QUANTIFICATION OF THE BELOWGROUND INPUTS OF ORGANIC CARBON BY THE ANNUAL PASTURE LEGUME BARREL MEDIC (*MEDICAGO TRUNCATULA* GAERTN.)

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ABSTRACT

In the cropping soils of southern Australia, pasture phases based on the annual legume barrel medic (*Medicago truncatula* Gaertn.) provide significant inputs of organic matter and lead to improvements in soil structure and fertility. This thesis aimed to quantify the belowground input of carbon by barrel medic and the effect of defoliation on this input. This aids the development of simulation models of soil organic carbon, and has practical implications regarding rotation choice and grazing management.

The major belowground input of carbon in annual species is found as root production. Measurement of total root biomass production, using a technique that accounted for root death and decomposition, found that it was 1.6 to 2.9 times the maximum live root biomass. In a year of very low rainfall, root production was a lot less than in a year of average rainfall. However, the ratio of total root production to total shoot production was higher. In both years, the total root biomass production and root:shoot ratio of barrel medic were greater than faba beans but similar to barley grass.

Total root biomass production of a frequently defoliated barrel medic pasture was estimated using a ^{13}C dilution technique where swards were pulse labelled with ^{13}C. Root biomass production following labelling was estimated from the subsequent dilution in ^{13}C enrichment during the remainder of the season. Although live root biomass of cut pasture was less than that of uncut pasture, total root biomass production was similar. ^{13}C allocation data showed that this was due to greater allocation of carbon belowground by cut plants. It was concluded there must be greater turnover of root material under cut pasture.

The effect of defoliation on the amount of carbon released to the soil through rhizodeposition was estimated by growing plants in pots in a labelled atmosphere in a growth cabinet. Plants were grown in a ^{14}C-labelled CO_2 atmosphere, defoliated and then grown in a ^{13}C-labelled CO_2 atmosphere. Distribution of ^{14}C and ^{13}C in the plants was compared with uncut plants. Defoliation did not have a significant effect on belowground allocation of carbon. The proportion of labelled carbon allocated belowground was 24 and 28 % for cut and uncut plants respectively. Total input of carbon into the soil was estimated to be 1.70 (cut) and 1.65 (uncut) times the amount of carbon recovered in root biomass.

Applying these correction factors to results from the field experiments, total annual belowground input of carbon ranged from 700 to 1880 kg C/ha. As a proportion of carbon in above ground production, carbon input belowground ranged from 0.40 to 0.77. Defoliation at the frequency and level imposed in these experiments had no adverse effect on total belowground inputs of carbon.

DECLARATION

I declare that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Michael Crawford Date

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Crawford, M.C., Grace, P.R. and Bellotti, W.D. (1995). Root production of a faba bean crop, a barrel medic pasture and a barley grass pasture. Proceedings of the 1995 Tillage Workshop, Adelaide, South Australia. pp. 18-19.

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CHAPTER 1

INTRODUCTION

The maintenance of soil organic matter (SOM) levels is considered by many to be necessary for sustainable agricultural activities, especially those involving some form of cropping (Jenkinson 1988). SOM can be defined as 'those components of soil that are products of microbial and chemical transformations of organic residues, including plant residues' (Clapp *et al.* 1990). Consequently, inputs to the SOM pool can include material such as crop residues (both aboveground and belowground), mulches, green manures, animal manures and sewage. As carbon is the 'backbone' of all organic matter, soil organic carbon is often presented as a surrogate measure of SOM.

Soil organic matter has a number of roles in the regulation and expression of soil characteristics. Physically, SOM promotes aggregation of primary soil particles, thereby improving tilth, aeration, and movement and retention of water (Emerson 1995). Chemically, it serves as a source of nitrogen, phosphorus, sulfur and trace elements essential for plant growth (Tiessen *et al*. 1994). It also provides exchange sites for buffering and nutrient holding capacity and enhances solubility and availability of minerals through chelation mechanisms (Oades 1989). Biologically, SOM affects activities of microflora and microfauna by serving as a source of energy for bacteria, actinomycetes, fungi and earthworms and it possibly acts as a growth-promoting substance for higher plants (Clapp *et al.* 1990).

Amounts and distribution of SOM are influenced by time, climate, vegetation, parent material and topography (Clapp *et al.* 1990). Organic matter does not accumulate

indefinitely in soils, but will attain an equilibrium with time that is governed by the other four factors. Climate is particularly important because it controls plant ecology, the quantity of plant material produced, the intensity of microbial activity in the soil and consequently, the decomposition rate. An important factor affecting the SOM content of agricultural soils is management. This encompasses such practices as tillage, fertilisation, grazing, residue return or retention and choice of cropping sequences.

In agricultural systems, declines in SOM are often associated with soil problems such as structural degradation, crusting, compaction, run-off and decreased fertility, all of which can lead to decreased crop yields. Decreases in soil organic carbon levels are well correlated with cultivation for extended periods (Russell and Williams 1982), lack of organic matter input (Jenkinson 1988) and increased proportions of cropping in the rotation (Grace *et al.* 1994).

To gain an increased understanding of the dynamics of SOM, and to assist in identifying management practices that are or are not sustainable in terms of maintaining SOM, researchers have developed models to simulate fluxes in SOM. Paustian (1994) reviewed the different approaches to modelling of SOM and presented the current state of knowledge in the area. Two of the more well known models are the Century (Parton *et al.* 1987) and Rothamsted (Jenkinson and Rayner 1977, Jenkinson 1990) models. Recently, a model (Socrates) has been specifically developed to predict changes in soil organic carbon in crop and pasture rotations in southern Australia (Grace and Ladd 1995).

Most models have been developed using empirical data from long term trials such as those at Rothamsted (Jenkinson and Rayner 1977) and the Waite Agricultural Research Institute (Grace *et al*. 1995). Where empirical data is lacking, most models generally include a number of assumptions. The accuracy of these assumptions can be tested retrospectively by comparing predicted values with observed values. Alternatively, research directed at investigating these assumptions can lead to the availability of accurate and realistic data to replace the assumptions.

One aspect of SOM modelling that remains an area of uncertainty relates to the belowground inputs of carbon by plants. Belowground root production is the main source of organic input for a wide variety of terrestrial ecosystems. However, the transfer of photosynthate belowground by roots is quantitatively the least understood of all the carbon cycling processes in soil (Oades 1995a). This has largely been because of the problems associated with observing roots, the simultaneous growth and death of root systems, and the difficulties associated with quantifying rhizodeposits that are secreted, exuded, lysed or respired from the roots (Böhm 1979).

This lack of understanding of belowground inputs of carbon applies to many natural and agricultural ecosystems. It is particularly relevant to the pasture phase of the rotations of southern Australia, where barrel medic (*Medicago truncatula* Gaertn.) is frequently sown. Barrel medic is one of the most important annual pasture legumes sown on the alkaline soils of Western Australia, South Australia and Victoria. It is a self-regenerating temperate species that is valuable for its nitrogen fixing ability and its high quality feed during autumn, winter and spring (Puckridge and French 1983).

Despite its value, very little is known about the belowground production of barrel medic and the contribution that it makes to the levels of organic matter in the soil, relative to other species commonly found in crop and pasture rotations in southern Australia. What is also unknown is the extent to which management can influence the amount of belowground input through manipulation of grazing intensity and frequency.

The aim of this study was to quantify the belowground input of organic carbon by barrel medic using techniques that account for root death and decomposition as well as root secretion and exudation. A secondary aim was to investigate the effect of defoliation on carbon allocation within the plant so as to determine the potential for optimising carbon input to the soil through grazing management.

The outcome of this research should be a better understanding of organic matter cycling in Australian agricultural soils and the ability to simulate these transformations more accurately through the use of models such as Socrates.

CHAPTER 2

LITERATURE REVIEW

1. Introduction

The purpose of this literature review is firstly, to examine the methodology directed at quantifying belowground inputs of organic carbon and secondly, to discuss some of the factors that affect the allocation of carbon belowground. Finally, some of the genetic, environmental and management factors that affect the belowground input of carbon will be reviewed in more detail. The focus is on annual legume pastures, particularly barrel medic (*Medicago truncatula* Gaertn.), but because of the lack of data in this area, many examples will be drawn from other agricultural species.

2. The role of barrel medic in the agricultural ecosystems of southern Australia

Barrel medic is one of the most important pasture legumes sown on the alkaline soils of Western Australia, South Australia and Victoria. Like subterranean clover (*Trifolium subterraneum* L.), it is a temperate annual legume species. It is self-regenerating. It germinates after the autumn break and produces high quality feed during the autumn, winter and early spring. In the spring, it flowers, sets seed and dies. The burrs and seed provide useful feed for sheep during the summer, but grazing should be managed so that enough seed is left to give a good germination in the following autumn. Barrel medic is easy to establish and persists well under heavy grazing (Cade 1984).

Barrel medic is often used in the cereal-ley farming systems of southern Australia (Puckridge and French 1983). This system of growing cereal crops in rotation with annual legume species was introduced to Australia in the 1950s (Donald 1965). The benefits of a pasture legume in the cropping rotation are well documented and have been reviewed by Puckridge and French (1983). This integrated pasture-livestock-crop system has led to increased crop yields, greatly increased livestock numbers, better protection of the soil and a more diverse and stable income. Increased crop yields have been due to increased soil fertility, especially nitrogen fixed by the legume pasture (Mullaly *et al.* 1967); greater water availability due to the improved soil structure resulting from the increased addition of organic matter (Greenland 1971); and reduced root disease severity following rotation with a non-host species (Rovira 1990).

3. The carbon cycle in agricultural plants

When considering the amount of carbon that is allocated belowground, it is useful to carefully define the pathway of carbon in agricultural ecosystems as it is photosynthetically fixed and distributed through the plant. This allows us to identify which processes are contributing to the belowground input of carbon and which processes are important to measure. Figure 2.1 represents a conceptual model of the partitioning and allocation of carbon by the plant and into the soil.

3.1 Aboveground allocation

As carbon is assimilated by the shoot through the process of photosynthesis, it first enters the metabolic pool (Figure 2.1). It is then either lost as shoot respiration,

incorporated in structural material as shoot growth or allocated belowground. Structural material is either grazed or harvested or it dies and begins to decompose. Depending on factors such as soil, climate, species and management strategies, some of this material decomposes on the surface of the soil and some of it may be incorporated belowground where it will add to the SOM pool. Where the aboveground material is grazed, it may be deposited as faeces. Eventually, it will experience a similar fate to plant material, and decompose on the surface of the soil or be incorporated belowground and added to the SOM pool.

3.2 Belowground allocation

Carbon allocated belowground by the plant first enters the metabolic pool of the roots (Figure 2.1). It is then either lost as root respiration, incorporated in structural material as root growth or lost to the soil as exudates or secretions. Exudates (such as sugars, amino acids, organic acids, hormones and vitamins) are water soluble and passively 'leak' from the root without the involvement of metabolic energy. Secretions (such as polymeric carbohydrates and enzymes) depend upon metabolic processes for their release (Whipps 1990). In some species, some carbon may be translocated back from the roots to the seed during the reproductive phase as grain filling or seedset occurs (Milthorpe and Moorby 1974). Translocation of carbon from roots to shoots may also occur during the vegetative stage if some sort of stress (*e.g.* defoliation) is applied to the shoots (Danckwerts and Gordon 1989).

Carbon that is incorporated into structural material continuously enters the SOM pool throughout the season as cells autolyse, as whole cells are sloughed off, and as roots die and begin to decompose. In the case of annual plants, there is a major input of

carbon at the end of the season as the plant senesces. In ecosystems composed of perennial species, there can be major inputs of carbon at different times of the year in response to different seasons, depending on the physiology and life cycle of the individual species.

In plants that exist in a symbiosis with mycorrhizal fungi or with rhizobia, a small proportion (about 2 to 8 %) of the metabolic carbon is directed to the maintenance of these symbiotic relationships (Kucey and Paul 1982, Rygiewicz and Anderson 1994). Depending on the technique used to estimate carbon inputs, it may or may not be possible to differentiate the carbon associated with these symbiotic relationships from that associated with the root. In those situations where it cannot be differentiated, mycorrhizal or nodule biomass that remains attached to the root is counted as root biomass and mycorrhizal or nodule respiration is counted as root respiration.

3.2.1 Rhizosphere respiration

Exudates and secretions provide an immediate source of energy for the microbial biomass (Whipps 1990). Much of the carbon in this form is quickly utilised by the rhizosphere micro-organisms and some is released by microbial respiration. The microbial biomass also acts on the dead plant residue in the soil and either mineralises it, incorporates it into biomass or assists in its physical and chemical protection.

Carbon allocated to the metabolic pool of the roots can be quickly lost as root respiration or it can be lost from the roots as exudates or secretions and quickly decomposed and mineralised by the microbial biomass. Whatever the mechanism, the end result is the same. When measuring respiration, it is generally difficult to

differentiate between root and microbial respiration. Consequently, the two sources collectively are called rhizosphere respiration (Whipps 1990). Techniques have been developed that attempt to individually quantify the two sources of root/soil respiration (Cheng *et al.* 1993, Swinnen 1994), but they are complex. Rouhier *et al.* (1996) developed and used a model accounting for the kinetics of exudate mineralisation so as to estimate respiration of rhizosphere microflora and roots separately. For sweet chestnut (*Castena sativa*) seedlings, they attributed 70 to 90 % of total respiration to root respiration. For wheat, Swinnen (1994) estimated that about 75 % of total respiration was directly derived from roots.

3.2.1.1 Inputs of carbon

The cumulative flux of these carbon allocation processes can be represented graphically. Figure 2.2 shows the hypothetical accumulation of carbon belowground in an annual species as the season progresses from germination and establishment through to flowering and senescence. The *live root biomass* generally shows a sigmoidal increase in growth, similar to that associated with shoots (Gregory 1994). Live root biomass reaches a maximum around flowering and then decreases as the plant fills its grain and then senesces.

(It is recognised that strictly speaking, the term 'biomass' means living mass (as opposed to 'necromass' - dead mass). However, general usage in the literature has led to the term biomass referring to material that once *was* living as well as material that *is* living. Hence, when live material is referred to specifically, it will be referred to as live root biomass.)

Total root biomass production is the summation of the increases in live root biomass throughout the season and it therefore includes all the roots that die and decompose during the season. In reference to the conceptual flow of carbon (Figure 2.1), it represents all the carbon that goes from the metabolic pool to the structural pool in the roots. The amount of biomass associated with this carbon is sometimes called the *net primary production* (Fogel 1985).

The *total input* of carbon into the soil organic matter pool can be defined as the summation of carbon in total root biomass production and carbon that is added to the soil as exudates and secretions (Figure 2.2). However, carbon in these rhizodeposits is generally rapidly turned over and lost as microbial respiration. *Total belowground allocation* of carbon is the summation of total input of carbon and root respiration carbon - *i.e.* all carbon that is allocated belowground by the plant. It is the total input of carbon that is of primary interest in this study, but because it is difficult to separate root-derived microbial respiration from root respiration as explained above, it can be difficult to estimate the total carbon input as such. A possible approach is to estimate total root biomass production and then to apply a correction factor to account for rhizodeposition (Swinnen 1994).

4. Techniques for measuring belowground inputs of carbon

Attempts to measure belowground inputs of carbon are essentially attempts to quantify the total root biomass production and the exudation and secretions that occur during the season. Because roots growing in soil are very much more inaccessible than shoots growing in the air, many of the methods available for their study are both time consuming and involve the destruction of the root environment itself (Gregory 1988).

Methods of studying the belowground input of carbon under crops and pastures can be classified into two broad areas. The first relates to the study of root biomass, encompassing all techniques which involve taking soil cores, washing the roots from the soil and measuring the biomass. The second is carbon isotope labelling, involving the use of isotopes such as ^{14}C, ^{13}C or ^{11}C to follow carbon allocation and distribution through the plant.

4.1 Root biomass studies

Techniques that relate to measurements of components of crop growth to determine belowground deposition of carbon can be collectively termed *crop studies* (Whipps 1990). This involves measurement of components of crop growth such as shoot biomass or root biomass. Carbon gained by photosynthesis and carbon lost from roots, shoots and soil respiration can be measured, thus enabling calculations of gross and net productivity. Crop study procedures can also measure changes in shoot and root masses and carbon contents of soils at various harvest intervals.

In practical terms, determination of shoot biomass involves harvesting and drying of shoot material whilst determination of root biomass involves taking soil cores and washing the roots out of the soil. This is perhaps the simplest way to determine belowground biomass, but it is often erroneous, as will be discussed later. These techniques are unable to quantify movement and distribution of carbon within the plant and into the rhizosphere with time. However, advantages include the flexibility and adaptability to examine a range of situations in the field with limited requirements for complex technical input.

4.1.1 Procedures

In studying root systems, measurements commonly used to describe root dynamics and distribution are number, mass, surface area, volume, diameter, root length, depth of rooting and number of root tips (Böhm 1979). In determining the properties to be monitored, the objectives of the research, as well as the time and labour involved in the analysis, need to be considered. Root mass is the most commonly used characteristic for studies of root growth, as it is a fundamental measure of photosynthate storage in a plant. In cases where the belowground productivity is to be determined, root dry weight is a good criterion for evaluation 'especially where the contribution of the roots to soil organic matter is to be studied' (Böhm 1979).

4.1.1.1 Sampling

Sampling techniques for determining root mass have many variations but basically consist of using an auger or sampling tube to take a core between 2.5 and 10 cm in diameter. This core is often separated into segments to enable the distribution of roots in the profile to be determined. Sampling procedure will vary with the type of crop or pasture. Where plant densities are low, roots can be sampled directly beneath the plants and corrections made to convert root length or mass to a unit area (Hamblin and Hamblin 1985, Pearson and Jacobs 1985). In high density situations, such as most annual pasture situations, individual plants cannot be separated. It is assumed that root distribution is uniform so cores are taken randomly and no corrections are necessary. However, because of the heterogeneous nature of soil, root distribution is not entirely uniform, so enough samples must be taken to ensure that a satisfactory estimation of the population is made.

In the case of row crops, root density decreases with increasing density away from the row. Sampling on the row will overestimate root mass whilst sampling midway between rows will underestimate root mass. Gajri *et al*. (1994) determined that for row crops, the best site for single-site augering is about one-third of the distance from the plant base to mid-way between the two rows.

Because root distribution within a plot can show a great deal of heterogeneity, it is common practice to obtain a number of soil core samples per plot and then bulk them (Böhm 1979). Schroth and Kolbe (1994) investigated the feasibility of subsampling of the homogenised bulk soil sample and determined that the required subsample size for a sample from a groundnut field was 5 to 10 % of the total soil sample. This procedure would save a lot of labour during subsequent sample processing in comparison with processing every sample in full. However, the optimum size ratio of subsample to total sample needs to be established for different plant communities under different conditions (Schroth and Kolbe 1994).

4.1.1.2 Separation of roots and soil

After sampling, roots must be separated from the soil. There are a variety of techniques to achieve this, depending on the texture and quantity of the soil. The greater the clay content, the more troublesome the washing procedure, so to facilitate the dispersion of soil particles, chemicals such as 0.27 % sodium pyrophosphate can be used (Böhm, 1979). The simplest technique is a washing process with a jet or spray of water, aided by hand manipulation. The soil-root sample is suspended in water and poured over fine mesh sieves where the roots are retained and collected for further cleaning.

Based on this principle, Cahoon and Morton (1961) developed and built a mechanical root separator that consists of special cans or buckets, fitted with tangential water jets at the base and a central overflow discharging on to a mesh screen which retains the roots. A modified version that was available commercially and that allowed buckets to be operated and emptied independently of each other was reported by Welbank (1975). This approach is better than washing root samples on a sieve because the roots are not continuously battered by water jets and stones, and coarse sand remains in the bucket and does not block the screen holes The method of Lauenroth and Whitman (1971), where soil samples are washed through a series of different size buckets, is based on a similar principle, although it appears that more manual labour is involved.

Alternatively, a flotation method can be used. This is less destructive for roots but takes longer. Air-dried soil-root samples are crushed through a 5 mm sieve and soaked, and the crushed small roots are collected by flotation (Böhm 1979). A modification of this that is now commercially available is the hydropneumatic elutriation system developed by Smucker *et al.* (1982). This is a mechanised system that separates roots from soil materials using the kinetic energy of pressurised spray jets and the low energy of air flotation. Quantitative separation of roots is achieved by a closed system of mechanical separations using water and air to isolate and deposit roots on a submerged sieve. A modified version of Smucker's system that could handle soil samples greater than 20 kg was developed by Chotte *et al*. (1995).

4.1.1.3 Separation of live roots and dead roots

Having separated the roots and other organic materials from the soil, it is then necessary to separate the roots from the organic debris. There is no easy way to achieve this and there are few reports in the literature on this process. The standard practice is to remove as much organic debris as possible by decanting (some of the organic debris may have a lower density) and then to float the sample in a shallow white dish and remove the impurities with tweezers (Schuurman and Goedewaagen 1971). This is a slow and laborious task.

It is often also necessary to distinguish and separate live and dead roots, but this is still one of the principal problems in root studies and one which requires time consuming work (Fiala 1990). There are a number of staining techniques used to assist in this process, such as the use of congo red (Ward *et al.* 1978) or triphenyltetrazolium chloride (Knievel 1973). However, these suffer from the disadvantage that it is difficult to quantify differences because of inconsistent intensity of staining. This is due to the several layers of cells in a root that differ in their rate of senescence. For example, when using congo red, dicot species are stained a lot less intensely than monocot species and there can be a range of staining intensities observed within a species (Ward *et al.* 1978). The other drawback is that staining techniques are laborious and not suited to large scale routine monitoring. And after using a staining technique to separate them, they still have to be physically separated by hand (Schuurman and Goedewaagen 1971).

Helal *et al.* (1996) proposed a technique that measured biochemical root constituents such as protein nitrogen, ATP and phosphatase activity as these were all correlated

with root age. Again, this technique is not suitable to large scale routine monitoring and the roots would be destroyed by the analytical procedure.

The most suitable technique for the separation of living and dead roots, on a relatively large scale, is still a morphologically based assessment similar to that described by Schuurman and Goedewaagen (1971). It is based on four major features: the elasticity of the root, its colour, the presence of a cortex and the presence of lateral roots. A dead root is far less elastic, is often greyer in colour, has either no cortex or a cortex that is ravelled and has lateral roots that have often been broken off, leaving stumps with frayed ends. The ease of assessment can be enhanced by suitable magnification. The degree to which these characteristics are expressed and their usefulness will vary with different species and environments.

4.1.1.4 Losses

Losses of biomass at each stage of handling is possible and attempts should be made to minimise potential sources of loss. In washing roots from soil samples, the proportion of fine rootlets lost will depend on the species and the mesh size that is used. Amato and Pardo (1994) compared a number of different mesh sizes and concluded that quantification of root length was more affected than quantification of biomass since losses from coarse screens were largely due to fine root fractions that have a high ratio of root length to biomass. Relative to a 0.2 mm sieve, a 1 mm sieve collected 75 % of the mass and a 2 mm sieve collected 55 % of the mass of wheat and faba bean roots. Böhm (1979) suggested that a mesh size of 0.71 mm (0.5 mm^2) was adequate for most situations.

Further losses of biomass and of carbon can occur during sampling, storing, washing and storage after washing. The remaining roots may lose part of their dry mass either by respiration before they die, by leakage of cell contents or by loss of part of the tissue. Van Noordwijk and Floris (1979) demonstrated that for wheat, these losses could amount to as much as 20 to 40 %. Grzebisz *et al.* (1989) found that for sugar beet, potential losses were even higher. They recommend that potential losses should be determined for each crop, time of sampling and methodology used, and results adjusted accordingly.

4.1.2 Estimation of total root biomass production

Many researchers measure root biomass only, usually at or around flowering. In an annual species, root biomass increases as the season progresses, until it reaches a maximum, somewhere between the onset of the reproductive phase and flowering (Gregory 1994). After this point, root biomass decreases as roots senesce and newly assimilated carbon is preferentially allocated to the reproductive organs. Consequently, the probability of sampling the root system at its moment of maximum biomass will depend upon the frequency and timing of sampling. Any deviation by more than a few days could lead to an underestimation of root biomass and root carbon.

Even if the timing is right, this approach grossly underestimates total root biomass production. During the season, there is usually a significant amount of root senescence and decomposition (Gregory 1994). Consequently, an estimation of root biomass at any one time will be a significant underestimation of cumulative or total

root biomass production to that time. Therefore, a method must be employed to derive total root biomass production from measurements of live root biomass.

Hansson and Steen (1984) explored the use of root sampling for calculating root biomass production in an annual crop in great detail. They proposed that the only way to monitor the fluctuations in root mass is to sample several times during the season. The sum of positive changes between successive sampling occasions gives an estimation of root biomass production which should be more accurate than determining the root maxima only.

However, this must be taken further. Production, death and decomposition of roots, along with translocation of stored material and possible grazing by animals such as earthworms, will occur simultaneously and at different rates. Consequently, a decrease in root biomass can occur despite production of new roots.

Hansson and Steen (1984) therefore calculated root biomass production in three ways:

1) The difference between the season's maximum and the previous autumn minimum amount of living roots (In annual systems, the autumn minimum will be zero);

2) The sum of the increases in living roots between sampling occasions (This is only different from the first method where there are positive and negative fluctuations in living root biomass during the season. This is unlikely to occur in most annual situations, except perhaps in grazed pastures); and

3) The sum of successive increases in both living roots and dead organic matter (This method may give the highest production because it may count root biomass production twice, first in living roots and then in dead organic matter in later sampling).

Using these three techniques on a barley (*Hordeum distichum*) crop, the first method yielded the lowest production (470 kg/ha) whereas the highest production was estimated using the third method (1370 kg/ha).

A problem with the first approach of Hansson and Steen (1984) is that the season's true maximum may not be correctly identified. One way of overcoming this is to increase the frequency of sampling. However, Milchunas *et al*. (1985) postulate that increasing sampling frequency in an attempt to better define maxima and minima may result in an overestimation of belowground net production. This is because of the artificial maxima and minima that can be generated by random sampling with variance - *i.e.* the more samples that are taken, the greater the probability of getting an extreme maximum or minimum.

In answering this claim, Vogt *et al*. (1986) concede that more frequent sampling can increase the opportunity for random fluctuations which can inevitably accumulate into extremely high estimates of root productivity. They argue that this problem is overcome by using statistical tests to distinguish between random fluctuations and potentially real changes, and then summing only significant changes in biomass and not non-significant changes.

Hansson and Steen (1984) surmise that all three calculation methods that they used still underestimate total root biomass production because losses through root mortality or root decomposition are not accounted for. They propose a more comprehensive method which involves obtaining the decomposition rates of soil litter and dead roots through incubation of the material in mesh bags in the soil. Using these decomposition rates, they outlined a series of equations that allow the calculation of

root mortality and the decomposition of dead roots which can then be added to increases in live root biomass to obtain the total root biomass production. This technique is discussed in more detail in Chapter 3.

Even if the suggested method of compensating for root mortality and decomposition (Hansson and Steen 1984) is used, total root biomass production could still be underestimated due to the loss of very fine roots and root hairs in the washing procedure and losses of carbon during sampling and storage before and after washing. Furthermore, in terms of belowground organic carbon input, the method still fails to take account of exudates and secretions from live, active roots.

Root biomass studies enjoy the advantage of being technically straightforward, and not reliant on expensive analytical equipment, but they are usually subject to large inputs of labour. Also, because of the losses associated with soil core sampling techniques and the inability to collect all the roots in the soil, it is not possible to directly calculate the total belowground input of carbon. To do this, techniques utilising isotopes of carbon need to be employed.

4.2 Carbon isotope labelling

The use of carbon isotopes, principally ^{14}C but sometimes ^{13}C or ^{11}C, enables us to study the pathway of carbon through a plant and into the soil (Warembourg and Kummerow 1991). The carbon isotope is incorporated through photosynthetic fixation of isotope-labelled CO_2 and then, following a given translocation period, measurements of the carbon isotope in various plant parts or soil are made. The methodology is based on the principle of isotope dilution, assuming that in any part of

the plant, a small amount of radioisotope, acquired through photosynthesis, is as evenly distributed as possible (Warembourg and Kummerow 1991).

4.2.1 Carbon isotopes

Approximately 98.89 % of carbon in nature is found as the stable isotope ^{12}C. The properties of the other isotopes which are relevant to this study are discussed in the following sections.

4.2.1.1 ^{14}C

Radioactive ^{14}C is the carbon isotope most commonly used for labelling studies in plant and soil science. Its low background concentration (only one atom in every 10^{12} carbon atoms is ^{14}C), long half-life (5730 years), beta emission and ease of detection by liquid scintillation counting make it very useful for isotope labelling. Being radioactive, there are restrictions on its use in the field and safety precautions that must be taken in its handling. These restrictions and precautions can make the use of ^{14}C impractical in some situations, especially in the field (Svejcar *et al*. 1990).

4.2.1.2 ^{13}C

The stable isotope ^{13}C comprises approximately 1.11 % of all carbon in nature. Many studies have used natural variations in the relative abundances of the two stable isotopes (^{13}C and ^{12}C) to make inferences about processes in plant and soil dynamics. In addition, plants can also be labelled with ^{13}C to assess carbon allocation (Svejcar *et al.* 1990). Being a stable isotope, it does not decay and it does not emit radiation,

therefore making it safe to use in all applications. A disadvantage is that because of the natural background concentration of ^{13}C, it is sometimes difficult to detect ^{13}C enrichment in the soil.

4.2.1.3 ^{11}C

An isotope that is rarely used in studies of carbon allocation in plants and soil is ^{11}C. It has a short half-life of 20.3 minutes, which makes it unsuitable for use in long term labelling studies and limits its use to specialised facilities where this short-lived isotope can be produced. ^{11}Carbon is a higher energy beta-emitter than ^{14}C and can be detected directly through several centimetres of tissue, thus allowing *in vivo* experiments studying carbon allocation (Magnuson *et al.* 1982). It has been used by Welker *et al.* (1985) to monitor carbon import by vegetative tillers of two bunchgrass species (*Paspalum plicatulum* and *Schizachyrium scoparium*). Because of its limited application in the quantification of carbon fluxes, the use of ^{11}C will not be discussed any further.

