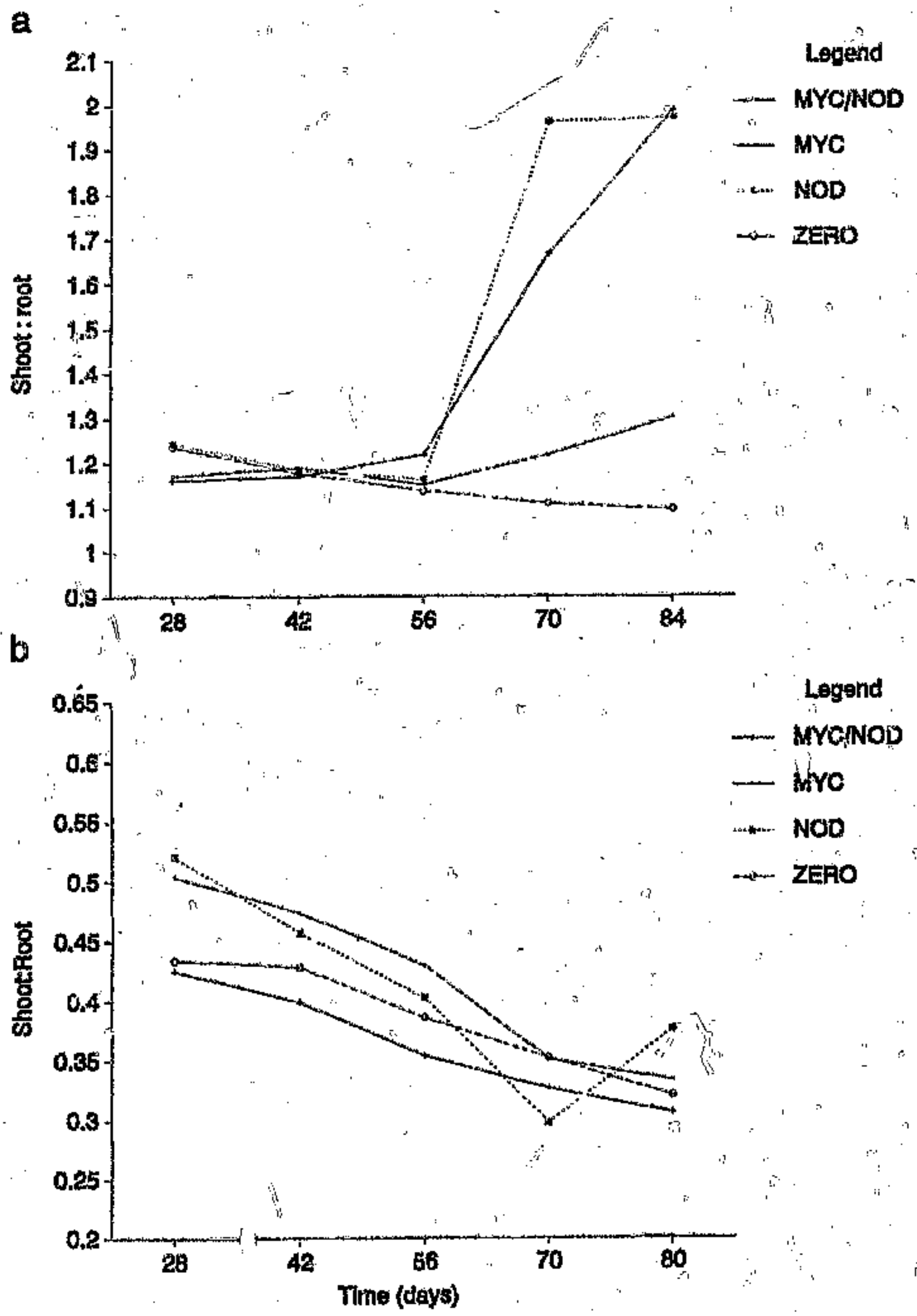

Fig 2.5. Shoot : root ratios for white clover (a) and Italian rye grass (b). Data are the means of three replicates.

by nodule-forming bacteria was confined to the +NOD treatments, confirming the effectiveness of the autoclaving in preventing establishment of rhizobial infection in the -NOD treatments. It is clear from table 2.2 that the establishment of nodules occurs in tandem with the occurrence of significant component biomass differences between the NOD treatments (ie from harvest 3 onwards). There were no significant differences in nodule biomass between the +MYC and -MYC treatments.

Table 2.2. Nodule biomasses (g) in the +NOD experimental treatments. Data are means of 3 replicates ± SEM.

	Treatment	
Harvest	+MYC	-MYC
1	0±0.000	0±0.000
2	0±0.000	0±0.000
3	0.1827±0.077	0±0.000*
4	0.2919±0.049	0.2246±0.078
5	0.3785±0.097	0.2946±0.060

* = Nodules were present, however, their dry masses were too small to be measurable.

2.3.3 NITROGEN CONTENTS

2.3.3.1 LEAF NITROGEN

In the case of clover (fig. 2.6 a) there were noticeable differences between the treatments. Leaf nitrogen of the -MYC-NOD treatment decreased over the growth period, while in the other treatments the leaf nitrogen generally increased (at least after the establishment of symbioses). Significant differences between the NOD treatments were detected in the fourth and fifth harvests (p=0.012 and 0.001 respectively), and between the MYC treatments in the last harvest (p=0.047). In the ryegrass on the other hand, there were no differences between the treatments and generally a decrease in the leaf nitrogen contents over the growth period (fig 2.6 b).

2.3.3.2 STEM NITROGEN

Stem nitrogen patterns in the clover were very similar to the leaf nitrogen patterns. There was a very slight decrease in the stem nitrogen content in the -MYC-NOD treatments over the growth period, while in the other treatments there was again a general increase (fig. 2.7 a). Significant differences were, however, only found between the NOD treatments (with a p value of 0.017 in harvest 4 and a p value of 0.007 in harvest 5), and not between the MYC treatments. For rye, the patterns (fig. 2.7 b) were also much the same as in leaf nitrogen, although the stem nitrogen contents were noticeably lower than the leaf nitrogen contents.

2.3.3.3 ROOT NITROGEN

Again, root nitrogen patterns were similar to leaf and stem nitrogen patterns in both clover (fig. 2.8 a) and rye (fig. 2.8 b). There was again a decline in the root nitrogen content in the -MYC-NOD treatments over the growth period, and an increase in the

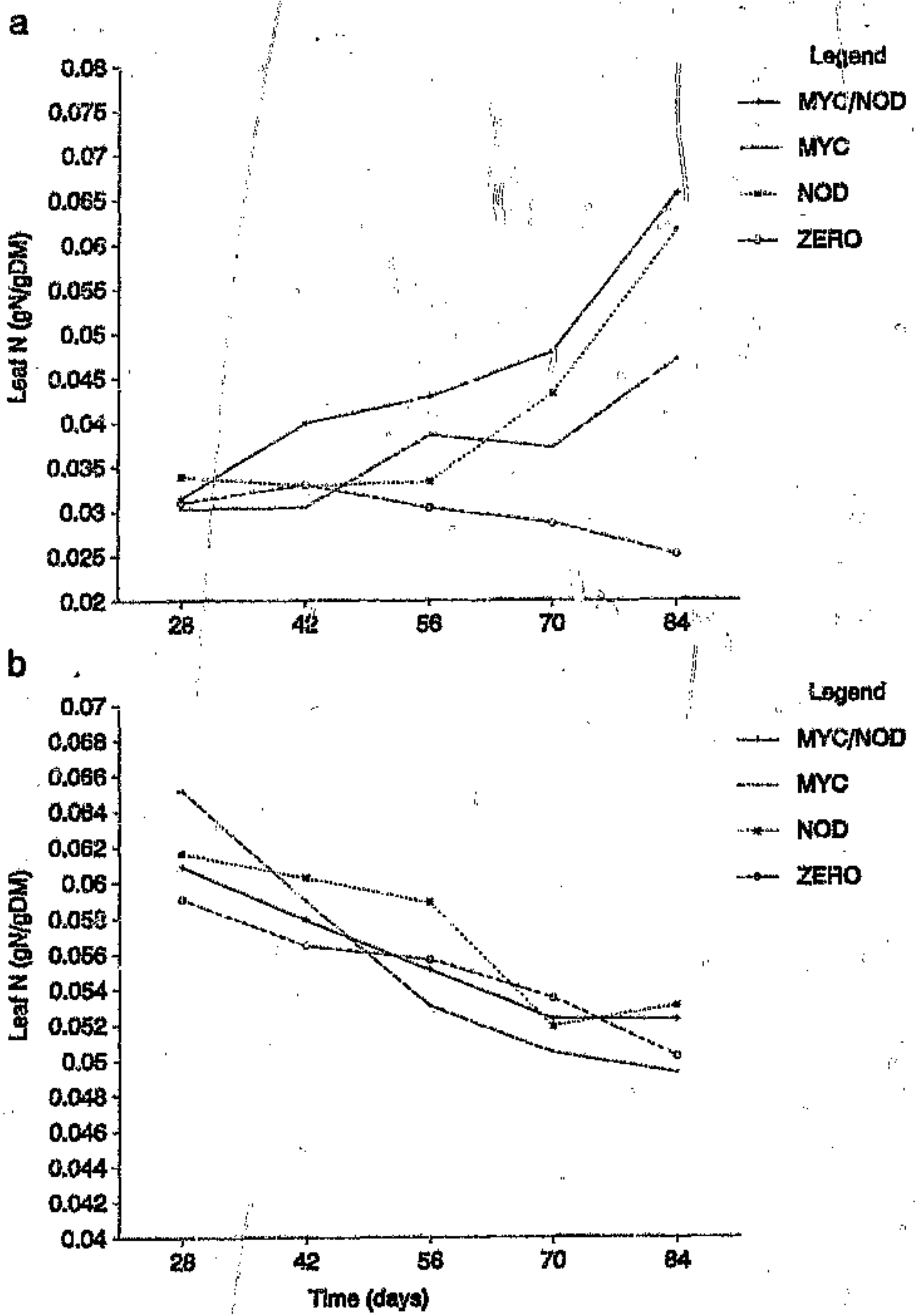

Fig 2.6. Leaf nitrogen content (gN/g dry matter) in white clover (a) and Italian ryegrass (b) over time. Data are the means of three replicates.

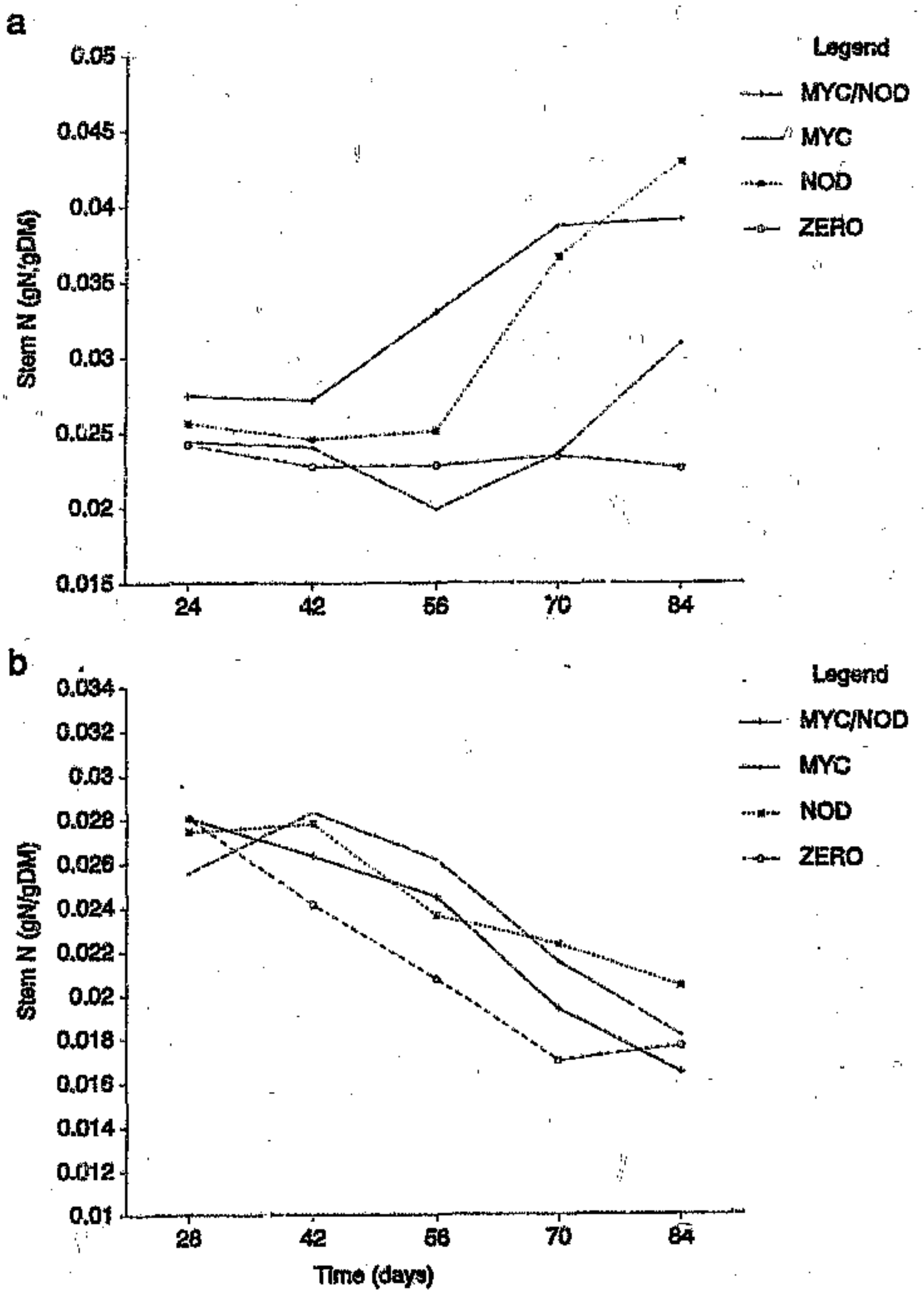

Fig 2.7. Stem nitrogen contents (gN/g dry matter) for white clover (a) and Italian ryegrass (b) over time. The data are the means of three replicates.

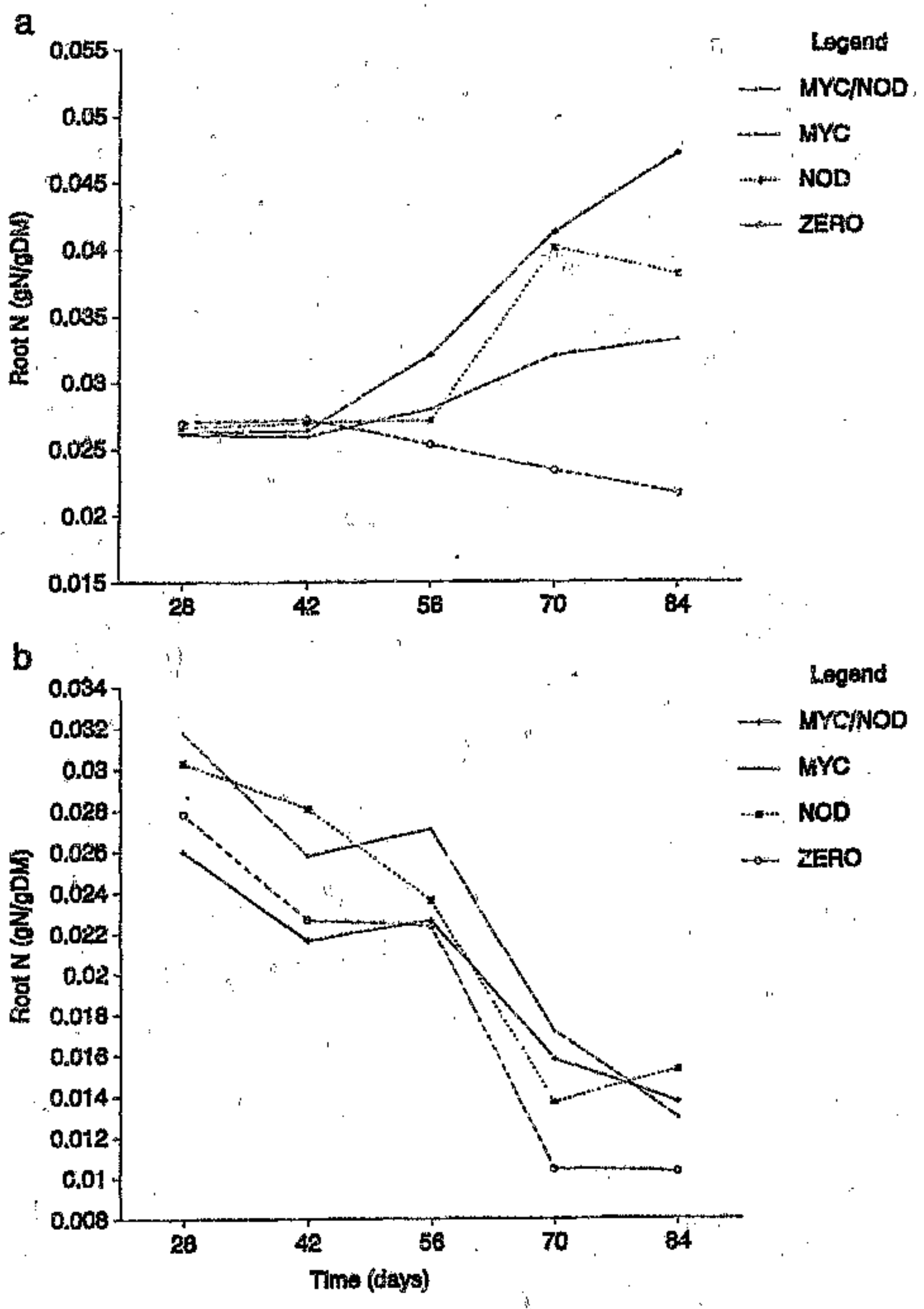

Fig 2.8. Root nitrogen contents (gN/g dry matter) for white clover (a) and Italian ryegrass (b) over time. The data are the means of three replicates.

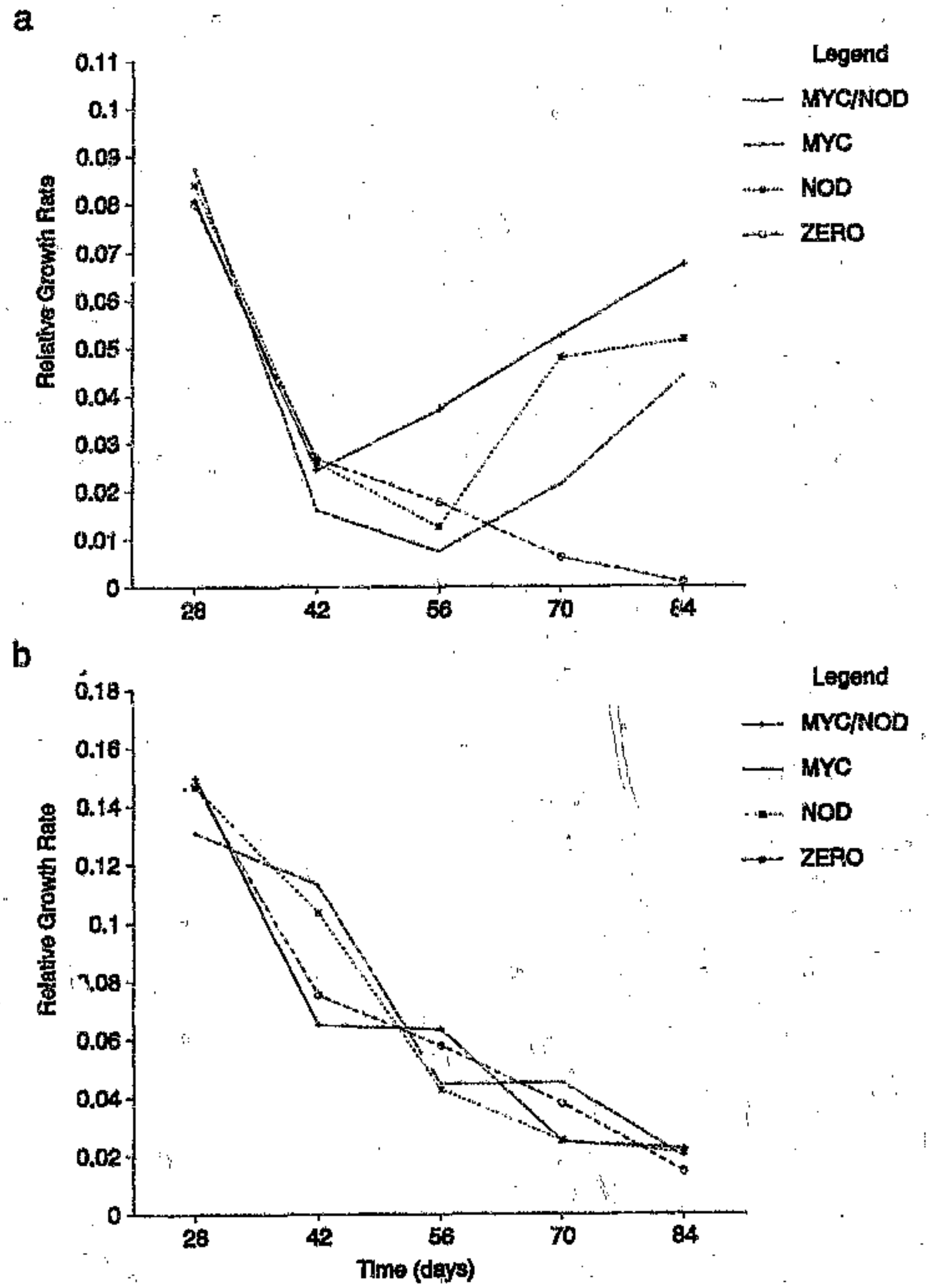

Fig 2.9. Mean relative growth rates (1/W* dW/dt) of white clover (a) and Italian ryegrass (b) over an 84 day growth period. The data were calculated on the basis of mean biomass.

content in the other treatments. In this case, significant differences were detected between both the NOD and the MYC treatments in the last harvest ($p=0.002$ and 0.016 respectively).

2.3.4 MEAN RELATIVE GROWTH RATES

The mean relative growth rates (RGR) for the intervals between harvests were calculated using the following equation (Hunt, 1978):

$$RGR = \frac{\ln W_{t2} - \ln W_{t1}}{t2 - t1} \tag{2.1}$$

where W_{t1} is the biomass at time t1, W_{t2} is the biomass at time t2.

In the case [illegible] clover, there were large differences in the trends of RGR between the treatments with symbionts and the -MYC-NOD treatment over the growth period (fig 2.9 a). In the -MYC-NOD treatment, there was a general decline in RGR over the growth period. In the other three treatments, there was an initial decline followed by an increase corresponding to the establishment of symbioses (fig 2.9 a). In the ryegrass, there was a general decline in the RGRs over the growth period in all treatments (fig 2.9 b).

In order to investigate the relationship between tissue nitrogen concentration and RGR, the leaf nitrogen concentrations were plotted against their respective RGRs in fig 2.10 a and b. Leaf nitrogen contents were chosen because of their role in photosynthesis. This is obviously of key importance in growth, since about 90% of a plants dry matter results form the products of photosynthesis (Poorter, Remkes and Lambers, 1990). The RGR's for the first growth period were omitted because the leaf nitrogen status within this time window was uncertain and the effects of seed nitrogen stores are likely to have played a large role. The simple linear regression that was carried out on these data yielded r values of r=0.857 for the clover and r=0.813 for the ryegrass. In both species, the fitted lines had a positive slope and negative y-intercept value.

2.3.5 BIOMASS VS NITROGEN CONTENTS

Previous studies for a wide range of crops have shown that, under conditions with sufficient nitrogen to permit maximum growth, the nitrogen content of crops declined sharply with increased biomass (Greenwood *et al.*, 1991). In order to investigate the relationship between biomass and nitrogen content in this system, the leaf, stem and root nitrogen contents were plotted against the respective component biomasses for all the treatments for both clover and rye (fig 2.11 and 2.12 respectively). In the case of clover, it can be clearly seen that the expected relationship does not hold. In the rye on the other hand there was a sharp decline in nitrogen content with increased biomass. This

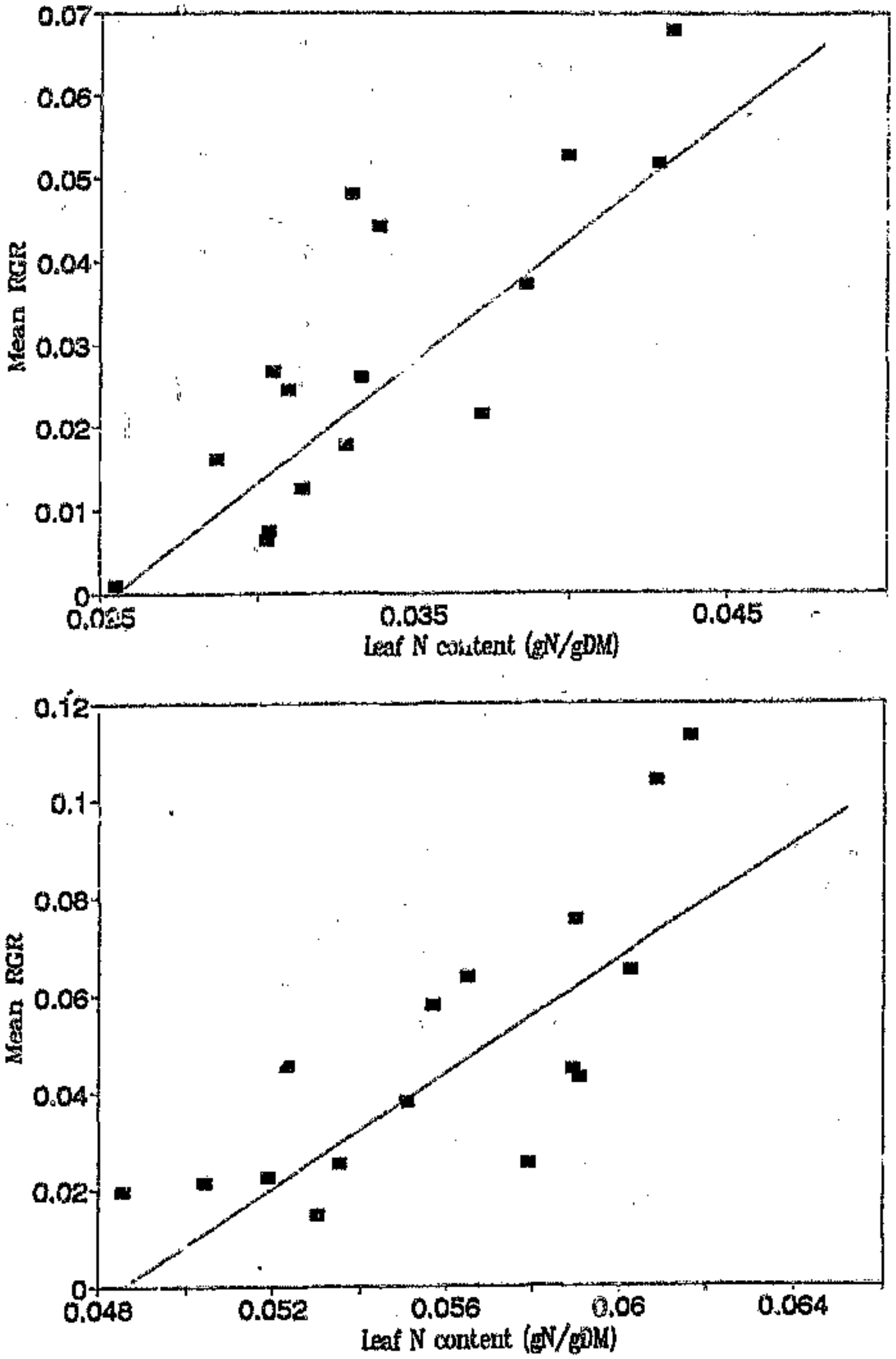

Fig 2.10. Scatter plots of mean RGR against leaf nitrogen content (LNC) (gN/gDM) for white clover (a) and Italian ryegrass (b). The equation for the linear regression fit for the clover is $RGR=2.94*LNC-0.075$ ($r=0.857$) and for the ryegrass is $RGR=5.909*LNC-0.287$ ($r=0.813$). Data are means of 3 reps.

