Segmental Morphology of Perennial Ryegrass (*Lolium perenne* L.): A Study of Functional Implications of Plant Architecture

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Abstract

This thesis investigated the structural and functional implications of segmental organisation of two hydroponically grown perennial ryegrass (*Lolium perenne* L.) cultivars, Alto and Aberdart in spring and autumn, for around 90 days in each season. The objectives included describing tiller axis morphology, studying leaf and root turnover pattern in a phyllochron (leaf appearance interval) time scale, and studying root-shoot and tiller-tiller functional relations. In the Spring experiment a total of 15 – 16 segments or phytomers developed, 10 – 11 of which bore roots. In the Autumn experiment, a total of 22 – 23 phytomers developed, 17 – 18 of which bore roots. New leaves appeared more frequently in autumn and achieved significantly greater final leaf length, dry weight and lamina area through a significantly faster rate of leaf extension, though with significantly shorter elongation duration compared to spring leaves. However, autumn leaves had significantly longer life span and lower specific leaf area. The individual leaves achieved maximum photosynthetic capacity between 12.5 and 14.8 days after appearance. The individual root-bearing phytomers in autumn bore a significantly higher number of roots (2.4) than in spring (1.7). At successively more developed phytomers root main axis length, root dry weight, root length including branches, surface area and volume increased linearly up to phytomer 6 – 7 for both of the cultivars in both seasons whereas dry matter deposition rate per phytomer per day and mean root diameter decreased gradually. Branching to quaternary order was observed during root development. Principal component analysis of root morphology data detected statistically significant morphological variation between genotypes of each cultivar but the basis for differentiation was not visually evident. Roots older than 10 leaf appearance intervals in autumn decreased gradually in volume while still increasing in total branch length. This was interpreted as evidence of root death in some branches while the remainder continued elongation. Tiller root:shoot ratio varied seasonally, possibly mediated by faster leaf than root appearance rate at successive phytomers in spring, and *vice-versa* in autumn. Excision of adult daughter tillers significantly reduced number of root-bearing phytomers of the main tiller which indicated slower new root appearance rate at the main tiller. A significant proportion of root derived N and assimilated C from daughter tillers was translocated to the main tillers and this may explain why daughter tillers remain smaller in size than their parent tillers. Evidence for a proposed oscillation of N concentration within the tiller axis of *Hordeum vulgare* L. linked to N uptake by successive developing leaves was also examined. A weak N concentration oscillation was detected, with the highest concentration just prior to each leaf appearance event. Evaluation of ryegrass root morphology from a segmental perspective, though logistically challenging, has provided previously unavailable information on the time course of root mass accumulation and of root branching. This methodology could be used in future to further explore the carbon economy of the root system and the factors that limit final root size.

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Dedication

I dedicate this thesis to my beloved mother

Ferdousi Begum

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Glossary of Abbreviations

A_{lf}	Leaf appearance interval/phyllochron
A_{lg}	Ligule appearance interval
ANOVA	Analysis of variance
A_{rt}	Root appearance interval/rhizochron
C	Carbon
C:N	Carbon-nitrogen ratio
CER	CO_2 exchange rate
CRD	Completely randomized design
d	Day(s)
d	Maximum net photosynthetic rate
de	Delay between leaf and root appearance at the same phytomer (node)
DM	Dry matter
DMD_p	Dry matter deposition rate per phytomer
$RL/RV^{1/3}$	Dimension corrected root length:root volume ratio
$RSA/RV^{2/3}$	Dimension corrected root surface area:root volume ratio
DT	Daughter tiller
DT-	Daughter tiller excised plants
DT+	Plants with two oldest daughter tillers
DTL	Daughter tiller labelled plant (refers to stable isotope labelling)
DW	Dry weight
EL	Elongating/emerging leaf
Exp	Experiment
f	A measure of curve-width of the log-normal curve in log-days
FLL	Final leaf lamina length
g	Leaf age when maximum net photosynthetic rate occurs
GDD	Growing degree days
Geno	Genotype
GLM	Generalized linear model
IGER	Institute for Grassland and Environmental Research
L	Leaf
LA	Leaf area
LA_i	Leaf area of the individual leaves
LA_t	Leaf area per tiller
LAR	Leaf appearance rate