4.2.2 Labelling techniques

Most of the literature relates to the use of ^{14}C, because ^{13}C has only come to prominence in recent years as the regulatory requirements associated with the use of radioactive materials have increased (Svejcar *et al.* 1990) and as the accuracy of detection by mass spectrometry has improved (Barrie *et al.* 1995). Nevertheless, the principles and assumptions related to labelling and detection in the plant are similar for both isotopes.

Isotope labelling, be it with ^{13}C or ^{14}C, requires (1) an exposure chamber, designed to cause as little alteration to plants or the environment as possible; (2) a system for delivering the labelled CO_2 so as to provide labelled carbon at an adequate rate for normal plant uptake; (3) a procedure for sampling and preparation of sample that aims to prevent any loss (*e.g.* by respiration or leaching); and (4) an instrument for measuring the enrichment in samples containing the carbon isotope, such as an isotope ratio mass spectrometer for ^{13}C or a liquid scintillation counter for ^{14}C (Boutton 1991, Warembourg and Kummerow 1991).

Two fundamentally different strategies for labelling plants with carbon isotope have been developed - pulse labelling and continuous labelling.

4.2.2.1 Pulse labelling

Pulse labelling or pulse-chase refers to those techniques where the shoot of the plant is exposed to high specific activity $^{14}CO_2$ or high enrichment of $^{13}CO_2$ for a relatively short time, and distribution of the isotope is followed with time as it spreads through the plant (Whipps 1990).

Pulse labelling techniques have been used by a number of workers to investigate the carbon dynamics of a range of species. Delivery of a pulse of isotope requires an exposure chamber which causes a minimum disturbance to the plant (Warembourg and Kummerow 1991). In the field, these can range from large plastic tents for labelling herbaceous vegetation such as tallgrass prairie (Dahlman and Kucera 1968) or oilseed rape (*Brassica campestris*) (Rood *et al.* 1984), to a polythene bag for labelling wheat (*Triticum aestivum*) (Keith *et al*. 1986, Martin and Kemp 1986).

Saggar and Searle (1995) used a rigid perspex chamber to label a grazed pasture. In the glasshouse or laboratory, individual soil microcosms consisting of a clear perspex shoot chamber (28 cm length, 7 cm diameter) connected to a PVC root chamber (20 cm length, 7.5 cm diameter) have been used (Meharg and Killham 1990c, Rattray *et al.* 1995). Techniques have also been developed to label young poplar (*Populus* × *euramericana*) trees (Bassman and Dickmann 1985) and chestnut coppices (Mordacq *et al.* 1986).

If total belowground carbon allocation and soil/root respiration is to be collected, then the root zone beneath the labelled area must be isolated (by a PVC tube for example) and the root atmosphere must be separated from the shoot atmosphere (by some sort of cap or seal) so that soil/root CO_2 can be collected (*e.g.* Keith *et al.* 1986, Swinnen *et al.* 1994a). In the case of pastures, which tend to have a high plant density and are more prostrate in habit relative to crop species, the isolation of above and belowground atmospheres may be virtually impossible to achieve. Furthermore, if pulse labelling plants with ^{13}C, it is generally difficult to detect the pulsed ^{13}C in the soil or belowground respiration, because of the small amount of enrichment relative to the large background concentration of ^{13}C at natural abundance.

Pulse labelling has the advantage of ease and rapidity of use. However, it produces a non-uniform distribution of label throughout the plant and only gives information on the movement and losses of recently fixed carbon. Therefore, it is not possible to accurately monitor dying parts of older roots. This can be overcome by using a series of overlapping pulses and harvests and then interpolating data in between pulses to give an estimate of total belowground input for the season (*e.g.* Keith *et al.* 1986, Gregory and Atwell 1991, Swinnen *et al.* 1994b).

Allocation of carbon within the plant can be assessed after the isotope has left the metabolic pool of the shoots and has been either incorporated in shoot or root structural material, lost to the soil as exudates or secretions or lost as shoot or root respiration. The amount of isotope recovered in each plant part at the time of sampling represents the net production of labelled compounds since the day of exposure. The relative proportions thus obtained represent the distribution pattern of total net production over the period after labelling.

The period allowed for distribution of the isotope throughout the plant varies with species and environment. For example, in a study by Ryle and Powell (1976), all respiration associated with the synthesis of new structures in barley and maize had taken place within 7 days after ^{14}C incorporation. Conversely, Swinnen *et al.* (1994a) harvested wheat plants after allocation periods of 5, 19 and 33 days and found that ^{14}C allocation in shoot tissue and soil/root respiration was not complete until after 19 days.

Climate can have an effect on the measurement of allocation. Warembourg and Paul (1977) observed the effect of soil moisture on respiration. When the soil moisture was below wilting point, nearly all the evolution of pulse labelled carbon occurred during the first week following assimilation. At higher soil moisture contents, $^{14}CO_2$ evolution occurred over a longer period of 3 to 4 weeks. Wetting of the soil increased the resistance to CO_2 diffusion and diminished the CO_2 flux at the soil surface. Therefore the period of allocation may need to be investigated for each situation.

Some pulse labelling or pulse-chase techniques assess the fate of the labelled isotope a number of times after labelling. The amount of isotope recovered from each plant part after increasing time periods following the first sampling, can be compared to amounts

measured previously, thus allowing an estimation of losses through respiration, translocation, remobilisation and senescence. If changes in biomass, rates of loss from each component and distribution patterns of net production are known, it is possible to estimate the carbon production of each compartment and therefore total production (Warembourg and Kummerow 1991).

An alternative method of calculating root biomass production is the dilution technique first proposed by Caldwell and Camp (1974). It is based on the dilution of ^{14}C:^{12}C ratios in structural carbon of root systems of ^{14}C pulse labelled plants during the course of the growing season, as the plants grow and assimilate only ^{12}C. Structural carbon is used because the movement of labile carbon in and out of the root systems could confound the use of total root carbon, especially in perennial systems. Investigations by Milchunas *et al.* (1985) suggested that this technique has potential field application if sampling is performed the year after labelling. However, in a later paper, Milchunas and Lauenroth (1992) expressed some concerns with the dilution technique. They concluded that the decomposition of non-uniformly labelled root tissue results in differential changes in the ratio of ^{14}C:^{12}C through time. This proved to be of large consequence in their study of a perennial shortgrass steppe because root biomass generally declined during the course of their study, indicating high rates of death and decomposition relative to production. Nevertheless, these problems appear to be of more importance in perennial systems, and would be largely avoided in annual systems that are labelled early in the season. Most labelled material would be of a similar age class and would be equally prone to death and decomposition and root biomass would still be increasing for the rest of the season.

Stewart and Metherell (1996) pulse labelled a clover (*Trifolium repens*) and ryegrass (*Lolium perenne*) pasture with ^{13}C and followed the allocation of carbon to roots at 1 hour, 3 weeks and 8 weeks after labelling. They planned to sample these plots again in future years and to use the dilution technique to determine the rate of turnover of carbon in roots and long term carbon inputs.

4.2.2.2 Continuous labelling

The other form of labelling is continuous or steady-state labelling. Plants are grown from seedlings in controlled environment growth chambers in an atmosphere containing constant specific activity $^{14}CO_2$ or specific atom % $^{13}CO_2$. Examples of such growth chambers are the Experimental Soil Plant Atmosphere System (ESPAS) described by Gorissen *et al.* (1996) and the controlled environment chamber of Martin *et al.* (1992). Plants are generally grown in repacked soil cores, although Martin *et al.* (1992) obtained intact soil cores (300 mm diameter x 1 m depth) from the field and transferred them to their chamber where they continuously labelled wheat from early tillering to anthesis. Continuous labelling in the field is difficult due to the technical complexity of maintaining a realistic environment (Saggar and Searle 1995). However, it has been attempted by Warembourg and Paul (1973) who labelled native grass in the field for up to 33 days.

Dual labelling of plants with isotopes was attempted by Johansson (1993) who continuously labelled plants in a growth cabinet with ^{14}C-labelled CO_2 in the atmosphere for a period, imposed defoliation treatments on the plants, and then regrew them for a further period with ^{13}C-labelled CO_2 in the atmosphere. This use of two

carbon isotopes allows the assimilation of carbon to be followed before and after cutting.

The major advantage of continuous labelling is the homogeneity of label distribution that it achieves and this allows ready calculation of the total input of carbon into the soil (Meharg 1994). Another advantage of continuous labelling with ^{14}C is that the soil microbial biomass may become highly labelled (assuming suitable activity of $^{14}CO_2$ has been fed to the plant). This allows a quantitative assessment of carbon inputs into the microbial biomass to be made (Liljeroth *et al*. 1990).

4.3 Other techniques

There are a number of other approaches to estimating the input of carbon in different agricultural systems. Theses include calculations based on the dilution or enrichment of natural ^{13}C abundance by C_3 or C_4 plants, calculations utilising the enrichment in atmospheric ^{14}C that resulted from thermonuclear testing in the 1960s, and calculations using models that have already been developed and validated.

4.3.1 Natural ^{13}C abundance

One approach makes use of the difference that exists in the natural ^{13}C abundance of plants that fix carbon via the C_4-dicarboxylic acid photosynthetic pathway (C_4 plants) compared with plants that fix carbon via the Calvin cycle pathway (C_3 plants) (Smith 1972). By growing C_4 plants on soils previously exposed only to C_3 species, or vice versa, this difference in isotope ratio may be utilised to quantify the input of carbon by the present species. This technique has been used by Skjemstad *et al.* (1990) and by

Balesdent and Balabane (1992, 1996). This technique is only possible where it is certain that only plants with one type of pathway have grown, yet it is still possible to grow plants with the other type of pathway. Cadisch and Giller (1996) proposed a modification of this technique to allow the estimation of the contribution of carbon by a C_3 legume in a mixed community of grasses with a C_4 pathway, where historically the vegetation was dominated by trees with a C_3 pathway. Their technique requires the existence of a pure grass pasture that was established next to the mixed pasture at the same time, to provide baseline measurements.

4.3.2 'Bomb' carbon

Another approach to estimating the belowground input of carbon is to utilise the doubling of ^{14}C content of the atmospheric CO_2 that occurred for a short time in the early 1960s as a result of thermonuclear testing. Jenkinson and Coleman (1994) determined the radiocarbon content of pre- and post-bomb samples from six experimental sites in southern England. Knowing the ^{14}C enrichment of the atmosphere and the decomposition rates of the soil organic matter, they were able to determine the annual input of organic matter at these sites. This approach relies on the availability of soil samples from the period before there was any enrichment of the soil. A method proposed by O'Brien (1984) allows for the calculation of carbon input rates without the historical record of ^{14}C enrichment in the soil.

4.3.3 Soil organic carbon models

Yet another approach to calculating the annual input of organic matter to the soil is to utilise models that have already been developed to describe the turnover of organic

carbon in soil. Instead of running the model to calculate the accretion of organic matter from known annual inputs, the model is run in reverse to calculate the annual input. This requires a knowledge of how much organic matter is in the soil, the history of the soil, its texture and the prevailing climate. Jenkinson *et al.* (1992) utilised the Rothamsted model (Jenkinson 1990) to do this and concluded that the approach has many advantages, including the integration of annual inputs over many years from a single sampling. To use this approach, it is firstly necessary to have a functional, well validated model that accurately models organic carbon turnover for the particular soil, climate and vegetation type.

5. Root:shoot ratios

Just as total shoot production can vary widely in response to many biotic and abiotic factors, so too can total root biomass production. As it is generally a lot easier to measure shoot production than root biomass production, it is sometimes useful to define root production in terms of shoot production *i.e.* as a root:shoot ratio. Hence, if the amount of shoot production is known, and an approximate root:shoot ratio is known from other studies, then it is possible to make an approximate estimate of root biomass production.

A discussion of some of the factors that affect root:shoot ratios will follow in the section on factors affecting the belowground allocation and input of carbon. It is known that the growth of roots and shoots are interdependent as each compartment supplies essential materials for the growth of the other (Klepper 1991). If it can be explained how this interdependency works, then it may be possible to make more accurate estimates of the belowground input of carbon in response to changes in biotic

and abiotic conditions. A number of different models have been proposed to explain how this works.

5.1 Allometric models

Pearsall (1927), Troughton (1956) and Chalmers (1987) all suggested that there is an allometric relationship between roots and shoots. They proposed that the relative growth rates of each compartment are proportional to each other for a given species in a particular environment. In other words, there is a general tendency for any species to maintain a characteristic relationship between root and shoot dry weight. Consequently, if a limiting factor affects root growth, then shoot growth will also be affected, so that the allometric relationship is maintained. When the limiting factor is removed, then root growth will be restored to its potential and shoot growth will compensate so that the allometric relationship is still maintained. Alternatively, when part of either root or shoot is removed (by grazing for example), the plant tends to compensate in such a way as to return to a root:shoot ratio characteristic of the species (Klepper 1991). The drawback of this approach is that this homeostatic phenomenon is an empirical relationship only and it makes no attempt to explain growth processes.

5.2 Functional equilibrium models

Brouwer (1962) proposed a functional equilibrium model such that the relationship between shoot and root is regulated by the activity of the compartment. Davidson (1969a) supported this stating that the product of root mass and rate of absorption was proportional to the product of shoot mass and the rate of photosynthesis. This means that the allocation of assimilate between the two compartments is not just a factor of

their relative size but also of their activity. Nevertheless, this again is an empirical model and 'no attempt is made to define the causal mechanism' (Davidson 1969a).

5.3 Thornley's partitioning model

Thornley (1972) proposed a mathematical model based on carbon and nitrogen uptake and transport. Later, this model was extended by Brugge and Thornley (1984) to a vegetative nodulated legume, such as clover or medic, whose nitrogen supply comes from nitrate uptake by the root and nitrogen fixation by the rhizobia, and where the rhizobia are supplied with carbohydrate by the host plant. This model reasons that the compartment that obtains the resource uses as much as it needs and the residue is then available for use by the other compartment. For instance, under nitrogen deficiency, the greater part of the nitrogen taken up is used by the root system for root growth, thus increasing the root:shoot ratio. Deficiencies of other minerals or water would have the same effect. Conversely, under carbohydrate effect (*i.e*. low light), the opposite mechanism would work.

A variation of this idea is the model of Van der Werf *et al*. (1993). They suggest that carbon and nitrogen is allocated to roots and shoots as a function of the nitrogen status of the plant. Under steady-state conditions, allocation of carbon and nitrogen to leaves is positively correlated with plant nitrogen concentration whereas allocation to roots is negatively correlated.

As these models are mechanistic and based on a theoretical framework, they do not suffer from the limitations associated with empirical models. However, despite predicting directions of change quite well, it is difficult to make accurate quantifiable

predictions because of the intensity of data needed at a plant process level. Furthermore, there still remains the question of how allocation functions are regulated or controlled.

5.4 Hormone models

It has often been suggested that root:shoot ratios are controlled by plant hormones (Scott Russell 1977). A hormone based model therefore would suggest that the root produces a hormone that controls the shoot and vice versa. However, despite there being evidence that exogenous application of hormones can affect root:shoot ratios (Stenlid 1982), there is still little evidence for how the production of hormones are stimulated and how they are detected and decoded by the target organ. Because of the potential complexity involved in a model of this type, it would be difficult to quantify root:shoot relationships using this approach.

To summarise this section, it appears that the above mentioned models are not totally adequate for use in predicting the root growth of a medic pasture, either because of their empirical nature or because of the complexity of specific data needed to run them. Nevertheless, after obtaining empirical data for a given species in a given situation, it may be valuable to see how they fit the models available as this gives an indication of the extent to which an empirically obtained root:shoot ratio can be extrapolated to other situations.

6. Factors affecting belowground allocation and inputs of carbon

Belowground carbon allocation has been studied either specifically or incidentally (as a result of root studies for other purposes) by a number of workers. The root systems of crop and pasture species have been studied to determine the effects of such factors as nitrogen (Hamblin *et al.* 1990, Johansson 1992), phosphorus (Davidson 1969b), potassium (Ozanne *et al*. 1965) and other fertilisers (Cakmak *et al*. 1994), pH (Meharg and Killham 1990c), air temperature (Meharg and Killham 1989), root temperature (Williams 1972), salinity (Gregory 1988, Cordovilla *et al.* 1994), CO_2 concentration (Paterson *et al.* 1996), anaerobiosis (Meharg and Killham 1990a), plant density (Pearson and Jacobs 1985), shading (Butler *et al.* 1959), tillage practices (Barber 1971) and heavy metal concentrations (Davies 1991).

Carbon distribution also varies with the age or stage of development of the plant. Generally, the root:shoot ratio decreases with increasing phenotypic development, with relatively less assimilated carbon being allocated belowground as the plants develop (Fernandez and Warembourg 1987, Meharg and Killham 1990b). Root biomass production and root:shoot ratios are also subject to genetic influences (Klepper 1991).

In this literature review, three major influences on root production will be examined in more detail - genetic effects (genotypic differences), environmental effects (moisture stress) and management effects (defoliation).

6.1 Genotypic differences

Hamblin and Hamblin (1985) tried to distinguish genotypic effects from environmental effects on the depth of rooting of a range of annual pasture and grain legumes. They did this by growing the same legumes at three sites where the only essential difference was rainfall. They found that maximum depth of rooting was significantly different between genotypes but non-significant between sites, hence implying that root length is genetically controlled. It is likely that root biomass production and belowground carbon input are subject to similar controls.

6.1.1 Perennial vs annual species

There are some fundamental differences in carbon allocation between perennial species and annual species. In fact, Monsi (1960) used carbon partitioning as the basis for classifying plant forms into annuals or perennials. Annuals must complete their whole life cycle in a very short time span. In order to accomplish rapid growth and large seed production, carbohydrates are mainly channelled into the formation of new leaves and reproductive structures, whilst root growth is relatively small (Schulze 1983). Many perennials on the other hand allocate a lot of their carbon to storage organs, especially belowground storage organs, and these can be utilised for aboveground growth at a later time (Schulze 1983). Consequently, only a small amount of carbon allocated belowground can actually be considered as an input of carbon to the soil.

The relative differences between annual and perennial forms of the same genus have been studied by Warembourg *et al.* (1990) who looked at carbon partitioning in an

annual bromegrass (*Bromus madrintensis*) and a perennial bromegrass (*Bromus erectus*). Nine months after establishment, the root:shoot ratio was 0.5 in the annual species and 1.0 in the perennial species. Daily rates of carbon allocation were determined four times during the season by pulse labelling plants with ^{14}C. Early in the season, when plants were in the vegetative stage, 60 to 70 % of carbon was partitioned belowground in both species. As the annual species reached the flowering stage, only 10 % of recently fixed carbon was allocated belowground compared to 40 % by the perennial species. However, of the amount allocated belowground by the annual species, the proportion allocated to the rhizosphere increased whilst that allocated to actual root growth decreased.

It appears that in determinate annual grasses, the roots stop growing after the stem extension stage and they begin to invest relatively more carbon in the rhizosphere, while the perennial species continue to increase their biomass. What happens in subsequent years when the perennial root system is well established and the perennial species undergoes a full reproductive period is uncertain (Warembourg *et al*. 1990).

In comparing the distribution of pulse labelled ^{14}C in an annual (*Lolium temulentum*) and a perennial (*Lolium perenne*) species of ryegrass, Ryle (1970) reported similar results to Warembourg *et al.* (1990). After labelling the youngest fully expanded leaf with ^{14}C, Ryle (1970) found that the source leaf retained 30 to 40 % of the labelled carbon and 20 to 25 % was translocated to the terminal meristematic region. Nearly all the remaining radiocarbon was translocated to tillers and roots in the perennial species. In the annual species, the tillers and roots shared this radiocarbon with the stem. But again, this experiment tells us little about what happens in subsequent years.

One possibility is that everything else being equal, an established perennial species will put more carbon belowground than an annual species, because it already has a large photosynthetic capacity at the beginning of the season and its growing season is longer. A more likely possibility is that over a number of years, a succession of annual plants will allocate more carbon belowground than a long-lived perennial plant because of the need to continually rebuild the root system each year. The extent to which this happens will depend on the species and whether the season is favourable for the growth of annual species (Ridley and Simpson 1994).

Differences in patterns of belowground allocation of carbon between annual and perennial species could be related to the degree of determinancy. Even within annual species, there exists large differences in the degree of determinancy. Gregory (1994) explained that in determinate species such as cereals, the mass of the root system rarely increases after flowering. Conversely, in many legumes (which are generally indeterminate) there can be an increase in the mass of the root system during early grain-filling. This means that if the conditions are favourable, indeterminate species continue to allocate a significant amount of carbon belowground late in the season.

6.1.2 Grasses vs legumes

Ozanne *et al*. (1965) showed that root:shoot ratios were mostly higher in grasses than in legumes, but they offer no explanation why. Davidson (1969a) found a similar pattern amongst some cool temperate pasture species. Boote (1977) suggests that this is because shoot growth of non-legumes is probably limited by the availability of nitrogen in the soil whereas legumes can fix atmospheric nitrogen if soil nitrogen is not available. Shinano *et al.* (1994), in a study of eight grasses and seven legumes,

found that root growth in grasses was generally higher than in legumes. They related the differences in root growth between grasses and legumes to the nitrogen absorbing ability of the roots. Dry matter accumulation per unit amount of nitrogen accumulated in the plant was higher in the grasses than in the legumes. However, as the experimental field was naturally fertile (Shinano *et al.* 1994), it is unlikely that nitrogen was a limiting factor, as it often is in Australian soils.

In contrast, Gregory (1988) surmised that the root:shoot ratio of grain legumes at maturity is probably in a similar range to that for many cereal crops, although during early growth, legumes appear to have heavier root systems relative to the shoot than do the cereals. If this was so, it might be related to seed size and the influence that this can have on early root growth (Asher and Ozanne 1966, Evans 1973). Evans (1977) found no consistent differences in root:shoot ratios in a study of three clovers and five pasture grasses after 8 weeks of growth.

6.1.3 C_3 vs C_4 species

The mechanism of photosynthetic carbon fixation, which is genetically determined, can also affect the root:shoot ratio. Above root temperatures of 20 °C, the tropical grasses (C_4 photosynthesis) have lower root:shoot ratios than the temperate grasses (C_3 photosynthesis) (Davidson 1969a). This general difference could be predicted by the better water use efficiency associated with the C_4 pathway of photosynthesis. In C_4 species, more carbon is fixed per unit of water than in C_3 plants and consequently, the same size root system can support a larger amount of shoot (Boote 1977). Below 20 °C, the response is the opposite as C_4 plants are not fixing carbon as effectively and

carbon that is fixed is preferentially allocated to the roots according to Thornley's partitioning model described in section 5.3 of this chapter (Bastow Wilson 1988).

6.1.4 Species and cultivar differences

Root growth also varies with species and cultivar. Within the *Medicago* genus, there exist a number of annual forage species. Derkaoui *et al*. (1990), in a study of biomass partitioning in some of these species, found that there were differences in root mass between species and that these differences changed with time. Early dissimilarities between species may have been related to seed size whereas differences at the end of the growth cycle were associated with maturation (Derkaoui *et al*. 1990). This is supported by Asher and Ozanne (1966) who found a strong correlation between seed mass and early rate of elongation.

Humphries and Bailey (1961) studied 13 species and varieties of *Trifolium* and found that root growth generally correlated with length of growing period and flowering date. Within a species, root biomass of subterranean clover (*T. subterraneum*) at flowering ranged from 1120 kg/ha for the early flowering Dwalganup variety to 2480 kg/ha for the late flowering Tallarook, indicating a developmental variation related to cultivar. Williams (1972) also found a difference in root growth and root:shoot ratios between four lines of subterranean clover, but there was a strong interaction with root temperature. Xu and Juma (1993, 1994) found a variation related to cultivar alone. They pulse labelled two cultivars of barley that had similar phenotypic development rates and found significant differences in carbon allocation, indicating that belowground allocation of carbon is controlled by the cultivar.

In contrast, Martin and Kemp (1980) found no significant differences in ^{14}C allocation between 11 continuously labelled wheat cultivars and Liljeroth *et al.* (1990) found no difference in ^{14}C in microbial biomass in the rhizospheres of two wheat cultivars labelled with ^{14}C. Nevertheless, where plant breeders have selected for differences in shoot morphology and yield, they have most likely inadvertently also selected for root properties as well (Klepper 1991). Siddique *et al.* (1990) found that modern varieties of wheat had lower root:shoot ratios than old varieties. This lower investment in root dry matter partly explained the increased harvest index and water use efficiency of the grain - traits that have been selected for in modern varieties.

Having established that significant and basic genotypic differences exist, it must be realised that the extent to which these are expressed in the field is greatly influenced by environmental factors, one of which is discussed in detail in the next section.

6.2 Moisture stress

The agricultural ecosystems of southern Australia are characterised by large and erratic variations in growing season rainfall (Puckridge and French 1983). When this is combined with summer rainfall that is sporadic and usually non-effective, the result is that soils can vary from very dry to very wet. More often, crops and pastures are exposed to periods of dry conditions, and this moisture stress impacts on plant growth, root growth and the belowground allocation of carbon.

6.2.1 Plant growth

The overall effect of soil moisture deficits in most cases is to decrease net production because water is essential for plant growth. Water stress affects practically every aspect of plant growth and causes changes in the anatomy, morphology, physiology and biochemistry of plants. Belowground, water stress can have an effect on root size, distribution, depth and length, and on the amount of carbon being released from the roots.

The most obvious effect, short of death, is a reduction in plant size. This is partly because the lower turgor of pressure potential in stressed plants results in less cell expansion and hence, in smaller organs. There is also a reduction in the rate of photosynthesis resulting from the closure of stomata, and under long term stress, there is a reduction in the capacity of the chloroplasts to carry out photosynthesis (Turner *et al*. 1984). In either case, the result is a smaller plant with less photosynthetic surface, and in turn, a smaller capacity to produce photosynthates and growth than is possessed by an unstressed plant. Consequently, in absolute terms, the response of root growth to moisture stress is negative (Kramer and Boyer 1995).

6.2.2 Carbon allocation

In contrast, the usual response of root:shoot ratios to deficits in soil moisture is to increase. A survey of many plants showed that in herbaceous species, there was a tendency for root:shoot ratios to increase from moist to mesic to xeric species (Bray 1963). Species growing in a dry environment generally had higher root:shoot ratios than the same species growing in a mesic or moist environment (Struik and Bray

1970). Wheat grown in a dry season had higher root:shoot ratios than when it was grown in a wetter year (Hamblin *et al.* 1990). *Lolium perenne*, *Trifolium repens* (Davidson 1969b) and wheat (Li *et al.* 1994) all had increased root:shoot ratios when grown at lower water contents than when grown at higher water contents. Furthermore, it has been shown that root:shoot ratios of faba beans (*Vicia faba*) increase during periods of moisture stress (El Nadi *et al.* 1969).

An explanation for this response to moisture stress can be derived from Thornley's partitioning model of root:shoot allocation (Thornley 1972). When water is in short supply, assimilate is directed more towards the root than when water is not limiting (Bastow Wilson 1988). This is as would be predicted by the partitioning model of Thornley, where the demands of the root (the source in this case) are met before the demands of the shoot (the sink). It appears that water deficits limit shoot growth more than photosynthesis, as an increase in carbohydrate concentration is often observed during periods of short term water stress (Bastow Wilson 1988).

6.2.3 Root exudation

It appears that moisture stress has an effect on both the amounts and the kinds of exudates of carbon from roots (Hale and Moore 1979). In a study of lodgepole pine (*Pinus contorta*), Reid and Mexal (1977) showed that trees subjected to a water stress of -400 kPa exuded more ^{14}C pulse labelled material through their roots than trees at 0 or -200 kPa. However, trees at 0 kPa exuded a greater proportion of the labelled material which was translocated to the roots than either the -200 or -400 kPa treatments. In the drier soil, more material was lost through the roots, but more material was also translocated to the roots. Martin (1977) investigated the effect of

soil moisture on the release of organic carbon from wheat roots and suggests that both the secretion of carbon as mucilage and the release of carbon into the soil by lysis of root tissue, is increased by water stress.

In summary, although there is little in the literature about the effect of moisture stress on total belowground inputs of carbon, especially by medic pastures, it appears that moisture stress leads to a decrease in the absolute amount of carbon contributed to the soil by plants, but an increase in the relative amount of assimilated carbon allocated belowground.

6.3 Defoliation

When examining the carbon dynamics and the belowground production of a pasture, the effects of defoliation must be considered, because, in southern Australia, it is for grazing that a pasture is primarily intended. Grazing has many complex effects upon a pasture, the most obvious of which is the removal of photosynthetic tissue. This is also the most important effect when considering the carbon dynamics of the system. Some other longer term effects of grazing upon a pasture include changes in pasture quality, botanical composition, soil structure (and hence water-holding and infiltration characteristics) and nutrient distribution (through the concentration of nitrogen in dung and urine), but these issues will not be directly addressed here.

The effect of grazing on most plant characteristics (*e.g.* photosynthesis, respiration, growth) can be conveniently represented by one of the three hypotheses shown in Figure 2.3, which has been adapted from a figure by McNaughton (1983). In the line

labelled A, grazing leads to an immediate detrimental effect on the plant and this effect increases as the frequency or intensity of grazing increases.

In the line labelled B, there is either no effect on the plant or the plant is able to compensate exactly so that the net effect on the plant is zero. This continues up to a point, beyond which the plant cannot adequately compensate, and there is then a negative effect on the plant which increases with increasing frequency or intensity of grazing. This critical level is likely to vary with species and environment.