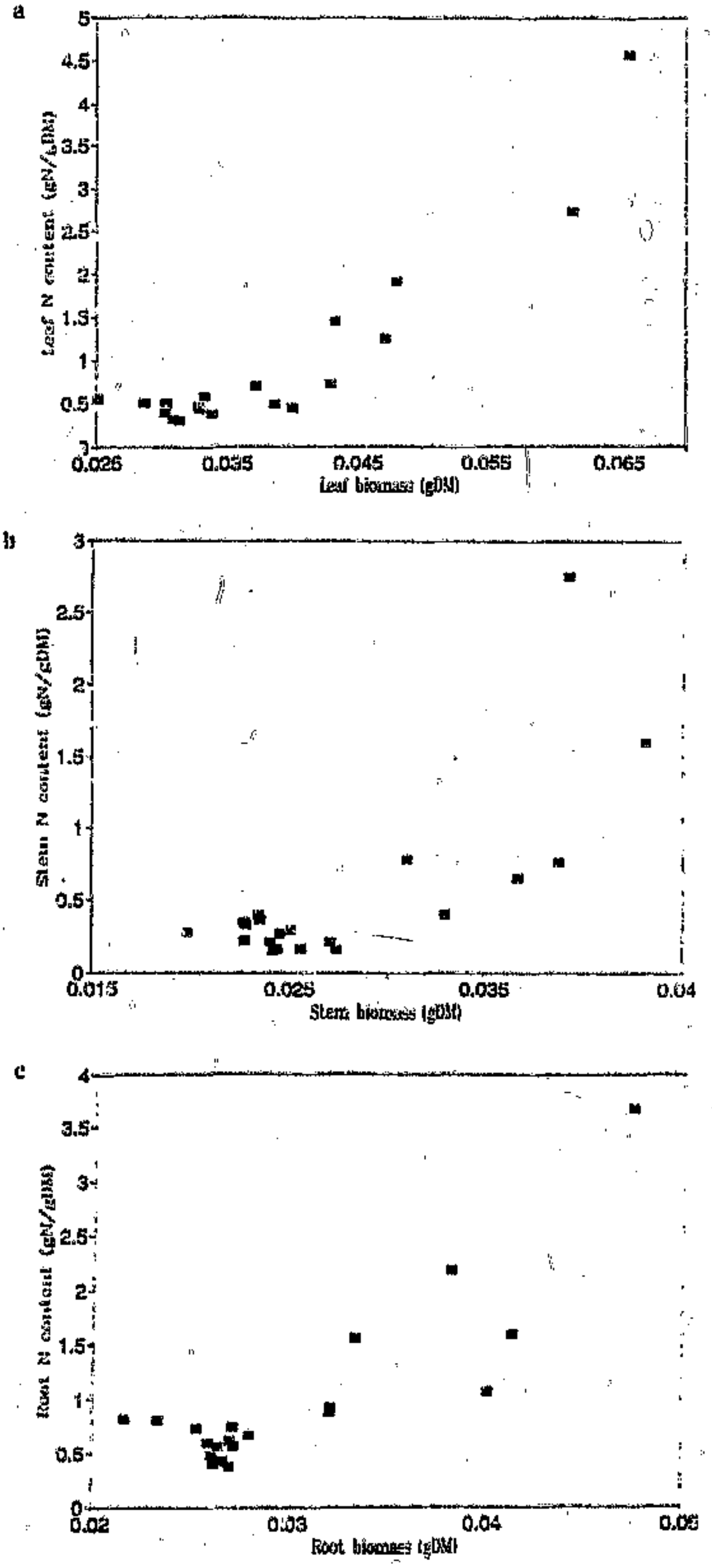

Fig 2.11. Nitrogen contents (gN/gDM) for the leaves (a), stems (b) and roots (c) plotted against their respective biomasses (gDM) for white clover. Data are the means of three replicates.

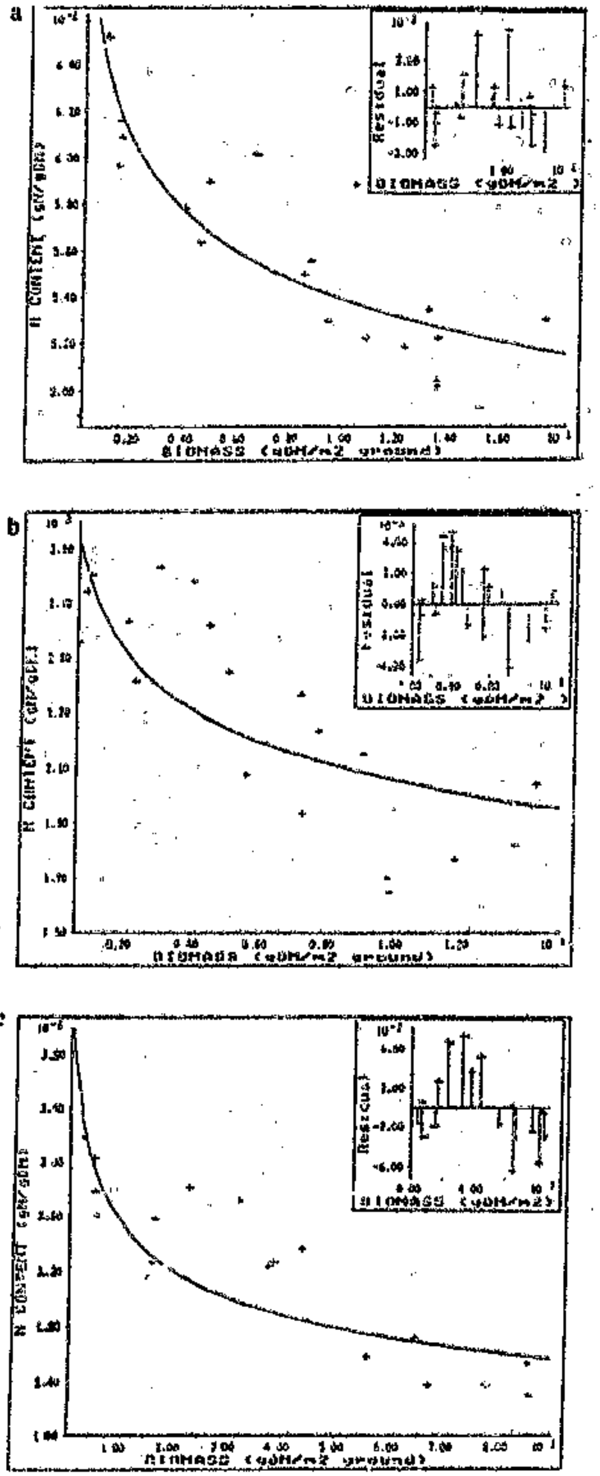

Fig 2.12. Nitrogen contents (N) (gN/gDM) of leaf (a), stem (b), and root (c) compartments plotted against their respective biomasses (W) (gDM) in Italian ryegrass. The equations for the regression fits are:

$$N=6.404E\text{-}2*W^{-7.43E\text{-}2}$$

$$N=2.276E\text{-}2*W^{-1.28E\text{-}1}$$

$$N=4.380E\text{-}2*W^{-2.28E\text{-}1}$$

respectively. Data are the means of three replicates.

relationship in the ryegrass was further investigated by fitting the equation (after Greenwood *et al.*, 1991):

$$N = a * W^b \qquad (2.2)$$

to the data (fig 2.12 a, b, and c), where N is the component nitrogen content, W is the component biomass and a and b are constants. The values for these constants are presented below, along with the variability that is accounted for by the regression (table 2.3).

Table 2.3. Estimates of the constants a and b (equn 2.2) relating component (COMP) nitrogen content to biomass in Italian ryegrass.

COMP	a	b	r
leaf	6.41E-2	-7.44E-2	0.85
stem	2.81E-2	-1.44E-1	0.78
root	5.01E-2	-2.78E-1	0.81

2.4 DISCUSSION

On the basis of previous work that was reviewed in chapter 1, there were a number of expectations (or hypotheses) for the experimental results presented above. And inevitably, some of these expectations were satisfied while others pose anomalies. In terms of the symbiotic components (the VA mycorrhizas and the nodules), there were two noteworthy findings. Firstly there was no infection of the ryegrass by VAM fungi.

There are two likely reasons for this: Firstly the fibrous root system of the Italian ryegrass may capture nutrients with sufficient effectiveness so as to nullify any advantageous effects of VA infection. It is thus possible that the costs of the mycorrhiza exceed the benefits (Koide and Elliot, 1989) and so the host might be expected (assuming it is able) to reject the infection. Secondly, Italian ryegrass is an annual and there seems to be some sort of correlation between so-called "non-host" species and annual and biennial plant species (Francis and Read, 1994). The implication of this non-mycorrhizal nature of the ryegrass was that there is no possibility that a common mycorrhizal network linking the two plant species investigated could have existed. For this reason, the objectives listed in 1.7 had to be modified to some extent.

The second interesting finding in terms of the symbionts was that there were no significant differences in levels of infection by either symbiont in response to the presence or absence of the other symbiont (with the exception of nodule biomass at harvest 3). There are inherent limitations to the statistical analysis used to investigate this (as discussed in 2.2.7), however, this finding was further confirmed by the lack of any significant MYC*NOD interactions in the plant variates.

This finding is rather unexpected. Intuitively it would be expected that the improved nitrogen status of the +NOD plants should result in an improved photosynthetic ability and thus a greater quantity of photosynthates which could become available to mycorrhizal fungal symbionts. In addition, the nitrogen fixing ability of nodules may be limited by phosphorus supply, and given the role of mycorrhizas in improved phosphorus nutrition, an interaction of sorts would be expected. A possible reason for this is the fact that it was only fairly late in the growth period that reasonable levels of infection (especially in the case of the mycorrhizal fungi) occurred. It is therefore possible that differences did not have time to manifest themselves in a significant way. This idea is supported by the visual differences in infection levels that are apparent (tables 2.1 and 2.2). In the case of the mycorrhizas, the means of the infection levels in the +NOD treatments were consistently (although not significantly) greater than those in the -NOD treatments. This was also the case in nodule biomass where mean biomasses were consistently higher in the +MYC treatments than in the -MYC treatment. It is possible that these differences might have been resolved with a greater number of replicates or longer growth trial.

In terms of the plant variates measured, the most obvious expectation is that all the biomasses should increase over the growth period unless competition is so great that there is thinning. In the case of the clover (figs 2.1a to 2.4a), it can be seen that this is generally the case. The only exception occurs in the -MYC-NOD treatments, where, towards the end of the growth period (particularly harvests 4 and 5), the component biomasses increase very slightly, as in the leaves (fig 2.2 a) and roots (fig 2.4 a), or decrease as in the case of the stem biomass (fig 2.3 a). This levelling off of the growth curves in the -MYC-NOD treatment is also reflected in the decline of relative growth rate (fig 2.9 a).

This also hold for the ryegrass (figs 2.1b to 2.4b) and here there were no differences between any of the treatments. This is contrary to the generalisation that the addition of mycorrhizal inoculum produces a positive effect upon the legume component in grass-legume mixtures at the expense of the grass (Francis and Read, 1994). A possible reason for this is that the planting densities were low, so the competitive effects may also have been low.

When considering the leaf nitrogen contents in relation to canopy RGR, the situation in clover and rye are not very different (fig. 2.10 a and b respectively). In the both cases, the relationship appears to be almost linear (in clover $r=0.857$ and in rye $r=0.813$) and both lines extrapolate to zero growth at positive nitrogen contents (ie have a positive x intercept) confirming the observations of Levin, Mooney and Field (1989). Levin *et al* (1989) use this as a justification for reformulating the growth model of Ågren and Igestad (1987) which was based on the equation :

$$d(\ln W)/dt = a.n \tag{2.3}$$

where $d(\ln W)/dt$ is the RGR; a is a growth constant; and n is the nitrogen content. The implication of this equation is that growth does not stop at very low nitrogen contents, despite the fact that at low nitrogen contents photosynthesis may be zero. One approach to circumventing this paradox is the approach proposed by Levin *et al* (1989). Another is to define growth as a function of either carbon or nitrogen substrate concentrations, depending on which is most limiting. In this way, if the photosynthetic rate becomes zero due to low nitrogen content, the carbon substrate content will decrease rapidly and growth will be limited. This latter approach is the approach used in the growth model described in chapter 4 (equns 4.21).

The apparently linear relationship between RGR and leaf nitrogen contents also leads to the prediction that in the ryegrass and in the clover -MYC-NOD treatment, the mean RGR should decrease over the growth period and this is in fact observed to be the case (fig 2.9). However, in the case of clover in the experimental treatments, the establishment of symbioses clearly perturbs the system. This perturbation leads to the breakdown of the expected relationship between biomass and nitrogen content as seen in fig. 2.11. There are noticeable increases in the nitrogen contents and therefore RGRs corresponding to the onset of infection (fig 2.9 a). The observed linear relationship provides a reason why clover responds so well to infection by symbionts. Growth is clearly limited by relatively poor nutrient acquisition rather than photosynthesis so that a large carbon substrate pool should develop which can supply nitrogen acquiring symbioses. This is as would be predicted: firstly the clover roots have to compete with the very efficient (Francis and Read 1994) and large (fig 2.4) fibrous roots of the ryegrass; and secondly the clover plants have higher potential photosynthetic rates (Papadopoulos 1992).

It is also expected that the nitrogen contents should decline over the growth period (Greenwood *et al* 1991). The reason being that as plants grow they contain increasing proportions of structural materials that contain little nitrogen (Lemaire *et al* 1992). The corollary to this is that with increasing biomass, nitrogen content should decrease. From fig 2.12 it is clear that the corollary holds fairly well in the case of rye, however, in the case of clover (fig 2.11), the expected relationship does not hold. The reason for this being that the formation of symbioses perturbed the nitrogen acquisition process.

Another generally observed trend in herbaceous plants, is that there is an increase in the shoot:root ratio as the plants grow (Wilson 1988). However, under suboptimum N-nutrition, root growth may be enhanced at the expense of shoot growth (Bèlanger, Gastal and Warembourg 1992; Smolders and Merckx 1992). This is in line with the suggestion that plants may be capable of optimal foraging (Gleeson 1993). The mechanism whereby this is achieved may simply be, that after nitrogen has been taken up, the roots retain what proportion they need, before any is partitioned to the shoots. Thus under conditions of suboptimum N uptake, the roots still retain their nitrogen requirements, leaving less nitrogen to be allocated above ground. Because growth is related to nitrogen content, this imbalance in the nitrogen contents manifests itself in the form of reduced above-ground growth relative to below-ground growth, and thus a decrease in the shoot:root ratio. However, when the system is perturbed from suboptimal N uptake (eg by adding a fertiliser boost, or by the formation of symbioses that facilitate improved resource acquisition), it is expected that plants should respond with increasing shoot:root ratios.

This situation appears to be the case in the results described above: there is a general decrease in shoot:root ratios over the growth period in rye (fig 2.5 b) as well as in the -MYC-NOD treatment clover (fig 2.5 a). However, there is a statistically significant trend towards increasing the shoot to root ratios associated with the +NOD treatments. In the case of the +MYC treatments the trend is not statistically significant, however, it is noticeable (fig. 2.5 a) and it is possible that the significance of this trend could have been established with a greater number of replicates, or a longer growth trial.

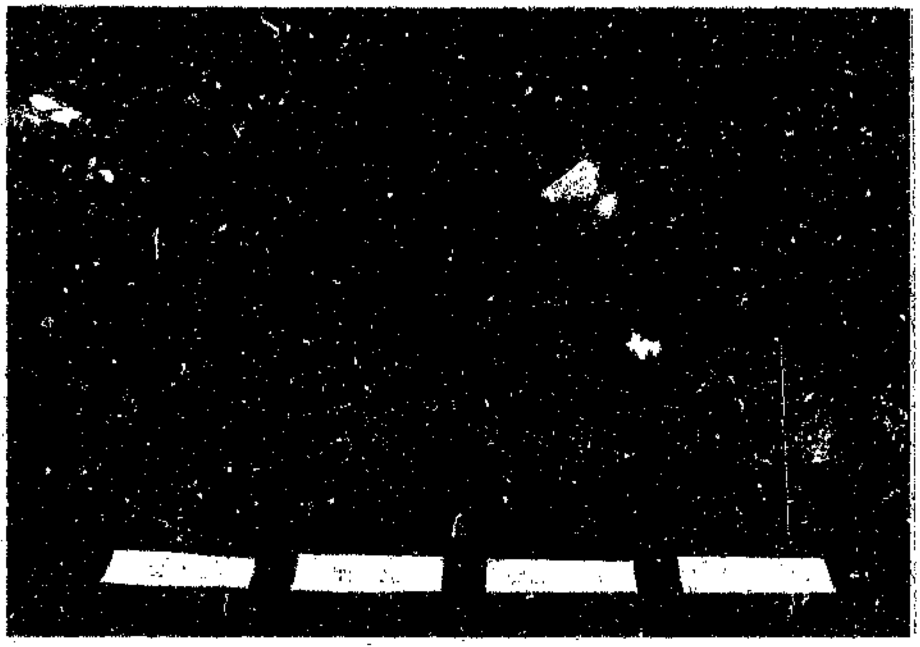

Plate 2. Photograph of the four treatments at harvest 5 showing the destinct above ground differences between treatments in the white clover plants, and no apparent differences between the Italian ryegrass.

To conclude, under the growth conditions described above (2.2.1), the performance of white clover in the mixture was significantly enhanced by both the establishment of active nodules and a vesicular-arbuscular mycorrhiza (plate 2). This was without any noticeable detrimental (or positive) effects on the Italian ryegrass component in the mixture. This result is difficult to assess in terms of real agricultural applicability, because of the obvious dangers associated with scaling up from pot data to field data. However, it is likely that in clover / ryegrass mixtures grown under nutrient-limited conditions, the formation of plant microbial symbioses may result in either improved performance of one or both of the components, or in shifting the competitive balance.

CHAPTER 3: CANOPY PHOTOSYNTHESIS IN A MIXED STAND

3.1 INTRODUCTION

Because plants derive energy and fixed carbon from photosynthesis, it is inevitable that studies of photosynthesis and the carbon economy should be at the forefront of comparative plant ecology (Osmond 1987). In addition, approximately 90% of a plants dry weight originates from the products fixed in photosynthesis (Poorter, Remkes and Lambers 1990). Much of the ecologically relevant work to date has been directed towards small- to large-scale integration of CO_2 acquisition (eg. from leaf to canopy photosynthesis) (Rastetter *et al* 1992). Models describing plant growth are frequently driven by such integrated canopy photosynthetic submodels (eg Johnson and Thornley 1983; Papadopoulos 1992).

In this chapter equations describing the canopy photosynthesis of the component species of equal ages in a binary canopy are derived. As will be discussed later, canopy photosynthesis is intimately related to leaf area distribution within the canopy. It is for this reason that the photosynthetic equations derived were combined with a canopy growth model which allows the description of changes in the distributions of leaves as photosynthesis progresses. These two components are incorporated into a computer program MICSIM (Appendix 1i) in order to simulate the processes of growth and photosynthesis. The variables, constants and parameters used in this model are listed and defined in appendix 3. Although the system in question is essentially continuous, difference equations were used to describe the dynamics. This discrete modelling approach is essentially the same as the Euler method for numerical integration and is therefore prone to truncation errors. These errors are, however, offset by the simplicity of the method (and therefore speed of processing).

3.2 THE GROWTH MODEL

Canopy architecture (and in particular leaf area distribution) is a crucial factor in determining the light attenuation properties through a canopy, particularly in a binary canopy (Rimmington 1984). There are a large number of possible configurations (see figure 2 in Rimmington 1984), however, for the purpose of this model it is assumed that there are only three: both species are equally high (fig 3.1 condition 3) or one of the species overshadows the other (fig. 3.1 conditions 1 and 2).

The canopy can thus be divided into two regions: the upper canopy where photosynthesis is the same as in a monoculture and the lower canopy where there is mixed canopy photosynthesis. The light incident on the upper canopy is simply "I" while the light incident on the lower canopy [I(iow)] is described by (Rimmington 1984):

$$I(low) = I*\exp(a)*\exp(b) \tag{3.1}$$

where a is -K1*L1UP and b is -K2*L2UP. Ki(i=1,2) is the canopy extinction coefficient of the leaf tissue for species i. LiUP (i=1,2) is the cumulative leaf area index of species

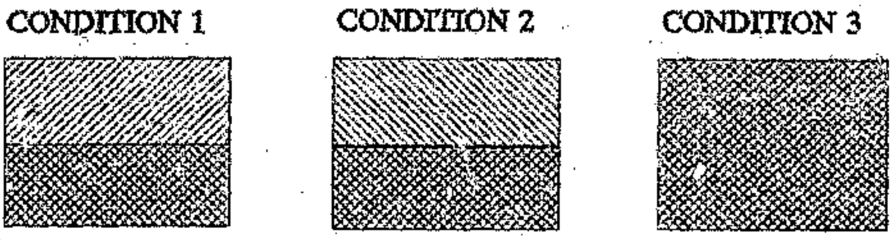

Fig. 3.1. Possible canopy configurations of a mixed stand considered in this model.

i in the upper canopy.

In order to describe changes in the canopy architecture the photosynthetic equations developed below must be combined with a dynamic growth model. The purpose of this model was not to simulate any particular data set, nor to reproduce quantitatively what occurs in a "typical" mixed canopy, but rather to assess the functioning of the photosynthetic equations developed below on a qualitative basis (ie were the trends biologically reasonable). For this reason the growth model used was a rather crude model with a number of important assumptions. Firstly, it was assumed that the activities of the canopy were independent of any below ground processes. It was also assumed that there were no changes in the light environment (either the intensity of light or the photoperiod) over the simulation period, and that the processes are independent of temperature fluctuations. Also the density of plants in the canopy was assumed to be constant (ie no thinning occurred) as was the allocation of photosynthate to leaves and stems.

Thirteen equations describe the growth of the canopies and the various components of these canopies. Changes in biomass are described by:

$$W_{i(t)} = W_{i(t-1)} + (Pi-M*W_{i(t-1)})*Y \quad (i=1,2) \tag{3.2}$$

Where $W_{i(t)}$ is the biomass of species i at time t, $W_{i(t-1)}$ is the biomass of species i at t-1, Pi is the photosynthetic inputs into the system over the time period 1, M is the maintenance respiration coefficient and Y is the efficiency with which photosynthates are converted to biomass. These latter two variables are assumed to be the same for both species in this model.

Changes in stem masses are described as follows:

$$S_{i(t)} = S_{i(t-1)} + (Pi-M*W_{i(t-1)})*Y*FSi \quad (i=1,2) \tag{3.3}$$

Where Si(t) is the stem mass of species i at time t, and Si(t-1) is the stem mass at time t-1. FSi is the stem allocation coefficient for species i.

Likewise, changes in the leaf masses are described by:

$$L_{i(t)} = L_{i(t-1)} + (Pi-M*W_{i(t-1)})*Y*FLi \quad (i=1,2) \tag{3.4}$$

Where Li(t) is the leaf mass of species i at time t, and Li(t-1) is the leaf mass at time t-1. FLi is the leaf allocation coefficient for species i.

Stem : Leaf ratios (SLi i=1,2) can then be defined:

$$SL_{i(t)} = S_{i(t)}/L_{i(t)} \ (i=1,2) \tag{3.5}$$

and the height of the two species' canopies can be calculated using allometric relationships between stem mass and height (Hi):

$$Hi = Bi^{*}(Si/Ni)^{Ci} \ (i=1,2) \tag{3.6}$$

Where Hi is the height of species i, Bi and Ci are allometric coefficients and Ni is the density of plants in the stand. Density of plants is incorporated here since the Si is the stem mass for the entire canopy, while the allometric coefficients are applicable to individual plants. This approach is similar to that taken by Hara (1984), except that here, height is related to stem mass rather than total biomass. The advantage of the approach used here is that it is possible to incorporate an optimization step for allocation to leaf and stem biomass, based on maximising photosynthesis (although this is not attempted in this model).