LDW	Leaf dry weight
LDW_i	Leaf dry weight of the individual leaves
LDW_t	Leaf dry weight per tiller
LDW_{DT}	Leaf dry weight of the two daughter tillers
LED	Leaf elongation duration
LER	Leaf elongation rate
LLS	Leaf life span
LW	Leaf lamina width
MES	2-(*N*-morpholino) ethanesulfonic acid, a pH stabilizer
MT	Main tiller
MTL	Main tiller labelled plant
N	Nitrogen
NEL	Number of visible elongating leaves per tiller
NLA	Number of leaf appearance events
NLL	Number of live leaves per tiller
NP	Number of phytomers per tiller
NPr	Number of root-bearing phytomers per tiller
NPR	Net photosynthetic rate
NR_t	Number of roots per tiller
p	Probability value
P (numeral)	Phytomer position using the emerging leaf as the reference point
PAR	Photosynthetically active radiation
PC	Principal component
PCA	Principal component analysis
PPFD	Photosynthetic photon flux density
Pr	Root-bearing phytomer using the youngest root as a reference point
PrAR	Root-bearing phytomer appearance rate
PVC	Polyvinyl chloride
R	Root
RA	Root axis
RAL	Root main axis length
RCBD	Randomized complete block design
RD	Root diameter
RDW	Root dry weight
RDW_i	Root dry weight of the individual roots
RDW_P	Root dry weight per phytomer
RDW_t	Root dry weight per tiller

RL	Root length
RL_i	Individual root length
RL_P	Root length per phytomer
RL_t	Root length per tiller
R_P	Number of roots per phytomer
RSA	Root surface area
RSA_i	Root surface area of the individual roots
RSA_P	Root surface area per phytomer
RSA_t	Root surface area per tiller
RT	Root tips
RT_i	Number of root tips of individual roots
RT_P	Number of root tips per phytomer
RV	Root volume
RV_i	Root volume of the individual roots
RV_P	Root volume per phytomer
SDW_t	Dry weight of leaf sheaths per tiller
SE	Standard error
SEM	Standard error of mean
SL	Senescing leaves
SLA	Specific leaf area
SRL	Specific root length
SRSA	Specific root surface area
SRV	Specific root volume
TADW	Tiller axis dry weight
TAR	Tiller appearance rate
T_{base}	Base temperature for GDD calculations
TD	Tissue density
TPA_t	Total photosynthetic assimilation per tiller
T_{max}	Maximum temperature
T_{min}	Minimum temperature
Treat	Treatment
YR	Young roots
$\delta^{13}C$	Carbon isotope mass ratio (^{13}C:^{12}C) per mill (‰)
$\delta^{15}N$	Nitrogen isotope mass ratio (^{15}N:^{14}N)

Chapter 1: Introduction

1.1 Background

The predominant land use in New Zealand is pastoral agriculture, with lowland and hill country pastures together covering around 14 m ha of a total land area of 27.6 m ha. About 53% of New Zealand's export income is provided by the pastoral industries (Ballingall and Lattimore, 2004; Statistics New Zealand, 2004). New Zealand's economy was strongly based on agricultural exports in the first half of the 20th century, and for a brief period in the late 1970s and early 1980s agricultural production was subsidised to encourage expansion. Despite vulnerability to domestic interest rate adjustments, increased exposure to global market forces and the removal of agricultural subsidies during the mid-1980's, meat and milk production from pastoral agriculture has increased over the last 20 years due to production intensification (Smith and Montgomery, 2004; MacLeod and Moller, 2006). The intensification has been driven directly by a fall over time in inflation-adjusted market prices for agricultural products. This intensification of agricultural production has placed pasture lands under pressure and has led to serious concerns related to soil quality (Sparling and Schipper, 2004; La Schipper et al., 2007), biodiversity (Moller et al., 2008) and other aspects of environmental sustainability (Mackay, 2008). The impacts on the wider landscape of production intensification on pastoral land have been the focus of some recent reviews (e.g. Monaghan et al., 2007; Williams et al., 2007; Mackay, 2008; Moller et al., 2008). According to Jay (2007) "the New Zealand dairy industry faces political and commercial pressure to improve its environmental performance on the one hand while maintaining economic efficiency and commercial competitiveness in a global marketplace on the other". The same pressures also face other sectors of the New Zealand pastoral industry. To allow farmers to adopt more environmentally friendly production practices, maintain their life-style, and also to remain competitive in the world market as a country, production will need to intensify through a combination of improved efficiencies, at all points in the production cycle, both on the farm and after product leaves the farm gate. At the fundamental level, better knowledge of sward dynamics and morphological determinants of productivity can assist breeders to develop superior genetic lines, and can help farmers to develop efficient fertilizer practices, and achieve efficiencies in other areas such as water use. During an earlier phase of agricultural intensification between the 1950s and the 1970s, following