In the line labelled C, moderate levels of grazing lead to an overcompensation by the plant so that there is a net increase in the variable being measured. Where line C is found to apply to aboveground production (or persistence), then the apex of the curve represents the level of optimal grazing from the perspective of the plant (Belsky 1986). In support of this hypothesis, Paige and Whitham (1987) concluded that some plants may benefit from the effects of herbivory. Eventually however, at higher levels of grazing, the effect on the plant becomes negative. Implicit in all three hypotheses is the observation that extreme levels of grazing (overgrazing) will ultimately lead to plant death (McNaughton 1983).

Within a plant, not all responses to grazing can be described by a single hypothesis. As will be seen in the following discussion, it is possible that one variable in a plant may immediately suffer a detrimental effect (A), whilst another variable may appear to be unaffected (B) whilst a third variable in the same plant may exhibit a positive compensation effect (C). In the same manner, it is possible that the response of a single plant may not be the same as the response of the population for a given variable. A simple example of this is that a decrease in leaf area on a single plant may be offset

by an increase in plant numbers so that the net effect on the population is no change in leaf area.

6.3.1 Aboveground production

In general, the aboveground response of plants to close or frequent defoliation is a reduction in total biomass production (Youngner 1972), although the extent of this reduction will be modified by other factors such as soil, climate, nutrients, light and time of clipping (Milchunas and Lauenroth 1993). Hence, aboveground production can generally be described by hypothesis A or B (Figure 2.3) except in instances where the removal of rank growth by grazing can allow an increase in photosynthesis and an increase in production (Doyle *et al*. 1993). The response of aboveground production to defoliation, and the importance of leaf area index and radiation interception is more fully discussed in a review by Simpson and Culvenor (1987).

6.3.2 Root biomass

It has often been observed that defoliation leads to a decrease in live root biomass (Youngner 1972) thus fitting hypothesis A or B (Figure 2.3). It is thought this is a direct result of the reduction in the amount of photosynthetically active tissue. Contrasting with this, in a review of 236 data sets worldwide, Milchunas and Lauenroth (1993) found the response of root mass to grazing was highly variable. In fact, more sites displayed a positive than a negative response to grazing in terms of root mass, suggesting that many populations could be following a response similar to hypothesis C. Whereas the effect of grazing on aboveground production averaged -23 %, the effect on root mass averaged +20 %. Furthermore, positive effects on root

mass occurred in 61 % of the sites where grazing had negative effects on aboveground production.

The findings of Milchunas and Lauenroth (1993) highlight a very important point with respect to measurement of root mass. Most of the studies that they reviewed made no distinction between live root biomass and total root biomass. The failure to do this impacts on the conclusion. What is likely to happen in response to grazing is that some root death may occur and hence, live root biomass may decrease. However, in many instances, the dead roots are unlikely to decompose or disappear at a rapid rate. The larger diameter roots which would have contributed greatly to the measured root mass in most of these studies, would be particularly slow to decompose and disappear. Hence, because these dead roots are still present at sampling, total root mass would be relatively unchanged in many of these studies. Furthermore, as the plant recovers from a defoliation event, new live roots may be produced, thus adding even more to the total root mass of a grazed pasture. Therefore, the conclusion of Milchunas and Lauenroth (1993) that defoliation can lead to increases in *total* root mass is not necessarily at odds with the statement of Youngner (1972) that defoliation reduces *live* root biomass.

6.3.3 Total root biomass production

As discussed earlier, live root biomass and even total root mass are likely to be underestimations of total root biomass production or total belowground input of carbon, because of the amount of root death and decomposition that occurs during a season. Milchunas and Lauenroth (1993), highlight that root mass, as measured in most of the studies they reviewed, underestimates total belowground production.

Nevertheless, they suggest that differences in root mass between grazed and ungrazed systems are probably indicative of root biomass production 'unless turnover is much less on grazed treatments' (Milchunas and Lauenroth 1993).

This statement of Milchunas and Lauenroth (1993) might not necessarily be true. Reports in the literature suggest that differences in root mass between grazed and ungrazed systems might *not* be truly indicative of root biomass production. Furthermore, it seems likely that turnover or root death may be *greater* on grazed treatments. Using a mini-rhizotron or root periscope, Richards (1984) observed a significant reduction of about 50 % in visible root length in Eurasian bunchgrass (*Agropyron desertorum)* following defoliation. Hodgkinson and Baas Becking (1977) grew wallaby grass (*Danthonia caespitosa*) in root observation boxes and found that relative to the uncut control, root mortality increased by about 10 % after defoliation to 40 mm height. Butler *et al.* (1959) showed defoliation led to a loss of roots and nodules in white clover, but this was more than overcome by new growth, leading to a rapid turnover of root and nodule tissue. In fact, under recurrent defoliation, the root systems of white clover, red clover (*Trifolium pratense*) and lotus (*Lotus uliginosus*) all underwent a cyclic pattern of decay and renewal (Butler *et al.* 1959).

The results of these studies lead to the conclusion that the net belowground input of carbon under grazing or defoliation can be different from an ungrazed or uncut situation, which is in contrast to what Milchunas and Lauenroth (1993) concluded above. In fact, total root biomass production and total belowground inputs of carbon can be greater under defoliation. In support of this, Sims and Singh (1978), in their survey of ten western North American grasslands, found that net root production was significantly higher on grazed treatments compared to ungrazed grasslands.

In conclusion, it seems that live root biomass, total root mass, root turnover and total root biomass production all respond differently to grazing or defoliation. The extent to which they respond will be partly determined by the intensity, frequency and timing of defoliation. To understand this better, it is necessary to consider the general physiological response of a plant in response to defoliation.

6.3.4 Physiological responses to defoliation

The immediate physiological response to defoliation will depend on the intensity of the defoliation, but some general trends can be observed. Total plant photosynthesis is immediately reduced because of the decrease in photosynthetic tissue available. This impacts on the roots and on carbon allocation within the plant as discussed in the following sections. Compensatory photosynthesis and translocation are two processes that may be found in some plants as a response to defoliation, and these are also discussed.

6.3.4.1 Root responses

Shortly after defoliation, root elongation and root growth slows considerably (Richards 1993). Davidson and Milthorpe (1966) showed that root extension stopped completely when cocksfoot (*Dactylis glomerata*) was cut to a height of 2.5 cm. In absolute terms, root respiration also decreases very quickly after defoliation. This was shown by Culvenor *et al.* (1989b) when they defoliated subterranean clover plants growing in swards in pots. They removed 80 % of shoot dry weight and found a decline of over 60 % in root respiration by the end of the first light period. This decline was associated with a decline in nitrogenase-linked respiration as well as root

and nodule maintenance respiration. Johansson (1993) also found that total rhizosphere respiration decreased immediately after cutting. Furthermore, it began to increase again after 7 to 8 days of regrowth. Because of the dual labelling approach that was used in this experiment, it was possible to determine that this regrowth was entirely due to photosynthate assimilated *after* defoliation. The plants had overcome the immediate effects of defoliation and were now in a recovery stage.

6.3.4.2 Carbon allocation

In the first 2 to 3 days immediately following defoliation, the supply of photosynthate to roots is reduced, not only because of reduced total photosynthesis, but also because of greater allocation to aboveground growth as the plant attempts to restore its lost leaf area (Richards 1993). Often there is a short-term decline in photosynthetic rates of the remaining leaves as well. Undamaged leaves on defoliated lucerne (*Medicago sativa*) had net photosynthetic rates 4 to 20 % lower than comparable leaves on undefoliated plants on the day of defoliation (Hodgkinson 1974).

Rate of regrowth after defoliation depends on whether or not the apical meristem has been removed; the level of carbohydrates in remaining organs; the potential photosynthetic rates of leaves that were previously shaded; root mass and activity; and the environment, especially temperature (Pearson and Ison 1987). Carbohydrate concentrations in the root are important because respiration, especially of roots, increases during early regrowth and small amounts of carbon move into the tissues which grow in the first 5 to 10 days after defoliation. Culvenor *et al.* (1989a) showed that in subterranean clover, root growth ceased for periods of greater than 5 days when plants were defoliated by even just 30 % of shoot dry weight. In the period after

defoliation, carbon allocation favoured leaf over branch and root, and in an 80 % defoliation treatment, there was a net loss of carbohydrate from the root. Culvenor *et al.* (1989b) also demonstrated that root respiration rates decreased immediately after defoliation, but in the period of early regrowth, during which photosynthetic capacity was being restored to pre-defoliation levels, root respiration comprised a relatively large respiratory cost of up to 75 % of net photosynthesis.

6.3.4.3 Compensatory photosynthesis

The next stage in the recovery of plants from defoliation sees a re-establishment of the whole-plant carbon balance. This occurs because of the growth of new leaves and shoots and also because of an increase in the photosynthetic capacity of the remaining leaves. Compensatory photosynthesis can be defined as an increase in the photosynthetic rates of foliage on partially defoliated plants, relative to foliage *of the same* age on undefoliated plants (Nowak and Caldwell 1984). Although not all species exhibit this phenomenon (*e.g*. uniculm *Hordeum vulgare*, Ryle and Powell 1975), compensatory photosynthesis response often occurs between 2 days and 2 weeks after defoliation (Richards 1993). It can be due to a change in light supply as the architecture and light interception of the sward is changed, as demonstrated by Woledge (1977) who looked at *Lolium perenne* (perennial ryegrass). Alternatively, it can also be due to a delay in leaf senescence and increased soluble protein concentrations (Nowak and Caldwell 1984).

Where compensatory photosynthesis does occur, it can mean that belowground carbon flows are rapidly restored to pre-defoliation levels. In relative terms, there may be preferential allocation of carbon to leaves and shoots (Richards 1993), but in absolute

terms, there may be a greater total allocation of carbon to roots to replace the roots that may have died following defoliation. Consequently, this could lead to a situation described by hypothesis C (Figure 2.3) where low intensity or frequency of grazing can lead to overcompensation and result in an increase in a plant function; in this case, total belowground allocation of carbon.

6.3.4.4 Translocation

A confounding factor in perennial systems is that there may be a significant amount of translocation of carbon from the roots back to the remaining stubble and newly expanding leaves in the period shortly after defoliation (Richards 1993). For example, Buwai and Trlica (1977) found only small differences in root mass among undefoliated and heavily defoliated western wheatgrass (*Agropyron smithii*) plants, but the defoliated plants had significantly less carbohydrate reserves than the undefoliated plants because of translocation aboveground. Johansson (1993) suggested that a decrease in labelled ^{14}C in the roots of meadow fescue (*Festuca pratensis*) after defoliation was due to translocation to new shoot tissue.

Perennial legume species such as lucerne, and to a lesser extent red clover, also rely on carbohydrate root reserves for regrowth after cutting (Smith 1962, Smith and Marten 1970) but this can depend on how much stubble is remaining after defoliation. Stubble leaves of lucerne can substitute, in part or completely, for the supply of carbohydrate to stubble shoots normally derived from reserves mobilised in the tap-root (Hodgkinson *et al.* 1972).

In annual legume species such as subterranean clover, there is less evidence for retranslocation of carbohydrate carbon. May and Davidson (1958) showed a slight reduction in non-structural carbohydrates in roots of subterranean clover following defoliation in August, but not following defoliation in September or October. Furthermore, they speculate that there was probably a translocation of carbohydrate *to* the roots as there was a large decrease in non-structural carbohydrate in remaining aboveground tissue following defoliation. It appears that in annual legume species, roots do not act as a carbohydrate reserve to nearly the same extent as in perennial species, but this can depend on the severity of the defoliation. Culvenor *et al.* (1989a) found that there was significant remobilisation of carbohydrate reserves from the roots of subterranean clover following removal of 80 % of the shoot dry weight but not following removal of 30 % of the shoot dry weight. They also concluded that mobilisation of nitrogenous compounds may be of equal or greater importance after severe defoliation.

7. Conclusions

Whipps (1990), in his review of carbon economy in the rhizosphere, summarised much of the work that has been done in the last two decades. He concluded that the belowground input of carbon by a wheat crop can lie somewhere between 1200 and 2900 kg C/ha/year. Van Veen *et al.* (1991) put the figure between 900 and 3000 kg C/ha/year. Based on a combination of estimates derived from carbon dioxide exchange rates and extrapolation from other results, Alston and Puckridge (1986) estimated the belowground translocation of carbon by a barrel medic pasture to be about 1200 kg C/ha/year. Apart from this single estimate, there is a general lack of

information in the literature about the belowground input of carbon by barrel medic pastures.

All techniques available to measure the amount of root growth and belowground inputs of carbon have advantages and disadvantages associated with them. Selection of an appropriate technique involves a trade off between accuracy, labour and technical complexity. A detailed discussion and explanation of the techniques used in this study is presented in Chapter 3.

Research areas of specific relevance to the agricultural ecosystems of southern Australian are the performance of barrel medic in relation to alternative rotations, the effect of moisture stress on the belowground inputs of carbon, and the effect of defoliation or grazing on carbon inputs. Chapter 4 details an experiment comparing the total root biomass production of a barrel medic pasture with that of a barley grass pasture and a faba bean crop. This experiment was conducted over two seasons including a very dry one, so the effect of moisture stress is also considered. In Chapter 5, the effect of defoliation on the total root biomass production of a barrel medic pasture is investigated. In Chapter 6, the effect of defoliation on the total carbon balance of barrel medic is examined in more detail through an isotope labelling experiment in a growth cabinet.

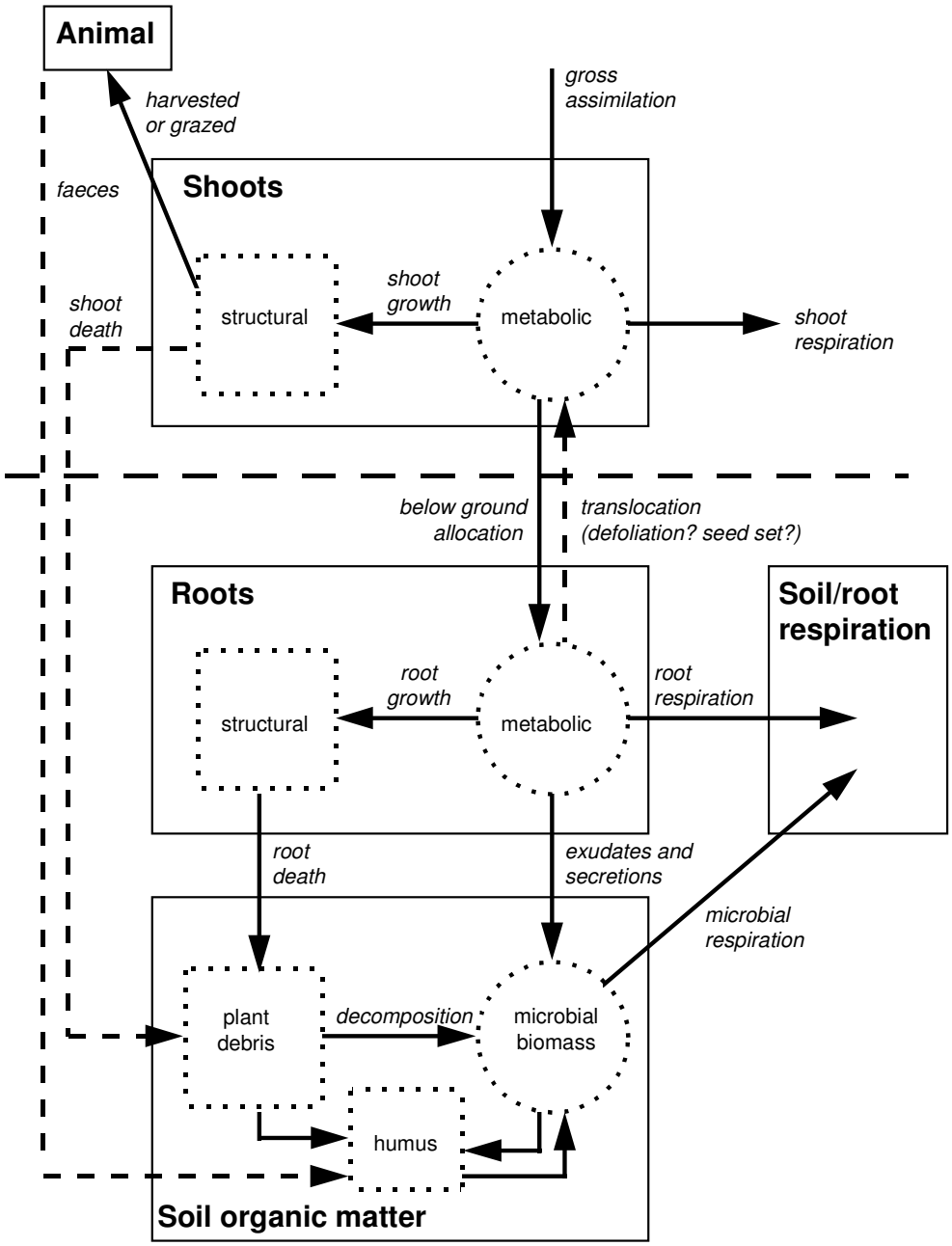

Figure 2.1. Conceptual model showing the allocation and partitioning of carbon in the plant and in the soil. (Adapted from Swinnen *et al*. 1994b.)

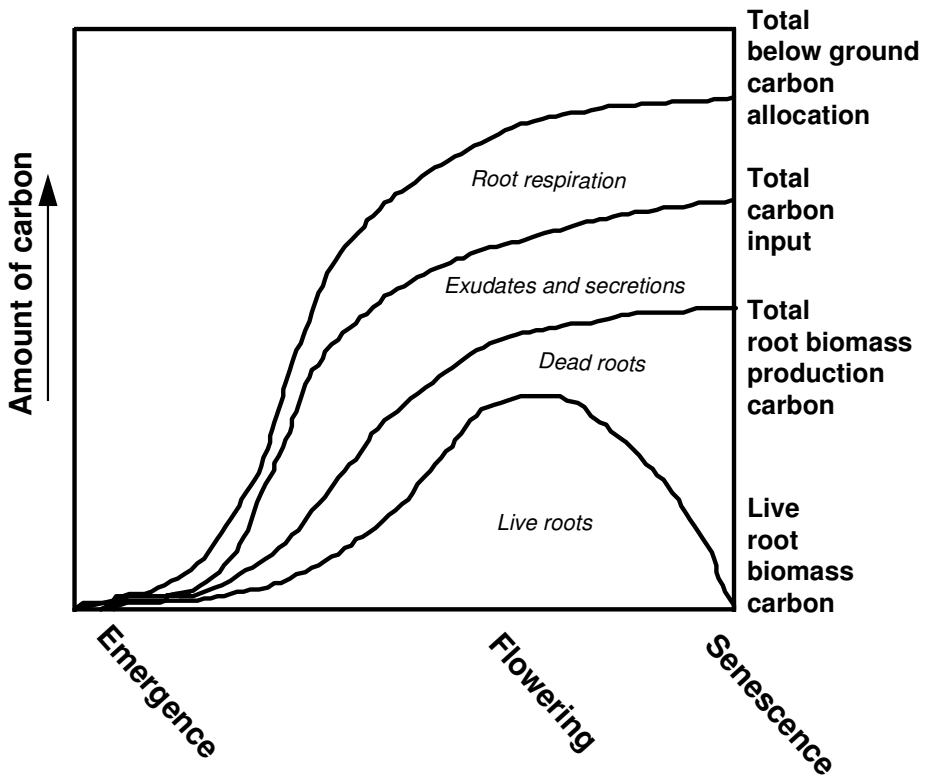

Figure 2.2. Conceptual accumulation of carbon belowground in an annual species as the season progresses from germination and establishment through to flowering and senescence.

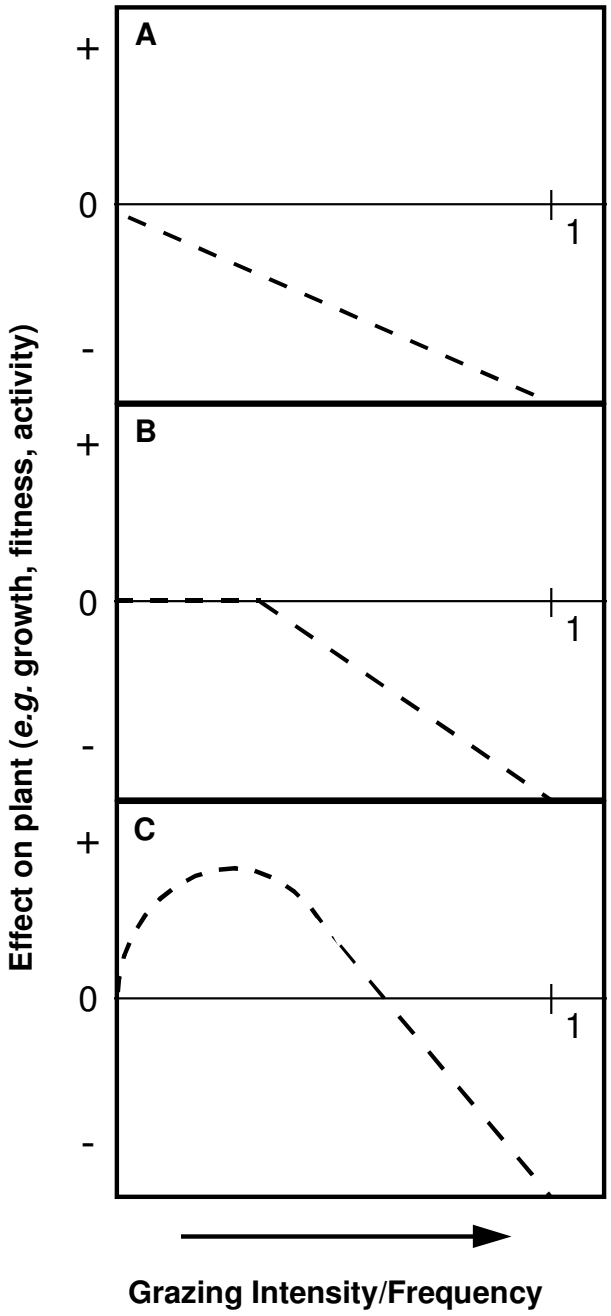

Figure 2.3. Three alternative hypotheses about how herbivory may affect plant characteristics. (Adapted from McNaughton 1983.)

CHAPTER 3

METHODOLOGY - THEORY AND CALCULATIONS

1. Introduction

In the previous chapter, a number of different approaches to measuring root biomass production and the belowground input of carbon were briefly discussed. In this chapter, a more detailed explanation of the techniques that were used in this study is presented, along with some discussion of the assumptions made. The techniques that will be discussed are the summation technique, the ^{13}C isotope dilution technique and the dual labelling technique.

2. Summation technique

2.1 Theory

The summation technique is the name given to the approach proposed by Hansson and Steen (1984) for measuring the root biomass production of an annual crop. It is a method of measuring root biomass production that also accounts for the losses of roots through death and decomposition.

A similar, but less comprehensive approach was presented by Persson (1978) and then developed further by Fairley and Alexander (1985) for the calculation of fine root production in forests. Using this similar approach, Persson (1978) estimated the annual fine root production of three perennial forest species in central Sweden as

1830, 300 and 1350 kg/ha respectively whilst the corresponding increase in fine root biomass only totalled 610, 160 and 590 kg/ha respectively. This means that root mortality was an extra 1.3 to 2.0 times the increase in root biomass during the season.

All summation methods originate from a basic equation adapted from population dynamics:

$$B_{t+1} = B_t + \text{production} - \text{mortality} \qquad \text{(Equation 3.1)}$$

where B is a measure of biomass and t is a time of sampling. This equation can be rearranged.

$$\text{Production} = B_{t+1} - B_t + \text{mortality} \qquad \text{(Equation 3.2)}$$

Often, an approximation of production is made by assuming mortality is equal to zero. Consequently, production is assumed to be the difference between biomass at the beginning of the period in question and biomass at the end.

$$\text{Production} = B_{t+1} - B_t \qquad \text{(Equation 3.3)}$$

In the case of an annual species, biomass at the beginning of the season is also equal to zero, which therefore leaves us with the following:

$$\text{Production} = B_t \qquad \text{(Equation 3.4)}$$

Obviously, this is not correct as root turnover during the season is a well recognised phenomenon in the field. Consequently, the methodology proposed by Hansson and Steen (1984) is very useful as it allows root mortality and disappearance to be accounted for. Despite this, reports of its use in the literature are infrequent. This is most probably because of the intensely laborious hand separation of live and dead roots that is necessary, as will be seen in the following detailed explanation.

In order to explain the calculations used in the summation technique, it is first necessary to describe the carbon flows in an annual arable crop or pasture (Hansson and Andrén 1986) (Figure 3.1). Carbon is fixed photosynthetically by the aboveground or living shoot compartment of the plant. Carbon that is not lost as respiration is either incorporated into shoot material or allocated belowground to root biomass production. Carbon allocated to shoot material is either grazed or harvested, or it forms part of the senescent shoot material fraction during the season or at the end of the season (Figure 3.1).

Carbon allocated to root biomass production becomes part of the macro-organic material fraction (MOM). MOM is defined as all the organic material captured on a 0.5 mm mesh sieve after wet separation. MOM consists of living and dead roots originating from the crop of the current year, as well as dead roots from previous years and aboveground residues previously ploughed under. MOM can be separated into living roots (LR) and dead organic material (DOM). DOM consists of old organic material (OOM) from previous years and dead roots (DR) from the present crop (Figure 3.1). In most instances, LR can be distinguished from DR because they are lighter in colour, more flexible and more intact than dead roots. Detailed separations

such as this require intensive manual procedures, as there are presently no satisfactory automated procedure available.

2.2 Calculations

If we follow the pathway of carbon through the plant (Figure 3.1), some of the carbon photosynthetically fixed in the plant aboveground is translocated belowground (F_1) to the living roots (LR), some of which then die (F_2) and become dead roots (DR). Dead roots then decompose (F_3) to form part of the soil humus with carbon eventually being released as CO_2. Immediately before the growing season of an annual pasture or crop, LR and DR are equal to 0 and all MOM that is present is from previous years (*i.e.* MOM = DOM = OOM).

The OOM fraction decreases during the growing season due to decomposition (F_4) and the only possible input to DR (and hence to DOM) comes from the living roots. If the amount of OOM (or DOM) at the beginning of the season is known and the disappearance rate of OOM is known, then the decreasing amount of OOM during the growing season can be calculated as follows:

$$OOM_{(t)} = OOM_{(t-0)} \times k_{OOM(t)} \qquad \text{(Equation 3.5)}$$

where t = sampling occasion and $k_{OOM(t)}$ = proportion of OOM incubated in litter bags at t=0 still remaining at time t.

The quantity of DR can then be calculated as the difference between DOM and OOM:

$$DR_{(t)} = DOM_{(t)} - OOM_{(t)} \quad \text{(Equation 3.6)}$$

However, this underestimates DR as there is decomposition of DR during the season (*i.e.* F_3). If we know the disappearance rate of DR, then we can determine the amount of dead root disappearance as follows:

$$F_3 = \sum_{t=1}^{n} \left(k_{DR} \times DR_{(t-1)} \right) \quad \text{(Equation 3.7)}$$

where k_{DR} = the proportion of dead roots that have disappeared between t and t-1 as measured using *in situ* litter bag incubations.

We can then calculate the amount of root death (F_2) by summing the differences between successive calculations of DR, and correcting for dead root decomposition (F_3):

$$F_2 = \sum_{t=1}^{n} \left(DR_{(t)} - DR_{(t-1)} \right) + F_3 \quad \text{(Equation 3.8)}$$

Total root biomass production (F_1) is then calculated by summing the differences between successive measurements of LR and correcting for root death (F_2):

$$F_1 = \sum_{t=1}^{n} \left(LR_{(t)} - LR_{(t-1)} \right) + F_2 \quad \text{(Equation 3.9)}$$

The factors k_{OOM} and k_{DR} are estimated by incubating OOM and DR material from the current year in nylon mesh bags. The incubation is made in the same field and

during the same growing season that LR is being measured. The other variables that must be measured are OOM at the start of the season and the amount of LR and DOM at each sampling occasion.

2.3 Discussion

One assumption that is made in this technique is that the flow of biomass carbon is one way, *i.e.* there is no translocation of carbon from the root back to the shoot (Figure 3.1). Translocation is known to happen in some perennial species as a response to defoliation (Danckwerts and Gordon 1989) or in annual species at the end of the season during the period of grain filling (Milthorpe and Moorby 1974). The extent to which this occurs in barrel medic in response to defoliation is examined further in the experiment reported in Chapter 6.

The major advantage of the summation technique is that it does not require elaborate technical facilities, yet it still allows a reasonably complete assessment of root biomass production. One disadvantage is the large input of labour required. Furthermore, the technique is based on the measurement of roots greater than a certain size (0.5 mm diameter in this case). Consequently, it underestimates production of finer roots and rootlets.

This technique is used in the experiment reported in Chapter 4 where the root biomass production of a barrel medic pasture, a barley grass pasture and a faba bean crop are compared.

3. ^{13}C dilution technique

3.1 Theory

The ^{13}C dilution technique is an adaptation of a technique that was first used by Caldwell and Camp (1974) to calculate belowground net primary productivity (BNPP) or total root biomass production. In the past, the ^{14}C isotope has been used for the dilution technique (Caldwell and Camp 1974, Milchunas *et al.* 1985, Milchunas and Lauenroth 1992). As there are now safety restrictions on the use of ^{14}C in the field, I have devised a method for the use of the stable ^{13}C isotope. It is based on the dilution of the ratio of $^{13}C_{excess}$ to ^{12}C (established by pulse labelling with ^{13}C) during subsequent growth when plants assimilate only ^{12}C and ^{13}C at natural abundance (Caldwell and Camp 1974). $^{13}C_{excess}$ is the amount of ^{13}C in excess of natural abundance.