Using the variables defined by these equations it is possible to define the architecture of the canopy. As mentioned above three configurations are considered (fig. 3.1):

$$H1U = \begin{cases} 0, & H1 \leq H2 \\ H1-H2, & H1 > H2 \end{cases} \tag{3.7}$$

$$H2U = \begin{cases} 0, & H2 \leq H1 \\ H2-H1, & H2 > H1 \end{cases} \tag{3.8}$$

$$H1L = \begin{cases} H1, & H1 \leq H2 \\ H2, & H1 > H2 \end{cases} \tag{3.9}$$

$$H2L = \begin{cases} H2, & H2 \leq H1 \\ H1, & H2 > H1 \end{cases} \tag{3.10}$$

Where H1U is the height of the upper canopy of species 1, H2U is the upper canopy of species 2, H1L is the height of the lower canopy of species 1 and H2L is the height of the lower canopy of species 2. Upper and lower canopy leaf area indices can then be calculated:

$$LA1U = \begin{cases} 0, & H1 \leq H2 \\ [(H1u/B1)^{1/C1}]*N1*SL1*SLA1, & H1 > H2 \end{cases} \tag{3.11}$$

$$LA2U = \begin{cases} 0, & H2 \leq H1 \\ [(H2U/B2)^{1/C2}]*N2*SL2*SLA2, & H2 > H1 \end{cases} \tag{3.12}$$

$$LA1L = [(H1L/B1)^{1/C1}]*N1*SL1*SLA1 \tag{3.13}$$

$$LA2L = [(H2L/B2)^{1/C2}]*N2*SL2*SLA2 \tag{3.14}$$

Where LA1U and LA1L are the upper and lower canopy leaf area indices of species 1 respectively, LA2U and LA2L are the upper and lower canopy leaf area indices of species 2. SLAi (i=1,2) is the specific leaf area of species i.

3.3 PHOTOSYNTHETIC EQUATIONS

In addition to canopy architecture considerations, a number of other factors need to be considered when scaling up from photosynthesis of individual leaves through many leaved elements to collections of leaf elements making up an entire canopy. The architecture of the canopy in terms of mean leaf inclination angle and the horizontal layers of leaves require a more complex description of canopy photosynthesis than that given by the product of photosynthetic rate per unit leaf area, the specific leaf area, the leaf allocation coefficient and the biomass of the canopy. The age structure of leaves on individual plants or in the canopy of a collection of plants also needs to be taken into account when fixing a value for canopy photosynthesis.

Photosynthesis for individual leaves (PI) in response to irradiance can be conveniently described by a rectangular hyperbola given by the expression (Johnson and Thornley 1983):

$$PI = AM*A*I/(AM + A*I) \qquad (3.15)$$

where, AM is the light saturated rate of photosynthesis; A is the quantum yield efficiency and I is the irradiance projected onto a horizontal surface.

From this point on the mixed canopy is assumed to be made up of two independent canopies: an upper and a lower canopy linked only by the fact that I(low) is dependent on the properties of the upper canopy (equation 3.1). Equations for canopy photosynthetic rates for the upper and lower canopy components of the two species can then be determined by substituting I into equation 3.15 and integrating throughout the canopy as follows:

From equn 3.1 above it is evident that the light absorbed by the upper canopy of species 1 (I1up) is:

$$I1up = \exp(-K1*L1UP) = u \qquad (3.16)$$

then by manipulation of this equation:

$$dL1UP = du/-K1*u \qquad (3.17)$$

so that

$$P1UP = \int_1^u \frac{A1*I*AM1}{A1*I + AM1} \cdot \frac{du}{-K1*u} \qquad (3.18)$$

Where P1UP is the instantaneous canopy photosynthesis of the upper canopy component of species 1.

Substituting I1up = Io exp (-K1*L1UP) into equation (3.18):

$$P1up = \int_1^u \frac{A1*I*exp(a)*AM1}{A1*I*exp(a) + AM1} \cdot \frac{du}{-K1*u} \tag{3.19}$$

$$= \int_1^u \frac{A1*I*AM1}{A1*I*exp(a) + AM} \cdot \frac{du}{-K1} \tag{3.20}$$

Let A1*Io = x and AM1 = y then

$$P1up = \int_1^u \frac{x*y}{x*u + y} \cdot \frac{du}{-K1} \tag{3.21}$$

$$= \frac{1}{K1} \int_u^1 \frac{x*y}{x*u + y} \cdot du \tag{3.22}$$

$$= \frac{x*y}{K1} \int_u^1 \frac{1}{x*u + y} \cdot du \tag{3.23}$$

$$= \frac{y}{K1} \ln \frac{x + y}{x*u + y} \tag{3.24}$$

Substituting for x, y and u:

$$P1up = \frac{AM1}{K1} \ln \frac{A1*I + AM1}{A1*I \exp (-K1*L1UP) + AM1} \tag{3.25}$$

ı species 2 were higher than species 1 then L1UP would be zero and so to would P1up.

Using a similar derivation it is possible to find an equation describing this canopy photosynthetic rate of the upper canopy component of species 2 (P2up):

$$P2up = \frac{AM2}{K2} \ln \frac{A2*I + AM2}{A2*I \exp (-K2*L2UP) + AM2} \tag{3.26}$$

For the lower canopy photosynthesis for the two species

I, the incident light flux density at a certain height in the canopy is given by (Rimmington 1984):

$$I = Ilo*\exp(-K1*L1LO-K2*L2LO) \quad (3.27)$$

or

$$I = Ilo*\exp(-K1*L1LO)*\exp(-K2*L2LO) \quad (3.28)$$

Let $c = -K1*L1LO$

$d = -K2*L2LO$

And let $\exp(c) = u$

Then by substituting for c:

$$dL1LO = du/-K1*u \quad (3.29)$$

As for the upper canopy, P2low (the instantaneous canopy photosynthesis of the lower canopy component of species 1) can be determined by substituting I into equation 3.15 and integrating throughout the canopy as follows:

$$P1lo = \int_{1}^{u} \frac{A1*Ilo*\exp(d)*u*AM1}{A1*Ilo*\exp(d)*u + AM1} \cdot \frac{du}{-K1*u} \quad (3.30)$$

Let $A1*Ilo*\exp(d) = x$ and $AM1 = y$ then

$$P1lo = \int_1^u \frac{x*y}{x*u + y} \cdot \frac{du}{-K1} \qquad (3.31)$$

$$= \frac{1}{K1} \int_u^1 \frac{x*y}{x*u + y} \cdot du \qquad (3.32)$$

$$= \frac{x*y}{K1} \int_u^1 \frac{1}{x*u + y} \cdot du \qquad (3.33)$$

$$= \frac{y}{K1} \ln \frac{x + y}{x*u + y} \qquad (3.34)$$

Substituting for x, y and u:

$$P1lo = \frac{AM1}{K1} * \ln \frac{A1*I*\exp(a+b)*\exp(d) + AM1}{A1*I*\exp(a+b)*\exp(c+d) + AM1} \qquad (3.35)$$

Similarly, for species 2:

$$P2lo = \frac{AM2}{K2} * \ln \frac{A2*I*\exp(a+b)*\exp(c) + AM2}{A2*I*\exp(a+b)*\exp(c+d) + AM2} \qquad (3.36)$$

This gives four equations that describe the instantaneous canopy photosynthesis of species 1 and 2. These are then converted to daily integral by assuming that I is constant throughout the day (Johnson and Thornley 1983):

$$P1UP = \frac{DAY*AM1}{K2} \ln \frac{A1*I/DAY + AM1}{A1*I/DAY \exp(-K1*L1UP) + AM1} \qquad (3.37)$$

$$P2UP = \frac{DAY*AM2}{K2} \ln \frac{A2*I/DAY + AM2}{A2*I/DAY \exp(-K2*L2UP) + AM2} \qquad (3.38)$$

$$P1LO = \frac{DAY*AM1}{K1} \ln \frac{A1*I/DAY*\exp(a+b)*\exp(d) + AM1}{A1*I/DAY*\exp(a+b)*\exp(c+d) + AM1} \quad (3.39)$$

$$P2LO = \frac{DAY*AM2}{K2} \ln \frac{A2*I/DAY*\exp(a+b)*\exp(c) + AM2}{A2*I/DAY*\exp(a+b)*\exp(c+d) + AM2} \quad (3.40)$$

Therefore:

$$P1=P1UP+P1LO \quad (3.41)$$

$$P2=P2UP+P2LO \quad (3.42)$$

3.4 SIMULATIONS

The model was written in FORTRAN (Etter, 1993) (see appendix 1i) and run on a 386dx personal computer. Values for the photosynthetic parameters for simulations were taken from a previous study of the growth of plants in a mixed canopy of Italian rye grass and white clover (Papadopoulos 1992). Species 1 represents the rye grass, and species 2, the white clover. The simulation was run at two different light intensities (vis. normal light and 1/2 normal light) to investigate the response of the canopy to different light regimes. Simulation results for these two scenarios are presented in figures 3.2 and 3.3.

3.5 DISCUSSION

The primary objective of this model was not to simulate growth of the plants in the

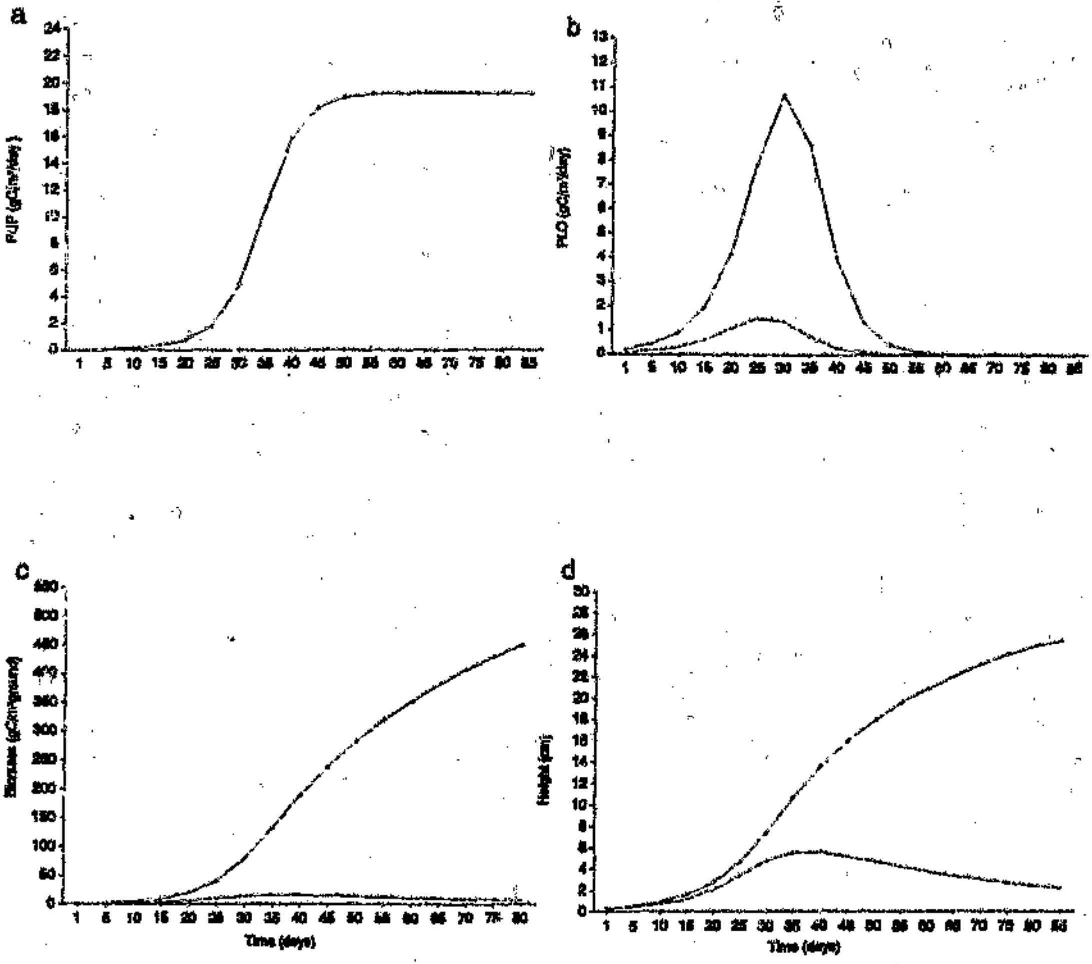

Fig 3.2. Simulation results for: a. upper canopy photosynthesis (PUP); b. lower canopy photosynthesis (PLO); c. biomass; and d. height with the value of I set at 1.87E+7J/m²/day (Papadopoulos 1992).

Grass —+—, Legume ——

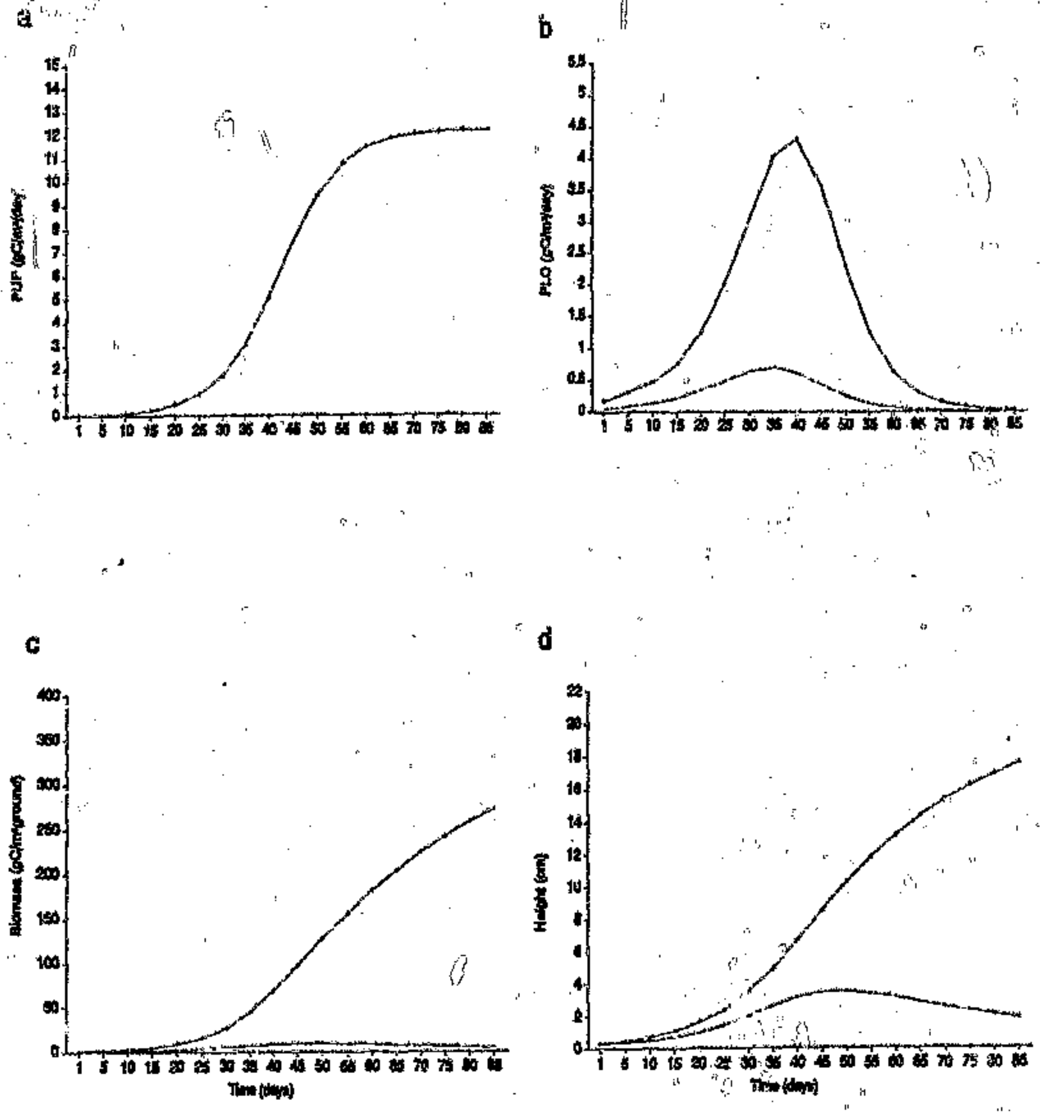

Fig 3.3. Simulation results for: a. upper canopy photosynthesis (PUP); b. lower canopy photosynthesis (PLO); c. biomass; and d. height with the value of I set at 9.35E+6J/m²/day.

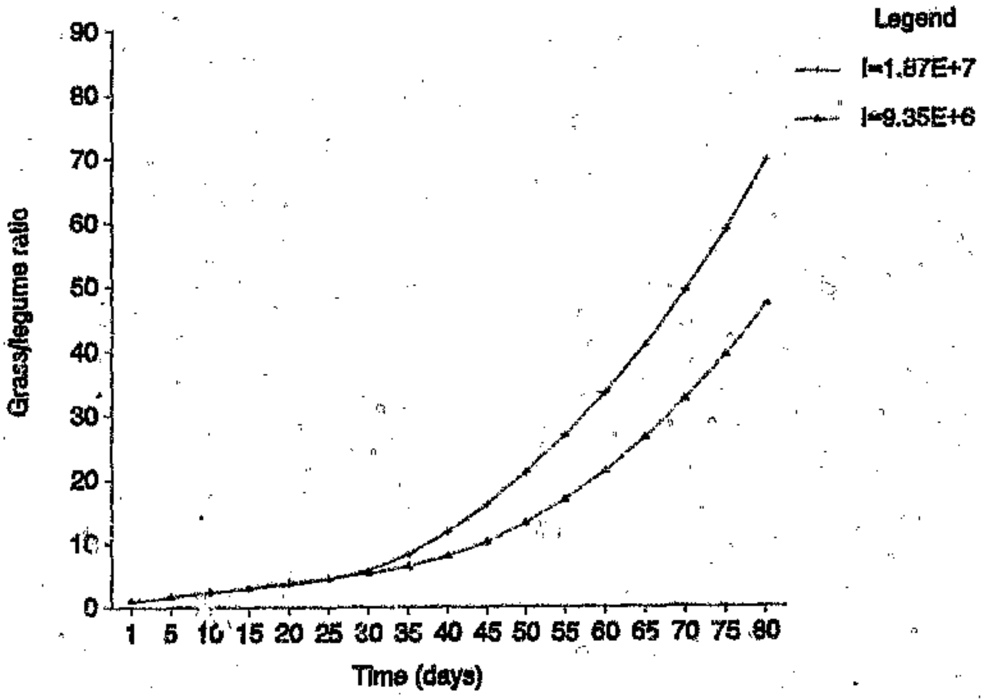

Fig 3.4. Grass:legume biomass ratio indicating the relative competitiveness of the grass and the legume in the canopy when simulations were carried out at two different light intensities (I).

particular binary canopy investigated in chapter 2 but rather to develop a description of photosynthesis in a mixed canopy which could be used in a more comprehensive growth model. In addition, the criterion for accepting the model was not that it simulate intricacies of growth in the real system but rather, the model was accepted on the basis that the simulations were intuitively reasonable. For this reason the output variables presented were not necessarily parameters that were measured experimentally or described in the previous chapter but were chosen to allow intuitive assessment of the description. Despite this, the values for parameters used in the simulations did come from experimental data and it is thus expected that broad trends, and in particular trends associated with different light regimes, should be simulated.

As is expected, the higher of the two species (species 1 in this case) came to dominate the canopy in terms of photosynthesis and biomass (fig 3.2 a and b). In terms of the model, height and growth are intimately linked: the species that is higher, will dominate photosynthesis as a result of shading. This is equivalent to a form of "interference" competition (Begon, Harper and Townsend 1986). The net result of this is that species 1 accumulates biomass more rapidly. In fact, towards the end of the simulation growth period, the biomass of species 2 began to decline, suggesting that senescence of plants or tissue was occurring due to the interspecific competition. As was noted above (3.4), species 1 was defined by parameters derived from rye grass, and species 2 from clover and so the general experimental trend of the grass being dominant in the canopy (see chapter 2) is reproduced by the model.

It has been also observed that when clover and rye are grown together under shaded conditions, that the grass may no longer be as dominant (Gray 1993 personal communication). From the simulation of growth at lower light (fig 3.3), it can be seen that this observation is duplicated by the model (at least qualitatively). This is more apparent when the grass:legume biomass ratios are compared for the two light intensities (fig 3.4). This is further evidence for the validity of the description. It can thus be seen that the simulations are intuitively reasonable, that the broad trends are in line with general experimental observations, and that the model canopy responds to changes in the light regime in a quantitatively similar fashion to observed tendencies. It is thus reasonable to assume that the model is suitable for incorporation into an integrated model.

CHAPTER 4: INTEGRATED PLANT GROWTH MODEL

4.1 INTRODUCTION

The plant growth model presented in the previous chapter represents the most simple form of plant growth model, where growth of all plant parts is set as dependent on a carbon substrate as supplied by photosynthesis. Basic to this approach is the assumption that there is no decoupling of growth rate from photosynthetic rate. From a purely theoretical point of view, such an approach has the disadvantage that the activities of all organs are dependent on the activity of one and this can lead to an exaggeration of the dominance of that organ in predictions of whole plant responses to the environment (Raper *et al* 1977).

In addition, although at first glance the simulations may resemble the actual data, closer inspection reveals that there are a number of noticeable differences. Firstly, there is a very large magnitude discrepancy between the final model plant biomasses and the real biomasses. It is possible that the magnitude gap could have been narrowed (either by incorporating a description of senescence or by calibration of the input variables). However, no amount of calibration would allow the reasonable simulation of the experimentally observed trends described in chapter 2.

Therefore, in this chapter, a more comprehensive growth model is described, in which growth is a function of both below ground and above ground processes. The basic structure of the model system is shown in fig 1.1. Unlike the Thornley (1991) forest growth model, no distinction is made between meristematic and non-meristematic tissue. However, a distinction is drawn between substrate and structure, thereby allowing the decoupling of growth from resource acquisition. Each of the various biotic compartments (i.e. roots, stems, leaves, nodules and mycorrhizal fungi) comprise two substrate pools (carbon and nitrogen), and a structure fraction. The sum of these three fractions makes up the total dry matter (DMi) of the component i (equn 4.1)

$$DMi=Wi+Ci+Ni \tag{4.1}$$

where Wi is the structural fraction (g structure.m^{-2}ground) of component i; Ci is the carbon substrate (gC substrate.m^{-2}ground) in component i; and Ni is the nitrogen substrate (gN substrate.m^{-2}ground) in component i. The substrate concentrations (CCi and CNi) in component i are then calculated as follows:

$$CCi=Ci/Wi \tag{4.2}$$

and

$$CNi=Ni/Wi \tag{4.3}$$

For output purposes, the total nitrogen concentration in component i (NDi) is also calculated:

$$NDi=(Wi*Qi+Ni)/DMi \tag{4.4}$$

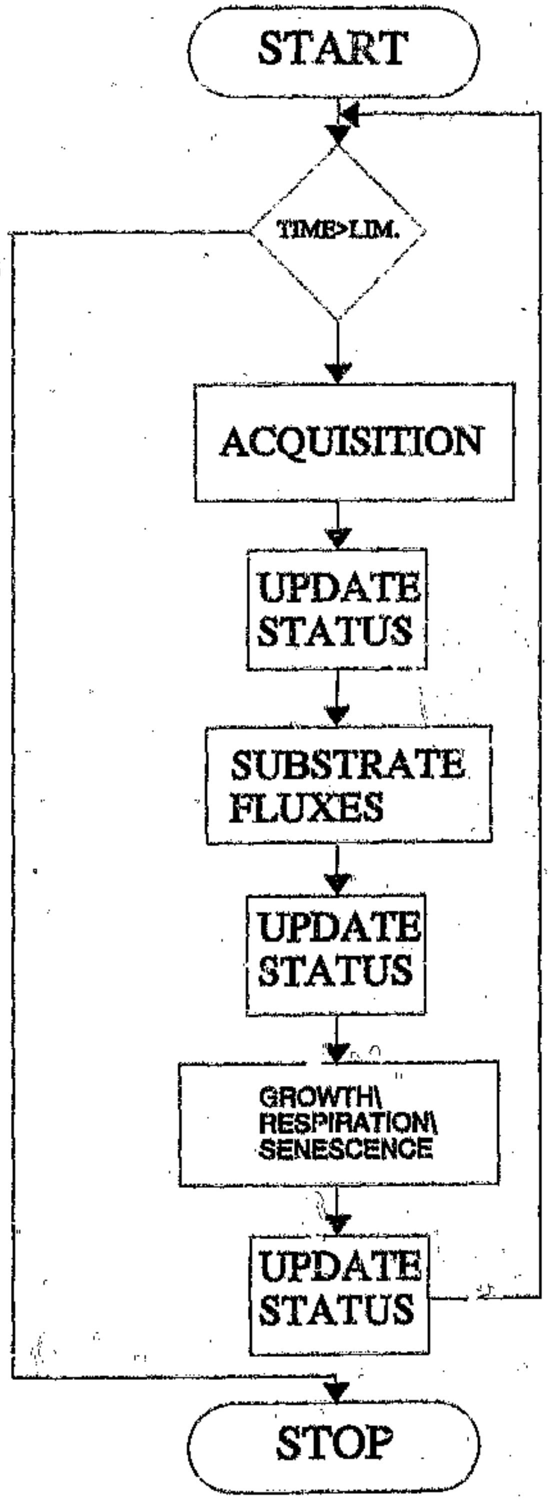

Fig 4.1. Flow chart for the sequence of events in the plant growth model.

where Qi is the nitrogen content of structure (gN.g structure^{-1}). This allows the direct comparison of the outputs with experimentally determined nitrogen contents.

The dynamics of the system are described by applying the algorithm summarised in fig 4.1 to each compartment. The algorithm iterated once per day. Because the status of each of the three pools in each compartment are updated three times per iteration. they are effectively updated three times every day. Day length is assumed not to change over time. This assumption can be justified for this particular model since the simulation period was only three months which is about one season. However, if the simulation period were to be extended, then it would be necessary to incorporate a function (eg. a sine function as used in Papadopoulos [1992]) describing seasonal changes in this parameter. The same would also apply to mean instantaneous light flux density above the canopy (I). In addition, temperature is assumed not to play any role in the rate at which various processes take place. This can again be justified by the fact that the simulation period falls within one season. Extending the applicability of the model is again a relatively simple exercise which can be achieved by including functions describing how certain rates may change with temperature as described by a sine function.