World War II (Molloy, 1980), a good number of such research projects were funded by various governments as part of the post-war recovery. Many of these studies conducted in the post war period tended to compartmentalise grass growth into a series of component processes that were studied separately, in particular root formation, tillering, and leaf turnover. Notable examples are reviewed in Chapter 2. Moreover, studies which consider the position of attachment and age of individual grass roots are almost unknown. However, funding emphases have shifted since the 1980s, and research of this type has been greatly reduced in volume, while at the same time technology developments have provided new opportunities to understand plant processes.

With this context in mind the present study was carried out with a broad aim of exploring the idea that a grass plant is best understood functionally in terms of the segmental morphology common to grasses as a botanical family. As will be demonstrated in Chapter 2, such an approach potentially integrates component processes like root or leaf formation, previously more commonly studied separately. Perennial ryegrass was chosen because it is the dominant pasture species in New Zealand and many other temperate countries of the world (Anderson, 1954; Belgrave et al., 1990).

The primary goal of the study was therefore to describe in detail the segmental morphology of the ryegrass tiller with a view to clarifying the coordination between different plant organs including roots and leaves on a parent tiller, and between parent and daughter tillers in order to provide a basis for enhancing the application of sward dynamics knowledge. As a result of such a study we could expect better understanding of grass eco-physiology especially in relation to understanding internal competition within the plant, as indicated by mass flow of resources and comparative size increases of different plant organs. Another potential application of such knowledge would be mechanistic computer modelling of the tiller unit and tiller populations.

1.2 Objectives

From the broad goals above, specific objectives for this study (in logical rather than chronological order) were:

i) To provide morphological data on segmental organisation of the tiller axis and numbers of live leaves and roots and their ages in two perennial ryegrass cultivars of contrasting breeding background;

ii) To study the pattern of leaf turnover on the tiller axis and photosynthetic efficiency of the individual leaves of known age;

iii) To study in detail the pattern of root development in relation to position on the tiller axis, including data on dry matter accumulation, degree of root branching, root diameter classes, length of individual root axes, total root length, total root volume, and root surface area, among others;

iv) To explore the hypothesis of Matthew *et al.* (1998; 2001) that plant architecture (specifically the physical separation of sites of root and leaf formation on the tiller axis) may provide a signal that spontaneously increases root:shoot ratio in spring and decreases root:shoot ratio in autumn;

v) To explore the exchange between parent tiller and daughter tiller of recently assimilated carbon (C) and recently acquired nitrogen (N), given that a daughter tiller is inserted on the tiller axis between the root and shoot systems of the parent;

vi) To explore the hypothesis of Irving (unpublished, see Appendix 1) that the segmental organisation of grasses, together with the recovery of N from Rubisco degradation in older leaves for reuse elsewhere in the plant (Irving and Robinson, 2006) should result in oscillation of tissue N concentration in the tiller axis that might have a signalling role in coordination of plant processes.

1.3 Experimental sequence

The chronological sequence of experimentation differed from the logical sequence above because initially it was planned (on advice of supervisors) to make a detailed investigation of the oscillation in N concentration of the tiller axis observed in unpublished data of Irving, as set out in Objective (vi) above. However, when observations of Irving were not reproduced in three experiments conducted between May 2007 and March 2008, it was

decided instead to make a broader study of the morphological organisation of perennial ryegrass tillers, exploring how the segmental organisation of the tillers contributes to coordination of root and shoot systems and the nature of exchanges between a parent tiller and a daughter tiller attached to the tiller axis between the root and shoot systems of the parent tiller.

Chronologically, six experiments were conducted over a period of 2½ years from May 2007 to September 2009. Five out of six experiments utilised plants in hydroponic culture as extraction of single roots of plants grown in soil to measure dimensions of individual roots would have been problematic. As mentioned above, Experiment 1 explored the evidence for N concentration oscillation in the tiller axis, mediated by Rubisco recovery from older leaves and recycling to younger leaves higher on the tiller axis. This experiment used seedling plants of barley (*Hordeum vulgare* L.). Two follow-up experiments, Experiment 2, and Experiment 3, again using barley seedlings in hydroponic culture and grown in soil, respectively were conducted between late 2007 and March 2008. Experiment 3 was unsuccessful and is not reported in this thesis.