3.2 Calculations

A turnover coefficient (TC) is calculated:

$$TC = [(^{13}C_{excess\ t1} / {}^{12}C_{t1}) / (^{13}C_{excess\ t2} / {}^{12}C_{t2})] - 1 \qquad \text{(Equation 3.10)}$$

where $t1$ is 7 days after labelling and $t2$ is at the end of the season. Live root biomass (LR) at $t1$ is then multiplied by the TC to give the amount of root biomass production for the period from $t1$ to the end of the season.

$$BNPP_{t2\text{-}t1} = LR_{t1} \times TC \qquad \text{(Equation 3.11)}$$

Where the pasture consists of an annual species, this root biomass production can be added to the biomass present at $t1$ to give an estimate of BNPP or total root biomass production for the growing season, so long as $t1$ is early enough in the season so that there is no significant root death and decomposition before $t1$.

$$BNPP = BNPP_{t2\text{-}t1} + LR_{t1} \quad \text{(Equation 3.12)}$$

3.3 Discussion

It is important that only living roots are used to determine the ratio of the two isotopes. If dead roots are included, there will be a tendency for the turnover coefficient to be underestimated (Caldwell and Camp 1974). At the first sampling (7 days after labelling), it would be expected that very little (if any) of the labelled isotope would be included in dead root material. Since the dead root component would be expected to decompose more quickly than that which was living at the first date, there would be a tendency for the dilution of the ratios to be less than if the ratio determinations were performed on living roots only.

A second important consideration is that an adequate period of time is allowed between labelling and the first sampling. If new ^{13}C is allocated to the roots during the time between the first and the end-of-season samplings, then the turnover coefficient will be underestimated because the ratio will not be diluted as much (Caldwell and Camp 1974). It is necessary to allow a sufficient period of time for carbon in the shoot to be allocated, be it to respiration or to structural material in the shoots or roots.

Caldwell and Camp (1974) and Milchunas *et al.* (1985) recommend the use of structural or cell wall material for the determination of the isotope ratio. This is because there is often a significant amount of flux of labile carbon in and out of the root system in perennial species which can lead to inconsistent amounts of the labelled isotope in the roots. In annual species however, the flow of carbon is more unidirectional with very little translocation of carbon from the roots to the shoots (as discussed in Chapter 2, section 6.3.4.4), so it is not necessary to use structural material for the determination of the isotope ratio.

A major assumption of the isotope dilution technique is that decomposition or disappearance of labelled root material occurs at the same rate as for unlabelled root material. This assumption has been criticised by Milchunas and Lauenroth (1992) who suggested that in a perennial system, non-uniform labelling and non-uniform decomposition are likely to occur. They argue that because labile ^{14}C is only incorporated into functional tissue, the incorporation of labile ^{14}C into structural tissue would be proportional to the growth rate of the tissue and its sink demand. Consequently, actively growing roots would be likely to receive more ^{14}C than older roots. Furthermore, these actively growing roots are more likely to die and decompose later than roots which received little or no ^{14}C.

These factors are unlikely to be so important in an annual system. Firstly, early in the season, most roots will be actively growing and hence, will have incorporated labelled isotope into structural tissue more uniformly. Secondly, in an annual system, the problem of younger roots dying later than older roots is less pronounced. The labelled isotope would be incorporated into freshly grown tissue which may contain a combination of primary, secondary and tertiary roots as well as root hairs. As the

season progresses, the secondary and tertiary roots are just as likely to disappear as roots formed earlier.

This technique is used in the experiment reported in Chapter 5, where the effect of repeated defoliation on the root biomass production of a barrel medic pasture is investigated. Subsequent to the completion of the experiment using this novel approach (Chapter 5), a report of the use of ^{13}C for pulse labelling in this manner has been published by Stewart and Metherell (1996). They are using the method to investigate the seasonality of carbon cycling in perennial pastures based on white clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*) and the influence of irrigation and phosphatic fertiliser on carbon inputs to the soil.

4. Dual labelling technique

Dual labelling (^{13}C and ^{14}C) of plants has a number of physiological applications (Meharg 1994) and is well suited to the study of carbon reallocation and allocation of new photosynthate following defoliation (Johansson 1993). It has been used for this purpose in the experiment described in Chapter 6 investigating the effect of defoliation on barrel medic. By continuously labelling plants with ^{14}C between the seedling stage and cutting, and with ^{13}C during the period of regrowth after cutting, it is possible to follow the utilisation for regrowth, both of residual plant carbon in roots and stubble, and of newly assimilated carbon. It is necessary to have a second set of plants that can be destructively sampled at the time of the first cut so that the enrichment and distribution of ^{14}C at this time can be determined.

4.1 Calculations

To make a carbon budget for the second growth period (when the plant contains both ^{14}C and ^{13}C), comparable units for ^{14}C-labelled C and ^{13}C-labelled C must be used. Consequently, the following calculations must be applied to convert ^{14}C-labelled C (kBq) and ^{13}C-labelled C (atom % ^{13}C excess) to ^{14}C-labelled C (mg) and ^{13}C-labelled C (mg) respectively.

Firstly, the amount of ^{14}C-labelled C (mg) in plant parts at the second cut can be calculated by relating the measured value for ^{14}C (kBq) of each plant part to the mean specific activity (SA) of ^{14}C (kBq/mg C) of the stubble plus roots at the time of the first cut, as carbon compounds in both stubble and roots could theoretically be utilised during regrowth.

$$^{14}\text{C-labelled C (mg)} = {}^{14}C_{sample}\ (\text{kBq}) \,/\, SA_{stubble + roots}\ (\text{kBq/mg C}) \qquad \text{(Equation 3.13)}$$

Secondly, amounts of ^{13}C-labelled C (mg) in samples can be calculated by relating to the atom (at) % ^{13}C excess in the ^{13}C-labelled atmosphere as follows:

$$^{13}\text{C-labelled C (mg)} = \frac{(\text{at \% } {}^{13}C\ \text{excess}_{sample} \times \text{total } C_{sample}\ (\text{mg}))}{\text{at \% } {}^{13}C\ \text{excess}_{atmosphere}} \qquad \text{(Equation 3.14)}$$

4.2 Discussion

To grow plants in a continuously labelled atmosphere (as is required by this technique), it is necessary to have a facility to control temperature, humidity and CO_2 level within a confined area. Due to the financial and technical requirements of such a facility, this can be considered a disadvantage of this technique. Where the facility is available, this technique can provide detailed information on the complete carbon budget and its response to defoliation. The one exception is that it may not be possible to detect enrichment of ^{13}C in the soil because of the high natural abundance of ^{13}C relative to the amount of ^{13}C that is released (Johansson 1993). This will depend on the specific activity of the ^{13}C in the atmosphere, the amount of carbon released from the roots and the volume of the soil.

The other disadvantage of this technique is that it cannot be undertaken in the field. Consequently, many of the artefacts associated with growing plants in repacked soil in pots apply. One potential way of combating this is to take undisturbed cores from the field and transfer them to a growth cabinet in a manner described by Martin *et al.* (1992).

This technique was used in the experiment described in Chapter 6 where the effect of a single defoliation on the carbon allocation of a sward of barrel medic plants growing in pots was investigated.

5. Conclusion

The three techniques described in this chapter range in technical complexity, in the accuracy and extent of information that they provide, and in their application. The summation technique, which allows the calculation of total root biomass production, can be performed in the field and requires no advanced facilities, although specialised root washing machines help to facilitate the separation of the macro-organic material from the soil. The ^{13}C dilution technique, which also allows the calculation of total root biomass production, can be readily carried out in the field provided special labelling chambers are constructed. However, analysis of ^{13}C enrichments in plant material requires access to an isotope ratio mass spectrometer. The dual labelling technique requires elaborate technical facilities for growth and labelling of the plants and for ^{14}C and ^{13}C analyses, but extensive information is obtained on the belowground allocation of carbon and its response to defoliation.

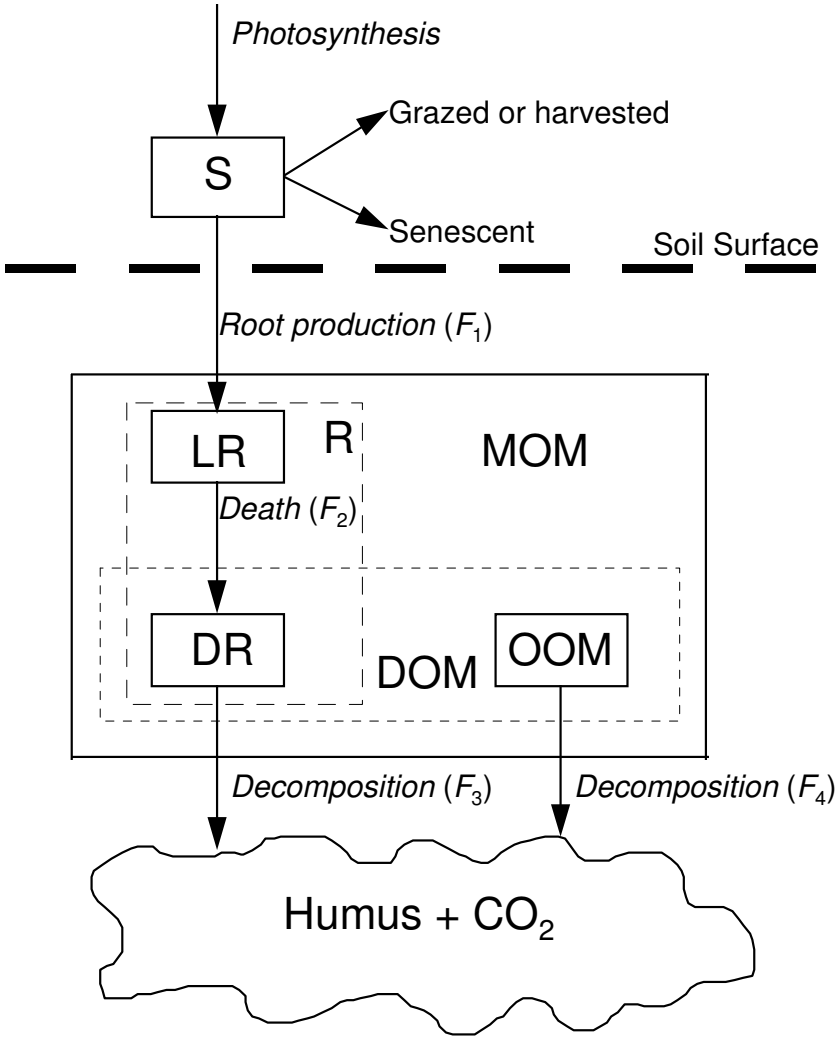

Figure 3.1. Conceptual model of carbon flow in an arable annual crop. (S=shoots, R=roots, LR=live roots, DR=dead roots, MOM=macro-organic material, DOM=dead organic material and OOM=old organic material) (From Hansson and Andrén, 1986).

CHAPTER 4

EXPERIMENT 1: TOTAL ROOT BIOMASS PRODUCTION OF A BARREL MEDIC PASTURE, A BARLEY GRASS PASTURE AND A FABA BEAN CROP

1. Introduction

In many arable systems, belowground inputs of carbon through the roots represent a major input into the soil organic carbon pool. In annual systems, a major input of carbon comes at the end of the growing season as the plants mature and senesce. There is also a significant input of carbon throughout the season as roots die or are sloughed off. In addition, there can be a significant amount of carbon released into the soil as exudates and secretions (Whipps 1990). Consequently, total root biomass production for a season can be difficult to measure.

Most measurements of root biomass fail to account for the turnover (death and decomposition) of roots during the season and are therefore only valuable in giving some idea of the biomass present at any one time. To measure total root biomass production, and to get a better estimate of the annual belowground input of organic carbon into the soil, it is necessary to use a technique that accounts for the death and decomposition of roots during the season. One such technique is that proposed by Hansson and Steen (1984). It estimates total root biomass production from repeated, simultaneous measurements of living roots, dead organic material and decomposition rates of dead roots and old organic material.

Under conditions found in the cereal/livestock zone of southern Australia, data on root production by different species is sparse, yet it is so important if we are to have a better knowledge of how management can affect soil organic carbon levels. There is some information on cereal crops (Schultz 1974, Keith *et al*. 1986) and grain legume crops (Gregory 1988) but little on the annual pasture species that might be grown in a cropping rotation. Humphries and Bailey (1961) studied the mass and distribution of roots at flowering of eight species of *Trifolium* grown in a sandy soil under high rainfall (about 900 mm). Hamblin and Hamblin (1985) investigated the maximum depth of rooting, the root length density and the distribution of root length with depth of a range of temperate grain and pasture legume species, again on sandy soils. Alston and Puckridge (1986) monitored root mass under a barrel medic pasture, but an estimation of total root biomass production was not within the scope of their work. Information on root biomass production by annual grass pastures is non-existent, yet many pasture phases still contain a significant proportion of grass pasture. Ridley and Simpson (1994) measured the root length density of annual ryegrass (*Lolium rigidum*) grown in a duplex red soil over two seasons with contrasting rainfall patterns and found that early growth is dependent on the timing of the opening rainfall.

The main purpose of this study was to use the sequential coring and summation technique of Hansson and Steen (1984) to quantify the total root biomass production of a barrel medic pasture. A further aim was to compare it with the root biomass production of some common alternative choices found in crop/pasture rotations in southern Australia, namely a grass pasture such as barley grass (*Hordeum leporinum*) and a grain legume crop such as faba beans (*Vicia faba*). The theory behind the summation technique and the calculations necessary to determine total root biomass production are detailed in Chapter 3.

2. Materials and methods

2.1 Trial details

The experiment was located at the Roseworthy campus of the University of Adelaide, approximately 60 km north of Adelaide, South Australia. The predominant soil on the experimental site was a Hypercalcic Calcarosol (medium, slightly gravelly, loamy/clay loamy, moderate) with some areas of Supracalcic, Red Chromosol (thick, non-gravelly, loamy/clay loamy, moderate) soil (Isbell 1996). According to the US classification system, it is a Calcic Rhodoxeralf (Soil Survey Staff 1994). The 0-100 mm layer of soil had an organic C content of 17 g/kg and a mean pH (H_2O 1:5) of 7.7.

The experiment consisted of three treatments: a barrel medic pasture (*Medicago truncatula* Gaertn. cv. Paraggio), a barley grass pasture (*Hordeum leporinum* Link) and a faba bean crop (*Vicia faba* L. cv. Fiord). Three replicates of each treatment were arranged in a complete randomised block design. Each plot was 100 x 50 m. The experiment was conducted in 1994 and then repeated in 1995 on an adjacent area where soil types were similar.

All treatments were established on land that had been under annual grass pastures for the previous two years. The medic pasture was established by direct drilling seed at 50 kg/ha before the opening rains. Seed did not germinate until after the opening rains, which fell in mid-June in 1994 and mid-May in 1995. The medic pasture was sprayed with selective herbicides to eliminate grass species and broadleaf weeds. The grass pasture consisted of volunteer annual grass species that germinated at the same time as the medic pasture. It was sprayed with a selective herbicide to eliminate all

broadleaf species. Measurement of botanical composition on September 15 in 1994 showed that the grass pasture was 99 % barley grass and the medic pasture was 95 % medic. Similar compositions were found in 1995. Medic and grass pastures were grazed by sheep at 10 dry sheep equivalents (DSE) per hectare from August 5 to October 20 in 1994 and from July 21 to November 3 in 1995. The faba beans were sown into a cultivated seedbed at 110 kg/ha after the opening rains. In 1994, they were sown on June 20 and in 1995, they were sown on May 17. In 1994, 50 % of the faba beans and the medic pasture was in flower by September 10 and 50 % of the grass pasture was in flower by September 16. Flowering dates in 1995 were September 5 for the medic and barley grass pastures and September 12 for the faba beans.

2.2 Soil water

Soil water content was measured to a depth of 500 mm using a neutron moisture meter. In 1994, measurements were taken at five times during the growing season. In 1995, only three measurements were taken, as technical difficulties prevented any measurements being made before October.

2.3 Aboveground production

Net aboveground production in the two grazed pasture treatments was estimated by summation of the difference in aboveground biomass between samplings in areas where animals were excluded by cages. There were five cages (1 m x 1 m) per plot and cages were moved every 5 weeks. Aboveground biomass was estimated by

cutting herbage within a 0.1 m^2 quadrat in each cage to ground level, drying at 60 °C and weighing.

Aboveground production for the faba beans was estimated by measuring biomass three times in September and October and taking the maximum value as a surrogate for total production. At each time, 20 quadrats (0.1 m^2), were cut, dried at 60 °C and weighed to determine aboveground biomass. Although estimation of aboveground production in this manner ignores any decomposition losses that might have occurred, it was assumed that these losses were minimal, based on observations of the crop.

2.4 Root sampling

Roots were sampled on the same dates (July 27, September 6, September 27 and October 17) in both 1994 and 1995. At each sampling time, cores of 43 mm diameter were taken to 50 cm depth. The exception to this was at the first sampling in 1994 when cores were taken to 30 cm depth. Five cores were taken per plot with a hydraulic corer and these were separated into 10 cm segments. Segments of the same depth in each plot were bulked.

Soil samples were stored in sealed plastic bags at 3 °C and processed within 2 to 5 days of sampling. Roots and dead organic material (DOM) were washed from the soil using root washing buckets (based on the design of Cahoon and Morton 1961) and captured on a sieve (0.5 mm mesh). Roots and dead organic material were then stored in plastic jars at -20 °C for 2 to 5 weeks until further processing. Samples were thawed at 3 °C and live roots were separated from dead roots and other DOM by hand (using tweezers). This was done on the basis of elasticity of the root, root colour and

the presence of lateral roots. Live roots were lighter in colour, more elastic and fleshier and possessed more lateral roots than dead roots. Nodule biomass was included with live root biomass where they were still attached to live roots.

Live roots and dead organic material were dried at 50 °C for 48 hours and weighed. Subsamples of all material were combusted at 600 °C for 4 hours to obtain the ash percentage. All results are presented on an ash-free basis to avoid inconsistencies associated with adhering soil (Van Noordwijk 1993).

2.5 Old organic material disappearance

Old organic material (OOM) (> 1 mm) was separated from the soil at the beginning of the season using the same method as above and dried at 50 °C for 48 hours. Nylon mesh bags (10 cm x 10 cm, 1.0 mm mesh) were filled with approximately 1.5 g of OOM and buried at 10 cm depth with as little disturbance of the surrounding soil structure as possible. Five bags were buried in each plot. Coinciding with each sampling time, one bag per plot was retrieved, except for the last sampling occasion, when the remaining two bags in each plot were retrieved. Bags were gently rinsed in water to remove adhering soil, and roots from the current season were carefully removed with tweezers. They could be easily identified using the same criteria for distinguishing live roots from DOM as above. The material was then dried at 50 °C for 48 hours, weighed, and the proportion of OOM remaining (k_{OOM}) was calculated.

2.6 Root disappearance

One week prior to each sampling occasion, root material was obtained from each treatment from between 0 and 20 cm depth, washed free of soil and dried at 50 °C for 48 hours. About 1 g of this material was placed in mesh bags (same specifications as above) and at the time of taking soil cores, three bags per plot were buried at 10 cm depth under the same treatments from which they had come. Bags were then retrieved at the next sampling time and subjected to the same procedure as the bags containing OOM. In this way, the proportion of dead roots (DR) that disappeared between t and t-1 (k_{DR}) was calculated for each sampling interval.

2.7 Chemical analyses

Live root fractions were finely ground in a ring mill and analysed for total C (%) and total N (%) in a Europa 20-20 mass spectrometer using an ANCA-*SL* elemental analyser. C:N ratios were calculated on a mass basis.

2.8 Statistical analyses

Data were subject to analysis of variance using Genstat 5.0 (Alvery *et al.* 1982). Where the F value was found to be significant, l.s.d.s were calculated for the 5 % significance level.

3. Results

3.1 Rainfall and soil water content

Total annual rainfall was 249 mm in 1994 and 382 mm in 1995. The long-term (105 years) average annual rainfall at the Roseworthy campus of the University of Adelaide (located 1 km east of the trial site) is 439 mm. Growing season rainfall (May to October) was 157 mm in 1994 and 278 mm in 1995 compared with the long term average of 289 mm. Growing season rainfall in 1994 was in decile 1 (the lowest 10 % of all years) and in 1995 it was in decile 5, making it close to an average season.

Figure 4.1 reveals the distribution of rainfall throughout the experimental period. In 1994, following a dry start to the year, the opening rains occurred in June. This was followed by below average rainfall in July, August, September and October. Soil water content (measured to 500 mm depth) was low at the beginning of the season and decreased further as the season progressed.

In 1995, the opening rains occurred in May and this was followed by above average rainfall in June and July. Rainfall in August and September was well below average. Due to technical problems, data for soil water contents for 1995 are only available from October and November. Nevertheless, the data show that the soil water content in October 1995 was much greater than at the corresponding time in 1994.

3.2 Live root biomass

In both years, live root biomass for all species was observed to have reached a maximum at the time of the third sampling (September 26), which was shortly after flowering (Figure 4.2). After this time, live root biomass decreased as the plants began to set seed and senesce. Due to sampling variance and the limited intensity of sampling, it was not possible to detect the exact point of maximum live root biomass.

In 1994, live root biomass for the medic pasture reached a maximum of 1050 kg/ha which was similar to the maximum for the barley grass pasture which was 1000 kg/ha (Figure 4.2). Both of the pasture species had a significantly higher maximum live root biomass than the faba beans (250 kg/ha). A similar trend occurred in 1995. In 1995, the maximum live root biomass of the medic pasture was 970 kg/ha, the barley grass was 1080 kg/ha and the faba beans was 310 kg/ha.

3.3 Total root biomass production

Total root biomass production was calculated using the summation technique as described in Chapter 3. In 1994, cumulative root biomass production by the medic and barley grass pastures closely paralleled each other (Figure 4.2) and was 1650 and 1780 kg/ha respectively (Table 4.1). This was significantly greater than the faba beans which had a total root biomass production of 410 kg/ha. A similar pattern was observed in 1995, although biomass production was much greater (Table 4.1). In 1995, total root biomass production was 2770 kg/ha for the medic pasture, 2480 kg/ha for the barley grass pasture and 1220 kg/ha for the faba bean crop.

The amount by which the total root biomass production exceeds the maximum live root biomass gives an indication of the amount of root turnover (death and decomposition) that occurs during the season. In 1994, total root growth was 1.57, 1.78 and 1.67 times the maximum live root biomass of the medic pasture, barley grass pasture and faba bean crop respectively. In 1995, the amount of turnover was far greater. Total root growth was 2.86, 2.30 and 3.94 times the maximum live root biomass of each species respectively. In considering these numbers, it must be highlighted that there was a greater variation in live root biomass and total root biomass production in 1995 compared to 1994.

3.4 Depth distribution

In general, there was a concentration of live root biomass in the top 10 cm and a decreasing concentration with depth (Figure 4.3). In 1994, at the time of maximum observed live root biomass (September 27), the faba beans had 85 % of its live root biomass in the top 20 cm, compared to 75 % for the barrel medic and 65 % for the barley grass (Figure 4.3). In 1995, root distribution at this time was more consistently distributed amongst the treatments with the faba beans and the barley grass both having 69 % and the barrel medic having 72 % of their live root biomass in the top 20 cm.

An increased concentration of calcrete gravel around 40 to 60 cm depth across most of the trial area prevented easy sampling beyond 50 cm. Occasional observational cores to depths beyond 50 cm suggested that there was very little root growth at these depths. It is estimated that no more than 5 % of root biomass would have been found beyond 50 cm. This applies to all treatments in both years.

At the first sampling of 1994 (July 26), samples were only taken to 30 cm depth. In retrospect, it is thought that roots would probably have been found in the 30 to 50 cm zone, but the amount would probably not have had a significant effect on the overall root biomass production for the season.

At the time of the first sampling in 1995, the barley grass pasture already had a well established deep root system as it was more than two months since the pasture had germinated. In absolute terms, it was much greater than the other treatments, but in relative terms, only 59 % of its biomass was in the top 20 cm. In both years, subsequent increases in live root biomass in this treatment were confined to the top 10 cm. For the other two species, increases were observed at most depths with time. Data for the last sampling (October 17) are not shown. In both years at this time, live root biomass had decreased relative to the previous sampling and decreases in live root biomass were found to be relatively constant at each depth.

3.5 Aboveground production

Shoot production tended to follow the same trends that were occurring belowground. In 1994, there was no significant difference between the two pasture treatments but they both had significantly higher aboveground biomass production than the faba beans (Table 4.1). The grain yield of the faba bean crop in 1994 was only 600 kg/ha, which was well below the long-term district average but not unusual for that year. Shoot production was much greater in 1995 for all treatments, but this time the medic pasture significantly outyielded the grass pasture. Again, shoot production of the faba bean crop was significantly lower than the other treatments (Table 4.1).

3.6 Root:shoot ratios

Root:shoot ratios were calculated as the ratio of total root biomass production to total shoot biomass production. For all treatments, root:shoot ratios were higher in the drier year of 1994 than in 1995 (Table 4.1). Root:shoot ratios of the pasture treatments were similar to each other in both years whilst the root:shoot ratios for the faba bean crop were significantly lower.

3.7 Carbon:nitrogen ratios and N contents

Carbon:nitrogen (C:N) ratios and nitrogen contents (% of dry weight) of roots were determined in 1994. The grain legume faba bean was richest in nitrogen and had a C:N ratio of about 12 to 14 at all depths and times (Table 4.2). The barrel medic (also a legume, but intermediate in N) had C:N ratios in the range of 15 to 18. The barley grass had the lowest N content and C:N ratios ranging from 17 to 33. At the first sampling date, the young root material of the barley grass had a N content similar to that of the medic pasture in the top 10 cm, but as the season progressed, the N concentration of the barley grass decreased, with resulting increases in C:N ratios. In addition, there was a significant trend to increasing C:N ratios with depth in the barley grass pasture. This trend was also significant at the first two sampling dates in the medic pasture, but later in the season, no significant differences between depth were found (Table 4.2).

4. Discussion

The summation technique was used in this experiment to estimate the total amount of root biomass produced. In discussing the results of this experiment, it is first necessary to examine the validity of the technique before making any conclusions based on these results.

The accuracy of this method cannot be known for certain, although the technique is considered more accurate than those where no account is made for death and decomposition of roots during the season (Van Noordwijk 1993). Nevertheless, it is likely that there is still some underestimation of total root biomass production arising from losses of fine roots that may have occurred during sampling and losses that might have occurred during washing and storage.

An attempt was made to estimate the amount of live root biomass that passed through a 0.5 mm sieve. Whilst washing the samples taken on September 6, 1994, a 0.25 mm sieve was placed under the 0.5 mm sieve. An extra 20 to 40 % of macro-organic material was captured on this sieve (data not shown). Because of its small size, it was too difficult to separate this material into live roots (LR) and DOM fractions, but a visual estimate suggested that about 20 % of this material may have been live root biomass. Consequently, it might be assumed that actual LR biomass was an extra 4 to 8 % above the measured figures. In support of this, Amato and Pardo (1994) investigated the effect of screen mesh sizes on biomass losses of wheat and faba bean roots. They found that a 2 mm sieve lost 45 % and a 1 mm sieve lost 25 % of the mass of roots that a 0.2 mm sieve collected. Interpolating between the figures leads to a loss similar to what is indicated in this study.

Another source of loss may be weight loss during sampling and storage. This could be due to respiration before the roots die, leaking out of cell contents or by loss of part of the tissue. As a percentage of an untreated control, wheat roots that were stored for 2 weeks at 4 °C prior to washing only lost an extra 1 % of their biomass (Van Noordwijk and Floris 1979) and the fine roots of sugar beet lost 4 % (Grzebisz *et al*. 1989). In this experiment, roots were stored at 3 °C for 2 to 5 days so losses here would have been minimal. After washing, the roots were stored in water at -20 °C, for 2 to 5 weeks which also leads to minimal losses of biomass (Schuurman and Goedewaagen 1971). However, the actual act of sampling and then transporting samples back to the laboratory led to a 19 % loss of biomass in wheat roots (Van Noordwijk and Floris 1979) and a 5 % loss in sugar beet roots (Grzebisz *et al*. 1989). Respiration and loss of cell contents during hand separation of LR and DOM (when roots were floating in water for 1 to 2 hours) may have led to further unknown losses.

Therefore, it appears likely that in this study, some losses may have occurred during sampling, washing and storage. However, because roots grown in soil cannot be collected in sufficient quantity without some storage, washing and handling of the samples, no adequate reference value can be obtained for directly estimating the effects of such treatments. In addition, the amount of loss will vary with species and sampling time or age of the roots (Grzebisz *et al*. 1989). Consequently, it is not possible to derive an accurate 'correction factor' for losses during sampling, washing and storage.

The estimation of total root biomass production using the sequential biomass sampling and summation technique requires an estimation of the decomposition of dead roots and also of old organic matter during the season. This was achieved by incubating

material in mesh bags buried in the field. Cogle *et al*. (1987) compared the mesh bag technique with the inherently more accurate technique of burial of ^{14}C-labelled material. They found that when wheat straw was cut into 2 cm lengths and placed in a bag with a mesh size of 2 mm, decomposition was underestimated relative to the per cent recovery of ^{14}C from ^{14}C-labelled straw. Possible reasons for this were the exclusion of meso- and macro-fauna by the mesh size, and poor soil/straw contact which would potentially hinder the microbial colonisation of the straw (Cogle *et al*. 1987). As these factors would potentially have played some role in this experiment as well, the decomposition rates of DR and OOM may have been underestimated. The alternative of using ^{14}C-labelled material was not possible in this experiment because of restrictions on the use of ^{14}C at this site and the difficulty in obtaining ^{14}C-labelled material sufficiently similar in quality to the OOM found at this site.

Furthermore, it is possible that the depth of placement of the mesh bags (at 10 cm) may have affected the rate of decomposition through the influence of wetting and drying on decomposition of plant material (Amato *et al.* 1984). Despite this, any errors in the estimation of decomposition rates of OOM and DR would not have a large bearing on the end result as they are reduced through the summation technique that was used to determine total root biomass production.