4.2 RESOURCE ACQUISITION

Photosynthesis - The photosynthesis submodel used in this plant growth model is almost identical to the model developed in the previous chapter. The only modification is that the two variables: quantum yield efficiency (A1 and A2) and the light saturated photosynthetic rate (AM1 and AM2) are not held constant, but rather vary with leaf nitrogen content (NDLi, i=1,2). It is assumed that the relationship is linear (as in Hirose and Werger 1987) (equns 4.5 and 4.6):

$$AMi=ci*NDLi-di \qquad (4.5)$$

$$Ai=ei*NDLi-fi \qquad (4.6)$$

where ci and ei are the slopes of the lines in species i, and di and fi are the y-intercepts. It is possible that this assumption may be a bit restrictive in terms of model flexibility. However, it can be justified on the basis of the growth conditions of the experimental plants. Were the model to be applied to a situation where nitrogen was available in abundance, then a rectangular hyperbolic type of function may be more appropriate.

Nitrogen uptake - To summarise section 1.4.1, there are a number of important factors to consider when attempting to model mineral acquisition: Firstly, it is necessary to incorporate some index of root size (often length), and its growth over time; secondly, nutrient uptake into the roots as a function of the nutrient concentration in the soil solution of the rhizosphere and root activity must be considered; and lastly, nutrient transport from the bulk soil solution into the rhizosphere is an important feature.

In order to account for zones of depletion and the importance of diffusion geometry, it is assumed that the soil is divided into two pools: The bulk soil, which is the ultimate source of all nitrogen (except for that which is fixed by the nodules); and the rhizosphere or depletion zone of soil which is within the sphere of influence of the absorbing surfaces of the roots and mycorrhizal fungal hyphae.

Flux of nitrogen between the bulk soil and the rhizosphere ($gN.day^{-1}$) (URH) is defined by the following equation:

$$URH = k3*(CNS-CNRH)/RES1 \tag{4.7}$$

Where CNS is the concentration of nitrogen in the soil ($gN.m^{-3}$ soil); CNRH is the concentration of nitrogen in the rhizosphere ($gN.m^{-3}$ soil); RES1 is the resistance to flux (day); K3 (m^3)is a scaling function (Thornley 1991):

$$K3=VS*VRH/(VS+VRH) \tag{4.8}$$

Where VS is the volume of soil (m^{-3}) (equn 4.11e); VRH is the rhizosphere volume (m^{-3}) (equn 4.11d).

The first step in developing a description of nutrient uptake from this soil system is to define the geometrical properties of below-ground compartments (roots, mycorrhizal fungal hyphae, rhizosphere and bulk soil). From the root and mycorrhizal biomasses the lengths ($m.m^{-2}$ground) of the roots of the two plant species, and of the fungal hyphae can be described as follows:

$$LR1(t)=R1(t)/(\pi*RR1^2*PR1) \quad (4.9a)$$

$$LR2(t)=R2(t)/(\pi*RR2^2*PR2) \quad (4.9b)$$

$$LM(t)=MYC(t)/(\pi*RM^2*PM) \quad (4.9c)$$

where R1,R2, and MYC are the structural biomasses (gDm.m^{-2}ground) of the roots of the two species and the mycorrhizal fungal hyphae respectively; RR1, RR2 and RM are the mean radii (m) of the roots of species 1, 2 and of the hyphae of the mycorrhizal fungi respectively; PR1, PR2 and PM are the densities (gC.m^{-3}) of the root tissue of species 1 and 2 and of the fungal tissue.

Surface areas (m^2) of the roots of the grass (SA1) and the legume (SA2) and of the mycorrhizal fungal hyphae (SAM) can also be calculated:

$$SA1(t)=2*\pi*RR1*LR1(t) \quad (4.10a)$$

$$SA2(t)=2*\pi*RR2*LR2(t) \quad (4.10b)$$

$$SAM(t)=2*\pi*RM*LM(t) \quad (4.10c)$$

Finally the volumes (m^3) of all the below ground compartments can be calculated:

$$VR1(t)=R1(t)/PR1 \quad (4.11a)$$

$$VR2(t)=R2(t)/PR2 \tag{4.11b}$$

$$VM(t)=MYC(t)/PM \tag{4.11c}$$

$$VRH(t)=(\pi*(RR1+D)^{2}*LR1(t)-VR1(t))+(\pi*(RR2+D)^{2}*LR2(t)-VR2(t))+(\pi*(RM+D)^{2}*LM(t)-VM(t)) \tag{4.11d}$$

$$VS(t)=T-(VRH(t)+VR1(t)+VF(t)+VM(t)) \tag{4.11e}$$

where VRi (i=1,2) is the volume of the roots of species i; VM is the volume of the fungal hyphae; VRH is the rhizosphere volume; VS is the soil volume; D is the mean thickness of the depletion zone (m); and T is the total volume of the below ground system (assumed to be 1m^{3} for this model).

Uptake (gN.m^{-2}ground.day^{-1}) by the roots of the grass and the legume species (U1 and U2 respectively) and by the mycorrhizal fungal hyphae (UM) can then be described by the following equations which are similar to those developed by Brugge (1985). They are based on Michaelis-Menten dynamics and allow for the uptake rates to be limited by either low carbon substrate levels (CCRi and CCM), or high nitrogen substrate levels (CNRi and CNM):

$$U1=\frac{VMAX1*CNRH}{(KM1+CNRH)}*SA1*\frac{CCR1}{KM4+CCR1}*\frac{1}{1+CNR1/JN1} \tag{4.12a}$$

$$U2= \frac{VMAX2*CNRH}{(KM2+CNRH)} * SA2 * \frac{CCR2}{KM5+CCR2} * \frac{1}{1+CNR2/JN2} \qquad (4.12b)$$

$$UM= \frac{VMAX3*CNRH}{(KM3+CNRH)} * SAM * \frac{CCM}{KM6+CCM} * \frac{1}{1+CNM/JNM} \qquad (4.12c)$$

where VMAXi (i=1-3) is maximal uptake at saturating levels of CNRH ($gN.m^{-2}$root surface.day^{-1}); KMi (i=1-3) = CNRH where V=1/2VMAX (V is the rate of uptake); SAi (i=1,2,M) is the absorbing surface area of the roots of either of the two plant species or of the mycorrhizal fungal hyphae (m^2absorbing surface.m^{-2}ground); KMi (i=4-6) is the concentration of carbon substrate in the roots of species i or in the fungal hyphae at which uptake is half the maximum rate; and JNi (i=1,2 or M) is nitrogen uptake inhibition constant for i.

The only source of nitrogen in the system other than the bulk soil is nitrogen fixed by the nodules associated with the legume. Fixation (FIX, $gN.m^{-2}$ground.day^{-1}) is described as the product of nodule biomass (DMN, $gDM.m^{-2}$ground) and the specific nitrogen fixation rate (SNFR, $gN.gDM^{-1}.day^{-1}$) (equn 4.13). This is based on the theory that fixation is not limited by carbon supply other than indirectly through growth limitation, but that it is in fact limited by other physical factors in the nodules (Vance and Heichel 1991) (see section 1.5.2).

$$FIX=DMN*SNFR \qquad (4.13)$$

In terms of the algorithm shown in fig 4.1, the status of all of the pools that are directly affected by acquisition processes of photosynthesis, nitrogen uptake and nitrogen fixation are updated as follows:

Carbon substrate levels -

$$CL1_{(t)}=CL1_{(t-1)}+P1 \tag{4.14a}$$

$$CR1_{(t)}=CR1_{(t-1)}-RU1*U1 \tag{4.14b}$$

$$CL2_{(t)}=CL2_{(t-1)}+P2 \tag{4.14c}$$

$$CR2_{(t)}=CR2_{(t-1)}-RU2*U2 \tag{4.14d}$$

$$CM_{(t)}=CM_{(t-1)}-RUM*UM \tag{4.14e}$$

$$CN_{(t)}=CN_{(t-1)}-(CFIX*FIX) \tag{4.14f}$$

where t represents the present status and t-1 represents the status after the preceding update; CL1 is the amount of carbon substrate in the leaves of the grass (gC.m^{-2}ground); CR1 is the amount of carbon substrate in the roots of the grass (gC.m^{-2}ground); RU1 is the utilization of carbon substrate for uptake in grass roots (gC.gN^{-1}), CL2 is the amount of carbon substrate in the leaves of the legume (gC.m^{-2}ground); CR2 is the amount of carbon substrate in the roots of the legume (gC.m^{-2}ground); RU2 is the utilization of carbon substrate for uptake in legume roots (gC.gN^{-1}); CM is the amount of carbon substrate in the mycorrhizal fungal hyphae (gC.m^{-2}ground); RUM is the utilization of carbon substrate for uptake in mycorrhizal hyphae (gC.gN^{-1}); CN is the amount of carbon substrate in the nodules (gC.m^{-2}ground); CFIX is the utilization of carbon substrate for nitrogen fixation in the nodules (gC.gN^{-1}).

Nitrogen substrate levels -

$$NR1_{(t)}=NR1_{(t-1)}+U1 \tag{4.15a}$$

$$NR2_{(t)}=NR2_{(t-1)}+U2 \tag{4.15b}$$

$$NM_{(t)}=NM_{(t-1)}+UM \tag{4.15c}$$

$$NN_{(t)}=NN_{(t-1)}+FIX \tag{4.15d}$$

$$NRH_{(t)}=NRH_{(t-1)}+URH-UM-U1-U2 \tag{4.15e}$$

$$NS_{(t)}=NS_{(t-1)}+F-URH \tag{4.15f}$$

where NR1 is the amount of nitrogen substrate in the roots of the grass (gN.m^{-2}ground); NR2 is the amount of nitrogen substrate in the roots of the legume; NM is the amount of nitrogen substrate in the VAM fungal hyphae; NN is the amount of nitrogen substrate in the nodules; NRH is the amount of nitrogen in the rhizosphere; NS amount of nitrogen in the soil; and F is the rate of fertilizer application ($gN.m^{-2}ground.day^{-1}$). It should be noted that no distinction was drawn between nitrate and ammonium in the soil or rhizosphere.

Where necessary, concentrations of carbon and nitrogen can then be calculated using equns. 4.2 and 4.3.

4.3 SUBSTRATE FLUXES

Fluxes of all substrates between compartments in the model plants are described in much the same way as fluxes between the bulk soil and the rhizosphere (equn 4.7). In the case of carbon:

$$Fij = \frac{Kij*(CCj-CCi)}{RSCij} \tag{4.16}$$

where Fij is the flux of carbon from compartment j to compartment i ($gC.m^{-2}ground.day^{-1}$); CCj is the concentration of carbon in compartment j; CCi is the concentration of carbon in compartment i; RSCij is the resistance to flux of carbon (day-time taken for 1g of carbon to move between compartment j to compartment i); and Kij is a scaling function (equn 4.17):

$$Kij=Wi*Wj/Wi+Wj \tag{4.17}$$

Where Wi is the structural biomass of compartment i and Wj is the structural biomass of compartment j.

For nitrogen the approach is the same:

$$FNij = \frac{Kij*(CNj-CNi)}{RSNij} \tag{4.18}$$

where FNij is the flux of nitrogen from compartment j to compartment i ($gN.m^{-2}ground.day^{-1}$); CNj is the concentration of nitrogen in j; CNi is the concentration of nitrogen in i; RSNij is the resistance to flux of nitrogen (day); and Kij is as above equn 4.8.

Again the status of all the pools are then updated in accordance with the algorithm (fig.4.1)

Carbon status -

$$CL1_{(t)}=CL1_{(t-1)}-FL1 \tag{4.19a}$$

$$CS1_{(t)}=CS1_{(t-1)}+FL1-FS1 \tag{4.19b}$$

$$CR1_{(t)}=CR1_{(t-1)}+FS1 \tag{4.19c}$$

$CL2_{(t)}=CL2_{(t-1)}-FL2$ (4.19d)

$CS2_{(t)}=CS2_{(t-1)}+FL2-FS2$ (4.19e)

$CR2_{(t)}=CR2_{(t-1)}+FS2-FN-FM$ (4.19f)

Where CS1 . CS2 ; FLi is the flux of carbon from the leaves of species i to the stems of species i; FSi is the flux of carbon from the stems of species i to the roots of species i; FN and FM are the fluxes from the roots of the legume to the nodules and fungal hyphae respectively.

Nitrogen status

$NL1_{(t)}=NL1_{(t-1)}+FNL1$ (4.20a)

$NS1_{(t)}=NS1_{(t-1)}+FNS1-FNL1$ (4.20b)

$NR1_{(t)}=NR1_{(t-1)}-FNS1$ (4.20c)

$NL2_{(t)}=NL2_{(t-1)}+FNL2$ (4.20d)

$NS2_{(t)}=NS2_{(t-1)}+FNS2-FNL2$ (4.20e)

$NR2_{(t)}=NR2_{(t-1)}+FNN+FNM-FNS2$ (4.20f)

$$NN_{(t)}=NN_{(t-1)}-FNN \quad (4.20g)$$

$$NM_{(t)}=NM_{(t-1)}-FNM \quad (4.20h)$$

where NSi is the amount of nitrogen substrate in the stems of species i (i=1,2); NLi is the amount of substrate nitrogen in the leaves of species i; FNLi is the flux of substrate nitrogen from the stems to the leaves of species i; FNSi is the flux of substrate nitrogen from the roots to the stems in species i; FNN and FNM are the fluxes of nitrogen from the nodules and VAM fungal hyphae respectively to the roots of the legume.

As with the previous status update, the substrate concentrations can then be calculated using equations 4.2 and 4.3.

4.4 GROWTH, RESPIRATION AND SENESCENCE

Growth of the various compartments in fig 1.1 (Gi, in g structure.day^{-1}) is set as the minimum (MIN) of two functions: one based on carbon substrate concentration and the other based on nitrogen substrate concentration:

$$Gi=MIN\begin{cases} Ii*CCi*Wi \\ Ii*CNi*(Ji/Qi)*Wi \end{cases} \quad (4.21)$$

Where Ii is a growth parameter (g structure .gC^{-1}) associated with the compartment i (i=L1, S1, R1, L2, S2, R2, NOD, MYC); Ji is the carbon content of structural material in compartment i (gC.g structure^{-1}); Qi is the nitrogen content of structural material in compartment i (gN.g structure^{-1}). Both Qi and Ji are assumed to be constants. In this way, growth is limited by whichever substrate is most limiting. When the two functions (growth as a function of carbon and as a function of nitrogen substrate concentration) are equal, the plants fulfil the criteria for balanced exponential growth, and can be said to be "optimally foraging". It is an important feature of the growth submodel, however, that balanced exponential growth is not assumed.

The utilization of substrates in growth is described by the following equations:

Carbon (gC.day^{-1}) -

$$UCLi=Ji*GLi/Yi \quad (4.22a)$$

$$UCSi=Ji*GSi/Yi \quad (4.22b)$$

$$UCRi=Ji*GRi/Yi \quad (4.22c)$$

$$UCN=Ji*GN/YN \quad (4.22d)$$

$$UCM=Ji*GM/YM \quad (4.22e)$$

where UCLi, UCSi, UCRi, UCN and UCM are the utilisation of carbon substrates in growth by the various compartments; Yi, YN and YM are growth respiratory coefficients.

Nitrogen (gN.day^{-1}) -

$$UNLi=Qi*GLi \tag{4.23a}$$

$$UNSi=Qi*GSi \tag{4.23b}$$

$$UNRi=Qi*GRi \tag{4.23c}$$

$$UNN=Qi*GN \tag{4.23d}$$

$$UNM=Qi*GM \tag{4.23e}$$

where UNLi, UNSi, UNRi, UNN and UNM are the utilisation of nitrogen substrates in growth of the various compartments. Maintenance respiratory costs are also calculated at this stage in the model. These are simply taken to be the product of a maintenance respiratory coefficient (Mj j=1, 2, MYC, and NOD) and the structural mass of the component:

$$Mi=Mj*I \tag{4.24}$$

where i refers to the relevant compartment (L1, S1, R1, L2, S2, R2, MYC, NOD).

Following this series of calculations, the status of the various pools are updated as follows ($i=1,2$):

Carbon substrate -

$CLi_{(t)}=CLi_{(t-1)}-UCLi-MLi$ (4.25a)

$CSi_{(t)}=CSi_{(t-1)}-UCSi-MSi$ (4.25b)

$CRi_{(t)}=CRi_{(t-1)}-UCRi-MRi$ (4.25c)

$CM_{(t)}=CM_{(t-1)}-UCM-MM$ (4.25d)

$CN_{(t)}=CN_{(t-1)}-UCN-MN$ (4.25e)

Nitrogen substrate -

$NLi_{(t)}=NLi_{(t-1)}-UCNi$ (4.26a)

$NSi_{(t)}=NSi_{(t-1)}-UNSi$ (4.26b)

$NRi_{(t)}=NRi_{(t-1)}-UNRi$ (4.26c)

$NM_{(t)}=NM_{(t-1)}-UNM$ (4.26d)

$$NN_{(t)}=NN_{(t-1)}+UNN \tag{4.26e}$$

Structural biomass -

$$Li_{(t)}=Li_{(t-1)}+GLi-(SNLi*Li) \tag{4.27a}$$

$$Si_{(t)}=Si_{(t-1)}+GSi-(SNSi*Si) \tag{4.27b}$$

$$Ri_{(t)}=Ri_{(t-1)}+GRi-(SNRi*Ri) \tag{4.27c}$$

$$NOD_{(t)}=NOD_{(t-1)}+GN-(SNN*NOD) \tag{4.27d}$$

$$MYC_{(t)}=MYC_{(t-1)}+GM-(SNM*MYC) \tag{4.27e}$$

where SNLi, SNSi, SNRi, SNN and SNM are senescence rates. Finally the substrate concentrations are updated in accordance with equations 4.2 and 4.3.

4.5 SIMULATIONS

The above equations were combined into a computer programs written in FORTRAN (see appendix 1 for source file). As was the case in the previous chapter, simulations were run for a period of 85 model time days on a 386 PC. All parameter and starting values used in the simulations are as listed in the source file. The simulation results are

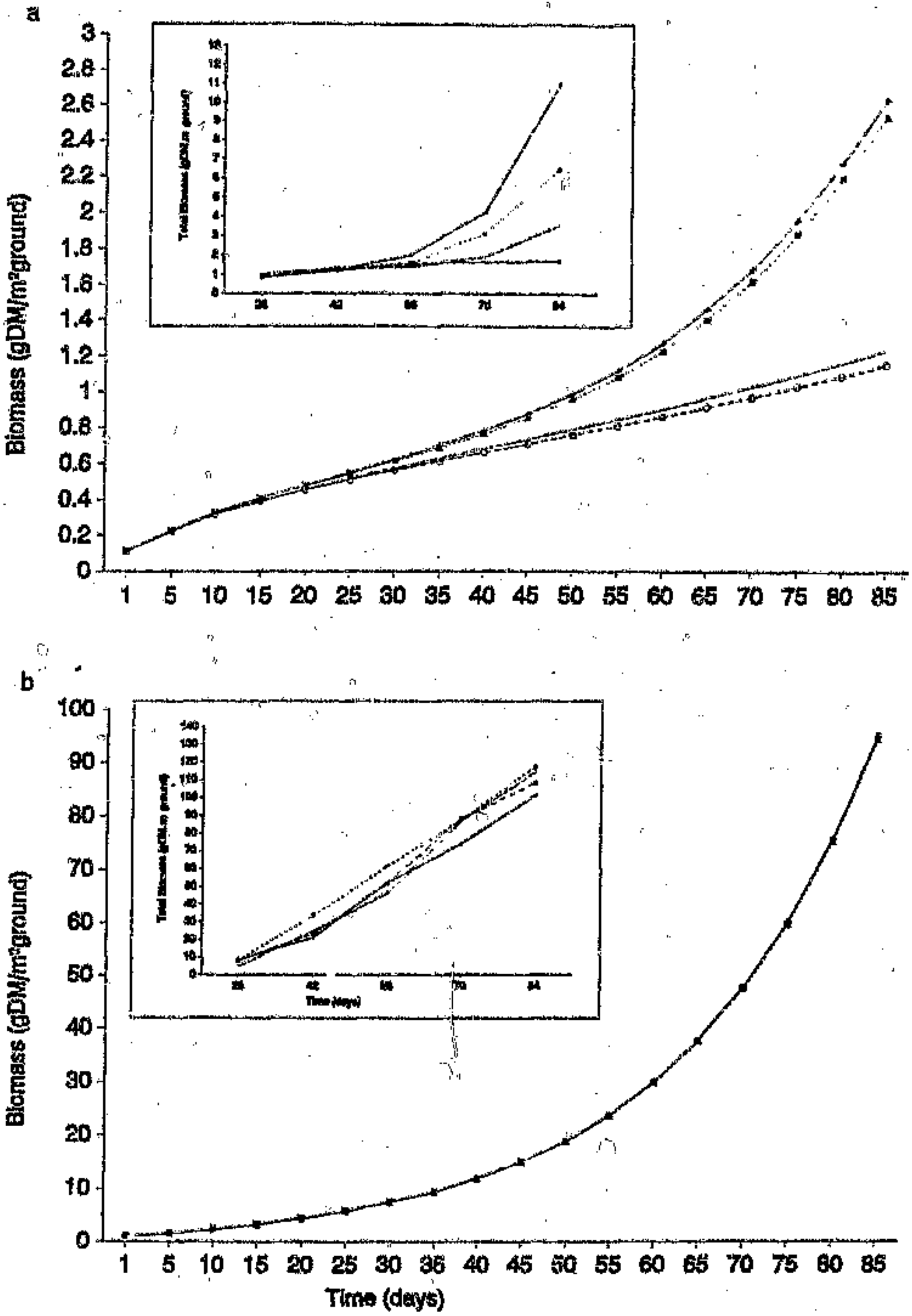

Fig 4.2. Computer simulations of total biomass (gDM/m²ground) in white clove (a) and Italian ryegrass (b). The inset graphs are the appropriate growth trial leaf biomass (gDM/m²ground) curves from chapter 2.

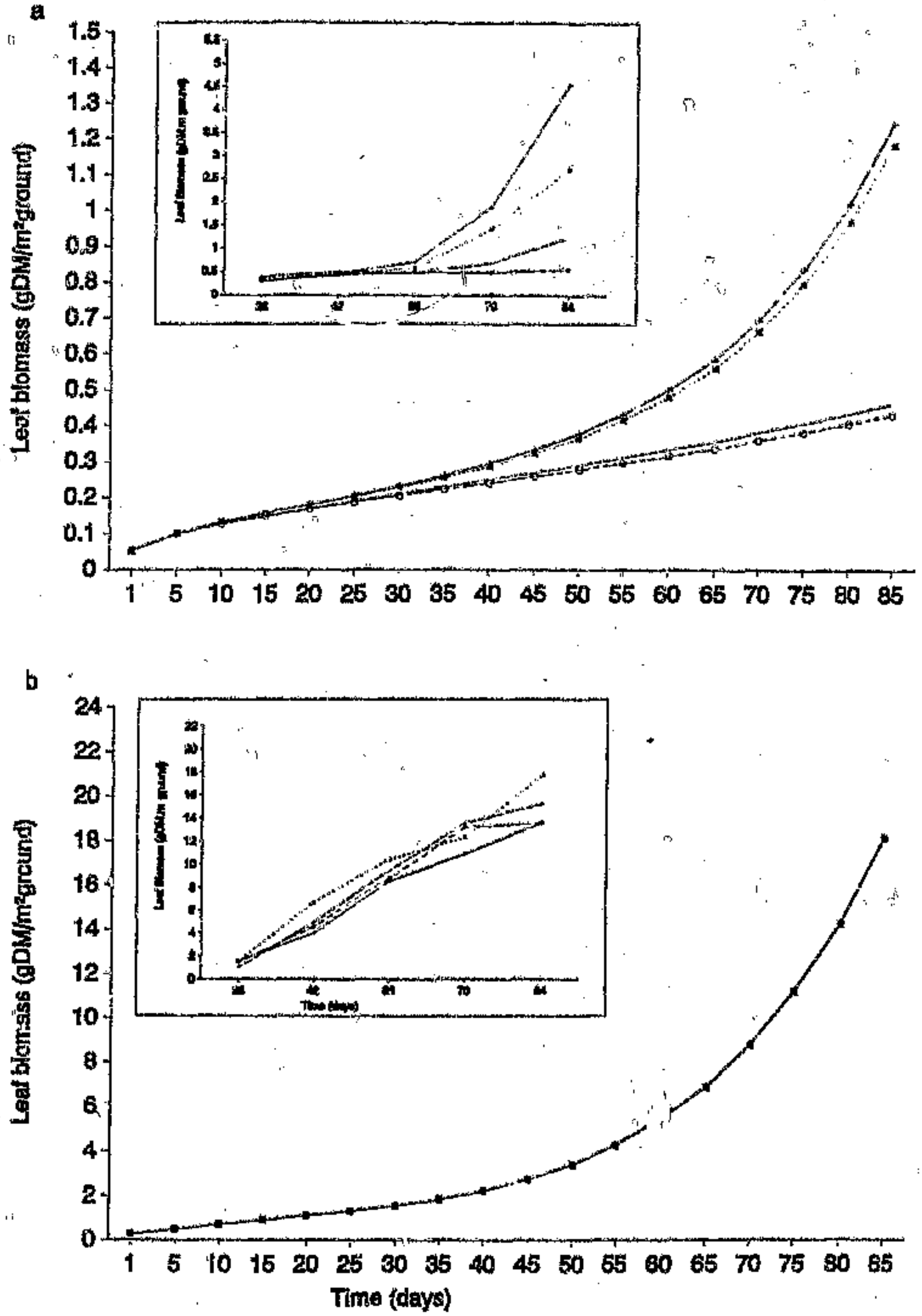

Fig 4.3. Computer simulations of leaf biomass (gDM/m²ground) in white clove (a) and Italian ryegrass (b). The inset graphs are the appropriate growth trial leaf biomass (gDM/m²ground) curves from chapter 2.

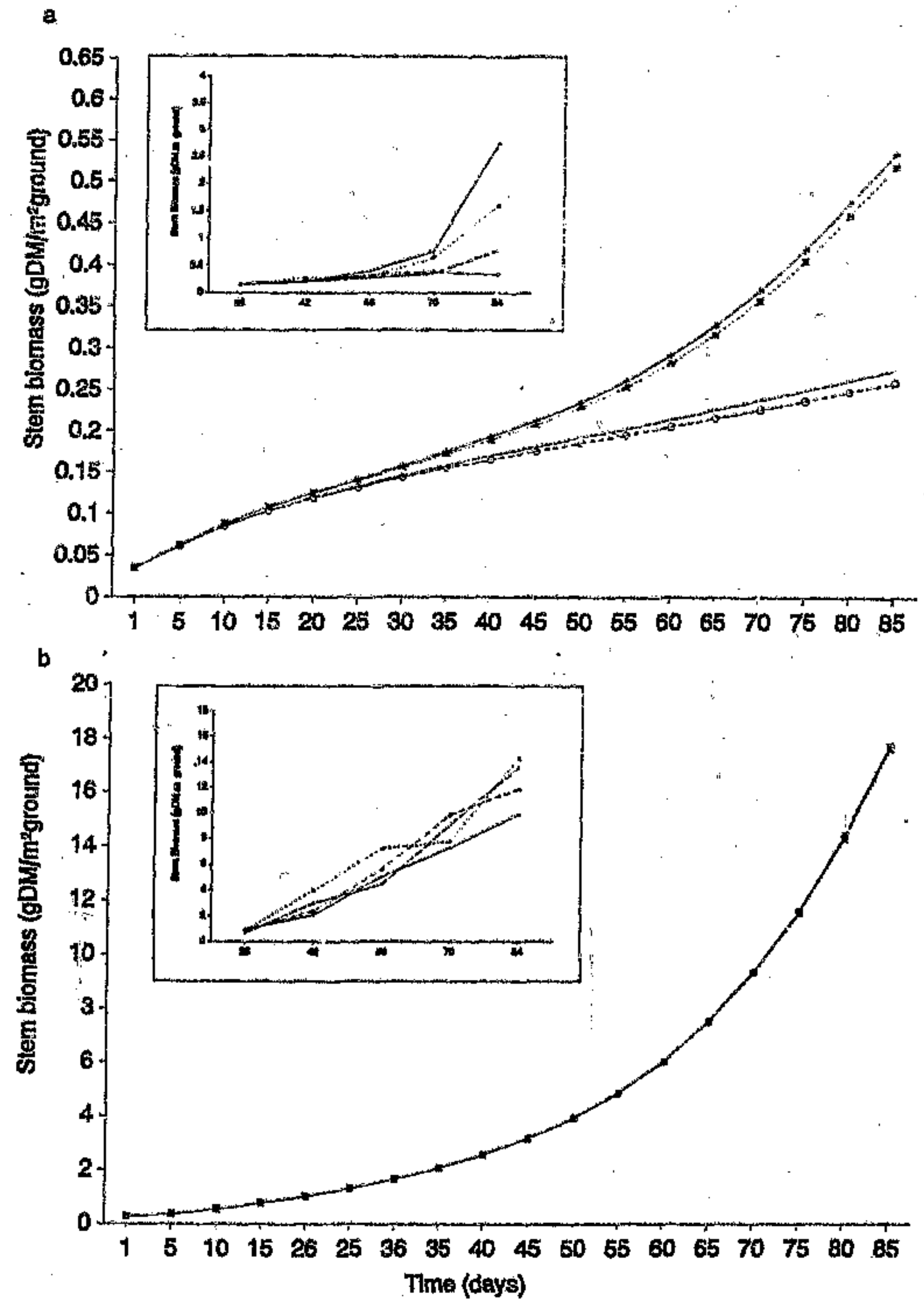

Fig 4.4. Computer simulations of stem biomass (gDM/m²ground) in white clove (a) and Italian ryegrass (b). The inset graphs are the appropriate growth trial stem biomass (gDM/m²ground) curves from chapter 2.

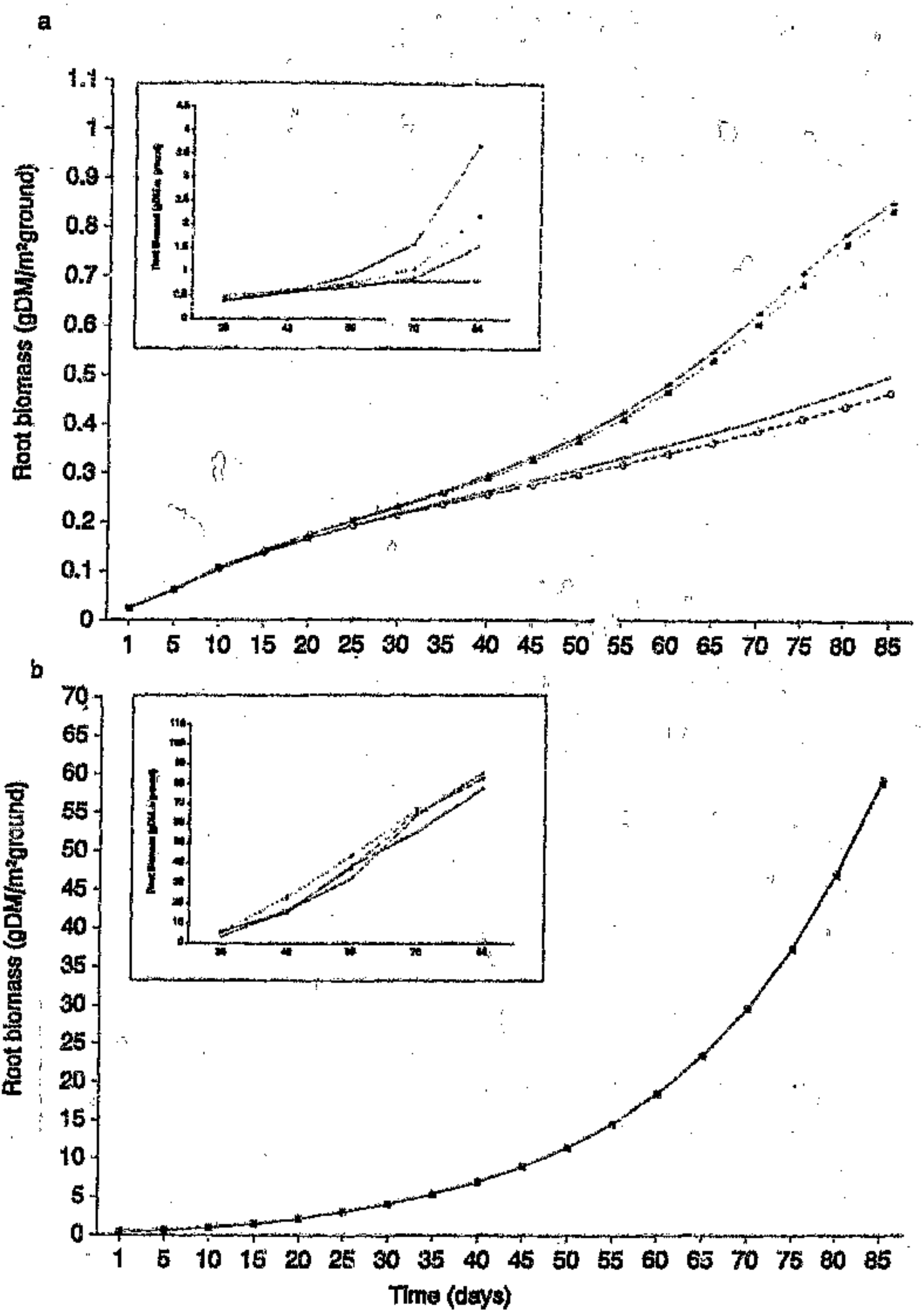

Fig 4.5. Computer simulations of root biomass (gDM/m²ground) in white clove (a) and Italian ryegrass (b). The inset graphs are the appropriate growth trial root biomass (gDM/m²ground) curves from chapter 2.

presented in the same form as the results from the growth trial (chapter 2): First the biomasses are presented (fig 4.2 to 4.5); then the shoot to root ratios are presented (fig 4.6). These are followed by the nitrogen contents (fig 4.7 to 4.9) and finally the relative growth rates are presented (fig 4.10). Inset into each of the simulation graphs is the appropriate graph from experimental data (chapter 2). This allows for direct comparison of the simulations and the experimental results.

4.6 DISCUSSION

It is apparent from comparison of the simulation results with experimental results that the model has mixed success in simulating the real growth system. In the case of the clover without any symbionts, the model describes all aspects of growth, nitrogen acquisition and allocation well (figs 4.2 to 4.10 a). In the simulations where nodules were included, the trends in biomass accumulation (figs 4.2 to 4.5 a) were similar to those observed experimentally, however, the impact in the model plants was not as great as was observed experimentally. This is despite very similar trends in the nitrogen levels (figs 4.7 to 4.9 a). Trends in both shoot:root ratio (fig 4.6 a) and mean relative growth rate (fig. 4.10 a) were both close to experimentally observed trends, while the magnitudes of these two variables were reasonably similar.

It is also apparent that the impact of mycorrhizal infection cannot be predicted with reference to nitrogen alone. The differences between the mycorrhizal and the non-mycorrhizal model clover plants in all of the output variables were slight in comparison

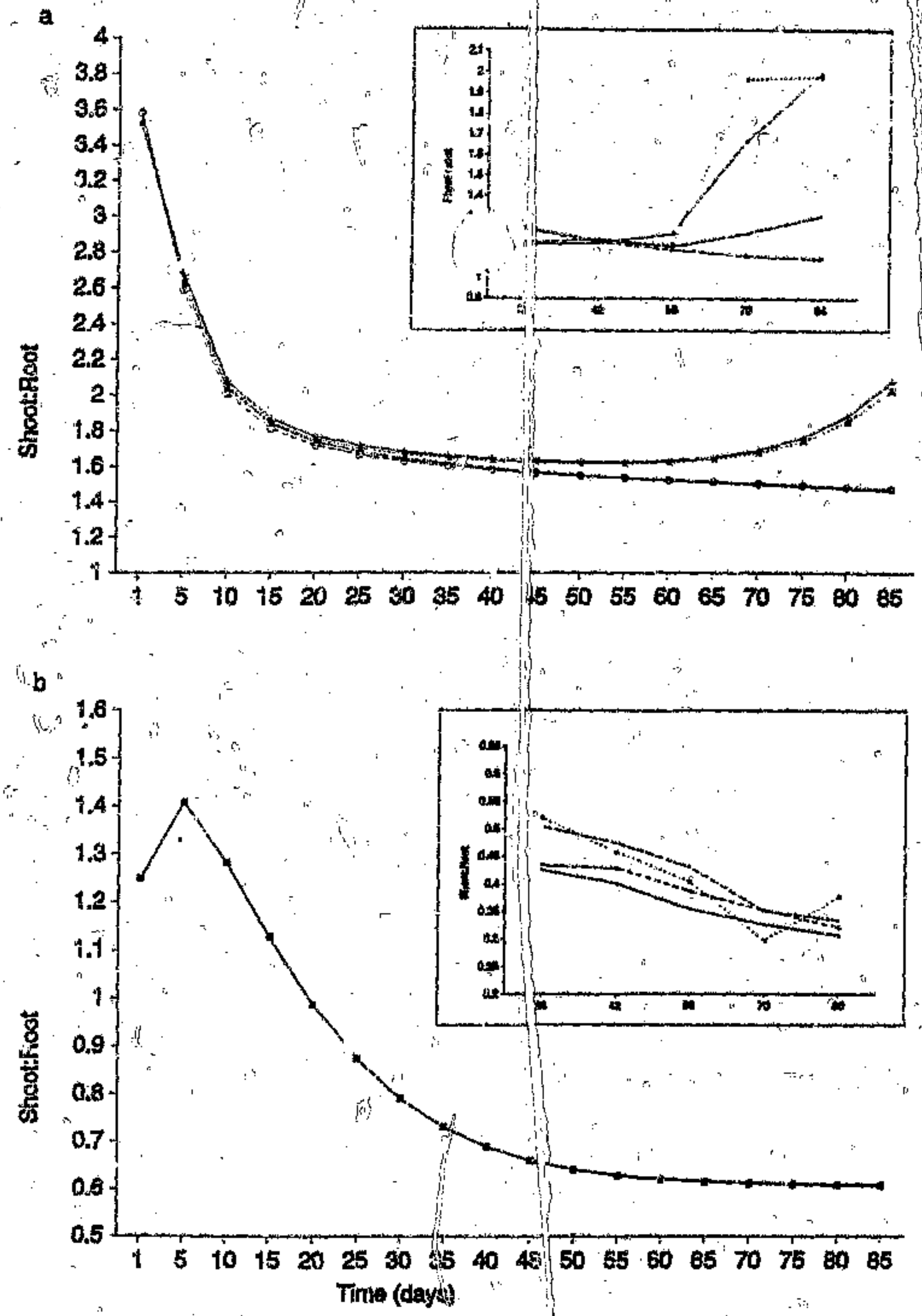

Fig 4.6. Computer simulations of the shoot:root ratio in white clove (a) and Italian ryegrass (b). The inset graphs are the appropriate growth trial shoot:root ratio curves from chapter 2.

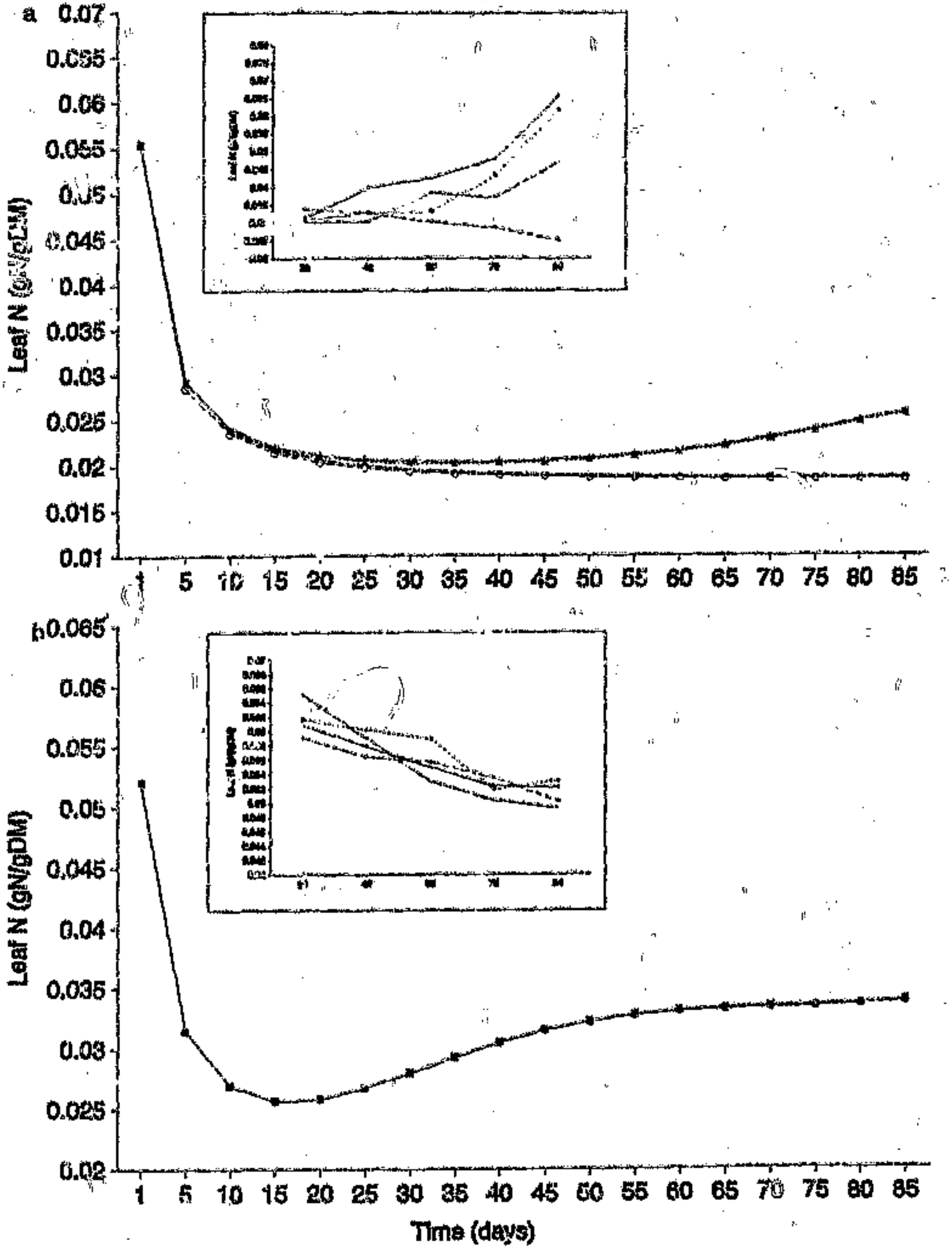

Fig 4.7. Computer simulations of leaf nitrogen content (gN/gDM) for white clover (a) and for Italian ryegrass (b). The inset graphs are the appropriate growth trial data for leaf nitrogen content from chapter 2.

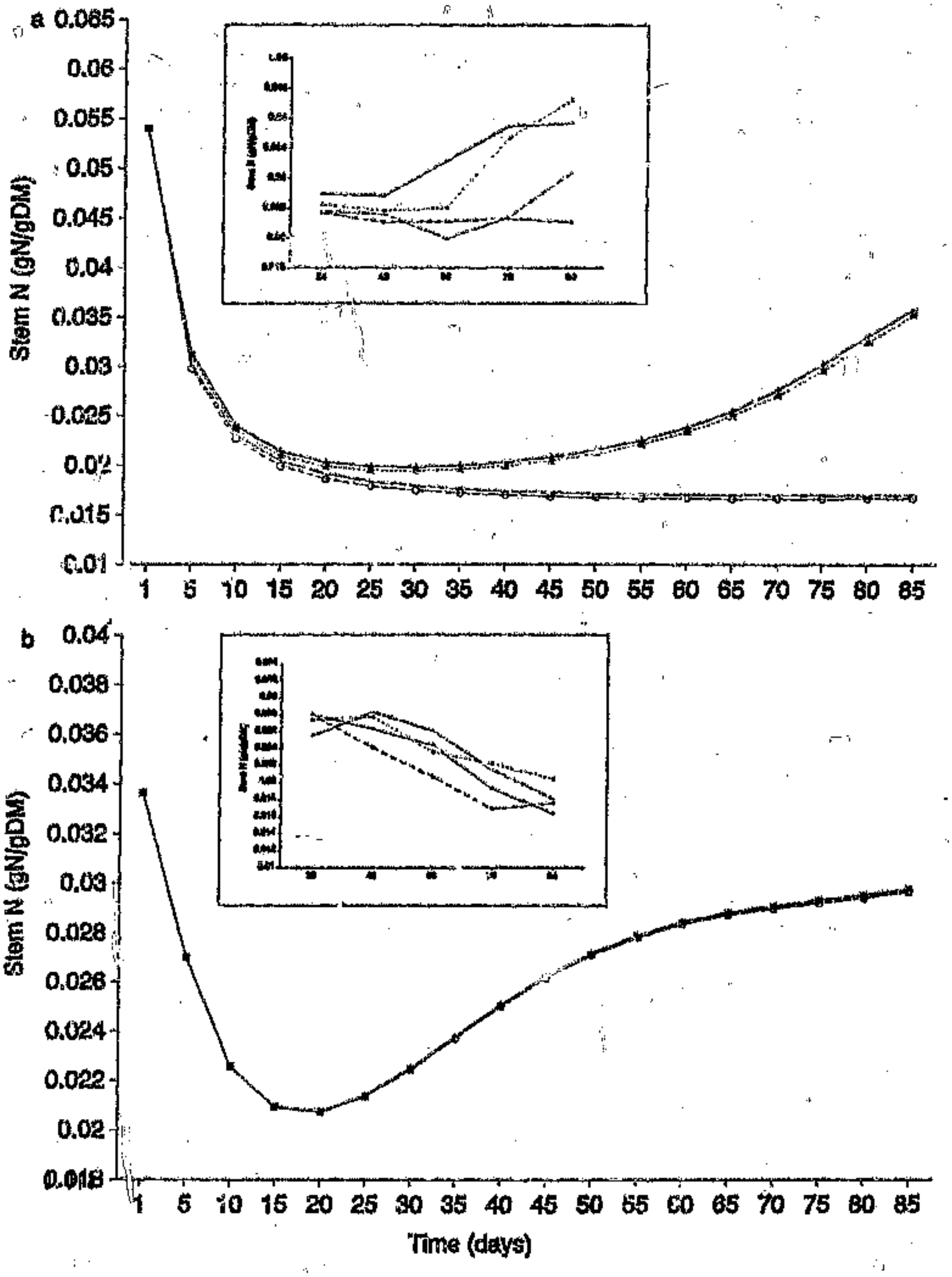

Fig 4.8. Computer simulations of stem nitrogen content (gN/gDM) for white clover (a) and for Italian ryegrass (b). The inset graphs are the appropriate growth trial data for stem nitrogen content from chapter 2.

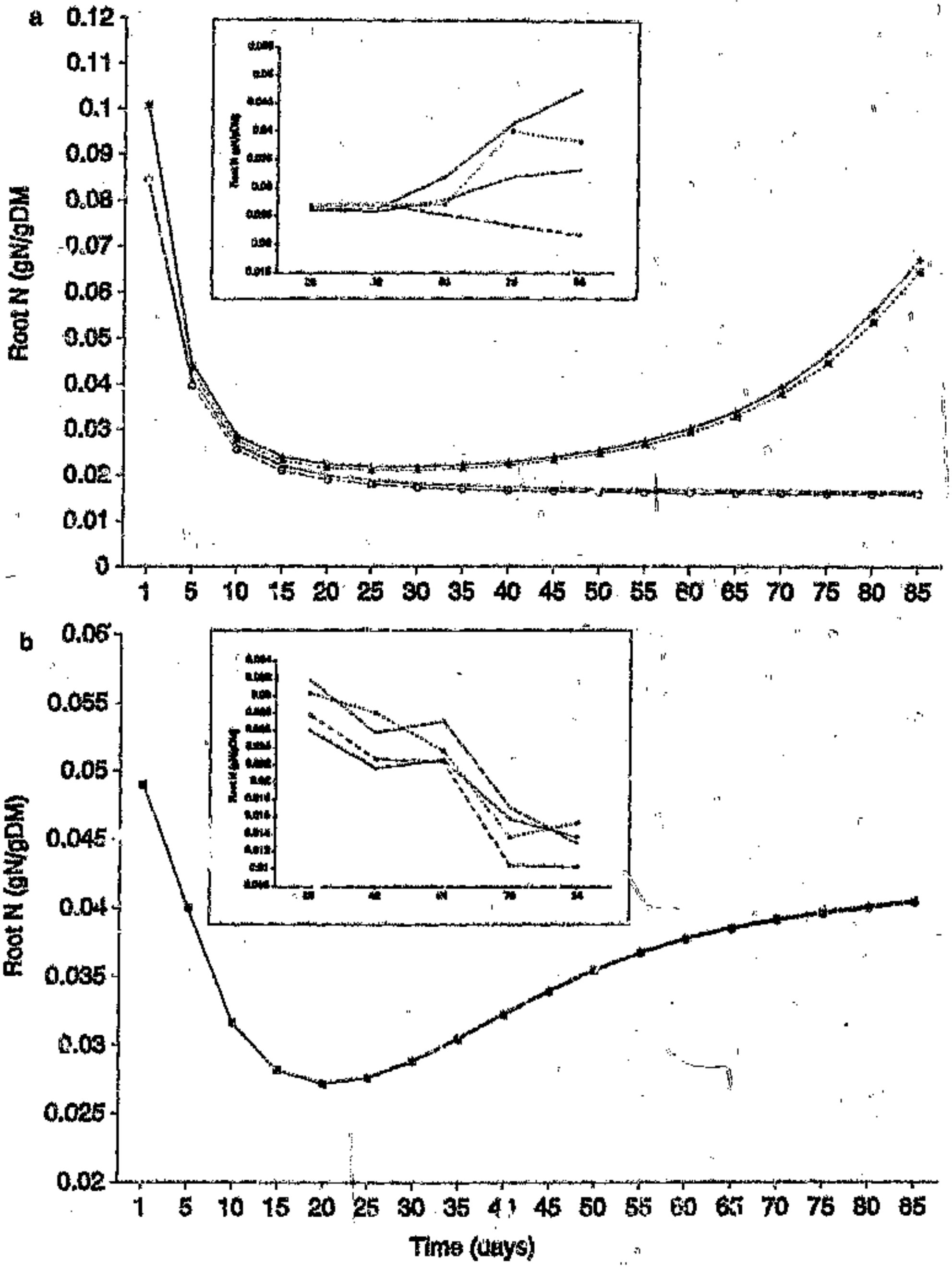

Fig 4.9. Computer simulations of root nitrogen content (gN/gDM) for white clover (a) and for Italian ryegrass (b). The inset graphs are the appropriate growth trial data for root nitrogen content from chapter 2.