In the calculations that were used to determine total root biomass production, it is assumed that live root disappearance is totally due to death and decomposition. It is possible that some translocation of organic material from the roots to the seed or grain might occur late in the season. Failing to take account of this would lead to a slight overestimation of root biomass production. This is unlikely to be significant as the

majority of carbohydrate in the seed comes from current assimilate or material stored in the aboveground parts of the plant (Milthorpe and Moorby 1974).

In summary, it can be concluded that the results do lead to a better estimate of total root biomass production than techniques where death and decomposition are not accounted for, but it is still best to read them as minimum values for root biomass and production.

The extent by which total root biomass production exceeded maximum live root biomass (Figure 4.2) gives some idea of the extent of root turnover (death and decomposition) during the season. Total root biomass production was 56 to 78 % in excess of maximum live root biomass in 1994, and 129 to 186 % greater in 1995. Using mini-rhizotrons to examine individual roots in detail, Cheng *et al.* (1990) estimated that total root biomass production was at least 67 % greater than the peak value of live roots. However, mini-rhizotron techniques consistently underestimate root growth in the upper 20 cm or so (Gregory 1994). Because it is a zone of major root and microbial activity (Figure 4.3), and is subject to the greatest environmental (temperature and moisture) fluctuations, it is expected that turnover would be greatest in the upper 20 cm. Consequently, it is feasible that the estimate of Cheng *et al.* (1990) should be greater and it appears reasonable to suggest that the figures reported here are valid.

In comparing the three species, it is clear that the root biomass production of the two pasture species was much greater than the grain legume (Table 4.1, Figure 4.2). This greater belowground input of organic matter under the pastures partly explains why soil organic matter is often higher under rotations with pastures than rotations with

crops alone (*e.g*. Chan *et al*. 1992, White *et al.* 1994). It is recognised that soil organic matter levels are also influenced by the rate of decomposition and that this is influenced by the conditions associated with the growth of different crops and pastures.

In the field, moisture stress often leads to a greater reduction in the growth of the shoots than the roots (*e.g*. Hamblin *et al.* 1990). This is thought to improve the ability of the plants to extract water and nutrients from the soil whilst limiting the potential for water loss by decreasing leaf area (Setter 1990). In this experiment, the two different seasons allowed a comparison of the response to rainfall and soil moisture to be made (Table 4.1, Figure 4.2). Although this experiment was not designed to isolate soil moisture as a variable, the results show that the lack of rainfall during the growing season in 1994 greatly reduced root and shoot biomass production, and increased root:shoot ratios compared to 1995 (Table 4.1).

Live root biomass and total root biomass production followed a sigmoidal pattern up until flowering (Figure 4.2). This is similar to what is commonly observed with shoots (Gregory 1994). For all three species, whilst live root biomass had decreased from their maxima by the time of the final sampling, the response of cumulative root biomass production was variable (Figure 4.2). In 1994, the barley grass exhibited no further root growth after flowering, whereas in 1995, encouraged by October rains and adequate soil moisture (Figure 4.1), there continued to be an increase in cumulative root biomass production. This occurred despite a decrease in live root biomass which suggests that there was still a lot more root senescence than root growth during this period. The medic pasture continued its root growth after flowering in both years, although in 1994, the increase was not significant. The faba beans exhibited a large

increase in root biomass production during the last sampling interval relative to previous growth. It is thought that the extent to which root growth after flowering might occur is related to the degree of determinancy of the particular species (Gregory 1994).

Flowering is a particularly important developmental stage after which assimilates are required to fill the growing grain, leaving little for roots (Gregory 1994). In most studies of determinate species such as cereals and annual grasses, the mass of the root system rarely increases after flowering and may decrease substantially depending on soil conditions (Gregory *et al*. 1978, Siddique *et al*. 1990, Gregory and Atwell 1991). In indeterminate species such as most annual legumes, there can be continued growth of the root system during early grain-filling (Mayaki *et al*. 1976, Sivakumar *et al*. 1977, Alston and Puckridge 1986, Gregory 1994). The degree of determinancy may influence the ability of the plant to increase root growth late in the season in response to favourable conditions.

The amount of organic matter in the soil not only depends on the quantity of the inputs but it also depends on the rate of decomposition and this is partly determined by the quality of the input and the depth of its placement. The ratio of carbon to nitrogen (C:N ratio) in the roots gives an indication of the quality of the input of organic material. Material that is rich in nitrogen (*e.g.* faba bean roots) is likely to decompose at a faster rate than material with a high C:N ratio (*e.g.* barley grass roots). The decomposition rates of the barrel medic roots are likely to be somewhere in between. Amato *et al*. (1984) reported C:N ratios of 10.4 for the roots of barrel medic, which is in contrast to the values of 15 to 18 found in this experiment (Table 4.2). The reasons for this difference are unclear but it is known that nitrogen contents vary in response to

soil nitrogen availability and effectiveness of nodulation and nitrogen fixation in the plant (Ledgard and Steele 1992). For instance, nitrogen fixation in legumes is highly sensitive to moisture stress (Ledgard and Steele 1992) and this might have led to higher C:N ratios in the dry year of 1994 when the analyses were performed. Differences in nitrogen content will influence the quality of the inputs and this will impact on the decomposition rates of the material in the future (Oades 1988).

The depth at which organic matter is placed in the soil is also important. There is evidence from ^{14}C dating of soils that mean residence times increase with depth (Oades 1989). The increased decomposition of organic material in the surface soil may be partly due to the increased amount of oxygen available at the soil surface. It may also be partly due to the increased frequency of wetting and drying cycles which promotes decomposition (Amato *et al*. 1984), presumably through an increased amount of microbial biomass activity. In this experiment, the barley grass pasture was notable for the amount of live root biomass found below 30 cm, relative to the barrel medic and faba beans (Figure 4.3). Although occasional observations had shown that the depth of rooting of the barley grass was greater than the other two species, the amount of root biomass beyond 50 cm was insignificant, relative to what was in the upper layers of the soil.

This study has provided useful quantitative information on root biomass production under a grazed annual legume pasture, a grazed annual grass pasture and a grain legume crop. It has come closer to identifying the total amount of root biomass production in a season, because root death and decomposition during the season has been accounted for. However, due to the inherent difficulties in isolating roots from the soil in which they grow, the true amount of root biomass production may still have

been underestimated by the methods used in this experiment. Both the barrel medic and barley grass pastures were grazed in this experiment and as discussed in the literature review (Chapter 2), defoliation may have an effect on root biomass production and on the belowground allocation of carbon. Consequently, the effect of defoliation on barrel medic is investigated further in the experiments reported in Chapters 5 and 6. Nevertheless, the information presented in this chapter is valuable for activities such as the validation and development of crop growth models, the construction of ecosystem budgets and in the development of models describing the dynamics of soil organic matter.

Table 4.1. Total root and shoot biomass production and root:shoot ratios for 1994 and 1995.

	Root production (kg/ha)		Shoot production (kg/ha)		Root:shoot ratio	
	1994	1995	1994	1995	1994	1995
Medic pasture	1650	2770	3100	7000	0.53	0.40
Grass pasture	1780	2480	3190	5940	0.56	0.42
Faba beans	410	1220	1330*	4880*	0.31	0.25
l.s.d.(*P*=0.05)	419	601	349	490	0.18	0.10

* Grain yield of faba beans was 600 kg/ha in 1994 and 2400 kg/ha in 1995.

Table 4.2. C:N ratio and nitrogen content (%) of live root biomass at each sampling time in 1994.

Depth	C:N ratio				Nitrogen content (%)			
(cm)	Jul 26	Sep 6	Sep 27	Oct 17	Jul 26	Sep 6	Sep 27	Oct 17
				Barrel medic				
0-10	14.9	15.3	17.8	17.1	2.08	2.40	2.02	2.27
10-20	15.6	16.0	17.0	17.2	2.15	2.29	2.24	2.19
20-50	17.2	16.6	16.8	17.4	2.04	2.11	2.16	2.03
				Barley grass				
0-10	16.6	19.9	22.3	23.8	1.77	1.68	1.47	1.50
10-20	18.4	22.1	27.7	27.7	1.85	1.58	1.28	1.32
20-50	19.5	26.0	32.8	29.0	1.81	1.29	1.16	1.33
				Faba beans				
0-10	11.6	12.2	12.2	12.9	2.57	2.67	2.87	2.79
10-20	n.a	13.2	12.0	14.5	n.a	2.53	2.89	2.55
20-50	n.a	12.2	11.8	14.1	n.a	2.74	2.79	2.50
l.s.d. (*P*=0.05)	1.83	0.95	1.68	1.99	0.40	0.25	0.36	0.25

n.a. = Not available - Insufficient material for analysis.

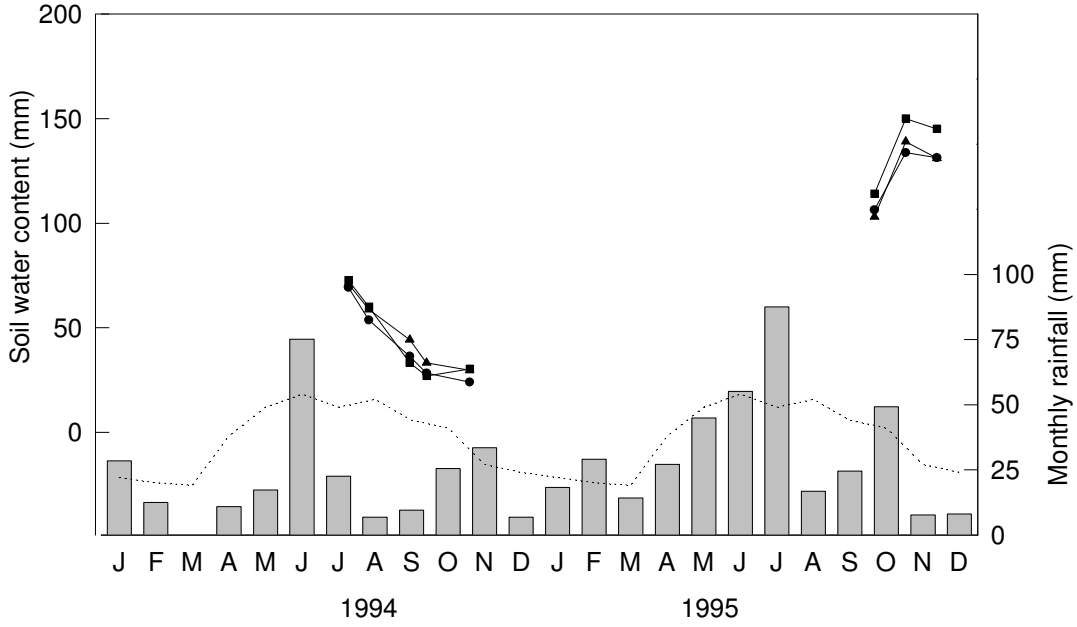

Figure 4.1. Soil water content (0-50 cm depth) under barrel medic (λ), barley grass (ν) and faba beans (σ) and monthly rainfall (shaded bars) for 1994 and 1995. Dotted line represents long term mean monthly rainfall.

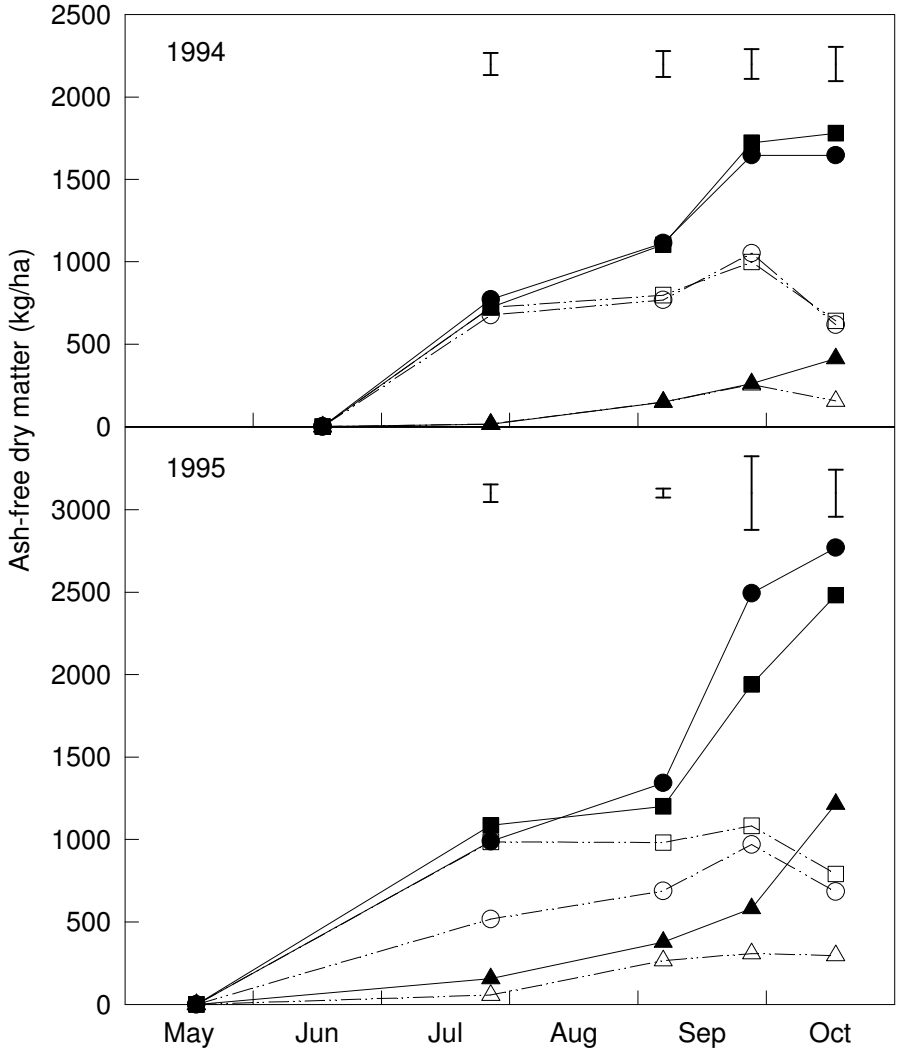

Figure 4.2. Live root biomass (dashed lines) and cumulative root biomass production (solid lines) for barrel medic (λ), barley grass (ν) and faba beans (σ). Error bars indicate least significant difference (*P*=0.05).

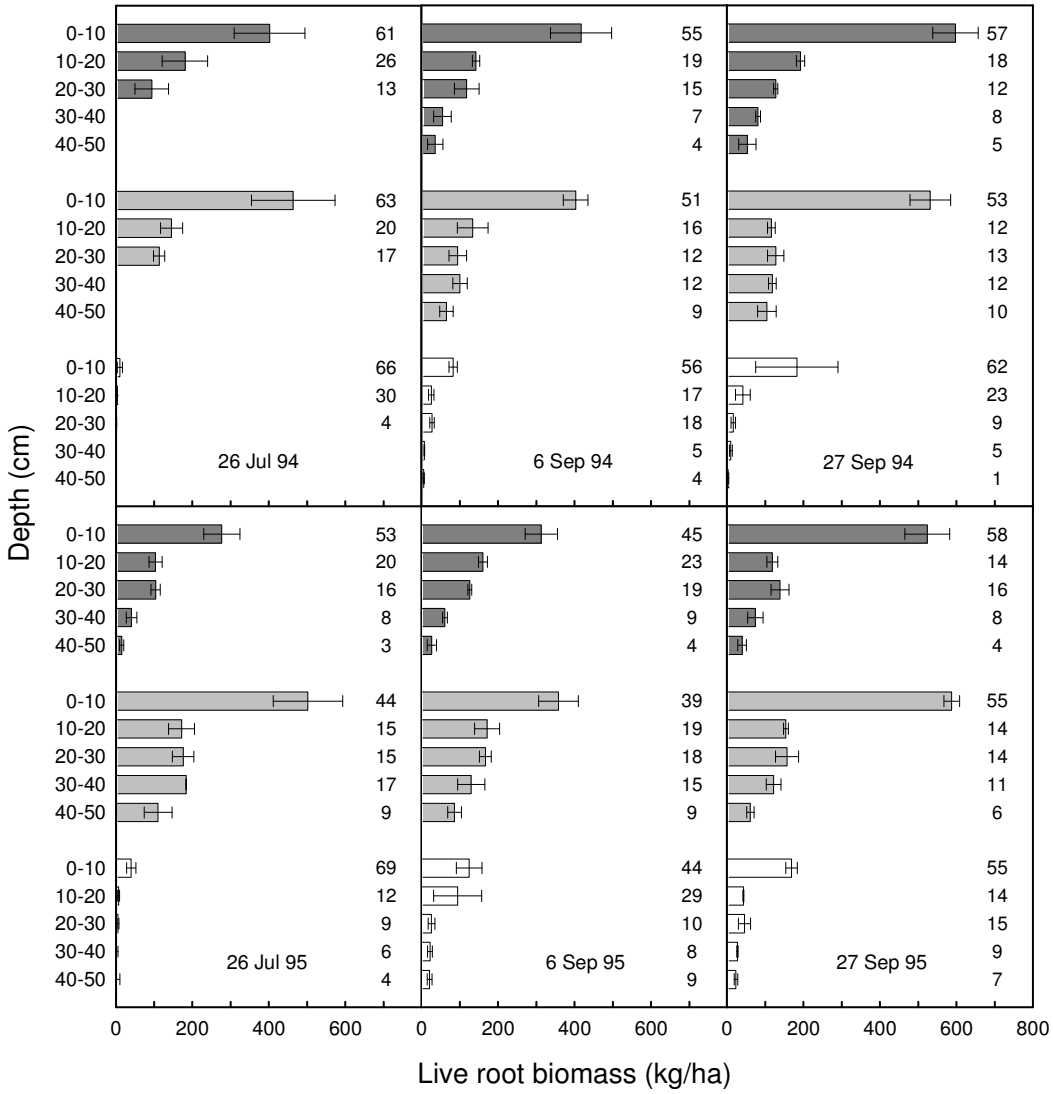

Figure 4.3. Distribution of live root biomass at different depth intervals for barrel medic (dark shaded bars), barley grass (light shaded bars) and faba beans (unshaded bars). Error bars indicate ± S.E. Numbers to the right of bars indicate % of total live biomass found at that depth interval.

CHAPTER 5

EXPERIMENT 2: EFFECT OF DEFOLIATION OF MEDIC PASTURES ON BELOWGROUND CARBON ALLOCATION AND TOTAL ROOT BIOMASS PRODUCTION

1. Introduction

Much of the world's agricultural environment is devoted to grazed grasslands and pastures (Lal and Logan 1995). In many cropping systems, a pasture ley is used as an opportunity to restore soil organic matter levels that may have been depleted under cropping (Grace *et al.* 1995, Greenland 1995). This is also the case in the cropping systems of southern Australia where legume pastures based on annual medic or clover species, are used in rotation with cereal, grain legume and oilseed crops to restore the structure and nitrogen status of the soil (Puckridge and French 1983). These pastures are most commonly grazed by sheep (and sometimes by cattle), although occasionally they are left ungrazed until shortly after flowering when they might be cut for hay (Doyle *et al*. 1993). There are few reports available on the effects of grazing on belowground inputs of carbon, especially with respect to medic pastures. The available information is incomplete and somewhat inconsistent as discussed in the literature review in Chapter 2.

The physiological response of plants after moderate to severe defoliation has two distinct phases. The first is a transient period of one to a few days and the second is a readjustment of physiological activity that occurs over several weeks (Richards 1993). During the period immediately after defoliation, root elongation may cease, root

respiration decreases and root growth slows down (Richards 1993). Rapidly reduced root growth and maintenance carbon costs contribute to the rapid recovery of the plant by allowing re-establishment of a balanced whole-plant carbon budget as soon as possible after defoliation. After this transient period, there is often a period of compensatory photosynthesis where photosynthetic rates of foliage on defoliated plants are often higher than those of foliage of the same age on undefoliated plants (Richards 1993). Furthermore, once photosynthetic rates start to exceed respiration, there appears to be a preferential allocation of carbon resources to leaves and shoots, especially in grasses.

The net effect of the physiological response to defoliation depends on the severity and frequency of defoliation and on the species. In an extensive review of the literature on the effect of grazing on perennial vegetation, Milchunas and Lauenroth (1993) could not find any consistent relationship between grazing and root mass. They found that although grazing generally had a negative effect on aboveground net primary production (ANPP), there were just as many positive effects as negative effects on root mass. However, the amount of root mass present at any one time can be very different from the amount of root growth for the season. Furthermore, in many of the studies, it is not clear whether it was live root biomass that was measured or if dead roots were also included. In addition, the physiology, phenology and consequent belowground carbon inputs of an annual pasture are potentially quite different from a perennial species (Warembourg *et al*. 1990).

In this chapter, the results of a field experiment aimed at quantifying the effect of simulated grazing episodes on the belowground carbon allocation and total root biomass production in a medic pasture are presented and discussed. Pulse labelling

with ^{13}C was used to determine the allocation of newly assimilated carbon to the roots and the ^{13}C dilution technique was used to calculate total root biomass production for the season. Details of the ^{13}C dilution technique, and the assumptions inherent in its use, were discussed in Chapter 3.

2. Materials and methods

2.1 Site details

The experiment was conducted at Roseworthy (441 mm mean annual precipitation, 16.5 °C mean air temperature), 60 km north of Adelaide, South Australia, on a site adjacent to the experiment in Chapter 4. The soil on the experimental site was a Hypercalcic Calcarosol (medium, slightly gravelly, loamy/clay loamy, moderate) (Isbell 1996). According to the US classification system, it is a Calcic Rhodoxeralf (Soil Survey Staff 1994). The 0-100 mm layer of soil had an organic C content of 16 g/kg and a mean pH (H_2O 1:5) of 7.7.

In May 1995, the site was sown to barrel medic (*Medicago truncatula* Gaertn. cv. Paraggio), a temperate annual pasture legume, at a rate of 50 kg/ha. The pasture was sprayed in early June to remove all grasses and broadleaf weeds. By September 5, 50 % of the plants had at least one flower.

2.2 Defoliation

The experiment was a randomised complete block design with four replicates. The two treatments were regular defoliation (*i.e.* simulated grazing, hereafter referred to as

'cut') and no defoliation ('uncut'). The experimental plot units were 4 m x 15 m. The defoliation plots were cut for the first time on July 21, after the plants had reached approximately 15 to 20 cm in height. Subsequent defoliations were at approximately 3 week intervals for the rest of the season (August 22, September 7, September 25 and October 13). At each defoliation time, the pasture was cut to 5 cm height with a rotary mower and the clippings weighed and removed. A subsample of the clippings was taken and dried to constant weight at 65 °C so that the dry weight of the herbage removed could be calculated. Quadrat cuts (4 x 0.1 m^2) of the stubble were then taken to ground level to determine the herbage remaining. At the same time, quadrat cuts were also taken from the uncut treatment to determine aboveground biomass. The intensity and frequency of defoliation resulted in a sward of similar height and growth habit to that of a barrel medic pasture that was being grazed by sheep at 10 DSE/ha in an adjacent paddock.

2.3 Labelling

Plots were pulse labelled with ^{13}C after defoliation. All plots were pulse labelled on August 10, August 29, September 15 and October 4. Although the first labelling took place 20 days after defoliation, subsequent labellings occurred at 7 or 8 days after the previous defoliation event. Due to unfavourable weather conditions, it was not possible to label plants any earlier in the season.

Samples were taken from these labelled areas 7 days after labelling. These samples were used for determining carbon allocation. Three of the replicates were also labelled with ^{13}C on August 11 and these labelled areas were sampled at the end of the growing season on November 1 when plants were senescing. These labels were used

for determining root biomass production using the ^{13}C dilution technique. Each label was applied to a previously unlabelled area of pasture, at least 2 m from a previous label.

Labelled $^{13}CO_2$ was made available to the plants using a rigid clear perspex chamber (30 x 30 x 30 cm) which was placed over the pasture sward and inserted approximately 2 cm into the soil. It was sealed at the base on all sides with fine moist sand. Hydrochloric acid was injected through a suba seal into a vial containing 0.0823 g $Na_2{}^{13}CO_3$ (10 mg ^{13}C) dissolved in 2 mL 0.5M NaOH, thus releasing labelled $^{13}CO_2$. Plants were allowed to assimilate $^{13}CO_2$ for 2 hours and then the chamber was removed. Plants were labelled on fine sunny days in the middle of the day when photosynthetic activity would have been at a maximum.

2.4 Sampling

To determine carbon allocation, labelled areas were destructively sampled 7 days after pulse labelling. Firstly, all aboveground plant material was cut to ground level (30 x 30 cm) and then, using a hydraulic corer, a 43 mm diameter core was taken to 50 cm depth in the centre of the labelled area. Previous investigations had shown that less than 5 % of medic roots were found below 50 cm in this soil (Chapter 4). Given sufficient knowledge of the root distribution of the barrel medic plants at this density, it was considered probable that all the roots in the core in the centre would belong to plants that were within the labelled area. Consequently, their enrichment would be representative of the belowground enrichment of ^{13}C. The long term dilution set of labelled areas was sampled at the end of the season as described above. At each

sampling time, samples were taken from a nearby unlabelled area in each treatment so as to obtain baseline ^{13}C abundances.

2.5 Chemical analyses

Shoot material was dried to constant weight at 65 °C and weighed. Roots and dead organic material (DOM) were washed from the soil core using a root washing machine similar to the design of Welbank (1975), and captured on a 0.5 mm sieve. Live roots were separated from DOM by hand, based on a combination of colour, elasticity, density and the presence of lateral roots. Live roots were lighter in colour, more elastic and fleshier and possessed more lateral roots than dead roots did. Nodule biomass was included with live root biomass where they were still attached to live roots. Each of these fractions was then dried at 50 °C and weighed. Subsamples of root material were ashed at 600 °C for 4 hours and the ash-free percentage calculated. All root biomass figures are presented on an ash-free basis.

Samples were finely ground in a ring mill and analysed for total C (%) and stable C isotopes (^{12}C and ^{13}C) in a Europa 20-20 mass spectrometer using an ANCA-*SL* elemental analyser.

Solid state high resolution ^{13}C nuclear magnetic resonance (NMR) with cross polarisation and magic angle spinning (CP/MAS) (Wilson 1987) was used to determine the overall chemical nature of the root material of each treatment at the first and last sampling times (August 17 and October 12). For each observation, material from the three replicates were combined for analysis.

The 50.309 MHz CP/MAS spectra of the four root samples were obtained on a Varian Unity 200 spectrometer with a 4.7 T wide-bore Oxford superconducting magnet. Samples were spun at 5 kHz in 7 mm diameter zirconia rotors with Kel-F caps in a Doty Scientific MAS probe. All spectra were attained with a 1 ms contact time and a 2.0 s recycle time. The number of transients acquired was 2048 for all spectra. Using the standard Varian pulse sequence, the free induction decays were acquired over a sweep width of 40 kHz over an acquisition time of 50 ms in a 4032 point database. All spectra were obtained with 8 k zero filling and 50 Hz Lorentzian line broadening and 0.010 s Gaussian broadening. Chemical shift assignments were externally referenced to the methyl resonance of hexamethyl benzene at 17.36 ppm.

2.6 Calculations

In hand separating the live root (LR) fraction from the dead organic matter (DOM) fraction, a conservative approach was taken such that if there was any doubt, material was allocated to DOM. Consequently, DOM was overestimated and LR biomass was underestimated. To correct for this, the following equation was applied to estimate the fraction ($f_{LR\ in\ DOM}$) of LR in DOM:

$$f_{LR\ in\ DOM} = (\delta_{DOM(L)} - \delta_{DOM(U)}) / (\delta_{LR(L)} - \delta_{DOM(U)}) \qquad \text{(Equation 5.1)}$$

where $\delta_{DOM(L)}$, $\delta_{LR(L)}$ and $\delta_{DOM(U)}$ are the $\delta^{13}C$ (‰) values of the labelled DOM, labelled LR and unlabelled DOM respectively. The amount of live root carbon in DOM ($C_{LR\ in\ DOM}$) was calculated by multiplying f_{LR} by the amount of carbon in DOM (C_{DOM}):

$$C_{LR\ in\ DOM} = f_{LR\ in\ DOM} \times C_{DOM} \quad \text{(Equation 5.2)}$$

This was added to the amount of carbon in LR to give a corrected value of carbon in live roots:

$$C_{LR(Total)} = C_{LR} + C_{LR\ in\ DOM} \quad \text{(Equation 5.3)}$$

LR biomass was then calculated by dividing the corrected value of carbon in roots by the percentage of carbon in the LR.

$$LR = C_{LR(Total)} / (\%C/100) \quad \text{(Equation 5.4)}$$

This method enabled a complete and accurate assessment of the amount of LR in DOM, provided that some preliminary hand separating was carried out beforehand. This corrected figure for root biomass was used in all subsequent calculations involving root biomass.

In order to calculate the amount of ^{13}C in the shoots and roots, $\delta^{13}C$ values were first converted to absolute ratios (R) and then to fractional abundances (F) as follows:

$$R = {}^{13}C/{}^{12}C = [(\delta^{13}C_{PDB}/1000) + 1] \times R_{PDB} \quad \text{(Equation 5.5)}$$

$$F = {}^{13}C/({}^{12}C + {}^{13}C) = R/(R + 1) \quad \text{(Equation 5.6)}$$

where $\delta^{13}C$ values are expressed relative to the PDB (Pee Dee belemnite) standard and R_{PDB} is the absolute ratio ($^{13}C/^{12}C$) of the PDB standard. The quantity of ^{13}C in each

compartment (*i.e.* roots or shoots) was calculated by multiplying the C content (biomass x %C) of that compartment by its corresponding fractional abundance.

$$^{13}C_{roots} = LR \times (\%C/100) \times F_{LR} \quad \text{(Equation 5.7)}$$

$$^{13}C_{shoots} = \text{Shoot Biomass} \times (\%C/100) \times F_{shoots} \quad \text{(Equation 5.8)}$$

The excess ^{13}C in each compartment attributable to labelling was then determined by subtracting the quantity of ^{13}C present at natural abundance (as determined on unlabelled control plants) from the total ^{13}C in each compartment (Svejcar *et al*. 1990).

$$^{13}C_{excess} = {}^{13}C_{labelled} - {}^{13}C_{unlabelled} \quad \text{(Equation 5.9)}$$

Allocation to the root compartment was calculated by dividing the mass of excess ^{13}C in the root by the total mass of excess ^{13}C in the plant.