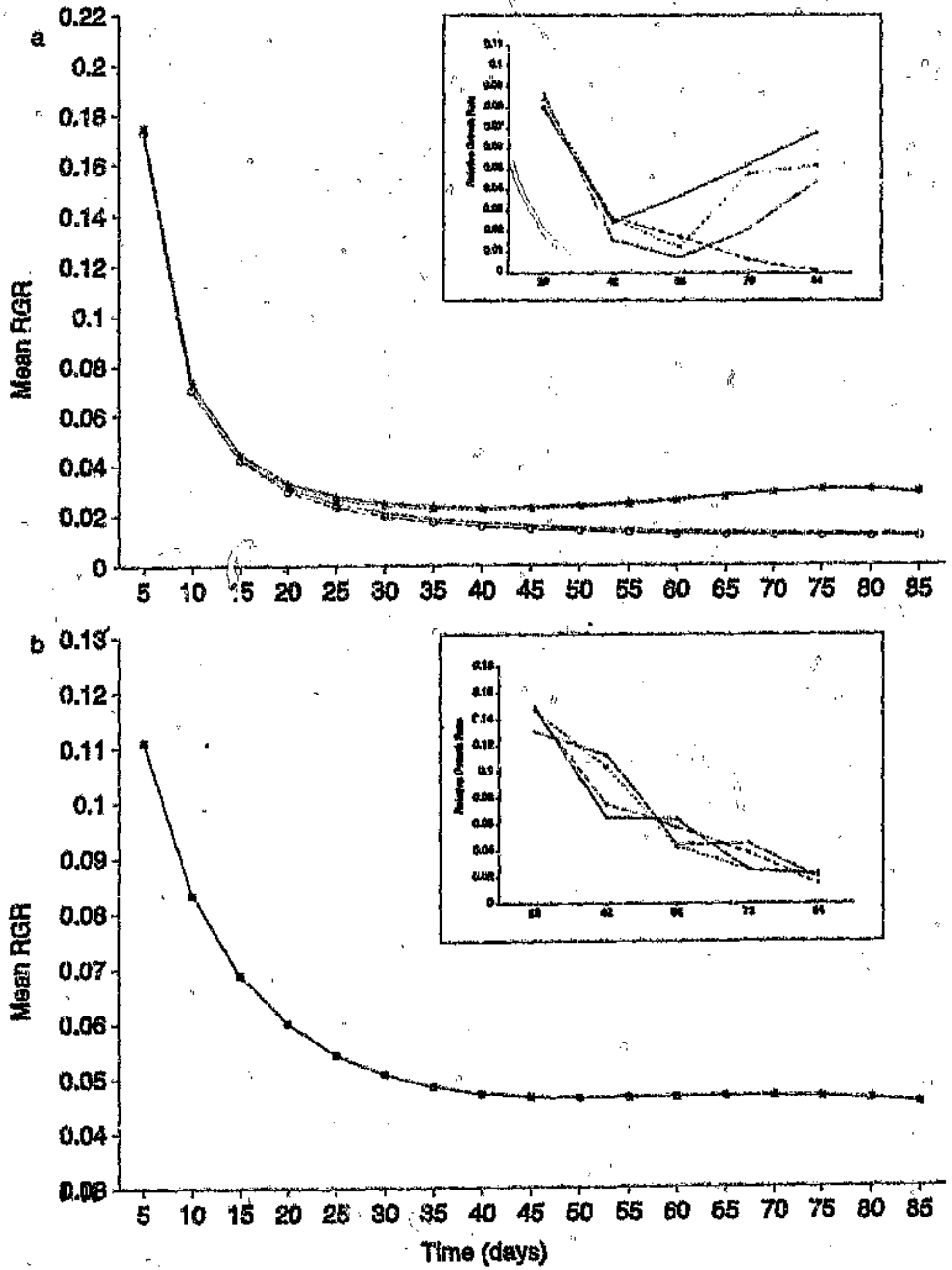

Fig 4.10. Computer simulations of relative growth rate for white clover (a) and for Italian ryegrass (b). The inset graphs are the appropriate growth trial data for relative growth rate from chapter 2.

with those observed in the experimental work (figs 4.2 to 4.10 a). This is despite the fact that the simulated biomass of mycorrhizal fungal hyphae was within the same range as reported in Rousseau, Sylvia and Fox (1993) (about 3% of root biomass). It is thus likely that the improved growth and nitrogen status of the clover plants in the mycorrhizal experimental treatments were due to an indirect effect. One likely possibility is that there was improved phosphorus uptake in the mycorrhizal treatments and that improved growth and nitrogen uptake were the indirect result of this. The geometrical properties of VAM fungal hyphae (eg. mean radius, surface area, length) are such that the hyphae are particularly suited to uptake of the less mobile nutrients, of which phosphorus is one (Clarkson 1985). It is apparent in the case of the more mobile nutrients (such as NO_3^- and NH_4^+) that geometrical considerations may be relatively less important in terms of uptake than the kinetic properties of the uptake sites (Gutschick 1993), and it is reasonable to assume that the uptake sites in mycorrhizal fungi are similar to those in plant roots. In the case of Italian ryegrass, although the final simulation values for biomasses were generally similar to the true values, the trajectories followed to achieve these final results were noticeably different (fig 4.2 to 4.5 b). While the growth curves in the simulations were of an exponential form (even when considered from day 28 to 85 rather than from day 1), the experimental growth curves were almost linear. It is in the simulations of nitrogen status of the ryegrass components, that the greatest shortcoming of the model are apparent. In the case of the leaves (fig 4.7 b), there are differences in both trend and magnitude. In the stems and roots, although the final magnitudes are similar, the trend is noticeably different (fig 4.9 and 4.10 b). Generally, the real plants exhibit a decrease in the nitrogen content, while the model plants initially show a sharp decline, followed by an increase. The differences in trends in the nitrogen contents do provide an explanation for why there were differences in the trends of biomass accumulation. The initial sharp decline in nitrogen content corresponds with the initial slow rate of biomass accumulation and the increase in rate of biomass accumulation corresponds to the increases in nitrogen content. In terms of the model, the most likely explanation for the inability to simulate the nitrogen status of the rye plants is the scaling functions (equn 4.17) which will restrict the flow of either substrate (carbon or nitrogen) between structurally small compartments and structurally large pools (such as the roots and stems).

CHAPTER 5: CONCLUSIONS

Despite any shortcomings of the model described in the previous chapter, the model does represent a significant improvement on previous attempts to model mixed canopies, particularly grass legume mixtures. And in at least four out of the five objectives of modelling laid down in Penning de Vreis (1983) (chapter 1.4) the exercise was a great success:

From the simulations carried out and presented in chapters 3 and 4, it is obvious that the model allows integrated information to be made operational. This was achieved through the incorporation of the models into the computer programs listed in appendix 1. As an attempt to help define and categorise the system, the model was also successful. As mentioned above, the formalisation of the system represents a significant improvement on previous attempts. In addition to improvements in the descriptions of plant processes (in particular photosynthesis in chapter 3), an attempt was also made to expand the description to include the impacts of two symbiotic associations, namely nodule-forming bacteria, and VAM-forming fungi. To my knowledge this has not been attempted before. Also, because of the submodel approach, the model very effectively categorizes the system into above and below ground processes, each of which are subdivided into a number of parts (fig.5.1). Despite this categorisation, the model still retains a high level of "integratability" as a result of feedback links, and a common upper hierarchical level.

Although a number of improvements have been made relative to previous models, the

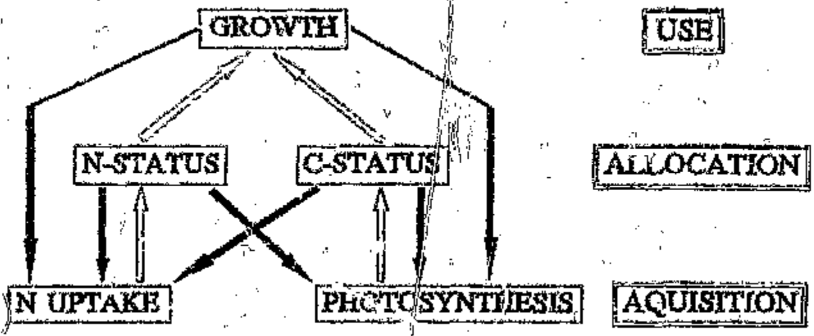

Fig. 6.1. Hierarchical conceptualisation of the system showing how below and above ground activities were combined into the interated model. The structure was based on three functional categories: acquisition, allocation and use.

model is not without its gaps, and these are manifest in the shortcomings of the model described in the previous chapter. For instance, a great deal of work still needs to be done in terms of describing the allocation of nitrogen. In addition, many of the parameters used were at best, borrowed from other systems, and at worst educated guesses, and this lack of knowledge needs to be remedied through further experimental work. The possibility that the observed impact of VA mycorrhizal formation on the clover growth and nitrogen status may be the result of indirect effects associated with improved phosphorous nutrition is an interesting hypothesis which needs to be tested, and if necessary, incorporated into the formalization. It can thus be seen that even the shortcomings of the model do have a positive contribution to make in terms of guiding future research.

In terms of the final objective described in section 1.2 (ie that the model provides a means for disseminating information) the models were at least partially successful in that the outputs (figs 3.2 to 3.4 and figs 4.2 to 4.10) represent a summary of the simulation ability of the models. However, because the models were "phrased" in terms of mathematics, the models readability is somewhat limited.

Finally, besides the general success of the modelling exercise, the results presented in chapter 2 provide further empirical data which may be added to existing data bases. A particularly interesting finding was the fact that the Italian ryegrass did not enter into any symbiotic association with VAM fungi. It was also interesting that the performance of the white clover in the mixture was significantly improved by the formation of symbiotic associations and that this improvement was not at the expense of decreased rye grass performance within the growth period defined by this experimental work. It is difficult, if not impossible to extrapolate this finding quantitatively to a larger scale. However, qualitatively, this finding must have management significance.

REFERENCES

Aapels, M.A. and Haaker, H. 1991. Glutamate oxaloacetate transaminase in pea root nodules. *Plant Physiology.* 95:740-747.

Ågren, G.I. and Ingestad, T. 1987. Root:shoot ratio as a balance between nitrogen productivity and photosynthesis. *Plant, Cell and Environment.* 10:579-586.

Anderson, C.P. and Rygiewicz, P.T. 1991. Stress interactions and mycorrhizal plant response: Understanding carbon allocation priorities. *Environmental Pollution.* 73:217-244.

Badoux, S. 1993. Utilization of tall fescue in a pure stand in association with white clover and in complex mixtures. *XVII International Grassland Congress. New Zealand and Queensland, Australia.*

Begon, M., Harper, J.L. and Townsend, C.R. 1986. *Ecology. Individuals, populations and communities.* Blackwell Scientific Publications; Oxford.

Bèlanger, G., Gastel, F. and Warembourg, F.R. 1992. The effects of nitrogen fertilization and the growing season on carbon partitioning in a sward of tall fescue (*Festuca arundinacea* Screb). *Annals of Botany.* 70:239-244.

Benjamin, L.R. and When, M.J. 1980. Root development and source-sink relations in carrot. *Daucus carota* L. *Journal of Experimental Botany*. 31:1139-1146.

Bethlenfalvay, G.J., Reyes-Solis, M.G., Camel, S.B. and Ferrera-Corrato. R. 1990. Nutrient transfer between the root zones of soybean and maize plants connected by a common mycorrhizal mycelium. *Physiologia Plantarum*. 82:423-432.

Boller, B.C. and Heichel, G.H. 1983. Photosynthate partitioning in relation to N2 fixation capability of alfalfa. *Crop Science*. 23:655-659.

Brugge, R. 1985. A mechanistic model of grass root growth and development dependent upon photosynthesis and nitrogen uptake. *Journal of Theoretical Biology*. 116:443-467.

Buwalda, J.G., Ross, G.J.S., Stribley, D.P. and Tinker, P.B. 1982. The development of endomycorrhizal root systems. III. The mathematical representation of the spread of vesicular-arbuscular mycorrhizal infection in root systems. *New Phytologist*. 91:669-682.

Carr, P.M., Schartz, B.G., Gardner, J.C. and Zwinger, S.F. 1992. Intercropping sorghum and pinto bean in a cool semiarid region. *Agronomy Journal*. 84:810-812.

Charles-Edwards, D.A., Doley, D. and Rimmington G.M. 1986. *Modelling plant growth and development*. Academic Press. Australia.

Chen, J-L, Reynolds, J.F., Harley, P.C and Tenhunen, J.D. 1993 Coordination theory of leaf nitrogen distribution in a canopy. *Oecologia*. 93:63-69.

Chiariello, N. and Roughgarden, J. 1984. Storage allocation in seasonal races of an annual plant: Optimal versus actual allocation. *Ecology*. 65(4):1290-1301.

Clarkson, D.T. 1985. Factors affecting mineral nutrient acquisition by plants. *Annual Review of Plant Physiology*. 36:77-115.

Cliquet, J-B, and Stewart, G.R. 1993. Ammonia assimilation in *Zea mays* L. infected with a vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatum*. *Plant Physiology*. 101:865-871.

Cohen, D. 1971. Maximizing final yield when growth is limited by time or by limiting resources. *Journal of Theoretical Biology*. 33:299-307.

Copeland, L., Vella, J. and Hong, Z. 1989. Enzymes of carbohydrate metabolism in soybean nodules. *Phytochemistry*. 28(1):57-61.

Cordero, A. and McCollum, R.E. 1979. Yield potential of interplanted annual food crops in Southeastern US. *Agronomy Journal*. 71:834-842.

Cralle, H.T. and Heichel, G.H. 1985. Interorgan photosynthate partitioning in alfalfa. *Plant Physiology*. 79:381-385.

Cralle, H.T., Heichel, G.H. and Barnes, D.K. 1987. Photosynthate partitioning in plants of alfalfa population selected for high and low nodule mass. *Crop Science.* 27:96-100.

Crookston, R.K. and Hill, D.S. 1979. Grain yields and land equivalent ratios from intercropping corn and soybeans in Minesota. *Agronomy Journal.* 71:41-43.

Davidson, R.L. 1969. Effect of root/leaf temperature differentials on root/shoot ratios in some pasture grasses and clover. *Annals of Botany.* 33:561-569.

Dabas, S., Swaraj, K. and Sheoran, I.S. 1988. Effect of source removal on functioning of the nitrogen fixation of pigeon pea (Cajanus cajan L.) nodules. *Journal of Plant Physiology.* 132:690-694.

Dorich, R.A. and Nelson, D.W. 1983. Direct colorimetric measurement of ammonium in potassium chloride extracts in soil. *Soil Science Society of America Journal.* 55:171-178.

Downie, J.A. and Johnston, A.W.B. 1988. Nodulation of legumes by rhizobium. *Plant, Cell and Environment.* 11:403-412.

Eason, W.R., Newman, E.I. and Chuba, P.N. 1991. Specificity of interplant cycling of phosphorus: The role of mycorrhizas. *Plant and Soil.* 137:267-274.

Eccles, N.S., Gray, V.M. and Straker, C.J. 1994. A mathematical description of the daily integral of photosynthesis in a binary canopy. *20th Annual Congress of the South African Association of Botanists. University of the Witwatersrand. Johannesburg. South Africa.*

Etter, D.M. 1993. *Structured FORTRAN 77 for engineers and scientists. Fourth edition,* The Benjamin/Cummings Publishing Company, Redwood City.

Farquhar, G.D., von Caemmerer, S. and Berry, J.A. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta.* 149:78-90.

Finn, G.A. and Brun, W.A. 1982. Effect of atmospheric CO2 enrichment on growth, nonstructural carbohydrate content and root nodule activity in soybean. *Plant Physiology.* 69:327-331.

France, R.C. and Read, C.P.P. 1983. Interactions of nitrogen and carbon in the physiology of ectomycorrhizae. *Canadian Journal of Botany.* 61:965-984.

Francis, R. and Read, D.J. 1994. The contribution of mycorrhizal fungi to the determination of plant community structure. *Plant and Soil.* 159:11-25.

Francis, R., Finlay, R.D. and Read, D.J. 1986. Vesicular-arbuscular mycorrhiza in natural vegetation systems. IV. Transfer of nutrients in inter- and intra-specific combinations of host plants. *New Phytologist.* 102:103-111.

Garcia-Garrido, J.M.. Garcia-Romera, I. and Ocampo. J.A. 1992. Cellulase production by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe. *New Phytologist*. 121:221-226.

George, E., Haussler, K.-U., Vetterlein, D., Gorgus, E. and Marschner, H. 1992. Water and nutrient translocation by hyphae of *Glomus mosseae*. *Canadian Journal of Botany*. 70:2130-2137.

Gianinazzi-Pearson, V., Smith, S.E., Gianinazzi, S. and Smith, F.A. 1991. Enzymatic studies on the metabolism of vesicular arbuscular mycorrhizas. V. Is H^+-ATPase a component of ATP-hydrolsing enzyme activeties in plant-fungus interfaces. *New Phytologist*. 117:61-74.

Gleeson, S.K. 1993. Optimization of tissue nitrogen and root-shoot allocation. *Annals of Botany*. 71:23-31.

Gleeson, S.K. and Tilman, D. 1992. Plant allocation and the multiple limitation hypothesis. *The American Naturalist*. 139(6):1322-1343.

Gordon, A.J., Ryle, G.J.A., Mitchell, P.F. and Powell, C.E. 1985. The flux of 14C-labelled photosynthate through soyabean root nodules during N2 fixation. *Journal of Experimental Botany*. 36(166):756-769.

Gray, V.M. 1994. Alfalfa: Source sink dynamics. In Press.

Greenwood, D.J., Gastai, J., Lemaire, G., Draycott, A., Millard, P. and Neeteson, J.J. 1991. Growth rate and % nitrogen of field grown crops: Theory and experiments. *Annals of Botany.* 67:181-190.

Guern, J. 1987. Regulation from within: The hormone dilemma. *Annals of Botany.* 60(*supplement*4):75-102.

Gutschick, V.P. 1993. Nutrient-limited growth rates: Roles of nutrient use efficiency and of adaptations to increase uptake rate. *Journal of Experimental Botany.* 44(258):41-51.

Hamel, C., Barrantes-Cartin, U., Furlan, V. and Smith, D.L. 1991 a. Endomycorrhizal fungi in nitrogen transfer from soybean to maize. *Plant and Soil.* 138:33-40.

Hamel, C., Nesser, C., Barrantes-Cartin, U. and Smith, D.L. 1991 b. Endomycorrhizal fungal species mediate 15N transfer from soybesn to maize in non fumigated soil. *Plant and Soil.* 138:41-47.

Hamel, C. and Smith, D.L. 1991 c. Interspecific N-transfer and plant development in a mycorrhizal field grown mixture. *Soil Biology and Biochemistry.* 27(7):661-665.

Hara, T. 1984. A stochastic model and the moment dynamics of growth and size distribution in plant populations. *Journal of Theoretical Biology.* 109:173-190.

Harkot, W. and Warda, M. 1993. Influence of leachates from seeds of three grass species on the germination and initial growth of white clover. *XVII International Grassland Congress. New Zealand and Queensland, Australia.*

Harley, J.L. 1989. The significance of mycorrhizas. *Mycological Research*. 92(2):129-139.

Haystead, A., Malajczuk, N. and Grove, T.S. 1988. Underground transfer of nitrogen between pasture plants infected with vesicular arbuscular mycorrhizal fungi. *New Phytologist.* 108:417-423.

Herbert, S.T., Putnam, D.H., Poos-Floyd, M.I., Vargas, A. and Grighton, J.F. 1982. Forage yields of intercropped corn and soybean in various planting patterns. *Agronomy Journal.* 76:507-510.

Hikam, S., Poneleit, C.G., Mackown, C.T. and Hildebrand, D.F. 1992. Intercropping maize and winged bean. *Crop Science*. 32:195-198.

Hilbert, D.W. 1990. Optimization of plant root:shoot ratios and internal nitrogen concentrations. *Annals of Botany*. 66:91-99.

Hilbert, D.W. and Reynolds, J.F. 1991. A model allocating growth among leaf proteins, shoot structure, and root biomass to produce balanced activity. *Annals of Botany*. 68:417-425.

Hirose, T. 1988. Modelling the relative growth rate as a function of plant nitrogen concentration. *Physiologia Plantarum.* 72:185-189.

Hirose, T. and Werger, M.J.A. 1987. Nitrogen use efficiency in instantaneous and daily photosynthesis of leaves in the canopy of a *Solodago altissima* stand. *Physiologia Plantarum.* 70:215-220.

Hirrel, M.C. and Gerdemann, J.N. 1979. Enhanced carbon transfer between onions infected with a vesicular-arbuscular mycorrhizal fungus. *New Phytologist.* 83:731-738.

Ho, I. and Trappe, J.M. 1975. Nitrate reducing capacity of two vesicular arbuscular mycorrhizal fungi. *Mycologia.* 67:886-888.

Hunt, R. 1978. *Plant Growth Analysis.* Edward Arnold; London.

Hunt, R. 1981. The fitted curve in plant growth studies. In: *Mathematics and plant physiology.* Eds: Rose, D.A. and Charles-Edwards, D.A. Academic Press; London.

Ingestad, T. and Ågren, G.I. 1988. Nutrient uptake and allocation at steady state nutrition. *Physiologia Plantarum.* 72:450-459.

Iwasa, Y. and Roughgarden, J. 1984. Shoot/root balance of plants: Optimal growth of a system with many vegetative organs. *Theoretical Population Biology.* 25:78-105.

Jeffers, J.N.R. 1988. *Practitioner's handbook on the modelling of dynamic change in ecosystems. Scope 34.* John Wiley & sons; Chichester.

Johansen, A., Jakobsen, I. and Jensen, E.S. 1992. Hyphal transport of ^{15}N-labelled nitrogen by a vesicular-arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. *New Phytologist*. 122:281-288.

Johnson, I.R., Melkonian, J.J., Thornley, J.H.M. and Riha, S.J. 1991. A model of water flow through plants incorporating shoot/root 'message' control of stomatal conductance. *Plant, Cell and Environment.* 14:531-544.

Johnson, I.R. and Thornley, J.H.M. 1983. Vegetative crop growth model incorporating leaf area and senescence, and applied to grass. *Plant Cell and Environment.* 6:721-729.

Johnson, I.R. and Thornley, J.H.M. 1984. A model of instantaneous and daily canopy photosynthesis. *Journal of Theoretical Biology.* 107:531-545.

Johnson, I.R. and Thornley, J.H.M. 1987. A model of shoot:root partitioning with optimal growth. *Annals of Botany* 60:133-142.

Jongbloed, R.H., Clement, J.M.A.M. and Borst-Pauwels, G.W.F.H. 1991. Kinetics of NH4+ and K+ uptake by ectomycorrhizal fungi. Effect of NH4+ on K+ uptake. *Physiologia Plantarum.* 83:427-432.

King, D. and Roughgarden, J. 1982. Multiple switches between vegetative and reproductive growth in annual plants. *Theoretical Population Biology.* 21:194-204.

Koide, R.T. and Elliott, G. 1989. Cost, benefit and efficiency of vesicular arbuscular mycorrhizal symbiosis. *Functional Ecology.* 3(2):4-7.

Koide, R.T. 1991. Nutrient supply, nutrient demand, and plant response to mycorrhizal infection. *New Phytologist.* 117:365 386.

Kouchi, H., Nakaji, K., Yoneyama, T. and Isizuka, J. 1985. Dynamics of carbon photosynthetically assimilated in nodulated soya bean plants under steady-state conditions. 3. Time-course study on 13C incorporation into soluble metabolites and respiratory evolution of 13CO2 from roots and nodules. *Annals of Botany.* 56:333-346.

Koske, R.E. and Gemma, J.N. 1989. A modefied procedure for staining roots to detect VA mycorrhiza. *Mycological Research.* 92(4):486-505.

Lemaire, G., Khaity, M., Onillon, B., Allirand, J.M., Chartier, M. and Gosse, G. 1992. Dynamics of accumulation and partitioning of nitrogen in leaves, stems and roots of lucerne (*Medicago sativa* L.) in a dense canopy. *Annals of Botany.* 70:429-435.

Leon, J.A. 1976. Life histories as adaptive strategies. *Journal of Theoretical Biology.* 60:301-335.

Levin, S.A., Mooney, H.A. and Field, C. 1989. The dependence of plant root:shoot ratios on internal nitrogen concentration. *Annals of Botany*. 64:71-75.

Loomis, R.S., Rabbinge. R. and Ng, E. 1979. Explanatory models in plant physiology. *Annual Revue of Plant Physiology*. 30:339-367.

Lu, J-L., Ertl, J.R. and Chen, C-M. 1992. Transcriptional regulation of nitrate reductase mRNA levels by cytokinin-abscisic acid interactions in etiolated barley leaves. *Plant Physiology*. 98:1255-1260.

Marshall, B. and Biscoe, P.V. 1980. A model for C_3 leaves describing the dependence of net photosynthesis on irradiance. I. Derivation. *Journal of Experimental Botany*. 31(120):29-39.

Martin, M.P.L.D and Field, R.J. 1984. The nature of competition between perennial ryegrass and white clover. *Grass and Forage Science*. 39:247-253.

Martins, M.A. 1993. The role of the external mycelium of arbuscular mycorrhizal fungi in the carbon transfer process between plants. *Mycological Research*. 97(7):807-810.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L. and Swan, J.A. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist*. 115:495-501.

Minchin, F.R., Summerfield, R.T., Hadley, P., Roberts, E.H. and Rawsthorne, S. 1981. Carbon and nitrogen nutrition of nodulated roots of grain legumes. *Plant. Cell and Environment.* 4:5-26.

Morton, J.B. 1993. Problems and solutionsfor the integration of glomalean taxonomy, systematic biology, and the study of endomycorrhizal phenomena. Mycorrhiza. 2:97-109.

Newman, E.I. and Ritz, K. 1986. Evidence on the pathways of phosphorus transfer between vesicular-arbuscular mycorrhizal plants. *New Phytologist.* 104:77-87.

Newman, E.I. 1988. Mycorrhizal links between plants: Their functioning and ecological significance. *Advances in Ecological Research.* 18:243-270.

Olsen, F.J., Kaiser, C.J. and Al-Maskari, A.Y. 1993. Forage yield and quality of three warm-season grasses mixed with legumes. *XVII International Grassland Congress. New Zealand and Queensland, Australia.*

Osmond, C.B. 1987. Photosynthesis and the carbon economy of plants. *New Phytologist.* 106(suppl.):161-175.

Papadopoulos, N. 1992. *A mathematical crop growth model for a mixed stand of Italian ryegrass (Lolium multiflorum L.) and white clover (Trifolium repens L.).* M.Sc. dissertation, University of the Witwatersrand. Johannesburg.

Pearcy, R.W., Björkman, O., Caldwell, M.M, Keeley, J.E., Monson, R.K. and Strain, B.R. 1987. Carbon gain by plants in natural environments. *Bioscience*. 37(1):21-29.

Penning de Vreis, F.W.T. 1983. Modelling growth and production. *In*: Lange, O.L., Nobel, P.S. and Osmond, C.B. eds., *Encyclopedia of Plant Physiology 12D. Physiological Plant Ecology*. Springer - Verlag; Berlin. pp. 118-150.

Perrin, N. 1992. Optimal resource allocation and marginal value of organs. *The American Naturalist*. 139(6):1344-1369.

Peters, W.S. and Felle, H. 1991. Control of apoplast pH in corn coleoptile segments. II. The effects of various auxins and auxin analogues. *Journal of Plant Physiology*. 137:691-696.

Poorter, H., Remkes, C. and Lambers, H. 1990. Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiology*. 94:621-627.

Raper, C.D. JR, Parsons, L.R., Patterson, D.T. and Kramer, P.J. 1977. Relationship between growth and nitrogen accumulation for vegetative cotton and soybean plants. *Botanical Gazette*. 138(2):129-137.

Rastetter, E.B., King, A.W., Cosby, B.J., Hornberger, G.M., O'Neill, R.V. and Hobbie, J.E. 1992. Aggregating fine-scale ecological knowledge to coarser-scale attributes of ecosystems. *Ecological Applications*. 2(1):55-70.

Reeves, M. 1992. The role of VAM fungi in nitrogen dynamics in maize - bean intercrops. *Plant and Soil.* 144:85-92.

Reynolds, J.F., Hilbert, D.W. and Kemp, P.R. 1993. Scaling ecophysiology from the plant to the ecosystem: A conceptual framework. In· *Scaling Physiological Processes: Leaf to Globe.* Academic Press.

Reynolds, J.F. and Thornley, J.H.M. 1982. A shoot:root partitioning model. *Annals of Botany.* 49:585-597.

Rimmington, G.M. 1984. A model of the effect of interspecies competition for light on dry-matter production. *Australian Journal of Plant Physiology.* 11:277-286.

Robinson, D. 1986. Limits to nutrient inflow rates in roots and root systems. *Physiologia Plantarum.* 68:551-559.

Rosendahl, L., Dilworth. M.J. and Glenn, A.R. 1992. Exchange of metabolites across the peribacteroid membrane in pea root nodules. *Journal of Plant Physiology.* 139:635-638.

Rousseau, J.V.D., Sylvia, D.M. and Fox, A.J. 1993. Contribution of ectomycorrhizae to nutrient absorbing surface area in pine. In: *Proceedings of the 9th North American conference on mycorrhizae, University of Guelph, Ontario, Canada.*

Ryle, G.J.A., Powell, C.E. and Davidson, I.A. 1992. Growth of white clover, dependent on N2 fixation, in elevated CO2 and temperature. *Annals of Botany.* 70:221-228.

Schubert, A., Wyss, P. and Wiemken, A. 1992. Occurrence of trehalosein vesicular-arbuscular mycorrhizal fungi and in mycorrhizal roots. *Journal of Plant Physiology.* 140:41-45

Smith, S. and Gianinazzi-Pearson, V. 1988. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. *Annual Revue of Plant Physiology and Plant Molecular Biology.* 39:221-244.

Smith, S.E. and Smith, F.A. (1990). Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. *New Phytologist.* 114:1-38.

Smolders, E. and Merckx, R. 1992. Growth and shoot:root partitioning of spinach plants as affected by nitrogen supply. *Plant, Cell and Environment.* 15:795-807.

Snell, F.J. and Quigley, P.G. 1993. Allelopathic effects of endophyte in perennial ryegrass residues on young subterranean clover plants. *XVII International Grassland Congress. New Zealand and Queensland, Australia.*

Sowokinos, J.R and Varns J.L. 1992. Induction of sucrose synthase in potato tissue culture: Effect of carbon source and metabolic regulators on sink strength. *Journal of Plant Physiology.* 139:672-679.

St John, J.V. and Coleman, D.C. 1983. The role of mycorrhizae in plant ecology. *Canadian Journal of Botany.* 61:1005-1014.

Stribley, D.P. and Read. D.J. 1974. The biology of mycorrhizae in the Ericaceae. iii. Movement of carbon-14 from host to fungus. *New Phytologist.* 73:731-741.

Sylvia, D.M. and Neal, L.H. 1990. Nitrogen affects the phosphorous response of VA mycorrhiza. *New Phytologist.* 115:303-310.

Thompson, B.D., Robson, A.D. and Abbott, C.R. 1990. Mycorrhizas formed by *Gigaspora calospora* and *Glomus fasciculatum* on subterranean clover in relation to soluble carbohydrate concentration in roots. *New Phytologist.* 114:217-225.

Thornley, J.H.M. 1991. A transport-resistance model of forest growth and partitioning. *Annals of Botany.* 68:211-226.

Thornley, J.H.M. 1972a. A balanced quantitative model for root : shoot ratios in vegetative plants. *Annals of Botany.* 36:431-441.

Thornley, J.M.H. 1972b. A model to describe the partitioning of photosynthate during vegetative plant growth. *Annals of Botany.* 36:419-430.

Twary, S.N. and Heichel, G.H. 1991. Carbon costs of dinitrogen fixation associated with dry matter accumulation an alfalfa. *Crop Science.* 31:985-992.

Vance, C.P. and Heichel, G.H. 1991. Carbon in nitrogen fixation: Limitation or exquisite adaptation. *Annual Revue Plant Physiology and Plant Molecular Biology.* 42:373-392.

Vance, C.P., Egli, M.A., Griffith, S.M. and Miller, S.S. 1988. Plant regulated aspects of nodulation and N2 fixation. *Plant, Cell and Environment.* 11:413-427.

Veenendaal, E.M., Monnaapula, S.C., Gilka, T. and Magole, I.L. 1992. Vesicular-arbuscular mycorrhizal infection of grass seedlings in a degraded semi-arid savanna in Botswana. *New Phytologist.* 121:477-485.

Whittingham, J. and Read, D.J. 1982. Vesicular-arbuscular mysorrhiza in natural vegetation systems. III. Nutrient transfer between plants via mycorrhizal interconnections. *New Phytologist.* 90:277-284.

Wilson, J.B. 1988. A review of evidence on the control of shoot:root ratio, in relation to models. *Annals of Botany.* 61:433-449.

APPENDIX 1. Source files for model programs

i.

```
*-----------------------------------------------------------------------*
    PROGRAM MICSIM
*
* This program is designed to simulate the growth and photosynthesis
* of two species in a mixed canopy.
* By Neil Eccles
*
    INTEGER TIME, LIMIT
    REAL W1, W2, S1, S2, L1, L2, A1, A2, AM1, AM2, I, FS1, FS2, FL1
    REAL FL2, SL1, SL2, B1, B2, C1, C2, H1, H2, H1U, H1L, H2U, H2L
    REAL LA1U, LA1L, LA2U, LA2L, P1U, P1L, P1, P2U, P2L, P2
    REAL N1, N2, SLA1, SLA2, M, Y, K1, K2, DAY
*
    OPEN (UNIT=1, FILE='b:\MICSIM.DAT', STATUS='OLD')
    OPEN (UNIT=2, FILE='a:MASS.DAT', STATUS='NEW')
    OPEN (UNIT=3, FILE='A:HEIGHT.DAT', STATUS='NEW')
    OPEN (UNIT=4, FILE='A:PHOTO.DAT', STATUS='NEW')
*
    PRINT*, 'How many days do you want to run for?'
    READ*, LIMIT
    TIME=0.0
*
    READ (1,*) W1, W2, S1, S2, L1, L2
    READ (1,*) H1, H2
    READ (1,*) P1, P2
*
* This is the end of the variables entry loop
* Now enter the input parameters
*
     PRINT*, 'Enter values for the following:'
     PRINT*, 'FS1, FS2, FL1, FL2'
     READ*, FS1, FS2, FL1, FL2
     PRINT*, 'N1, N2'
     READ*, N1, N2
     PRINT*, 'K1, K2'
     READ*, K1, K2
     PRINT*, 'I'
     READ*, I
*
   1 IF (TIME.LT.LIMIT) THEN
      A1=1.95E-06
      A2=1.22E-06
      AM1=0.00026
      AM2=0.00041
      SLA1=0.1
      SLA2=0.07
      M=0.032
      Y=0.76
      B1=40
      B2=80
      C1=.7
      C2=.9
      DAY=31325.43
```

```
*
       W1=W1+((P1-M*W1)*Y)
       W2=W2+((P2-M*W2)*Y)
       S1=S1+((P1-M*W1)*Y*FS1)
       S2=S2+((P2-M*W2)*Y*FS2)
       L1=L1+((P1-M*W1)*Y*FL1)
       L2=L2+((P2-M*W2)*Y*FL2)
       SL1=L1/S1
       SL2=L2/S2
       H1=B1*(S1/N1)**C1
       H2=B2*(S2/N2)**C2
      IF (H1.GT.H2)THEN
         H1U=H1-H2
         H1L=H2
         H2U=0.0
         H2L=H2
         LA1U=((H1U/B1)**(1/C1))*N1*SL1*SLA1
         LA1L=((H1L/B1)**(1/C1))*N1*SL1*SLA1
         LA2U=0.0
         LA2L=((H2L/B2)**(1/C2))*N2*SL2*SLA2
*
         P1U=DAY*(AM1/K1)*LOG((AM1+A1*K1*I/DAY)/(AM1+A1*K1*(I/DAY)*
     +    EXP(-K1*LA1U)))
*
         P1L=DAY*(AM1/K1)* LOG((AM1+A1*((I*EXP(-K1*LA1U-K2*LA2U))/DAY)
     +    *(EXP(-K2*LA2L)))/(AM1+A1*((I*EXP(-K1*LA1U-K2*LA2U))/DAY)*
     +    (EXP(-K2*LA2L-K1*LA1L))))
*
         P2L=DAY*(AM2/K2)* LOG((AM2+A2*((I*EXP(-K2*LA2U-K1*LA1U))/DAY)
     +    *(EXP(-K1*LA1L)))/(AM2+A2*((I*EXP(-K2*LA2U-K1*LA1U))/DAY)*
     +    (EXP(-K1*LA1L-K2*LA2L))))
*
         P2U=0.0
         P1=P1U+P1L
         P2=P2U+P2L
      ELSE IF (H1.LT.H2) THEN
         H1U=0.0
         H1L=H1
         H2U=H2-H1
         H2L=H1
         LA1U=0.0
         LA1L=((H1L/B1)**(1/C1))*N1*SL1*SLA1
         LA2U=((H2U/B2)**(1/C2))*N2*SL2*SLA2
         LA2L=((H2L/B2)**(1/C2))*N2*SL2*SLA2
*
         P1U=0.0
*
         P1L=DAY*(AM1/K1)* LOG((AM1+A1*((I*EXP(-K1*LA1U-K2*LA2U))/DAY)
     +    *(EXP(-K2*LA2L)))/(AM1+A1*((I*EXP(-K1*LA1U-K2*LA2U))/DAY)*
     +    (EXP(-K2*LA2L-K1*LA1L))))
*
         P2U=DAY*(AM2/K2)*LOG((AM2+A2*K2*I/DAY)/(AM2+A2*K2*(I/DAY)*
     +    EXP(-K2*LA2U)))
*
         P2L=DAY*(AM2/K2)* LOG((AM2+A2*((I*EXP(-K2*LA2U-K1*LA1U))/DAY)
     +    *(EXP(-K1*LA1L)))/(AM2+A2*((I*EXP(-K2*LA2U-K1*LA1U))/DAY)*
     +    (EXP(-K1*LA1L-K2*LA2L))))
*
```

```
      P1=P1U+P1L
      P2=P2U+P2L
    ELSE
      H1U=0.0
      H1L=H1
      H2U=0.0
      H2L=H2
      LA1U=0.0
      LA1L=((H1L/B1)**(1/C1))*N1*SL1*SLA1
      LA2U=0.0
      LA2L=((H2L/B2)**(1/C2))*N2*SL2*SLA2
*
      P1U=0.0
*
      P1L=DAY*(AM1/K1)* LOG((AM1+A1*((I*EXP(-K1*LA1U-K2*LA2U))/DAY)
   +   *(EXP(-K2*LA2L)))/(AM1+A1*((I*EXP(-K1*LA1U-K2*LA2U))/DAY)*
   +   (EXP(-K2*LA2L-K1*LA1L))))
*
      P2U=0.0
*
      P2L=DAY*(AM2/K2)* LOG((AM2+A2*((I*EXP(-K2*LA2U-K1*LA1U))/DAY)
   +   *(EXP(-K1*LA1L)))/(AM2+A2*((I*EXP(-K2*LA2U-K1*LA1U))/DAY)*
   +   (EXP(-K1*LA1L-K2*LA2L))))
*
      P1=P1U+P1L
      P2=P2U+P2L
    END IF
     WRITE (2,*) TIME, W1, W2
     WRITE (3,*) TIME, H1, H2
     WRITE (4,*) TIME, P1U, P1L, P2U, P2L
     TIME=TIME+1.0
     GO TO 1
    END IF
    END

    END
```

ii.

```
*--------------------------------------------------------------------------------*
    PROGRAM MOD
*
* This program is designed to simulate the growth, photosynthesis
* and nutrient uptake of two species in a mixed canopy.
* Species 1 is the grass, while sp 2 is the legume.
*
* By Neil Eccles   1994
*
    INTEGER TIME, LIMIT, ANM, ANN
    REAL S1, S2, L1, L2, A1, A2, AM1, AM2, I, MYC, NOD
    REAL SL1, SL2, B1, B2, B3, B4, H1, H2, H1U, H1L, H2U, H2L
    REAL LA1U, LA1L, LA2U, LA2L, P1U, P1L, P1, P2U, P2L, P2
    REAL N1, N2, SLA1, SLA2, DAY, PI, N, S
    REAL R1, R2, RR1, RR2, RM, PR1, PR2, PM, SA1, SA2, SAM
    REAL LR1, LR2, LM, VR1, VR2, VM, VRH, VS, T
    REAL NS, NRH, NM, NN, NR1, NR2, NS1, NS2, NL1, NL2
    REAL CNS, CNRH, CNM, CNN, CNR1, CNR2, CNS1, CNS2, CNL1, CNL2
    REAL CL1, CS1, CR1, CL2, CS2, CR2, CN, CM
```

```
      REAL CCL1, CCS1, CCR1, CCL2, CCS2, CCR2, CCN, CCM
      REAL SNM, SNN, SNR1, SNR2, SNS1, SNS2, SNL1, SNL2
      REAL GL1, GS1, GR1, GL2, GS2, GR2, GN, GM
      REAL GCL1, GCS1, GCR1, GNL1, GNS1, GNR1
      REAL GCL2, GCS2, GCR2, GNL2, GNS2, GNR2
      REAL GCN, GCM, GNN, GNM
      REAL I1, I2, I3, I4, I5, I6, I7, I8
      REAL UCL1, UCS1, UCR1, UCL2, UCS2, UCR2, UCN, UCM
      REAL J1, J2, J3, J4, J5, J6, J7, J8
      REAL UNM, UNN, UNR1, UNR2, UNS1, UNS2, UNL1, UNL2
      REAL Q1, Q2, Q3, Q4, Q5, Q6, Q7, Q8
      REAL M1, M2, MNOD, MMYC, Y1, Y2, YN, YM
      REAL ML1, MS1, MR1, ML2, MS2, MR2, MN, MM
      REAL K1, K2, K4, K3, K5, K6, K7, K8, K9
      REAL KM1, KM2, KM3, KM4, KM5, KM6, JN1, JN2, JNM
      REAL RS1, RS2, RS3, RS4, RS5, RS6, RS7, RS8
      REAL RS9, RS10, RS11, RS12, RS13
      REAL FL1, FS1, FL2, FS2, FN, FM
      REAL U1, U2, VMAX1, VMAX2, VMAX3
      REAL DML1, DMS1, DMR1, DML2, DMS2, DMR2, DMM, DMN
      REAL NDL1, NDL2, NDS1, NDS2, NDR1, NDR2
      REAL RU1, RU2, RUM, FIX, CFIX, SNFR
*
      OPEN (UNIT=1, FILE='A:COMP1.DAT', STATUS='OLD')
      OPEN (UNIT=2, FILE='A:COMP2.DAT', STATUS='OLD')
      OPEN (UNIT=3, FILE='A:SYM.DAT',STATUS='OLD')
      OPEN (UNIT=4, FILE='A:NITRO1.DAT', STATUS='OLD')
      OPEN (UNIT=5, FILE='A:NITRO2.DAT',STATUS='OLD')
      OPEN (UNIT=6, FILE='A:COMP.DAT', STATUS='OLD')
      OPEN (UNIT=7, FILE='A:COMP3.DAT', STATUS='OLD')
      OPEN (UNIT=8, FILE='A:COMP4.DAT', STATUS='OLD')
      OPEN (UNIT=9, FILE='A:COMP5.DAT', STATUS='OLD')
*
* Specify number of iterations
*
      PRINT*, 'How many days do you want to run for?'
      READ*, LIMIT
      TIME=0.0
*
* Characterize system: myc or nod?
*
      PRINT*, 'Enter 1 for +MYC or 0 for -MYC:'
      READ*, ANM
      PRINT*, 'Enter 1 for +NOD or 0 for -NOD:'
      READ*, ANN
*
* Enter the starting conditions
*
*
* Starting values for state variables and other driving variables
* biomasses
*
      L1=0.1
      L2=0.0175
      S1=0.1
      S2=0.014
      R1=0.25
      R2=0.021
```

```
      MYC=0.001
      NOD=0.0004
*
* nitrogen status
*
      NS=750
      NRH=7.5E-6
      NM=7E-5
      NN=4.5E-4
      NR1=0.03
      NR2=.0015
      NS1=0.008
      NS2=0.001
      NL1=0.008
      NL2=0.002
*
* carbon status
*
      CL1=0.12
      CL2=0.0053
      CS1=0.016
      CS2=0.002
      CR1=0.2
      CR2=0.002
      CN=1.44E-4
      CM=9.5E-5
*
* Calculate substrate concentrations (substrate/structure)
* carbon
*
      CCL1=CL1/L1
      CCS1=CS1/S1
      CCR1=CR1/R1
*
      CCL2=CL2/L2
      CCS2=CS2/S2
      CCR2=CR2/R2
*
      CCN=CN/NOD
      CCM=CM/MYC
*
*  nitrogen
*
      CNL1=NL1/L1
      CNS1=NS1/S1
      CNR1=NR1/R1
*
      CNL2=NL2/L2
      CNS2=NS2/S2
      CNR2=NR2/R2
*
      CNN=NN/NOD
      CNM=NM/MYC
*
    1 IF (TIME.LT.LIMIT) THEN
*
        TIME=TIME+1.0
*
```

```
* LIST CONSTANTS
*
      SLA1=0.1
      SLA2=0.07
      M1=0.032
      M2=0.032
      MMYC=0.032
      MNOD=.032
      Y1=0.76
      Y2=0.76
      YM=0.76
      YN=0.76
      RU1=4
      RU2=4
      RUM=4
      B1=40
      B2=80
      B3=.7
      B4=.9
      DAY=31825.43
      N1=100
      N2=100
*
* growth constants
*
      I1=0.18*3/5
      I2=0.0464*0.5
      I3=0.45/10
      I4=0.017
      I5=0.00664
      I6=0.009
      I7=0.05
      I8=0.06
*
* C concentrations in structural matter
*
      J1=0.4
      J2=0.4
      J3=0.4
      J4=0.4
      J5=0.4
      J6=0.4
      J7=0.4
      J8=0.4
*
* N concentrations in structural matter
*
      Q1=0.06
      Q2=0.009
      Q3=0.009
      Q4=0.07
      Q5=0.04
      Q6=0.04
      Q7=0.08
      Q8=0.04
*
      K1=0.25
      K2=0.5
```

```
      I=1.87E+7
      D=1E-3
      T=1
      PR1=5.3E+6
      PR2=5.3E+6
      PM=5.3E+6
      PI=22/7
      RR1=1E-4
      RR2=1.2E-4
      RM=6E-6
      F=0.01
      VMAX1=50
      VMAX2=40
      VMAX3=50
      KM1=100
      KM2=100
      KM3=100
      KM4=0.5
      KM5=0.5
      KM6=0.5
      JN1=0.006
      JN2=0.006
      JNM=0.006
*
* resistance to flux
*
      RS1=2
      RS2=1.2
      RS3=1.2
      RS4=1.2
      RS5=1.2
      RS6=2
      RS7=1.2
      RS8=1.02
      RS9=1.02
      RS10=1.02
      RS11=1.02
      RS12=1.02
      RS13=1.02
*
* senescence rates
*
      SNL1=0.007
      SNS1=0.007
      SNR1=0.007
      SNL2=0.007
      SNS2=0.007
      SNR2=0.007
      SNN=0.007
      SNM=0.007
*
      N=0.75
      S=0.4
      SNFR=0.03
      CFIX=6.14
*
* CALCULATE UPTAKES/ACQUISITION
* Calculate photosynthetic inputs
```

```
*
      DML1=L1+CL1+NL1
      DMS1=S1+CS1+NS1
      DMR1=R1+CR1+NR1
*
      DML2=L2+CL2+NL2
      DMS2=S2+CS2+NS2
      DMR2=R2+CR2+NR2
*
      DMN=NOD+CN+NN
      DMM=MYC+CM+NM
*
      NDL1=(NL1+L1*Q1)/DML1
*
      NDL2=(NL2+L2*Q4)/DML2
*
      SL1=DML1/DMS1
      SL2=DML2/DMS2
      H1=B1*(DMS1/N1)**B3
      H2=B2*(DMS2/N2)**B4
*
      A2=(1.08E-4/6)*NDL2-2.97E-7
      A1=(6.7E-5/2)*NDL1-1.35E-7
      AM1=(0.0144)*NDL1-2.88E-5
      AM2=(0.0227)*NDL2-4.54E-5
*
     IF (H1.GT.H2)THEN
       H1U=H1-H2
       H1L=H2
       H2U=0.0
       H2L=H2
       LA1U=((H1U/B1)**(1/B3))*N1*SL1*SLA1
       LA1L=((H1L/B1)**(1/B3))*N1*SL1*SLA1
       LA2U=0.0
       LA2L=((H2L/B2)**(1/B4))*N2*SL2*SLA2
*
       P1U=DAY*(AM1/K1)*LOG((AM1+A1*K1*I/DAY)/(AM1+A1*K1*(I/DAY)*
     +  EXP(-K1*LA1U)))
*
       P1L=DAY*(AM1/K1)*LOG((AM1+A1*((I*EXP(-K1*LA1U-K2*LA2U))/DAY)
     +  *(EXP(-K2*LA2L)))/(AM1+A1*((I*EXP(-K1*LA1U-K2*LA2U))/DAY)*
     +  (EXP(-K2*LA2L-K1*LA1L))))
*
       P2L=DAY*(AM2/K2)*LOG((AM2+A2*((I*EXP(-K2*LA2U-K1*LA1U))/DAY)
     +  *(EXP(-K1*LA1L)))/(AM2+A2*((I*EXP(-K2*LA2U-K1*LA1U))/DAY)*
     +  (EXP(-K1*LA1L-K2*LA2L))))
*
       P2U=0.0
       P1=P1U+P1L
       P2=P2U+P2L
     ELSE IF (H1.LT.H2) THEN
       H1U=0.0
       H1L=H1
       H2U=H2-H1
       H2L=H1
       LA1U=0.0
       LA1L=((H1L/B1)**(1/B3))*N1*SL1*SLA1
       LA2U=((H2U/B2)**(1/B4))*N2*SL2*SLA2
```

```
        LA2L=((H2L/B2)**(1/B4))*N2*SL2*SLA2
*
        P1U=0.0
*
        P1L=DAY*(AM1/K1)*LOG((AM1+A1*((I*EXP(-K1*LA1U-K2*LA2U))/DAY)
     +   *(EXP(-K2*LA2L)))/(AM1+A1*((I*EXP(-K1*LA1U-K2*LA2U))/DAY)*
     +   (EXP(-K2*LA2L-K1*LA1L))))
*
        P2U=DAY*(AM2/K2)*LOG((AM2+A2*K2*I/DAY)/(AM2+A2*K2*(I/DAY)*
     +   EXP(-K2*LA2U)))
*
        P2L=DAY*(AM2/K2)*LOG((AM2+A2*((I*EXP(-K2*LA2U-K1*LA1U))/DAY)
     +   *(EXP(-K1*LA1L)))/(AM2+A2*((I*EXP(-K2*LA2U-K1*LA1U))/DAY)*
     +   (EXP(-K1*LA1L-K2*LA2L))))
*
        P1=P1U+P1L
        P2=P2U+P2L
      ELSE
        H1U=0.0
        H1L=H1
        H2U=0.0
        H2L=H2
        LA1U=0.0
        LA1L=((H1L/B1)**(1/B3))*N1*SL1*SLA1
        LA2U=0.0
        LA2L=((H2L/B2)**(1/B4))*N2*SL2*SLA2
*
        P1U=0.0
*
        P1L=DAY*(AM1/K1)*LOG((AM1+A1*((I*EXP(-K1*LA1U-K2*LA2U))/DAY)
     +   *(EXP(-K2*LA2L)))/(AM1+A1*((I*EXP(-K1*LA1U-K2*LA2U))/DAY)*
     +   (EXP(-K2*LA2L-K1*LA1L))))
*
        P2U=0.0
*
        P2L=DAY*(AM2/K2)*LOG((AM2+A2*((I*EXP(-K2*LA2U-K1*LA1U))/DAY)
     +   *(EXP(-K1*LA1L)))/(AM2+A2*((I*EXP(-K2*LA2U-K1*LA1U))/DAY)*
     +   (EXP(-K1*LA1L-K2*LA2L))))
*
        P1=(P1U+P1L)
        P2=(P2U+P2L)
      END IF
*
* Calculate N-uptake
*
       LR1=R1/(PI*(RR1**2)*PR1)
       LR2=R2/(PI*(RR2**2)*PR2)
       LM=MYC/(PI*(RM**2)*PM)
*
       SA1=2*PI*RR1*LR1
       SA2=2*PI*RR2*LR2
       SAM=2*PI*RM*LM
*
       VR1=R1/PR1
       VR2=R2/PR2
       VM=MYC/PM

       IF (T.GT.VRH) THEN
```

```
        VRH=(PI*((RR1+D)**2)*LR1-VR1)+(PI*((RR2+D)**2)*LR2-VR2)+
     +   (PI*((RM+D)**2)*LM-VM)
        VS=T-(VRH+VR1+VR2+VM)
       ELSE
        VRH=VRH-VR1-VR2-VM
        VS=0
       END IF
*
       IF (VS.GT.0) THEN
       CNS=NS/VS
       ELSE
       CNS=0
       END IF
*
       CNRH=NRH/VRH
*
       K3=VS*VRH/(VS+VRH)
*
       K4=S1*L1/(S1+L1)
       K5=S1*R1/(S1+R1)
*
       K6=S2*L2/(S2+L2)
       K7=S2*R2/(S2+R2)
*
       K8=MYC*R2/(MYC+R2)
       K9=NOD*R2/(NOD+R2)
*
       URH=K3*(CNS-CNRH)/RS1
*
       IF (CNRH.GT.0) THEN
        U1=(VMAX1*CNRH/(KM1+CNRH))*SA1
     +   *(CCR1/(KM4+CCR1))*(1/(1+CNR1/JN1))
        U2=(VMAX2*CNRH/(KM2+CNRH))*SA2
     +   *(CCR2/(KM5+CCR2))*(1/(1+CNR2/JN2))
        UM=((VMAX3*CNRH/(KM3+CNRH))*SAM)*ANM
     +   *(CCM/(KM6+CCM))*(1/(1+CNM/JNM))
       ELSE
        U1=0
        U2=0
        UM=0
       END IF
*
* Calculate fixation
*
*       FIX=(CN*SNFR/CFIX)*ANN
      FIX=DMN*SNFR*ANN
*
* UPDATE STATUS
* new substrate levels
* Carbon
*
      CL1=CL1+P1
      CS1=CS1
      CR1=CR1-RU1*U1
*
      CL2=CL2+P2
      CS2=CS2
      CR2=CR2-RU2*U2
```

```
*
      CM=CM-RUM*UM
      CN=CN-(CFIX*FIX)
*
* Nitrogen
*
      NL1=NL1
      NS1=NS1
      NR1=NR1+U1
*
      NL2=NL2
      NS2=NS2
      NR2=NR2+U2
*
      NM=NM+UM
      NN=NN+FIX
*
      NRH=NRH+URH-UM-U1-U2
      NS=NS-URH
*
* Calculate substrate concentrations
* carbon
*
       CCL1=CL1/L1
       CCS1=CS1/S1
       CCR1=CR1/R1
*
       CCL2=CL2/L2
       CCS2=CS2/S2
       CCR2=CR2/R2
*
       CCM=CM/MYC
       CCN=CN/NOD
*
*  nitrogen
*
       CNL1=NL1/L1
       CNS1=NS1/S1
       CNR1=NR1/R1
*
       CNL2=NL2/L2
       CNS2=NS2/S2
       CNR2=NR2/R2
*
       CNM=NM/MYC
       CNN=NN/NOD
*
       WRITE (9,*) TIME, CNL1, CNS1, CNR1
*
*
* CALCULATE FLUXES/ALLOCATION
* Carbon
*
      FL1=K4*(CCL1-CCS1)/RS2
      FS1=K5*(CCS1-CCR1)/RS3
*
      FL2=K6*(CCL2-CCS2)/RS4
      FS2=K7*(CCS2-CCR2)/RS5
```

```
*
      FN=ANN*K9*(CCR2-CCN)/RS6
      FM=ANM*K8*(CCR2-CCM)/RS7
*
* Nitrogen
*
      FNL1=K4*(CNS1-CNL1)/RS8
      FNS1=K5*(CNR1-CNS1)/RS9
*
      FNL2=K6*(CNS2-CNL2)/RS10
      FNS2=K7*(CNR2-CNS2)/RS11
*
      FNN=ANN*K9*(CNN-CNR2)/RS12
      FNM=ANM*K8*(CNM-CNR2)/RS13
*
* UPDATE STATUS
* Calculate the new substrate levels
* Carbon
*
      CL1=CL1-FL1
      CS1=CS1+FL1-FS1
      CR1=CR1+FS1
*
      CL2=CL2-FL2
      CS2=CS2+FL2-FS2
      CR2=CR2+FS2-FN-FM
*
      CN=CN+FN
      CM=CM+FM
*
* Nitrogen
*
      NL1=NL1+FNL1
      NS1=NS1+FNS1-FNL1
      NR1=NR1-FNS1
*
      NL2=NL2+FNL2
      NS2=NS2+FNS2-FNL2
      NR2=NR2+FNN+FNM-FNS2
*
      NN=NN-FNN
      NM=NM-FNM
*
* Calculate substrate concentrations
* carbon
*
       CCL1=CL1/L1
       CCS1=CS1/S1
       CCR1=CR1/R1
*
       CCL2=CL2/L2
       CCS2=CS2/S2
       CCR2=CR2/R2
*
       CCN=CN/NOD
       CCM=CM/MYC
*
*  nitrogen
```

```
*
      CNL1=NL1/L1
      CNS1=NS1/S1
      CNR1=NR1/R1
*
      CNL2=NL2/L2
      CNS2=NS2/S2
      CNR2=NR2/R2
*
      CNN=NN/NOD
      CCM=NM/MYC
*
      WRITE (8,*) TIME, CNL1, CNS1, CNR1
*
*
* CALCULATE GROWTH AND USE OF SUBSTRATES
* growth
*
      GCL1=I1*CCL1*L1
      GNL1=I1*CNL1*J1/Q1*L1
      IF (GNL1.GT.GCL1) THEN
        GL1=GCL1
      ELSE
        GL1=GNL1
      END IF
*
      GCS1=I2*CCS1*S1
      GNS1=I2*CNS1*J2/Q2*S1
      IF (GNS1.GT.GCS1) THEN
        GS1=GCS1
      ELSE
        GS1=GNS1
      END IF
*
      GCR1=I3*CCR1*R1
      GNR1=I3*CNR1*J3/Q3*R1
      IF (GNR1.GT.GCR1) THEN
        GR1=GCR1
      ELSE
        GR1=GNR1
      END IF
*
      GCL2=I4*CCL2*L2
      GNL2=I4*CNL2*J4/Q4*L2
      IF (GNL2.GT.GCL2) THEN
        GL2=GCL2
      ELSE
        GL2=GNL2
      END IF
*
      GCS2=I5*CCS2*S2
      GNS2=I5*CNS2*J5/Q5*S2
      IF (GNS2.GT.GCS2) THEN
        GS2=GCS2
      ELSE
        GS2=GNS2
      END IF
*
```

```
      GCR2=I6*CCR2*R2
      GNR2=I6*CNR2*J6/Q6*R2
      IF (GNR2.GT.GCR2) THEN
        GR2=GCR2
      ELSE
        GR2=GNR2
      END IF
*
      GCN=I7*CCN*NOD
      GNN=I7*CNN*J7/Q7*NOD
      IF (GNN.GT.GCN) THEN
        GN=GCN
      ELSE
        GN=GNN
      END IF
*
      GCM=I8*CCM*MYC
      GNM=I8*CNM*J8/Q8*MYC
      IF (GNM.GT.GCM) THEN
        GM=GCM
      ELSE
        GM=GNM
      END IF
*
* respiration
*
      ML1=M1*L1
      MS1=M1*S1
      MR1=M1*R1
*
      ML2=M2*L2
      MS2=M2*S2
      MR2=M2*S2
*
      MN=MNOD*NOD
      MM=MMYC*MYC
*
*
* use of substrates for growth
* carbon
*
      UCL1=J1*GL1/Y1
      UCS1=J2*GS1/Y1
      UCR1=J3*GR1/Y1
*
      UCL2=J4*GL2/Y2
      UCS2=J5*GS2/Y2
      UCR2=J6*GR2/Y2
*
      UCN=J7*GN/YN
      UCM=J8*GM/YM
*
* nitrogen
*
      UNL1=Q1*GL1
      UNS1=Q2*GS1
      UNR1=Q3*GR1
*
```

```
      UNL2=Q4*GL2
      UNS2=Q5*GS2
      UNR2=Q6*GR2
*
      UNN=Q7*GN
      UNM=Q8*GM
*
* UPDATE STATUS
* Calculate the new substrate levels
* Carbon
*
      CL1=CL1-UCL1-ML1
      CS1=CS1-UCS1-MS1
      CR1=CR1-UCR1-MR1
*
      CL2=CL2-UCL2-ML2
      CS2=CS2-UCS2-MS2
      CR2=CR2-UCR2-MR2
*
      CN=CN-UCN-MN
      CM=CM-UCM-MM
*
* Nitrogen
*
      NL1=NL1-UNL1
      NS1=NS1-UNS1
      NR1=NR1-UNS1
*
      NL2=NL2-UNL2
      NS2=NS2-UNS2
      NR2=NR2-UNS2
*
      NN=NN-UNN
      NM=NM-UNM
*
* Change in structural material
*
      L1=L1+GL1-(SNL1*L1)
      S1=S1+GS1-(SNS1*S1)
      R1=R1+GR1-(SNR1*R1)
*
      L2=L2+GL2-(SNL2*L2)
      S2=S2+GS2-(SNS2*S2)
      R2=R2+GR2-(SNR2*R2)
*
      NOD=NOD+GN-(SNN*NOD)
      MYC=MYC+GM-(SNM*MYC)
*
* Calculate substrate concentrations
* carbon
*
      CCL1=CL1/L1
      CCS1=CS1/S1
      CCR1=CR1/R1
*
      CCL2=CL2/L2
      CCS2=CS2/S2
      CCR2=CR2/R2
```

```
*
      CCN=CN/NOD
      CCM=CM/MYC
*
*  nitrogen
*
      CNL1=NL1/L1
      CNS1=NS1/S1
      CNR1=NR1/R1
*
      CNL2=NL2/L2
      CNS2=NS2/S2
      CNR2=NR2/R2
*
      CNN=NN/NOD
      CCM=NM/MYC
*
      WRITE (7,*) TIME, CNL1, CNS1, CNR1
*
*
* CALCULATE MEASURED PARAMETERS
* dry mass
*
      DML1=L1+CL1+NL1
      DMS1=S1+CS1+NS1
      DMR1=R1+CR1+NR1
*
      DML2=L2+CL2+NL2
      DMS2=S2+CS2+NS2
      DMR2=R2+CR2+NR2
*
      DMN=NOD+CN+NN
      DMM=MYC+CM+NM
*
* nitrogen content
*
      NDL1=(NL1+L1*Q1)/DML1
      NDS1=(NS1+S1*Q2)/DMS1
      NDR1=(NR1+S1*Q3)/DMR1
*
      NDL2=(NL2+L2*Q4)/DML2
      NDS2=(NS2+S2*Q5)/DMS2
      NDR2=(NR2+S2*Q6)/DMR2
*
* Now comes the output!
*
      WRITE (1,*) TIME, DML1, DMS1, DMR1
      WRITE (2,*) TIME, DML2, DMS2, DMR2
      WRITE (3,*) TIME, DMM, DMN
      WRITE (4,*) TIME, NDL1, NDS1, NDR1
      WRITE (5,*) TIME, NDL2, NDS2, NDR2
      WRITE (6,*) TIME, CNRH, NRH, CNS, NS
*
     GO TO 1
    END IF
    END
```