2.7 ^{13}C dilution technique - theory

The ^{13}C dilution technique was used to calculate total root biomass production. It is based on the dilution of the ratio of $^{13}C_{excess}$ to ^{12}C after pulse labelling when plants assimilate only new ^{12}C and ^{13}C at natural abundance (Caldwell and Camp 1974). A detailed discussion on the theory of the technique, its assumptions and calculations, and some of its advantages and disadvantages is presented in Chapter 3.

2.8 Statistical analyses

Where applicable, cut and uncut treatment means were compared using a two-tailed paired *t*-test with an assumption of equal variance where standard errors of the means differed by less than a factor of 2 (Maindonald 1992). Where standard errors of the means differed by a factor greater than 2, a *t*-test was performed using the assumption of unequal variances.

Alternatively, the standard error of the mean is presented where applicable.

3. Results

3.1 Shoot biomass production

Aboveground biomass of the uncut control increased as the season progressed, reaching a maximum of 4590 kg/ha towards the end of September, shortly after flowering (Figure 5.1). This peak standing biomass in the uncut treatment was used as an estimate of aboveground biomass production as suggested by Milchunas and Lauenroth (1993) (Table 5.1). It was considered probable that the true shoot biomass production would not have been significantly greater as there was not a great amount of leaf death and decomposition during the short growing season.

The effect of defoliation every 2 to 3 weeks was to maintain a standing biomass in the range from 750 to 1750 kg/ha (Figure 5.1). The amount of stubble left after each cut increased as the pasture became more dense and prostrate with regular cutting. The aboveground biomass production of the cut treatment was calculated by summing the

increases in biomass between each defoliation. The aboveground biomass production for the cut treatment was 3430 kg/ha, significantly less than the uncut treatment (Figure 5.1, Table 5.1).

3.2 Live root biomass

Live root biomass was variable, although the effect of defoliation was to decrease the live root biomass relative to the uncut control (Figure 5.1). This difference existed at each sampling time, although it was only significant at the September 6 sampling. The maximum live root biomass was 1240 kg/ha for the uncut control and 1030 kg/ha for the defoliated treatment. Live root biomass of both pastures reached a maximum at about the same time as when aboveground biomass of the uncut control was at a maximum.

3.3 Total root biomass production

Total root biomass production was calculated by the ^{13}C dilution technique as outlined in Chapter 3. There was no significant difference between the two treatments (Table 5.1). Both treatments contributed approximately the same amount of root biomass to the soil during the season. The amount of carbon allocated to total root biomass production was calculated by multiplying belowground biomass production by carbon content. It was found that 670 kg C/ha was utilised in root biomass production by the medic pasture, irrespective of defoliation regime (Table 5.1).

3.4 Root:shoot ratios

The root:shoot ratio was calculated as the amount of carbon allocated to root production relative to the amount of carbon allocated to shoot production over the season. The root:shoot ratio was greater for the cut treatment at 0.49 compared to 0.37 for the uncut control (Table 5.1), but because of variability associated with root production and the small number of replicates, the difference was not significant.

3.5 ^{13}C allocation

3.5.1 Unlabelled plots

At each time of sampling, δ^{13}C values of plant material harvested from unlabelled plots were determined for use as baseline measurements. Mean unlabelled δ^{13}C values of cut shoots and roots were slightly higher than that of uncut shoots and roots, although the differences were not significant (Table 5.2). The mean δ^{13}C values of the unlabelled shoots were also slightly higher than the roots, although again the differences were not significant.

3.5.2 ^{13}C enrichment

The relative δ^{13}C enrichment 7 days after labelling was calculated as the difference between the δ^{13}C values of labelled and unlabelled plant material at each time of sampling. The δ^{13}C enrichment of the cut treatment was always greater than the uncut treatment (Figure 5.2). This was true for both shoots and roots. The relative enrichment in δ^{13}C was always greater in shoots than in roots. Maximum enrichment

occurred in the cut treatment labelled on August 29 when the labelled shoots had a $\delta^{13}C$ value of 58.80 ‰. The $\delta^{13}C$ value for unlabelled shoots at the same time was –27.15 ‰.

3.5.3 ^{13}C recovery

At each labelling date, 10 mg of ^{13}C was released in each labelling chamber. Figure 5.3a shows that between 3.4 and 5.9 mg of released ^{13}C was recovered in plant material (shoots and roots) 7 days later, depending on the labelling date and the treatment. This figure gives an indication of the relative amounts of net carbon fixation at each time. There was no obvious relationship between ^{13}C recovery and labelling date (Figure 5.3a).

At all times, the total amount of ^{13}C recovered in cut plants was equal to or slightly less than the amount of ^{13}C recovered in uncut plants (Figure 5.3a). Specific ^{13}C recovery (amount of ^{13}C recovered per unit amount of shoot biomass) shows that the cut plants had a much greater rate of net carbon fixation per unit of shoot biomass than did the uncut plants (Figure 5.3b). Specific ^{13}C recovery of uncut plants decreased after the first labelling date, whereas it increased and remained high in cut plants at subsequent labellings.

3.5.4 Allocation of ^{13}C to roots

The net allocation of the ^{13}C isotope to the roots 7 days after each labelling was calculated from the $\delta^{13}C$ values and biomass of the shoots and roots. At all sampling times, the cut treatment had both a greater amount (Figure 5.4a) and a greater

proportion (Figure 5.4b) of the assimilated ^{13}C in the roots than did the uncut control. For both treatments, the proportion of ^{13}C allocated to the roots was greatest at the time of the first label (August 10) and decreased to be lowest at the time of the last label (October 4) (Figure 5.4b).

3.6 NMR analyses

NMR analyses were used to investigate the effect of defoliation on the chemical nature of the roots. The NMR spectra show that the general chemical nature of the roots was similar for both treatments both earlier in the season (August 17) and at the end of the season (October 12) (Figure 5.5a). The major signal of all samples was from 46 to 110 ppm which is in the O-alkyl range (Wilson 1987). The peaks at around 75 ppm arise mainly from the oxygenated carbon of carbohydrates. The presence of carbohydrates in all samples was confirmed by the peaks at 105 ppm which arise from the dioxygenated 'linkage' carbon of polysaccharides. The peaks at 175 ppm derive from acid or ester linkages indicating the presence of proteins or acid materials such as fatty acids (Golchin *et al*. 1994).

The spectra in Figure 5.5b were obtained by subtracting the spectrum of the cut treatment from that of the uncut treatment at each time. The resulting spectrum for August 17 indicates a difference between the two treatments arising at about 75 ppm with smaller differences arising at 110 and 175 ppm. This suggests that the uncut treatment was slightly more enriched in carbohydrates and protein than the cut treatment at this time. The existence and greater resolution of the peaks in the 80 to 105 ppm region in the absolute difference spectrum of August 17 (Figure 5.5b) indicates that the roots of the uncut treatment had a higher amount of crystalline

cellulose than the roots of the cut treatment. The roots of the cut treatment appear to have had a higher amount of unstructured amorphous material. The spectrum for October 12 indicates almost no difference between the two treatments except for a small negative peak at 75 ppm. This suggests that there was slightly more carbohydrate in the roots of the cut treatment at the end of the season.

4. Discussion

Live root biomass was depressed by defoliation, relative to the uncut control, although the difference was only significant at the September 6 sampling (Figure 5.1). A reduction in live root biomass under defoliation could have resulted from either of two situations.

Firstly, defoliation may have led to a decrease in root biomass production such that live root biomass was less under defoliation. This response would fit hypothesis A as proposed by McNaughton (1983), where low levels of grazing begin to have a detrimental effect on the plant (see Chapter 2 and Figure 2.3).

Alternatively, the total root biomass production for both treatments may have been similar, but a greater amount of root senescence under defoliation may have led to a decreased live root biomass. In this situation, the response of root *senescence* to defoliation could be described by hypothesis C (McNaughton 1983 and Figure 2.3) where low levels of grazing can lead to a stimulation of plant responses. Furthermore, the response of root *biomass production* to cutting would fit hypothesis B (McNaughton 1983 and Figure 2.3) where low levels of grazing have no net effect on plant response.

In this experiment, the total root biomass production for both treatments was about the same (Table 5.1), but live root biomass was slightly less under defoliation (Figure 5.1) Therefore, it must follow that the second hypothesis, as postulated in the previous paragraph, must apply. The rate of root senescence must have been slightly greater for the cut plants. The mechanisms and physiology of this response are complex but an insight into how this may have occurred is given by the ^{13}C allocation results.

In discussing the general physiology of plants recovering from defoliation, Richards (1993) states that immediately following cutting there is a transient period of a few days during which time little or no carbon is allocated to the roots. Total photosynthesis decreases as a result of lower leaf area and carbon that is fixed is preferentially allocated to shoots so as to restore leaf area and photosynthetic capacity. There are also a number of reports of mortality of roots following defoliation (Butler *et al.* 1959, Hodgkinson and Baas Becking 1977, Richards 1984). If root senescence occurs, live root biomass decreases (as was shown in this experiment). Concurrently, the rate of carbon allocation belowground decreases (Richards 1993).

However, this experiment shows that decreased root allocation immediately after defoliation might be compensated for, to some extent, by increased root allocation some time later. The allocation of ^{13}C to roots in the 7 days after labelling (which in turn was 7 to 8 days after defoliation), indicated that the allocation of new assimilate to root growth was greater in the cut treatment than in the uncut treatment (Figures 5.4a and 5.4b). Not only was the relative proportion of ^{13}C that was found in the roots greater in the cut treatment than the uncut treatment, but the absolute amount of ^{13}C found in the roots of cut plants was also greater. This suggests that there was compensatory allocation of carbon belowground 7 days after defoliation.

Consequently, this led to the total root biomass production being almost the same for both treatments (Table 5.1).

It is conceivable that the greater allocation of ^{13}C to the roots by the cut plants was made possible by compensatory photosynthesis and that defoliation led to a stimulation of photosynthesis, in accordance with hypothesis C of McNaughton (1983) (Figure 2.3). Even though there was significantly less shoot biomass in the cut treatment at the time of labelling, it was able to fix almost as much ^{13}C as the uncut treatment, as indicated by the total amount of ^{13}C recovered in the plants (Figure 5.3a). When expressed as a factor of the amount of shoot biomass already present, the cut treatment was significantly more efficient at fixing the labelled carbon (Figure 5.3b). This may be due to changes in light supply as the architecture and light interception of the sward was changed (Woledge 1977) or due to a greater proportion of new leaves with higher photosynthetic potential (Nowak and Caldwell 1984). Leaves that develop in shade within canopies of high leaf area index exhibit reduced photosynthetic potential when exposed to high light intensities (Boller and Nosberger 1985).

Alternatively, it is possible that the amount of ^{13}C recovered in each treatment was limited by the amount of ^{13}C available for assimilation, but this is unlikely. No measurements were made of the ^{13}C enrichment in the chambers at the end of the 2 hour sampling period, so it cannot be ascertained whether there was full uptake of the ^{13}C or not. However, within the volume of the labelling chamber (~27 L), there would have initially been about 9450 mg ^{12}C with a further 10 mg added as ^{13}C. For the CO_2 level to deplete to 150 μL/L (where the compensation point of the leaves would be attained and respiration would dominate) within 2 hours of enclosure would require a photosynthetic rate of 7 mg $CO_2/m^2/s$ which is extremely high (Milthorpe and Moorby

1974). Of the 10 mg of ^{13}C that was available for fixation at each labelling, between 3.4 and 5.8 mg was recovered in roots and shoots (Figure 5.3a). The mean recovery was 41 % in the cut treatment and 46 % in the uncut treatment. Keith *et al.* (1986) labelled wheat plants a number of times throughout the season with ^{14}C and they recovered between 54 and 82 % of the ^{14}C in plant biomass. Generally, rates of recovery in this experiment were below that of Keith *et al.* (1986) which suggests that maximum uptake of available ^{13}C was not achieved in the 2 hour labelling period.

It is also likely that some of the ^{13}C that was assimilated within the 2 hour labelling period was lost within the following 7 days as either shoot respiration, or as root respiration, exudation or secretion, or was lost in the root washing and retrieval process and was not recovered. The magnitudes of loss via respiration and soil deposition in this experiment could have been very different from those reported by Keith *et al*. (1986), and this would affect the amount of carbon found in the plant. Defoliation may also have had a positive or negative effect on the proportion of carbon retained in the plant. Losses via respiration would probably have been different for the cut and uncut treatments. The effect of defoliation of barrel medic plants on allocation to soil and rhizosphere respiration is examined in detail in the experiment reported in Chapter 6.

To further investigate whether increased photosynthesis per unit of shoot biomass in cut medic plants was truly a means of compensating for reduced belowground allocation immediately after defoliation, one would need to maintain an excess of ^{13}C-enriched CO_2 in the labelling chamber during the labelling period. This would be possible as the cost of ^{13}C is not prohibitive (10 mg costs approximately A$5) and

because ^{13}C is not harmful or radioactive, it does not have to be fully recovered. If this was done, then it could be sure that ^{13}C was not limiting.

Irrespective of whether the availability or fixation of ^{13}C was or was not a limiting factor, the relative allocation of recovered ^{13}C to roots is still a valid observation, and this clearly indicates that cut plants had a higher proportion of ^{13}C in their roots than uncut plants (Figure 5.4*b*). Hence, the evidence still supports the basic proposition that the decreased belowground allocation immediately after cutting is offset somewhat by comparatively greater relative belowground allocation a week or two after cutting. The extent to which this occurs will depend on the severity and frequency of grazing or defoliation. If too much leaf area is removed, or it is removed too often, then it is likely that defoliation will eventually result in reduced photosynthesis, reduced carbon allocation belowground and reduced total root biomass production, as befitting hypothesis C of McNaughton (1983) (Figure 2.3).

Residual leaf area is a major determinant of regrowth potential (Simpson and Culvenor 1987). Cutting the medic pasture to 5 cm height every 2 to 3 weeks decreased the total shoot biomass production for the season relative to the uncut control (Figure 5.1). After the August 22 defoliation, there was only 750 kg/ha of stubble remaining, but as the season progressed, the cut pasture developed a more prostrate growth habit and more biomass and leaf area remained below 5 cm after each cut. At the time of the last cut, there was 1500 kg/ha remaining in the stubble below 5 cm. Consequently, the subsequent regrowth was greater each time. This is in accordance with the observed behaviour of other pasture legumes such as subterranean clover (*Trifolium subterraneum*) (Rossiter and Collins 1980).

As the residual biomass increased with subsequent defoliations, it is likely that shorter periods were required to recover net photosynthetic rates to those of pre-cut levels. Culvenor *et al*. (1989b) found that when 30 % of leaf area was removed, recovery of photosynthetic rates to those achieved by uncut swards took 4 days compared to 12 days when 80 % of leaf area was removed. This would mean that compensatory belowground allocation could be instigated within a shorter time.

In retrospect, measurements of leaf area index would have been useful to determine if the residual leaf area index was below the critical leaf area index. This is the leaf area at which 95% of incident light is intercepted (Simpson and Culvenor 1987). In order to maximise regrowth after defoliation, a pasture must not be harvested to leaf area indices below its critical leaf area index (Simpson and Culvenor 1987).

Defoliation had an interesting effect on the $\delta^{13}C$ value of both the shoots and roots of the unlabelled barrel medic plants. Although the differences were not significant, there was a trend to increasing $\delta^{13}C$ values with defoliation (Table 5.2). These trends are in agreement with some preliminary findings of Lorna Mackie-Dawson (personal communication). It is likely that this phenomenon is related to the increased irradiance available to the plant as shading leaves are removed. Farquhar *et al.* (1989) concluded that increased light affects the photosynthetic discrimination of C_3 plants, thus leading to higher $\delta^{13}C$ values. This phenomenon may potentially be used as an integrated indicator of the intensity or severity of grazing, when making comparisons between different grazing treatments. Additionally, comparisons of the ratios of shoot and root $\delta^{13}C$ values before and after grazing may give some idea of the allocation of carbon belowground after grazing. In this experiment, not enough observations of unlabelled plants were made to make any statistically valid conclusions.

Defoliation also had an interesting effect on the overall chemical nature of the roots as revealed by solid state ^{13}C CP/MAS NMR spectroscopy (Figure 5.5a). Generally, NMR analyses showed that the chemical nature of the root material was very similar to that of plant fragments in soil (Oades 1995b). Further examination (Figure 5.5b) reveals small differences between treatments earlier in the season (August 17) which disappeared or were slightly reversed by the end of the season (October 12). The spectra indicate that at the time of the August 17 sampling, there was a lower amount of crystalline cellulose or a higher proportion of amorphous unstructured material in the roots of the cut treatment. This suggests that the roots of the cut treatment were subject to a higher degree of oxidation or oxidative damage. This is likely to be to a result of increased microbial attack and decomposition of the root material of the cut treatment. A response of this type would occur if there was a higher amount of root exudation and lysate release in response to defoliation. This supports the hypothesis that defoliation leads to a greater turnover of root material during the season.

This phenomenon disappeared by the end of the season because the dominating process at this time was the maturation and senescence of the plant. Roots of both the cut and uncut treatments were beginning to senesce and decompose and defoliation would have had relatively little effect on the chemical composition of the roots at this stage. In fact, the roots of the cut treatment showed a lower degree of unstructured amorphous carbon than the uncut treatment (Figures 5.5a and b). This is consistent with frequent defoliation of prostrate legume pastures leading to a delay in flowering (Collins and Aitken 1970) and consequently, leading to a delay in maturity and senescence.

In conclusion, the results of this experiment have shown that defoliation, at an intensity and frequency commonly found in grazing situations, does not adversely affect the total root biomass production of a barrel medic pasture. However, it appears that root turnover could be greater under regular defoliation, and this may have implications for nitrogen fixation and mineralisation during the pasture phase. This requires further investigation.

The similar amount of belowground carbon input through root biomass production under the two treatments has implications for the management of ley pastures in a cropping rotation such as those of southern Australia, if inputs of carbon are to be maximised. Where annual medic pastures are grown, it appears that it is possible to graze them without adversely affecting the input of carbon through root biomass production, provided that enough leaf area is maintained to enable plants to re-establish photosynthetic rates within a week or so. This recommendation is also the recommended management strategy for maximising shoot growth rate, which should in turn lead to maximum livestock production (Simpson and Culvenor 1987). Further investigations of the effect of defoliation on the *total* belowground input of carbon are presented in Chapter 6.

Table 5.1. Total shoot and root biomass production, total root biomass carbon and root:shoot ratio for cut and uncut barrel medic pasture.

	Cut	Uncut	Significance
Shoot biomass production (kg/ha)	3430	4590	$P<0.05$
Root biomass production (kg/ha)	1560	1640	n.s.
Root biomass carbon (kg C/ha)	671	669	n.s.
Root:shoot ratio*	0.49	0.37	n.s.

* Ratio of root biomass carbon to shoot biomass carbon. Shoot biomass carbon calculated as 40 % of shoot biomass production.

Table 5.2. Mean $\delta^{13}C$ values (‰) of unlabelled roots and shoots of cut and uncut barrel medic pasture (Standard error of the mean in brackets; n=4).

	Cut	Uncut
Shoots	-26.66 (0.49)	-27.15 (0.75)
Roots	-27.06 (0.40)	-27.29 (0.37)

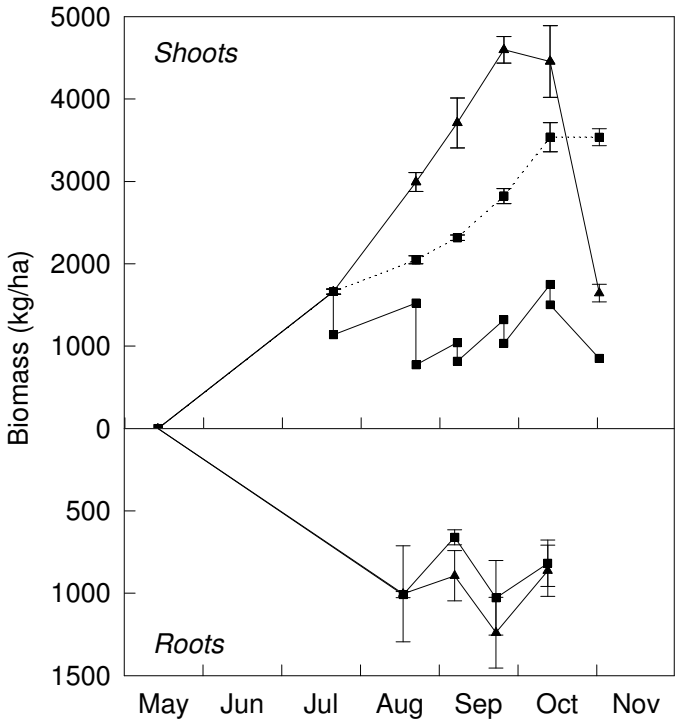

Figure 5.1. Shoot biomass (above the line) and corrected ash-free live root biomass (below the line) of cut (ν) and uncut (σ) treatments. The dashed line represents the cumulative sum of the increases in shoot biomass of the cut treatment. Vertical bars represent ± SE (standard error of the mean; $n = 4$).

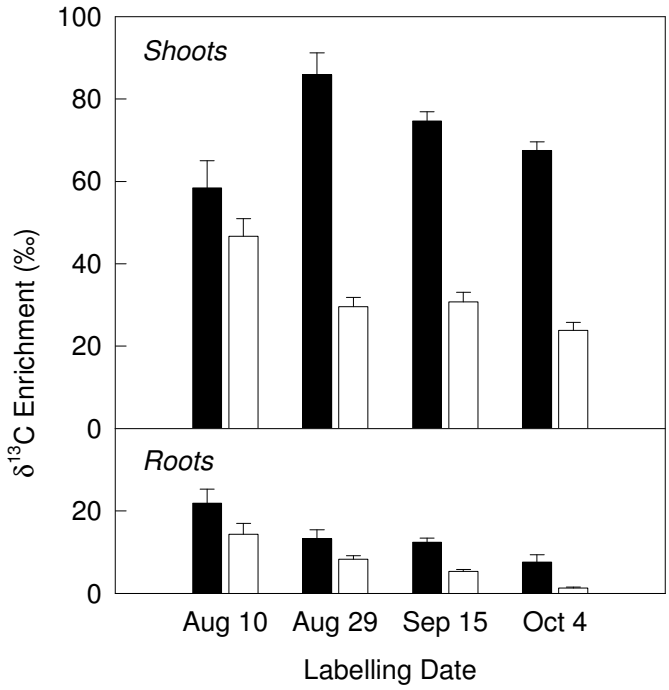

Figure 5.2. Relative enrichment of ^{13}C ($\delta^{13}C$ labelled - $\delta^{13}C$ unlabelled) in shoots and roots of cut (dark bars) and uncut (light bars) barrel medic pasture, 7 days after labelling. Vertical bars represent ± SE (standard error of the mean; $n = 4$).

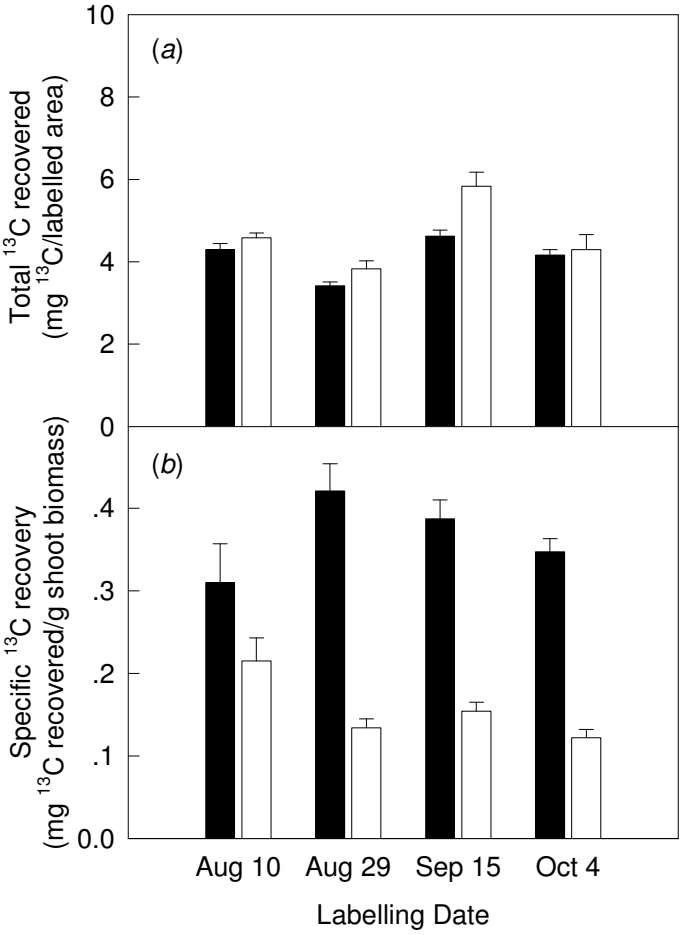

Figure 5.3. (*a*) Total ^{13}C recovered in shoots and roots (mg ^{13}C/labelled area) and (*b*) specific ^{13}C recovery (mg ^{13}C/g shoot biomass) of cut (dark bars) and uncut (light bars) barrel medic pasture, 7 days after labelling. Vertical bars represent ± SE (standard error of the mean; $n = 4$).

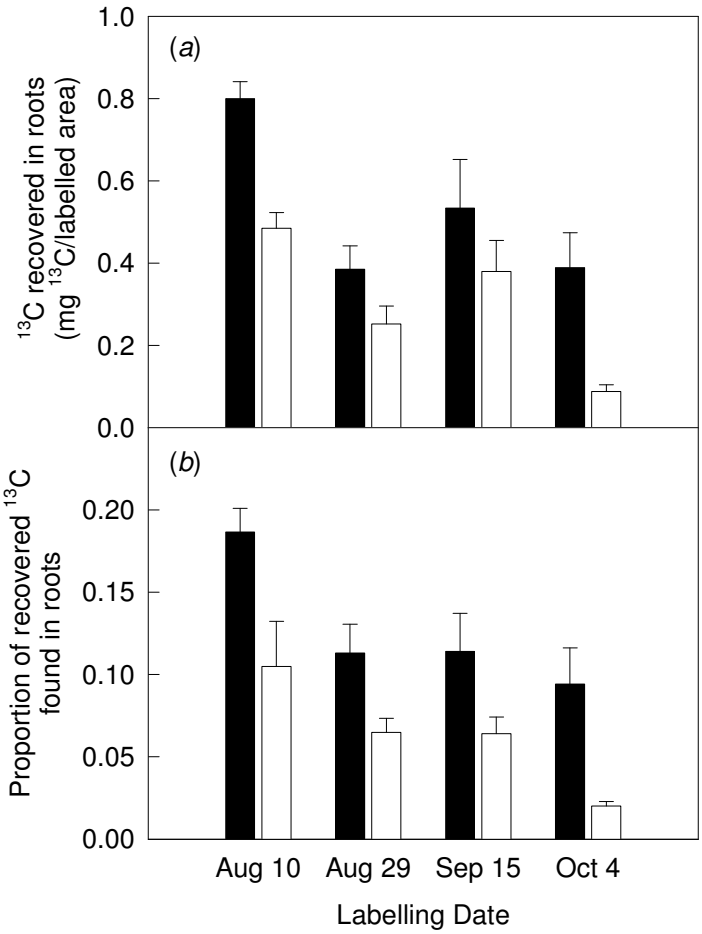

Figure 5.4. (*a*) ^{13}C recovered in roots (mg ^{13}C/labelled area) and (*b*) proportion of recovered ^{13}C found in roots of cut (dark bars) and uncut (light bars) barrel medic pasture, 7 days after labelling. Vertical bars represent ± SE (standard error of the mean; $n = 4$).

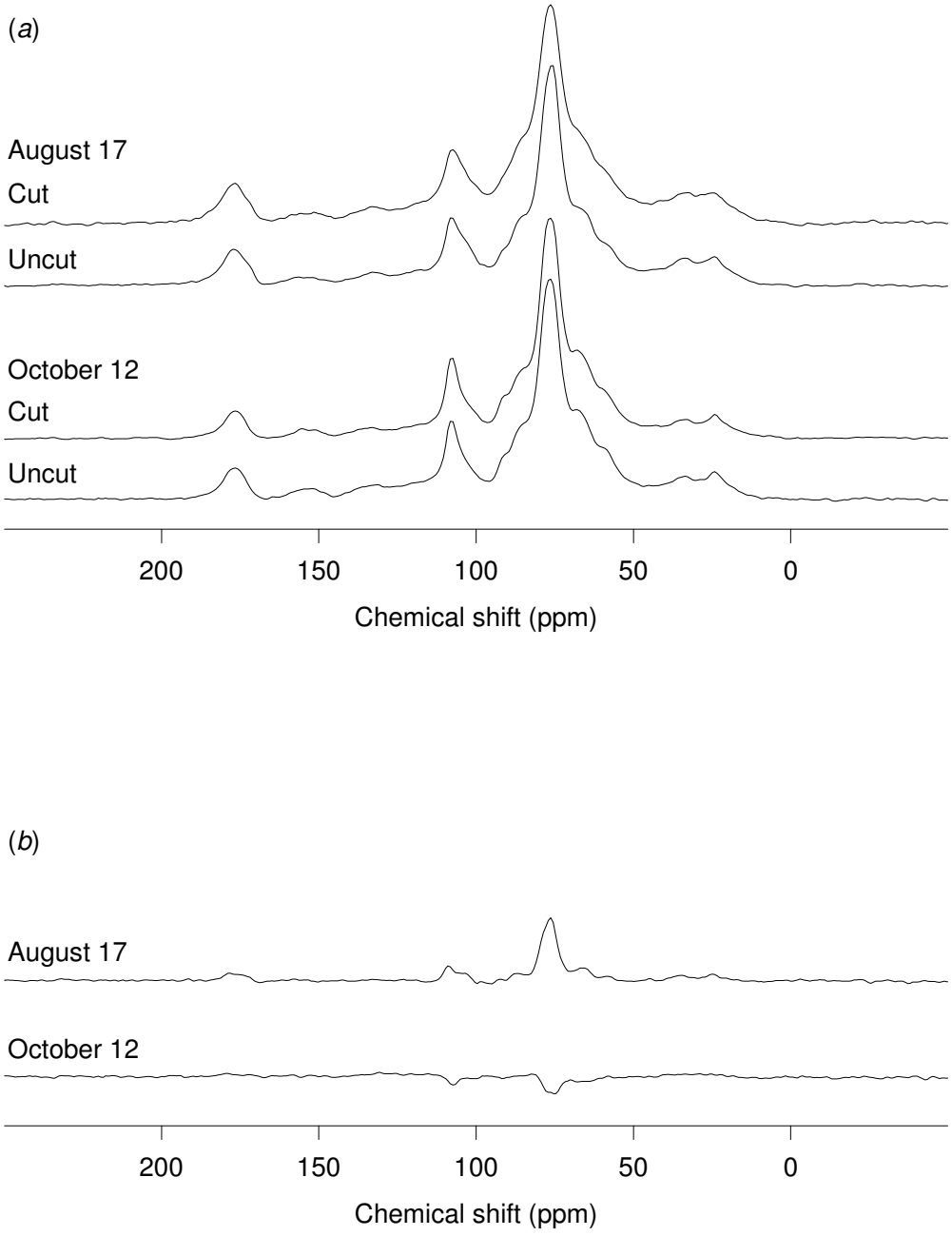

Figure 5.5. (*a*) Solid state CP/MAS ^{13}C-NMR spectra and (*b*) absolute difference (uncut - cut) between solid state CP/MAS ^{13}C-NMR spectra of roots of cut and uncut barrel medic pasture at August 17 and October 12.

CHAPTER 6

EXPERIMENT 3: ALLOCATION OF CARBON IN BARREL MEDIC BEFORE AND AFTER DEFOLIATION

1. Introduction

Previous experiments focused on the total root biomass production of a grazed barrel medic pasture (Chapter 4) and the effect of defoliation on the belowground allocation of carbon in a barrel medic pasture (Chapter 5). Results indicate that the total root biomass production is underestimated when based on root biomass sampling alone. This is because of the amount of root death and decomposition that occurs during the season (Chapter 4). Furthermore, although defoliation had no significant effect on total root biomass production, root turnover appeared to be greater when the pasture was regularly defoliated, (Chapter 5). However, because of the techniques used in these experiments, information was not obtained about the effect of defoliation on the total belowground allocation of carbon or on the net input of carbon into the soil.

There are a number of questions that were raised in the literature review (Chapter 2) relating to the allocation of carbon within barrel medic and the effect of defoliation or grazing on this allocation. What proportion of the assimilated carbon is allocated belowground? How much is released into the soil? What proportion of the assimilated carbon is released as respiration from the roots and soil? What is the effect of defoliation on belowground allocation of carbon? How is soil/root respiration affected by defoliation? Is carbon translocated from the roots to the stubble to assist in the recovery of leaf area and photosynthetic capacity? It is

proposed that the experiment reported in this chapter will answer many of these questions.

An effective way to obtain information relating to the total allocation of carbon within a plant is to grow it in pots in an atmosphere enriched in a carbon isotope such as ^{14}C. This approach has often been used to investigate the allocation of carbon within the plant and into the soil, especially in response to environmental variables (see Chapter 2, Section 4.2.2 for a discussion of the literature on this topic). Using this approach, it is possible to detect the amount of carbon in the soil and the amount of soil/root respiration derived directly or indirectly from the plant.

Continuous labelling with ^{14}C was used in this experiment to investigate the allocation of carbon in barrel medic. In addition, by continuously labelling plants with ^{13}C after defoliation, it was possible to isolate and quantify the individual components of carbon dynamics in a defoliated plant-soil system. This approach allowed the distribution of carbon before cutting, the distribution of newly assimilated carbon during regrowth, the utilisation of residual plant carbon during regrowth and the release of residual root carbon during regrowth to be individually quantified.

The experiment reported in this chapter is similar in design to that of Johansson (1993) who worked with meadow fescue grass (*Festuca pratensis*). This experiment differs from Johansson's in two fundamental ways. Firstly, an extra treatment was included so the effect of defoliation could be compared with the absence of defoliation. Secondly, the plant under investigation was a legume and not a grass, and it is possible that this might have implications for allocation of carbon after defoliation.

The aim of this experiment therefore, was to investigate the carbon dynamics of barrel medic and its response to defoliation and to determine the net effect of defoliation on the belowground input of carbon to the soil. In an attempt to replicate as closely as possible the conditions experienced in the field, plants were grown in swards in pots containing soil collected from the field site at which Experiments 1 (Chapter 4) and 2 (Chapter 5) were conducted.

2. Materials and methods

2.1 Soil

The soil, a clay loam, was collected from the top 15 cm at a site at Roseworthy, 60 km north of Adelaide, South Australia. The soil at the site is a Supracalcic, Red Chromosol (thick, non-gravelly, loamy/clay loamy, moderate) (Isbell 1996). According to the US classification system, it is a Calcic Rhodoxeralf (Soil Survey Staff 1994). The soil had an organic C content of 18 g/kg and a mean pH (H_2O 1:5) of 7.7. Air-dry soil was sieved (4 mm), fertilised, and wetted to a water potential of about -50 kPa (about 18 % w/w). The fertiliser supplied 87 mg N/pot, 18 mg P/pot and 29 mg K/pot as well as trace elements. The amount of water necessary to attain the desired water potential was determined from moisture retention data obtained using pressure plate apparatus. Soil was packed to a density of 1.18 g dry soil/cm^3 (about 4800 g dry soil/pot).

2.2 Labelling in the growth cabinet

Seeds of barrel medic (*Medicago truncatula* Gaertn. cv. Paraggio) were pre-soaked on moist filter paper in a Petri dish, inoculated with *Rhizobium meliloti* (Group A inoculant), and transferred to pots 2 days later. Pots were made of PVC tubing and were 25 cm long and 15 cm in diameter (Figure 6.1). The pots were designed such that the root-soil atmosphere was separated from the shoot atmosphere. This allowed the CO_2 respiration originating from the roots and soil to be isolated and collected. The air-tight lid had seven 2 cm diameter holes in it in which were secured small PVC cylinders (3 cm long) into which the seeds were planted. These cylinders projected 0.5 cm into the soil and 0.5 cm above the top of the lid and there was a 2 cm headspace between the top of the soil and the lid from where CO_2 could be collected.

Initially, three seeds were placed in each cylinder. Five days later, after the seedlings had emerged, these were thinned to one plant per cylinder. Consequently, there were seven equally spaced plants in each pot. This was an attempt to simulate sward conditions (plant density about 400 plants/m^2). After a further 7 days, when the spade leaf had fully emerged, plant stems were sealed into the cylinders with silicon rubber (Dow Corning Silastic, 738 RTV, Brussels, Belgium). A further 5 days later, when all pots were totally air-tight, they were placed in a growth cabinet.

The growth cabinet (2 m long x 1 m wide x 1 m high) was located in a glasshouse and hence daylength and radiation were not controlled. The experiment was conducted during May and June, a time of shortening daylength and generally low radiation in the southern hemisphere. This corresponds to when barrel medic would typically be germinating in the field. Other environmental conditions were able to be controlled

and were maintained as follows: day temperature 18-22 ºC, night temperature 13-15 ºC, day humidity 60-80 %, night humidity 75-95%, and CO_2 concentration 340-360 µL/L.

Initially, 16 pots were placed in the growth cabinet and exposed to a ^{14}C-labelled CO_2 atmosphere (specific activity 3.3 kBq/mg C). Twelve pots were planted with barrel medic, two contained soil only and two were left empty. After 30 days, when stolons of the barrel medic were 20 to 30 cm in length, the ^{14}C phase was brought to an end. At this time, three barrel medic pots were removed and abandoned because some plants had died, thus leaving nine barrel medic pots to be allocated to the treatments.

Of the remaining pots, three barrel medic pots were removed and destructively harvested to determine the distribution of ^{14}C, three barrel medic pots were defoliated to 5 cm height, and three barrel medic pots were kept intact as uncut controls. The latter six pots, plus the two soil pots and two empty pots, were then returned to the growth cabinet and grown in a ^{13}C-labelled CO_2 atmosphere (enrichment 3.6 atom % ^{13}C excess) for a further 19 days. The experimental design is expressed diagrammatically in Figure 6.2.

$^{14}CO_2$ or $^{13}CO_2$ was generated by pumping labelled Na_2CO_3 into a flask containing phosphoric acid, whenever CO_2 levels fell below 350 µL/L. The liquid in the flask was continuously agitated by bubbles produced by an aquarium pump and a fan rapidly dispersed the labelled CO_2 throughout the chamber. Trays of Purafil, a commercial preparation of acid permanganate on activated alumina (Purafil Inc., Chamblee, Georgia, U.S.A.), were placed near the fan and in each corner of the cabinet to absorb potentially toxic ethylene produced by the plants (Martin and

Sinnaeve 1987). Four of the pots were located on gravimetric balances that could be read from outside the cabinet and water was supplied when needed to all pots as indicated by the mean weight of the pots on balances. Water was supplied from outside the cabinet by a plastic tube (3 mm diameter).

CO_2 respiration from the soil and roots was collected by means of two tubes connected to the top of the pots (Figure 6.3). Air was continuously pumped through a soda lime scrubber to remove all CO_2 and then into each pot individually at the rate of 5 mL/min. The second tube leading away from each pot led to a CO_2 trap where sinter sticks bubbled it through 50 mL of 2 M NaOH. These traps were changed every 3 days, except for the first 8 days after defoliation when traps were changed every 2 days. Tubes led from these primary traps into a combined back-up NaOH trap to ensure that no $^{14}CO_2$ escaped into the atmosphere. Regular samples of this trap showed that very little CO_2 was present. This indicates that the primary traps were very effective in trapping all of the respiration evolving from the soil and roots.

2.3 Harvesting and sample preparation

When it was time for pots to be destructively harvested, shoots were cut as close as possible to the soil surface as the silastic seal would allow (about 2 cm from the soil surface). At the time of the first defoliation, the pots that were destructively harvested were first treated in a similar way to the cut treatment (*i.e.* cut at 5 cm from the base) so that the distribution of ^{14}C in the stubble (shoot material remaining after defoliation) and in the leaf (shoot material removed by defoliation) could be determined. All shoot material was dried at 50 ºC and weighed. All roots and nodules that could be seen with the naked eye were carefully removed from the soil using

tweezers. Roots were gently washed in a minimum volume of water (about 400 mL/pot in total) and the wash water was then poured onto the remaining soil and thoroughly mixed. Roots were also dried at 50 ºC and weighed and a subsample of soil was dried at 90 ºC and weighed. The separation of roots from soil took a day for each pot so pots were stored at 4 ºC whilst awaiting processing.

2.4 Chemical analyses

Total CO_2-C collected in 2 M NaOH was determined by titration with HCl after precipitation with excess $BaCl_2$. ^{14}C-labelled CO_2-C was determined by liquid scintillation counting, using Ready Gel™ (Beckman) as the scintillation cocktail. ^{13}C-labelled CO_2-C was determined by adding excess $BaCl_2$ to the NaOH and collecting the precipitated $BaCO_3$ by centrifuging and drying. The atom % ^{13}C excess in the precipitate was then determined in a Europa 20-20 mass spectrometer using an ANCA-*SL* elemental analyser. NaOH traps connected to the two empty pots were used as blanks when determining total CO_2-C evolution. The traps connected to the two pots containing soil only were used as blanks for the purposes of determining ^{14}C- and ^{13}C-labelled CO_2-C.

All plant and soil material was ground in a ring mill and total C and ^{14}C were determined after wet digestion using the method of Amato (1983). ^{13}C in ground plant and soil material was determined using the Europa 20-20 mass spectrometer.

2.5 Calculations

In order to compare amounts of carbon in each compartment derived from pre- and post-defoliation assimilation, comparable units for ^{14}C-labelled C and ^{13}C-labelled C must be used. The following calculations were applied to convert ^{14}C-labelled C (kBq) and ^{13}C-labelled C (atom % ^{13}C excess) to ^{14}C-labelled C (mg) and ^{13}C-labelled C (mg) respectively.

The amount of ^{14}C-labelled C (mg) in each compartment was calculated by relating the measured value for ^{14}C (kBq) to the specific activity (SA) of the atmosphere in which the plants were grown.

$$^{14}\text{C-labelled C (mg)} = {}^{14}\text{C (kBq)} / \text{SA}_{\text{atmosphere}} \text{ (kBq/mg C)} \qquad \text{(Equation 6.1)}$$

The amount of ^{13}C-labelled C (mg) in each compartment was calculated by relating the amount of ^{13}C in the sample to the atom (at) % ^{13}C excess in the ^{13}C-labelled atmosphere as follows:

$$^{13}\text{C-labelled C (mg)} = (\text{at \% } {}^{13}\text{C excess}_{\text{sample}} \times \text{total C}_{\text{sample}} \text{ (mg)}) / \text{at \% } {}^{13}\text{C excess}_{\text{atmosphere}}$$

(Equation 6.2)

2.6 Statistical analyses

Where applicable, cut and uncut treatment means were compared using *t*-tests with an assumption of equal variance where standard errors of the means differed by less than

a factor of 2 (Maindonald 1992). Where standard errors of the means differed by a factor greater than 2, a *t*-test was performed using the assumption of unequal variances.

3. Results

3.1 Biomass

At the first harvest, defoliation resulted in the removal of 1.03 g/pot from the cut treatment as leaf material (Table 6.1). Before being destructively harvested, the pots that were sacrificed at this time were also clipped in the same manner and 0.60 g/pot was removed as leaf material (Table 6.1). It is apparent that the pots that were destructively harvested for the purpose of estimating the distribution of ^{14}C in the remaining pots were in fact, not representative of the remaining pots. The plants were actually slightly smaller in size.

As a consequence of this, an estimation was made of the dry weight and the ^{14}C content of plant parts in the remaining plants based on the dry weight and ^{14}C content of the leaf fraction of the defoliated treatments. It has long been observed that there is an allometric relationship between the sizes of growing plant organs such that the logarithm of the size of one organ is directly proportional to the logarithm of the size of the other organ (Pearsall 1927). Based on this assumption, dry weight and ^{14}C content of stubble and roots and allocation of ^{14}C to the soil in the defoliated plants was estimated using the logarithmic relationship of these parts in the harvested plants. The estimated values of dry matter and ^{14}C content for the cut plants at the first harvest are listed in Tables 6.1 and 6.3 alongside the actual values of the harvested

plants. These estimated values were subsequently used in all pre- and post-defoliation comparisons.

Between the first and final harvests, shoot dry weight increased by 1.47 g/pot in the cut treatment and 2.58 g/pot in the uncut treatment over their estimated values at the first harvest (Table 6.1) but the difference was not significant. After allowing for the 1.03 g/pot of leaf material that was removed in the cut treatment at the first harvest, total shoot production was 2.96 g/pot which was still less than in the uncut treatment, but not significantly so. Belowground, there was a 0.10 g/pot increase in dry weight in the roots of the cut treatment and a 0.37 g/pot increase in the roots of the uncut treatment over the estimated values at the first harvest (Table 6.1). Again, the difference was not significant.

3.2 ^{14}C content

At the end of the first growth period, the specific activities (^{14}C content expressed as kBq/mg C) of the leaf and stubble material in the harvested pots were similar and both were slightly higher than the roots (Table 6.2). The specific activity of the leaf material of the defoliated plants at the first harvest was 1.02 kBq/mg C which was almost the same as that of the harvested plants. Specific activity of all plant parts was less than the atmosphere in which they were grown.

During the second growth period, dilution with ^{13}C-labelled C resulted in reduced ^{14}C specific activities in the shoots and roots of both treatments (Table 6.2). At the final harvest, there was no significant difference between the specific activities of the shoot material of the two treatments. However, the specific activity of the roots was

significantly less in the uncut treatment (Table 6.2) as the greater root biomass accumulation resulted in a greater dilution of ^{14}C in the roots (Table 6.1).

At the end of the first growth period, 72.6 % of the net assimilated ^{14}C was found in the plants and 27.4 % was recovered in the soil and as rhizosphere respiration (Table 6.3). In total, 38.4 % of assimilated ^{14}C was allocated belowground and was recovered in roots, soil or as rhizosphere respiration. Of the ^{14}C allocated belowground, 28.6 % was recovered in the roots, 12.0 % was recovered in the soil and 59.4 % was released as rhizosphere respiration.

At the final harvest, the amount of ^{14}C content in each plant part of both treatments was less than that estimated to be in the respective plant parts at the time of the first harvest (Table 6.3). Most importantly, the ^{14}C content of the shoots of the cut plants decreased from 292 kBq/pot at the first harvest to 227 kBq/pot at the final harvest, thus indicating no net retranslocation of ^{14}C from the roots back to the shoots after defoliation. There was an increase in soil ^{14}C in both treatments and a continued release of ^{14}C as rhizosphere respiration, although not to the same extent as during the ^{14}C growth period (Table 6.3).

With the exception of the shoot compartment, no significant difference was observed in ^{14}C contents of each compartment of the two treatments at the final harvest. The significant difference that was observed was the direct result of 424 kBq/pot being removed at the first harvest by the defoliation treatment (Table 6.3).

The estimated amount of ^{14}C remaining in the cut plants at the first harvest was 403 kBq/pot. At the final harvest, 324 kBq/pot was recovered in the plants, leaving a

shortfall of 79 kBq/pot, most of which was released to the soil and as root respiration. The difference in total ^{14}C contents of the uncut plants between the first (827 kBq/pot) and final (550 kBq/pot) harvests was 277 kBq/pot. Of this, only an extra 82 kBq/pot was recovered in the soil and as rhizosphere respiration (Table 6.3), thus leaving a relatively large amount unaccounted for.

The results detailed in the paragraph above are reflected in the total amount of ^{14}C recovered at the end of the final harvest relative to that present in the pots at the first harvest. In the cut treatment, there was 441 kBq/pot at the first harvest, and 407 kBq/pot was recovered in shoots, roots, soil and rhizosphere respiration at the final harvest, leaving a shortfall of 34 kBq/pot (Table 6.3). In the uncut treatment, there was a shortfall of 195 kBq/pot, that being the difference between the estimated residual in the pot at the first harvest (865 kBq/pot) and the total amount of ^{14}C recovered at the final harvest (670 kBq/pot).

3.3 ^{13}C content

At the end of the second growth period, during which the plants were grown in a ^{13}C labelled atmosphere, the ^{13}C enrichment of the shoots and roots of both treatments was determined (Table 6.4). There was no significant difference between treatments in enrichment of shoot material, but enrichment in the roots of the cut treatment was significantly less than that of the uncut treatment. Within the uncut treatment, enrichment with ^{13}C was uniformly distributed between shoots and roots, whereas in the cut treatment, the shoots were significantly more enriched than the roots (Table 6.4).

Table 6.5 details the distribution of ^{13}C-labelled C within the pots. Primarily because of the large variation in dry weight between replicates, there was no significant difference between treatments in the absolute amounts of ^{13}C-labelled C found in each compartment. The total amount of ^{13}C-labelled C recovered from the pots of the uncut treatment was 86 % greater than the amount recovered from the pots of the cut treatment, but the difference was not significant.

The relative distribution of the ^{13}C-labelled C that was recovered from each pot was very similar for each treatment (Table 6.5). The proportion of ^{13}C allocated belowground was 19.9 and 23.5 % for the cut and uncut treatments respectively. Of the ^{13}C-labelled C allocated belowground, 52 % was found in the roots, 20 % was found in the soil and 28 % was recovered as rhizosphere respiration, irrespective of whether the shoots had been defoliated 19 days earlier or not.

3.4 Rhizosphere respiration

Labelled CO_2 evolution from the roots and soil was followed and compared by converting kBq of ^{14}C and atom % excess ^{13}C to mg of labelled CO_2 as detailed previously. Cumulative ^{14}C-CO_2 evolution increased exponentially from the time the plants were placed in the ^{14}C-labelled atmosphere to the time they were removed (Figure 6.4). The maximum rate of ^{14}C-CO_2 evolution was achieved immediately prior to the end of the ^{14}C labelling period and averaged 3.21 mg of ^{14}C labelled CO_2-C/day over the last 3 days. Cumulative ^{14}C-labelled CO_2-C up until defoliation is derived from the mean of the six pots that were grown for the whole experimental period and does not include the three pots that were destructively harvested at the first harvest.

Defoliation decreased ^{14}C-CO_2 evolution relative to the uncut control but the difference was not significant (Table 6.6). At the end of the ^{13}C growth period, 19 days after defoliation and 22 days after removal from the ^{14}C atmosphere, the rate of ^{14}C-CO_2 evolution was 0.36 and 0.66 mg of ^{14}C-labelled CO_2-C/day for the cut and uncut treatments respectively (averaged over the last 5 days). At no time after defoliation was there a significant difference between pots in the rate of ^{14}C-CO_2 evolution (Table 6.6).

The effect of defoliation on respiration of carbon assimilated after defoliation is visibly demonstrated by the differences in cumulative evolution of ^{13}C-CO_2 (Figure 6.4). In the first 2 days after defoliation, ^{13}C-CO_2 was being released from the pots of the uncut plants at the rate of 0.80 mg of ^{13}C-labelled CO_2-C/day which was significantly greater than the 0.11 mg of ^{13}C-labelled CO_2-C/day being released from the pots of the cut plants (Table 6.6). There continued to be a significant difference in the rate of ^{13}C-CO_2 evolution between the treatments up until 8 days after defoliation. Nineteen days after defoliation, the rate of ^{13}C-CO_2 evolution was 2.87 and 4.26 mg of ^{13}C-labelled CO_2-C/day for the cut and uncut treatments respectively (averaged over the last 5 days), but the difference was not significant (Table 6.6).

In the uncut control, the rate of evolution of 'new' (^{13}C) carbon from the rhizosphere began to exceed that of 'old' (^{14}C) carbon about 2 days after being placed in the ^{13}C-CO_2 atmosphere, whereas this took 8 days in the defoliated treatment (Table 6.6).

Total labelled CO_2 evolution was determined as the sum of ^{13}C-labelled and ^{14}C-labelled CO_2-C. After being placed in the ^{13}C atmosphere, there was a drop in the rate of total labelled C in both treatments because the pots were growing in an unlabelled

atmosphere for 3 days prior to entering the ^{13}C atmosphere whilst preparations were made for defoliation. Consequently, it took 6 days for the rate of total labelled rhizosphere respiration to exceed the levels that were measured in the last days of the ^{14}C growth regime. In the cut treatment, the rate of total labelled C evolution decreased immediately after defoliation (Figure 6.4). It was not until 14 days after defoliation that the rate of total labelled CO_2 evolution exceeded that of pre-defoliation levels. This was 8 days later than the uncut treatment.

3.5 Total carbon allocation

By converting ^{14}C-labelled C and ^{13}C-labelled C to comparable units (mg labelled C/pot), the total allocation of carbon for each treatment was calculated. This allowed the determination of the combined contribution of pre- and post-defoliation assimilate to belowground pools of carbon. Although there were greater total amounts of labelled carbon in all compartments of the uncut control at the final harvest, no significant differences were observed (Table 6.7). Most importantly, the total belowground allocation of labelled carbon in the cut treatment was not significantly different from that of the uncut control.

4. Discussion

4.1 Distribution of carbon before defoliation

The distribution of assimilated ^{14}C in the plants after 30 days of growth in a ^{14}C labelled atmosphere (42 days after emergence) was 85 % in the shoot and 15 % in the root (Table 6.3). The distribution of biomass at this time was similar, with 83 % of

dry matter in the shoot and 17 % in the root (Table 6.1). Similar distribution figures are often found for annual legume species. Following a review of the literature, Gregory (1988) stated that in general, root weight represents 10 to 15 % of total plant dry weight for annual legume species.

The difference between the distribution of ^{14}C (*i.e.* carbon assimilated between 12 and 42 days after emergence) and the distribution of dry matter (*i.e.* seed carbon and carbon assimilated between 0 and 42 days after emergence) in the plant arises from the decreasing proportional allocation of carbon to the roots as the plant develops. For example, Whipps (1987) found the proportion of plant ^{14}C found in the roots of pea (*Pisum sativum*) seedlings decreased from 32 % after 14 days to 13 % after 28 days growth in a continuously ^{14}C-labelled atmosphere.

At the end of the ^{14}C labelling period, the amount of ^{14}C found in the soil was 28 kBq/pot (Table 6.3). In quantifying the input of carbon into the soil, this amount should be considered as well. ^{14}C in the soil is likely to be derived from either fragments of root that were too small to be picked out during the root removal procedure, secretions or mucilage exuded from the roots, or from root-derived carbon that has been incorporated into the soil microbial biomass or humus. If this amount is included, then about 20 % of the carbon found in the pot and the plant at the first harvest was recovered belowground.

During the 30 days of growth in the ^{14}C labelled atmosphere, a further amount of ^{14}C was recovered in rhizosphere respiration. It is uncertain how much of this carbon was derived directly from the root as root respiration and how much consisted of respiration from the breakdown of carbon material that had been deposited into the

soil by the root. In an experiment with 3 week old wheat plants, Cheng *et al*. (1993) found that 59 % of total rhizosphere respiration could be attributed to microbial respiration. This could be an overestimation, because in their technique, a short ^{14}C allocation period meant that ^{14}C root respiration may have been incomplete. Using a different methodology, Swinnen (1994) suggested that no more than 25 % of rhizosphere respiration evolving from field-grown wheat and barley could be attributed to microbial respiration of root-derived carbon.

If it is assumed that 25 % of the rhizosphere respiration in this experiment was derived from the breakdown of products released from the roots into the soil, then the total input of organic carbon into the soil can be estimated. Using this approach, the ratio of belowground input to aboveground production after 30 days in the ^{14}C-labelled atmosphere was 0.35. This figure is compatible with the ratios presented in Chapters 4 and 5, but as discussed in the literature review (Chapter 2), this figure can vary greatly with plant size, stage of development, moisture, nutrition and other biotic and abiotic factors.

4.2 Biomass production after defoliation

At the final harvest, there was no significant difference in shoot or root biomass between the cut and uncut treatments (Table 6.1). In the field (Chapter 5), regular defoliation had a statistically significant detrimental effect on both aboveground biomass production and live root biomass. The inability of this experiment to show statistically significant differences (at $P = 0.05$) between treatments was probably related to the small number of replicates, given the large variability between replicates.

Plant mortality in some of the pots had reduced the number of useable replicates to three per treatment.

Biomass production before and after defoliation can be affected by plant density (Simpson and Culvenor 1987). In this experiment, seven plants were grown in each pot (at an equivalent density of about 400 plants/m^2) in an attempt to simulate sward conditions found in the field. Regrowth under sward conditions has not been widely studied as most reports have focused on single or spaced plants (Culvenor *et al*. 1989a). It is widely recognised that canopy architecture can have a significant effect on regrowth and on the photosynthetic capacity of emerging leaves through its influence on light quantity and quality (Simpson and Culvenor 1987). Presumably, plant density and canopy architecture also has an effect on belowground fluxes of carbon as well. For example, Pearson and Jacobs (1985) showed that in subterranean clover, the amount of root per unit area increased with population.

4.3 Distribution of newly assimilated carbon after defoliation

The distribution of newly assimilated carbon (^{13}C) after defoliation was similar to the distribution of assimilated carbon before defoliation, in that 89 % of ^{13}C found in the plant was found in the shoot and 11 % was found in the roots. The slight shift in favour of the shoots is in line with the increasing allocation aboveground as plant development continues (Keith *et al.* 1986, Whipps 1987) and was unlikely to be related to the effect of defoliation. As evidence for this, Table 6.5 shows that the relative allocation of ^{13}C within the plant was not significantly different from that within the uncut control.

If the total amount of ^{13}C allocated to the soil is estimated as above (Section 4.1 of this chapter) by including all of the ^{13}C found in the soil and 25 % of ^{13}C released as rhizosphere respiration, then the ratio of belowground input to aboveground production for carbon assimilated after defoliation can be estimated at 0.20. The corresponding figure for the uncut control is 0.25 but the difference is not significant.

4.4 Distribution of residual plant carbon after defoliation

The distribution of ^{14}C in the cut treatment before and after defoliation indicates no net translocation of carbon from the roots to the shoots after defoliation (Table 6.3). At the final harvest, there was less ^{14}C in the shoots than in the stubble at defoliation and there was less ^{14}C in the roots than at defoliation, but there was more ^{14}C in the soil and an amount of ^{14}C had continued to evolve as rhizosphere respiration. This all indicates that the net flow of ^{14}C continued to be downwards through the roots and into the soil. The uncut control exhibited a distribution of ^{14}C similar to that of the cut treatment.

In a similar experiment using meadow fescue (*Festuca pratensis*), Johansson (1993) separated aboveground growth into shoot (above 15 mm) and crown (below 15 mm) compartments at each harvest. Because there was ^{14}C present in shoot material at the final harvest, Johansson (1993) concluded that this must have been translocated from the residual plant parts (crowns and/or roots) after the first cut. However, because grasses grow from the base of the plant, it is possible that ^{14}C was carried with the newly expanding leaves in the crown rather than actively translocated from remaining plant material to 'new' plant material after defoliation.

In this experiment, no attempt was made at the final harvest to separate leaf material into stubble (crowns) and leaf (shoots). This is because it was uncertain which leaves developed before defoliation and 'expanded' into the newly available space (taking residual ^{14}C with them) and which leaves developed after defoliation and were the depositories for translocated ^{14}C. Any attempt to partition shoot ^{14}C into stubble and leaves at the final harvest would have involved considerable guesswork.