APPENDIX 2. Reagents.

i. Fertiliser solutions and applications:

	Stock conc (g.l^{-1})	ml stock.5l^{-1}
KH_2PO_4	136.083	0.5
KNO_3	101.097	3
NH_4NO_3	80.052	3
$Ca(NO_3)_2$	164.088	4
KCl	74.551	7.5
$MgSO_4$	36.9	
$CaSO_4.5H_2O$	0.024	
$MnSO_4$	0.223	
$ZnSO_4.7H_2O$	0.030	50
H_3BO_3	0.186	
$(NH_4)_6Mo_7O_3.4H_2O$	0.004	
$CoSO_4.7H_2O$	0.003	
NaCl	0.585	

ii. Rhizobium selection and growth media (pH 6.8-7.0):

K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	0.2 g
NaCl	0.1 g
Mannitol	10.0 g
Yeast extract	0.4 g
*Agar	15.0 g
Distilled H_2O	1.0 l
*Congo red (0.25% mv)	10.0 ml

* These components were not included in the liquid growth medium.

iii. Reagents for nitrogen assays:

Digestion mixture:

420 ml concentrated H_2SO_4 carefully added to 350 ml H_2O_2 (100 vol.) on ice. This was stored at 4°C.

Staining solutions:

Solution A:

12.5 g EDTA (ethylene-diamine-tetra-acetic acid) was dissolved in 500 ml de-ionized water and the pH was adjusted to 10 with 2N NaOH. 10 ml of 0.25% methyl red solution was then added.

Solution B:

70 ml phenol and 340 mg of sodium nitroprusside were mixed. This was then made up to 1000 ml with de-ionized water.

Solution C:

14.8 g NaOH, 49.8 g Na_2HPO_4, and 200 ml sodium hypochloride (5%) were mixed and then made up to 1000 ml with de-ionized water.

Ammonium sulphate stock solution:

0.25 M $(NH_4)_2SO_4$

APPENDIX 3

i. PARAMETERS AND CONSTANTS[4]:

SNLi	Leaf senescence rate for sp.i	$gST.m^{-2}.day^{-1}$
SNSi	Stem senescence rate for sp.i	$gST.m^{-2}.day^{-1}$
SNRi	Root senescence rate for sp.i	$gST.m^{-2}.day^{-1}$
SNN	Nodule senescence rate	$gST.m^{-2}.day^{-1}$
SNM	VAM hyphal senescence rate	$gST.m^{-2}.day^{-1}$
SNFR	Specific nitrogen fixation rate	$gN.gDM^{-1}.day^{-1}$
CFIX	Carbon cost of nitrogen fixation	$gC.gN^{-1}$
RS1	Resistance to mineral nitrogen flux between the soil and the rhizosphere	day
RSi(i=2-7)	Resistance to substrate carbon fluxes	day
RSi(i=8-13)	Resistance to substrate nitrogen fluxes	day
Yi	Growth respiratory coefficient	

---

[4] For values used see FORTRAN source file listing in Appendix 1

	for sp.i	
YN	Growth respiratory coefficient for the nodules	
YM	Growth respiratory coefficient for the VAM fungi	
RUi	Respiratory cost of nitrogen uptake and assimilation in sp.i	gC.gN^{-1}
RUM	Respiratory cost of nitrogen uptake and assimilation in sp.i	gC.gN^{-1}
DAY	Day length	s
Ni	Density of plants of sp.i in the canopy	plants.m^{-2}
Ii	Growth constants for the various components	
Ji	Carbon concentration in structural dry matter	gC.gDM^{-1}

Qi	Nitrogen concentration in structural dry matter	$gN.gDM^{-1}$
Mi	Maintenance respiration coefficient for sp.i	
MNOD	Nodule maintenance respiration coefficient	
MMYC	Mycorrhizal fungal maintenance respiration coefficient	
Ki(i=1,2)	Canopy extinction coefficient of sp.i	
F	Rate of fertiliser application	$gN.m^{-2}.day^{-1}$
Bi(i=1,2)	Allometric constant for relating height and biomass	
Bi(i=3,4)	Allometric constant for relating height and biomass	
SLAi	Specific leaf area of sp.i	$m^2.g^{-1}$
T	Total volume of the below ground	m^3

	system	
I		
RRi	Mean root radius of sp.i	m
RM	Mean VAM hyphal radius	m
PRi	Density of root tissue of sp.i	g.m^{-3}
PM	Density of the VAM hyphal tissue	g.m^{-3}
VMAXi	Maximal uptake rate at saturating nitrogen levels	gN.m^{-2}.day^{-1}
KMi(i=1-3)	Michealis-Menten constant	gC.gST^{-1}
KMi(i=4-6)	Root activity parameter	gC.gST^{-1}
JNi(i=1,2)	Nitrogen uptake inhibition constant for species i	gN.gST^{-1}
JNM	Nitrogen uptake inhibition constant for the VAM hyphae	gN.gST^{-1}

ii. VARIABLES:

Li	Leaf structural dry mass of sp. i	gST[5].m^{-2}
Si	Stem structural dry mass of sp. i	gST.m^{-2}
Ri	Root structural dry mass of sp.i	gST.m^{-2}
NOD	Nodule structural dry mass	gST.m^{-2}
MYC	Structural dry mass of the VAM fungi	gST.m^{-2}
CCLi	Carbon substrate concentration in the leaves of sp. i	gC.gST^{-1}
CCSi	Carbon substrate concentration in the stems of sp. i	gC.gST^{-1}
CCRi	Carbon substrate concentration in the roots of sp. i	gC.gST^{-1}
CCN	Carbon substrate concentration in the nodules	gC.gST^{-1}
CCM	Carbon substrate concentration in the VAM fungi	gC.gST^{-1}
CNLi	Nitrogen substrate concentration in the leaves of sp. i	gN.gST^{-1}
CNSi	Nitrogen substrate concentration in the stems of sp. i	gN.gST^{-1}

---

[5] ST=structure

CNRi	Nitrogen substrate concentration in the roots of sp i	gN.gST^{-1}
CNN	Nitrogen substrate concentration in the nodules	gN.gST^{-1}
CNM	Nitrogen substrate concentration in the VAM fungi	gN.gST^{-1}
CNS	Mineral nitrogen concentration in the soil	gN.m^{-3}soil
CNRH	Mineral nitrogen concentration in the rhizosphere	gN.m^{-3}soil
CLi	Amount of carbon substrate in the leaves of sp. i	gC
CSi	Amount of carbon substrate in the stems of sp. i	gC
CRi	Amount of carbon substrate in the roots of sp. i	gC
CN	Amount of carbon substrate in the nodules	gC
CM	Amount of carbon substrate in the VAM fungi	gC
NLi	Amount of nitrogen substrate in the leaves of sp. i	gN

NSi	Amount of nitrogen substrate in the stems of sp. i	gN
NRi	Amount of nitrogen substrate in the roots of sp. i	gN
NN	Amount of nitrogen substrate in the nodules	gN
NM	Amount of nitrogen substrate in the VAM fungi	gN
DMLi	Total leaf biomass of sp.i	$gDM.m^{-2}$
DMSi	Total stem biomass of sp.i	$gDM.m^{-2}$
DMLi	Total leaf biomass of sp.i	$gDM.m^{-2}$
DMRi	Total root biomass of sp.i	$gDM.m^{-2}$
DMN	Total nodule biomass	$gDM.m^{-2}$
DMM	Total VAM fungal biomass	$gDM.m^{-2}$
NDLi	Total nitrogen content in the leaves of sp. i	$gN.gDM^{-1}$
NDSi	Total nitrogen content in the stems of sp. i	$gN.gDM^{-1}$
NDRi	Total nitrogen content in the roots of sp. i	$gN.gDM^{-1}$
NDN	Total nitrogen content in the nodules	$gN.gDM^{-1}$

NDM	Total nitrogen content in the nodules	$gN.gDM^{-1}$
VS	Soil volume	m^3
VRH	Rhizosphere volume	m^3
VRi	Volume of the roots of sp.i	m^3
VM	Volume of the VAM fungal hyphae	m^3
LRi	Root length of sp.i	m
LM	VAM hyphal length	m
SAi	Surface area of the roots of sp.i	m^2
SAM	Surface area of the VAM fungal hyphae	m^2
Ai	Quantum yield efficiency of sp.i	
AMi	Light saturated photosynthetic rate of sp.i	$gC.m^{-2}.day^{-1}$
SLi	Stem:leaf ratio of sp.i	
LAiU	Upper canopy leaf area of	m^2

	sp.i	
LAiL	Lower canopy leaf area of sp.i	m^2
PiU	Upper canopy daily photosynthetic rate of sp.i	$gC.m^{-2}.day^{-1}$
PiL	Lower canopy daily photosynthetic rate of sp.i	$gC.m^{-2}.day^{-1}$
Pi	Total daily canopy photosynthetic rate for sp.i	$gC.m^{-2}.day^{-1}$
Hi	Canopy depth of sp. i	m
HiU	Upper canopy depth of sp.i	m
HiL	Lower canopy depth of sp.i	m
URH	Flux of nitrogen from the bulk soil to the rhizosphere	$gN.day^{-1}$
Ui	Nitrogen uptake rate of the roots of sp.i	$gN.m^{-2}.day^{-1}$
UM	Nitrogen uptake rate of the VAM fungi	$gN.m^{-2}.day^{-1}$
Ki	Scaling factors	m^3
Mi	Maintenance respiration costs	$gC.m^{-2}.day^{-1}$

	of the leaves of sp.i	
MSi	Maintenance respiration costs of the stems of sp.i	gC.m^{-2}.day^{-1}
MRi	Maintenance respiration costs of the roots of sp.i	gC.m^{-2}.day^{-1}
MN	Maintenance respiration costs of the nodules	gC.m^{-2}.day^{-1}
MM	Maintenance respiration costs of the VAM fungi	gC.m^{-2}.day^{-1}
FLi	Flux of carbon substrate from the leaves to the stems of sp.i	gC.day^{-1}
FSi	Flux of carbon substrate from the stems to the roots of sp.i	gC.day^{-1}
FN	Flux of carbon substrate from the roots of the legume to the nodules	gC.day^{-1}
FM	Flux of carbon substrate from the roots of the legume to the VAM fungal hyphae	gC.day^{-1}
FNLi	Flux of nitrogen substrate from the stems to the leaves	gN.day^{-1}

	of sp.i	
FNSi	Flux of nitrogen substrate from the roots to the stems of sp.i	gN.day^{-1}
FNN	Flux of nitrogen substrate from the nodules to the roots of the legume	gN.day^{-1}
FNM	Flux of nitrogen substrate from the VAM fungae to the roots of the legume	gN.day^{-1}
UCLi	Expendature of carbon substrate in leaf growth of sp.i	gC.day^{-1}
UCSi	Expendature of carbon substrate in stem growth of sp.i	gC.day^{-1}
UCRi	Expendature of carbon substrate in root growth of sp.i	gC.day^{-1}
UCN	Expendature of carbon substrate in nodule growth	gC.day^{-1}
UCM	Expendature of carbon substrate in VAM fungal growth	gC.day^{-1}
UNLi	Expendature of nitrogen substrate in leaf growth of sp.i	gN.day^{-1}
UNSi	Expendature of nitrogen substrate in stem growth of sp.i	gN.day^{-1}

UNRi	Expendature of nitrogen substrate in root growth of sp.i	$gN.day^{-1}$
UNN	Expendature of nitrogen substrate in nodule growth	$gN.day^{-1}$
UNM	Expendature of nitrogen substrate in VAM fugal growth	$gN.day^{-1}$
GLi	Leaf growth rate for sp.i	$gST.m^{-2}.day^{-1}$
GSi	Stem growth rate for sp.i	$gST.m^{-2}.day^{-1}$
GRi	Root growth rate for sp.i	$gST.m^{-2}.day^{-1}$
GN	Nodule growth rate	$gST.m^{-2}.day^{-1}$
GM	Mycorrhizal growth rate	$gST.m^{-2}.day^{-1}$
FIX	Nitrogen fixation rate	$gN.m^{-2}.day^{-1}$

ADDENDUM: ***Revised equations 3.35 (A7) and 3.36 (A8) and their derivation.***

First consider the lower canopy with Io the incident light, which has leaf area components L1lo and L2lo. The light level within the canopy is given by (Rimmington, 1984)

$$I=Io*e^{(-K1*l1-K2*l2)} \tag{A1}$$

Where $l1$ and $l2$ are dummy variables which vary between 0 and L1lo and 0 and L2lo respectively. Assuming the canopy is homogeneous in the two components and if h is the height of the canopy and x is the distance from the top of the canopy, then

$$l1=x/h\ L1lo \quad \text{and} \quad l2=x/h\ L2lo \tag{A2}$$

Substituting equations A2 into A1

$$I=Io*e^{-K1 \quad (L1lo+K2*L2lo)} \tag{A3}$$

The photosynthetic of component 1 of the canopy is

$$P1lo=\int_0^{L1lo} \frac{A1*I*AM1}{A1*I+AM1}\, dl1 \tag{A4}$$

Substituting

$$dl1=\frac{dx}{h} \tag{A5}$$

and equations A3 into equation A4

$$P1lo=\frac{L1lo}{h}\int_0^h \frac{AM1*A1*Io*e^{-x/h*(K1*L1lo+K2*L2lo)}}{AM1+A1*Io*e^{-x/h*(K1*L1lo+K2*L2lo)}}\, dx \tag{A4}$$

$$=\frac{L1lo*AM1*A1*Io}{h}\int_0^h \frac{Io*e^{-x/h*(K1*L1lo+K2*L2lo)}}{AM1+A1*Io*e^{-x/h*(K1*L1lo+K2*L2lo)}}\, dx \tag{A5}$$

$$=\frac{L1lo*AM1*A1*Io}{h}\ \ln\left[AM1+A1*Io*e^{-x/h*(K1*L1lo+K2*L2lo)}\right]*\left[\frac{-h}{A1*Io(K1*L1lo+K2*L2lo)}\right] \tag{A6}$$

from x=0 to x=h

$$\mathrm{P1lo} = \frac{\mathrm{AM1*L1lo}}{\mathrm{K1*L1lo+K2*L2lo}} * \ln\left[\frac{\mathrm{AM1+A1*I*exp(-K1*L1up-K2*L2up)}}{\mathrm{AM1+A1*I*exp(-K1*L1up-K2*L2up-K1*L1lo-K2*L2lo)}}\right] \tag{A7}$$

similarly, for species 2

$$\mathrm{P2lo} = \frac{\mathrm{AM2*L2lo}}{\mathrm{K1*L1lo+K2*L2lo}} * \ln\left[\frac{\mathrm{AM2+A2*I*exp(-K1*L1up-K2*L2up)}}{\mathrm{AM2+A2*I*exp(-K1*L1up-K2*L2up-K1*L1lo-K2*L2lo)}}\right] \tag{A7}$$

Author: Eccles Nell Stuart.
Name of thesis: The use of mathematical models to investigate carbon and nitrogen relations of a Mycorrhizal grass-legume mixed stand.

PUBLISHER:
University of the Witwatersrand, Johannesburg
©2015