The role of root reserves in assisting regrowth of barrel medic after defoliation has not been specifically investigated before. Research with graminaceous species suggests that in some species, especially perennial species, root reserves can be an important part of the plant's response to defoliation (Ryle and Powell 1975, Richards 1993). The role of carbohydrate root reserves in the re-establishment of leaf area after defoliation in the perennial legume lucerne (*Medicago sativa*) is also well known (Smith 1962).

The story concerning annual legume species is less clear. May and Davidson (1958) suggest any loss of carbohydrate from the roots of subterranean clover (*Trifolium subterraneum*) following defoliation is associated not with translocation to the shoots but with its utilisation as a respiratory substrate. Culvenor *et al*. (1989a) found that mobilisation of nitrogenous compounds from the roots of subterranean clover can be of equal or greater importance than carbohydrates after severe defoliation. This is similar to wheat, where it has been found that root carbon can be retranslocated to the shoots as amino acids (Lambers *et al.* 1982). In this experiment, there was no evidence of translocation of carbon from the roots to the shoots in response to defoliation.

At the final harvest, the amount of ^{14}C found in the soil in both treatments had increased since the first harvest (Table 6.3). This indicates that 'old' carbon continued to be released into the soil, either through root senescence or through exudation. Importantly, at the final harvest, there was no significant difference in soil ^{14}C between treatments, so the hypothesis that defoliation leads to increased root mortality (Chapter 5) was not confirmed in this experiment. One possible explanation for this could be that because the plants were younger than those in Experiment 2, root mortality would not have occurred as easily. A further possibility is that the more favourable growing conditions (*i.e.* water, nutrients and temperature) in the growth cabinet would also have made the roots less likely to die in response to defoliation.

4.5 Respiration

The rate of rhizosphere respiration increased up until defoliation as the plants continued to increase in size (Figure 6.4). After being placed in a ^{13}C-labelled atmosphere, ^{14}C-CO_2 continued to evolve from the soils of both treatments. This could be derived from either the microbial decomposition of 'old' ^{14}C-labelled organic material in the soil or 'new' ^{14}C-labelled root material released during the second growth period. In this experiment, it was not possible to separate the two possible sources of this respiration. Nevertheless, at no time was there any difference between the two treatments in the rate of ^{14}C-CO_2 evolution from the rhizosphere (Table 6.6).

After defoliation, there was a decrease in the rate of total rhizosphere respiration from the soil of the cut plants. The significant difference in ^{13}C-CO_2 evolution between treatments after defoliation indicates that this decrease was largely attributable to a decrease in the belowground flux of newly assimilated carbon immediately after

defoliation (Table 6.6). This is in accordance with the hypothesis that immediately after defoliation, plants preferentially allocate newly assimilated carbon to aboveground components in an attempt to restore leaf area (Richards 1993). For 8 days after defoliation, the cut treatment exhibited significantly lower rates of ^{13}C-CO_2 evolution than the uncut control. This suggests that it took this long for the effect of defoliation to be overcome and for the plant to resume its normal pattern of allocation.

In Chapter 5, it was postulated that after the recovery phase, defoliated plants enter a phase of compensatory belowground allocation as photosynthetic rates aboveground match or exceed those of uncut plants. If there was a significantly greater amount of carbon being allocated belowground in the cut treatment, it would be expected that this would be reflected in greater rhizosphere respiration, especially in the current assimilate (^{13}C) component. However, the results of this experiment do not confirm this hypothesis as there was no significant difference between treatments in the absolute rate of rhizosphere respiration after this recovery phase.

4.6 Total carbon balance

One of the most important results of this experiment is that relative to the uncut control, defoliation did not have a significantly detrimental effect on belowground carbon allocation (Table 6.7). This is in accordance with the results presented in Chapter 5, where the total root biomass production of the defoliated pasture was not significantly different from that of the uncut pasture. Defoliation resulted in a reduced rate of rhizosphere respiration (presumably as a result of a short-term decreased belowground flux of carbon), but it was not great enough to significantly affect total belowground inputs of carbon.

4.7 Conclusion

It is recognised that the extent to which defoliation affects carbon dynamics within the plant may still depend on the severity of defoliation. The amount of residual leaf area remaining, or the time taken to re-attain a critical leaf area index at which irradiance is absorbed, will affect the time taken for the sward to resume its normal pattern of carbon allocation (Simpson and Culvenor 1987). Additionally, the extent to which the results of this experiment can be extrapolated to the field will depend on the interaction of defoliation with other environmental factors such as moisture and nutrient stress.

Nevertheless, this experiment and the field experiment in Chapter 5 confirm that a certain amount of defoliation can be tolerated without having a significant negative effect on the belowground input of carbon by barrel medic. Further research could be directed to elucidating the effect of interactions of moisture and nutrient stress with different intensities of defoliation on the belowground allocation of carbon.

Table 6.1. Dry weights of different plant parts. (n.s. = not significant.)

	First harvest		Final harvest		
	Actual (g/pot)	Estimated[1] (g/pot)	Cut (g/pot)	Uncut (g/pot)	Significance
Leaf	0.60	1.03			
Stubble	0.46	0.86			
Shoots[1]	1.06	1.89	1.93	3.64	n.s.
Roots	0.22	0.36	0.46	0.73	n.s.

[1]Estimated dry weights of biomass in remaining plants based on dry weight of leaf. See text for details.

[2]At the first harvest, shoots equals the sum of leaf and stubble.

Table 6.2. Specific activities of different plant parts after growth in $^{14}CO_2$ atmosphere (first harvest) followed by growth in $^{13}CO_2$ atmosphere (final harvest).

(n.s. = not significant, * = P<0.05.)

	First harvest	Final harvest		
		Cut	Uncut	Significance
	(kBq/mg C)	(kBq/mg C)	(kBq/mg C)	
Leaf	1.03			
Stubble	0.97			
Shoots		0.28	0.33	n.s.
Roots	0.88	0.55	0.32	*

Table 6.3. ^{14}C content in plant parts, soil, rhizosphere respiration and residual pools at the first and final harvests after growth in $^{14}CO_2$ atmosphere between seedling stage and first harvest. (Standard error of the means in parenthesis, n=3, n.s. = not significant, * = $P<0.05$.)

	First harvest			Final harvest		
	Harvested		Estimated[1]	Cut	Uncut	Significance
	(kBq/pot)	(%)	(kBq/pot)	(kBq/pot)	(kBq/pot)	
Leaf	217 (36)	35.8	424			
Stubble	157 (31)	25.8	292			
Shoots[2]	374 (53)	61.6	716	227 (55)	471 (140)	*
Roots	67 (16)	11.0	111	97 (35)	79 (32)	n.s.
Soil	28 (12)	4.6	38	52 (6)	73 (42)	n.s.
Rhizosphere respiration	137 (7)	22.8		31 (5)	47 (18)	n.s.
Total ^{14}C recovered	606			407	670	
Residual ^{14}C in pot[3] (cut)	252		441			
Residual ^{14}C in pot[4] (uncut)	469		865			
Residual ^{14}C in plant[5] (cut)	224		403			
Residual ^{14}C in plant[6] (uncut)	441		827			
Total ^{14}C in plant[7]				324	550	

[1]Estimated distribution of ^{14}C in remaining plants based on ^{14}C in leaf. See text for details.

[2]The sum of ^{14}C in leaf and stubble.

[3]The sum of ^{14}C in stubble, roots and soil.

[4]The sum of ^{14}C in leaf, stubble, roots and soil.

[5]The sum of ^{14}C in stubble and roots.

[6]The sum of ^{14}C in leaf, stubble and roots.

[7]The sum of ^{14}C in shoots and roots at the final harvest.

Table 6.4. ^{13}C enrichment (atom % excess) of different plant parts after growth in $^{13}CO_2$ atmosphere between first and final harvests.

(n.s. = not significant, ** = $P<0.01$, *** = $P<0.001$.)

	Cut (Atom % excess)	Uncut (Atom % excess)	Significance
Shoots	1.80	1.71	n.s.
Roots	1.07	1.61	**
Significance	***	n.s.	

Table 6.5. ^{13}C-labelled C in plant parts, soil and rhizosphere respiration of cut and uncut plants at the final harvest after growth in $^{13}CO_2$ atmosphere between first and final harvests. (n.s. = not significant.)

	^{13}C-labelled C (mg/pot)			^{13}C-labelled C (%)		
	Cut	Uncut	Significance	Cut	Uncut	Significance
Shoots	437	770	n.s.	80.1	76.5	n.s.
Roots	56	134	n.s.	10.3	12.4	n.s.
Soil	22	45	n.s.	4.0	4.6	n.s.
Rhizosphere respiration	30	64	n.s.	5.6	6.5	n.s.
Total	545	1013	n.s.			

Table 6.6. Rate of evolution of labelled C from rhizosphere of pots in cut and uncut treatments during growth in ^{13}C-CO_2 atmosphere.

(n.s. = not significant, * = $P<0.05$, ** = $P<0.01$.)

Days after defoliation	^{14}C-labelled C (mg/pot/day)			^{13}C-labelled C (mg/pot/day)		
	Cut	Uncut	Significance	Cut	Uncut	Significance
0-2	0.69	0.78	n.s.	0.11	0.80	*
2-4	0.40	0.80	n.s.	0.39	2.05	**
4-6	0.59	0.77	n.s.	0.52	2.27	*
6-8	0.55	0.84	n.s.	1.20	3.68	*
8-11	0.59	0.67	n.s.	1.78	3.63	n.s.
11-14	0.54	0.86	n.s.	1.98	4.78	n.s.
14-19	0.36	0.66	n.s.	2.87	4.26	n.s.

Table 6.7. Total labelled C (sum of ^{14}C-labelled C and ^{13}C-labelled C) in different plant parts, soil and rhizosphere respiration at final harvest. (n.s. = not significant.)

	Cut	Uncut	Significance
	(mg labelled C/pot)	(mg labelled C/pot)	
Shoots	644	913	n.s.
Roots	85	158	n.s.
Soil	38	67	n.s.
Rhizosphere respiration	84	124	n.s.
Total belowground allocation[1]	207	349	n.s.

[1]The sum of labelled C in roots, soil and rhizosphere respiration.

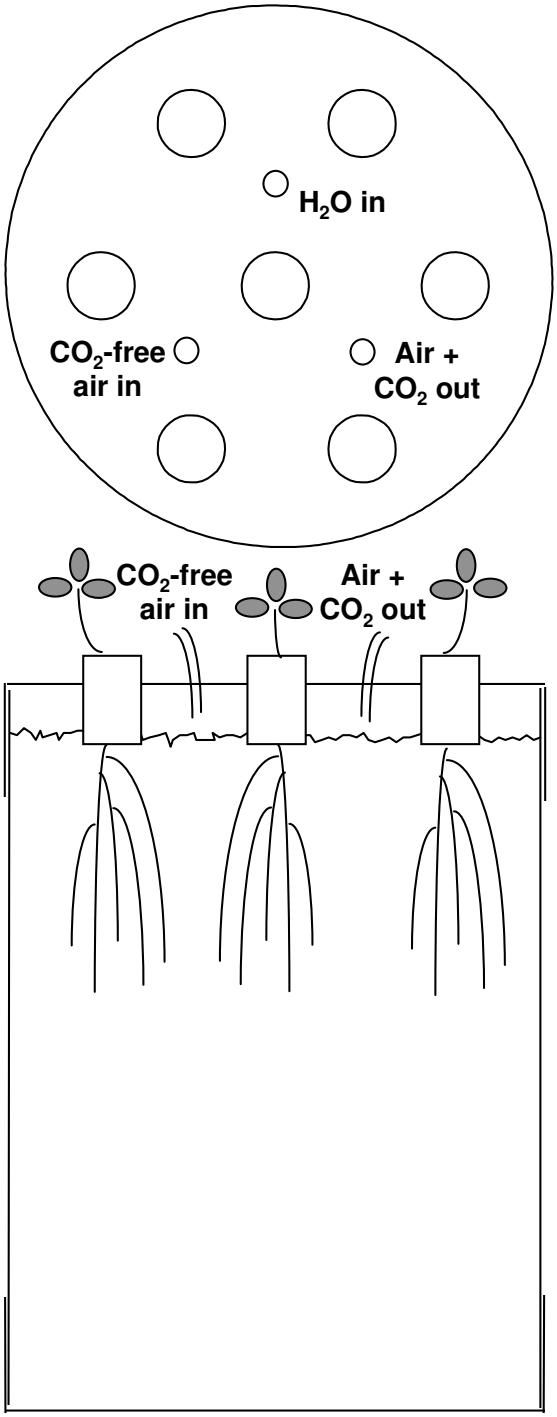

Figure 6.1. Top and side views of pot showing individual plant tubes and ports for irrigation and continuous aeration and collection of CO_2.

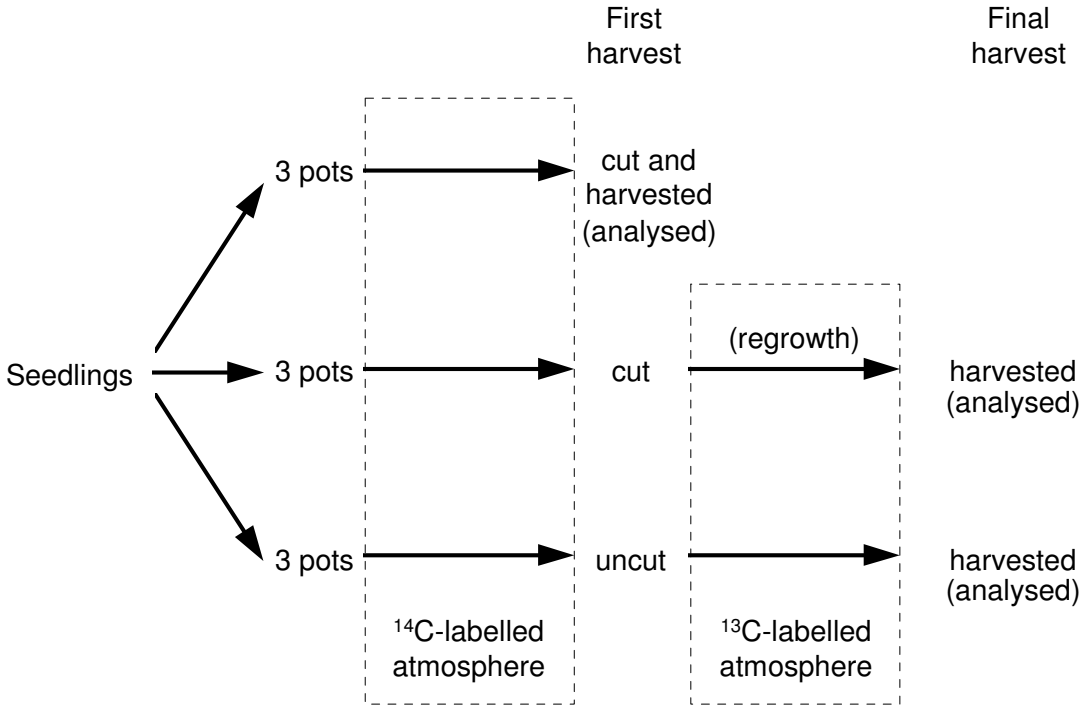

Figure 6.2. Schematic diagram of experimental design showing labelled atmosphere and harvest times.

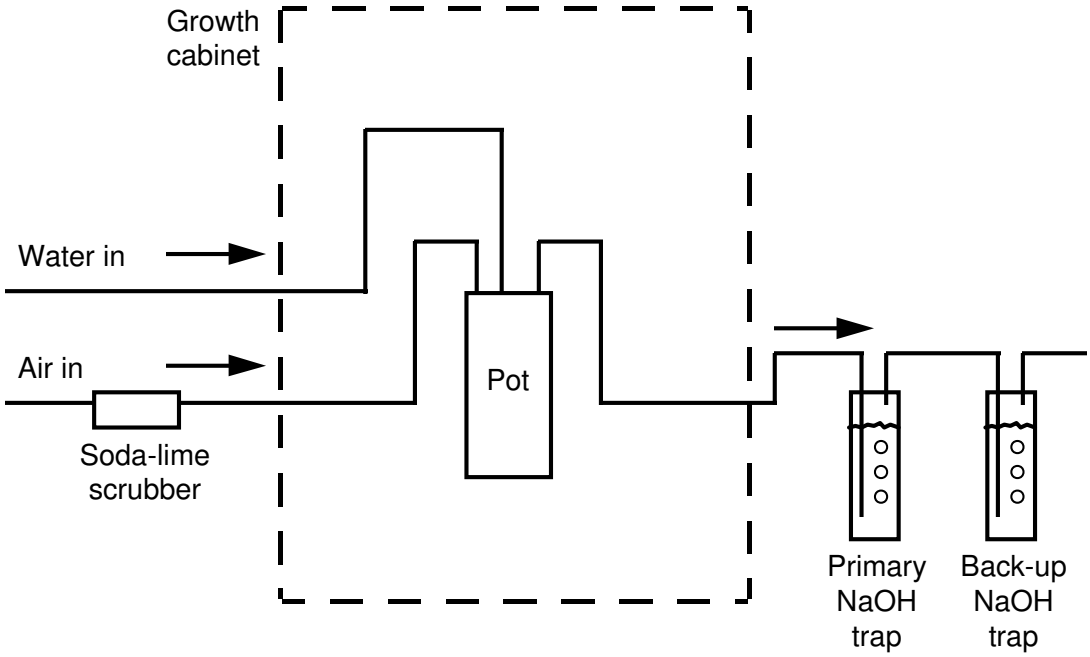

Figure 6.3. Schematic representation of collection system for rhizosphere respiration.

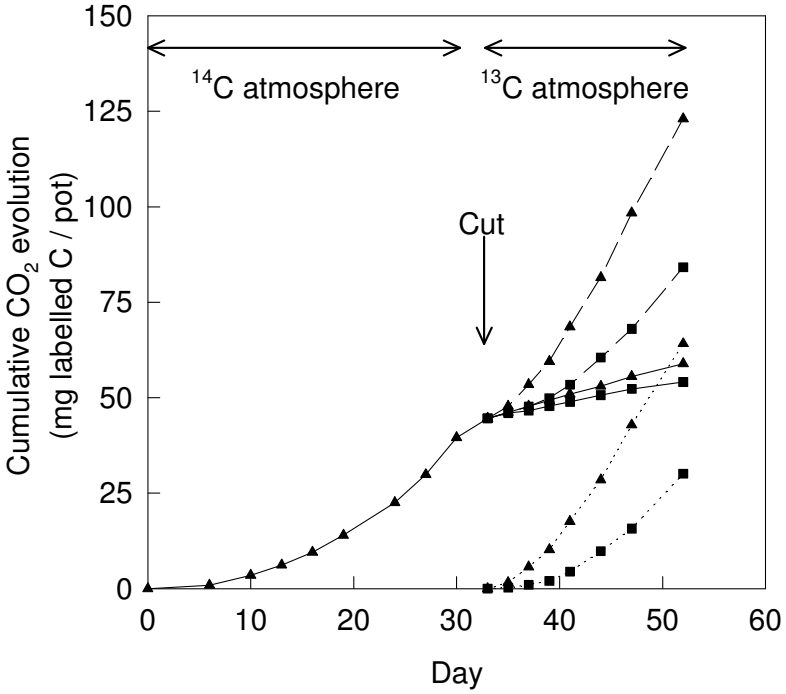

Figure 6.4. Cumulative evolution of ^{14}C-labelled (solid lines) and ^{13}C-labelled (dotted lines) CO_2-C and total rhizosphere respiration C (dashed lines) collected from the root-soil compartment of cut (ν) and uncut (σ) barrel medic plants.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

The general aim of this study was to quantify the belowground input of organic carbon by the annual pasture legume barrel medic. This information is important in the development and validation of simulation models that predict the dynamics of soil organic matter in the crop/pasture rotations of southern Australia. The study also investigated the effect of defoliation on the amount of carbon allocated belowground. The extent to which farmers can use grazing management to influence the input of organic carbon by barrel medic pastures is unknown.

Belowground inputs of carbon derive from carbon allocated to the roots of crops and pastures. Carbon enters the soil organic matter pool either in the form of root carbon as roots die, or as exudates or secretions from living roots. In annual species, a large amount of root input comes at the end of the growing season as the plants mature and senesce. There can also be a significant amount of root input before then if there is a high degree of root senescence during the growing season. Carbon is also released from the roots as respired CO_2, but as this does not pass through the soil organic matter pool, it is not considered as an input of carbon.

The significance of root death during the season as an important source of carbon was demonstrated in Experiments 1 and 2 (Chapters 4 and 5). In Experiment 1, measurements that accounted for root death during the season were made of barrel medic, barley grass and faba beans. These results showed that total root biomass production could be as much as 1.6 to 2.9 times the maximum observed live root

biomass, depending on the species and the season (Chapter 4). In Experiment 2, a different technique was used to measure the total root biomass production. In this experiment, total root biomass production was 1.5 and 1.3 times the maximum live root biomass for cut and uncut barrel medic pasture respectively (Chapter 5). The conclusion that can be drawn from this is that any measurement of live root biomass alone will greatly underestimate the contribution that has been made to the soil organic matter pool for that season.

Experiment 3 (Chapter 6) investigated the contribution made to the belowground input of organic carbon in a form either too small to be visibly identified (exudates, secretions and root fragments not visibly recognisable) or in a form that is relatively short-lived (as evidenced by respiration derived from the soil-root atmosphere). Inclusion of plant-derived carbon found in the soil in the estimation of the total input of carbon resulted in a 40 to 45 % increase above root biomass (Chapter 6). In addition, it is necessary to include that proportion of root/soil or rhizosphere respiration that is attributable to mineralisation of rhizosphere deposits. Estimates by Swinnen (1994) suggest that 25 % of rhizosphere respiration could be attributed to microbial respiration of root-derived carbon. If this is also included in the estimation of total input of carbon, then an extra 65 to 70 % of that already found in roots should be added to the root biomass carbon to most accurately estimate the total amount of carbon contributed belowground (Chapter 6).

Application of a correction factor to the results obtained in Experiments 1 and 2 to allow for root carbon left behind in the soil and microbial decomposition of root-derived material, is difficult. Because of the different techniques used for isolating root biomass, it is difficult to compare the amounts of carbon unaccounted for by each

technique. In Experiments 1 and 2, root material was isolated by retaining material on a 0.5 mm sieve and then separating live and dead roots. Subsequently, estimates of total root biomass production were based on the amount of live root material recovered. In Experiment 3, isolation of root material included live and dead roots but did not include roots that had decomposed to such an extent that they were not visibly recognisable as roots. In this experiment, estimates of total inputs of carbon were based on recovery of isotope-labelled carbon.

Consequently, if a correction factor is applied to the maximum live root biomass obtained in Experiments 1 and 2, then the total carbon input will be underestimated because of the failure to account for carbon in dead roots. If this correction factor is applied to the total root biomass production obtained in Experiments 1 and 2, then the total carbon input will be overestimated because the contribution of dead roots will be counted twice.

Applying a correction factor of 1.7 (derived from the cut treatment in Experiment 3) to the maximum live root biomass yields of barrel medic reported in Experiment 1 results in a total input of carbon of 710 kg C/ha in 1994 and 660 kg C/ha in 1995 (assuming roots contain an average of 40 % of carbon). The estimate for 1995 can be modified because it is known from Experiment 1 that in 1995, the input of carbon through root biomass production alone was at least 1110 kg C/ha. Applying the same correction factor to the total root biomass production estimated in Experiment 1, the total input of carbon is 1120 kg C/ha in 1994 and 1880 kg C/ha in 1995. For the reasons explained in the previous paragraph, it is most likely that the real amount lies somewhere between the two extremes.

The same procedure can be applied to the results of Experiment 2. Estimates can be improved by applying correction factors of 1.70 to the cut treatment and 1.65 to the uncut treatment (as obtained in Experiment 3). This results in estimations of total input of carbon of between 700 and 1060 kg C/ha for the cut treatment and 810 and 1080 kg C/ha for the uncut treatment, depending on whether maximum live root biomass or total root biomass production is used as the basis.

These results are comparable with those recorded for other annual species under Australian conditions as presented in Table 7.1. Strict comparisons are difficult because of the variety of conditions that the plants were grown under and the different techniques that were used to estimate the belowground input of carbon.

A convenient form for presenting an estimation of the amount of carbon input belowground is to represent it as a fraction of the amount of carbon in aboveground plant material. Aboveground production of biomass is a lot easier to measure than belowground production and a lot easier to measure more accurately. Having measured aboveground production, a ratio can be used to estimate the amount of carbon input belowground. In Experiment 1, the ratio of carbon input belowground to carbon in aboveground production for a grazed medic pasture was 0.57 to 0.90 in a dry year and 0.40 to 0.67 in a season of average rainfall (Table 7.1). In Experiment 2, in a season of average rainfall, the ratio was 0.51 to 0.77 for a regularly defoliated pasture and 0.44 to 0.59 for an uncut pasture (Table 7.1). A range of figures is given to allow for the minimum estimate based on live root biomass plus rhizodeposits and the maximum estimate based on total root biomass production plus rhizodeposits, as explained above.

The findings of this thesis suggest that when estimating belowground carbon input as a proportion of aboveground carbon, estimates can be made more accurate by varying the ratio to suit the conditions. Firstly, Experiment 1 showed that generally, the amount of belowground carbon input as a proportion of carbon in aboveground production appears to be higher under pasture species than under a grain legume species. The ratio for barrel medic pastures also appears to be generally higher than under cereal crop species such as wheat or barley (Table 7.1). Secondly, the ratio is increased when plants are placed under stress. Less soil moisture in 1994 led to higher ratios for all three species than in 1995 (Chapter 4). As discussed in the literature review (Chapter 2), this effect has often been reported elsewhere as well. Thirdly, defoliation or grazing, whilst reducing aboveground production, can lead to a higher ratio of belowground carbon input to aboveground carbon (Chapter 5).

As a result, this information can be used to refine soil organic carbon simulation models such as Socrates (Grace and Ladd 1995) to more accurately represent inputs of carbon under different environmental and management conditions. Within the Century model (Parton *et al*. 1987), a plant sub-model is used to estimate the input of carbon. Aboveground production is calculated from stored water and precipitation and then carbon is allocated to roots, grain and straw according to a fixed allocation pattern. For example, in modelling the production of wheat varieties in Century, Parton and Rasmussen (1994) set the portion of total production found in roots at 25 % for medium tall wheat varieties and 20 % for semi-dwarf varieties. They suggest that if the prediction capability of the plant growth sub-model is to be improved, it would be necessary to add a dynamic carbon allocation model.

Socrates also uses a plant sub-model to calculate the annual input of carbon input. As a result of the findings of this thesis, a preliminary attempt has been made to increase the responsiveness of Socrates to different conditions. Under annual pastures, the belowground input of carbon has been set at 50 % of aboveground production. This increases to 55 % when the pasture is grazed. A belowground allocation of 40 % of aboveground production is used for all cereal and grain legume crops. This has increased the accuracy of the model when tested against existing data sets.

A possible area of further research arising from this work is the relationship of nitrogen to carbon inputs in grazed barrel medic pastures. From Experiment 2 (Chapter 5), it appears that the turnover of roots in the field is greater under regularly defoliated pastures than undefoliated pastures. What is the effect of this on the production and input of nitrogen under a legume pasture? Does the legume fix more nitrogen in proportion to the extra root growth or is the nitrogen in decomposing roots and rhizodeposits quickly mineralised and incorporated into new root growth within the current season? Research in this area could have implications for the management of legume pastures in cropping rotations where the aim is to maximise the input of nitrogen for the benefit of the following crop.

Another opportunity for further research relates to the use of the ^{13}C dilution technique (Chapters 3 and 5) to estimate the total root biomass production of annual crop and pasture species. At its simplest, it requires a single pulse labelling in the early part of the season and sampling a short time after labelling and at the end of the season. This technique allows for the turnover of roots during the season to be estimated, hence allowing an estimate of total root biomass production to be made based on the live

root biomass early in the season. Within crops, it can be used to estimate the effect of different management options on root turnover and total root biomass production.

In conclusion, this thesis has shown that the total annual belowground input of carbon by barrel medic pastures ranged from 700 to 1880 kg C/ha, depending on the year and the technique used to estimate it. As a proportion of carbon in aboveground production, carbon input belowground mostly ranged from 0.40 to 0.77, with one estimate as high as 0.90. This ratio tended to increase with environmental stresses such as moisture stress and defoliation. The ratio for barrel medic was comparable to that of another pasture species (barley grass) but higher than for a grain legume such as faba beans. These results are valuable in the development of more accurate soil organic carbon simulation models and will ultimately lead to a better understanding of the effect of management on the sustainable use of cropping soils in southern Australia.

Table 7.1. Estimated annual belowground carbon input for a range of annual species and conditions in Australia.

			Input (kg C/ha)	Ratio[1]	Reference
Barrel medic	1994	grazed	710-1120[2]	0.57-0.90	Chapter 4
"	1995	"	1110-1880[2]	0.40-0.67	"
"	1995	cut	700-1060[2]	0.51-0.77	Chapter 5
"	"	uncut	810-1080[2]	0.44-0.59	"
Barley grass	1994	grazed	>710[3]	>0.56	Chapter 4
"	1995	"	>990[3]	>0.42	"
Faba beans	1994		>160[3]	>0.31	"
"	1995		>490[3]	>0.25	"
Barrel medic	Adelaide SA	not grazed	1200	0.34	Alston and Puckridge (1986)
Wheat	"		1500	-	Martin and Puckridge (1982)
"	"		1305	0.30	Keith *et al.* (1986)
"	East Beverley WA		480	0.25	Gregory and Atwell (1991)
Barley	"		580	0.20	"

[1]Ratio of carbon input belowground to carbon in aboveground production.

[2]Lower value based on correction to maximum live root biomass. Higher value based on correction to total root biomass production. See text for further details.

[3]Based on total root biomass production only. Does not include rhizodeposits.

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