Experiments 4 and 5 then addressed the broader implications of segmental morphology for the ecophysiology of perennial ryegrass. These experiments utilised adult tillers of perennial ryegrass obtained by breaking up mature plants dug up from field swards of two cultivars with widely different breeding backgrounds to extract adult or 'dominant' tillers for further study in hydroponic culture. In this way it was possible to have an experiment design structure of different plant genotypes clonally replicated. Experiment 4 was conducted during the winter-spring period (June – December 2008) and Experiment 5 was conducted during autumn-winter (March – September 2009). Subsets of plants were harvested from these two experiments for specific purposes to address objectives (i), (ii) and (iii) above. Although it was recognised that care is needed when comparing results of different experiments, it was expected that comparison of results from plants grown in increasing day length in Experiment 4 and decreasing day length in Experiment 5, would allow preliminary evaluation of the hypothesis that the segmental plant architecture would result in root:shoot ratios differing seasonally (Objective iv). Experiment 6 (C and N labelling experiment) was conducted in autumn 2009. Objective (v) was addressed by feeding ^{12}C-enriched CO_2 to leaves or ^{15}N enriched ammonium sulphate to roots of main

or daughter tillers and using isotope ratio mass spectrometry to detect movement of the isotopic tracers from main to daughter tillers or *vice-versa*.

1.4 Thesis structure

The results from the four experiments reported are presented in this thesis in nine chapters, sequenced logically rather than chronologically, to address the 6 objectives set out above. The introductory chapter (Chapter 1) is followed by a review of literature (Chapter 2).

Chapter 3 provides 'tiller axis maps' similar to those of Yang *et al.* (1998) for the two perennial ryegrass cultivars studied in Experiment 4 (and also Experiment 5). The data for Chapter 3 came from an early harvest of a small subset of plants in Experiment 4. Chapter 3 is provided so as to define the plant structure occurring under the particular growing conditions, and also to orientate the reader to the data presentation conventions used for the remainder of the thesis to indicate the point of attachment on the tiller axis of particular leaves or roots. In particular, Chapter 3 reports the numbers of leaves and roots present on the tiller axis, the positions on the tiller axis where leaf senescence and root initiation occurred and the size and status of leaves and roots at each position (phytomer) on the tiller axis.

Chapter 4 and Chapter 5 describe the turnover of leaves and roots, respectively, under increasing day length in a winter-spring experiment (Experiment 4) and under decreasing day length in an autumn-winter experiment (Experiment 5). Chapter 6 presents root-shoot relations and evaluates whether there is evidence of seasonal change in shoot:root ratio (Objective iv). Chapter 7 presents results obtained with stable isotope tracers (Objective v). Additionally and unexpectedly, when measuring ratios of ^{15}N:^{14}N and ^{13}C:^{12}C in individual leaves and roots, isotopic fractionation within the shoot and root systems was detected, and this too is reported in Chapter 7. Chapter 8 explores the question of N concentration oscillation in the tissues of barley plants based on results of Experiment 1 and Experiment 2 (Objective vi). The results are collectively discussed in Chapter 9. Table 1.1 below summarises the layout of the thesis.

Table 1.1 Chapter structure of the thesis

Chapter Theme	Contents
1. Introduction	Context and overview of the research.
2. Literature review	Review of earlier research findings and identification of gaps in present understanding.
3. Tiller morphology	Tiller axis maps of leaf and root size by phytomer number on the axis are compiled for two perennial ryegrass cultivars as a basis for further investigation and discussion.
4. Leaf turnover and photosynthesis	Leaf number and length described according to phytomer position for two perennial ryegrass cultivars of contrasting breeding background when grown hydroponically under increasing or decreasing day length.
5. Dynamics of root production	Root dimensions at successive phytomers when grown under increasing or decreasing day length quantified and root production rates inferred for two perennial ryegrass cultivars of contrasting breeding background.
6. Root-shoot interrelations and seasonal morphogenetic variations	Root-shoot relations in changing seasonal environment explored. Morphogenetic variations associated with seasonal variations investigated. Daughter tillers' contribution to main tiller's roots explored.
7. Functional implications of segmental organisation	C-N relations in segmental organisation explored. Evidence of ^{15}N:^{14}N and ^{13}C:^{12}C fractionation in the root system detected.
8. Evidence of N concentration oscillation	Evidence for oscillation in tissue N concentration as a result of N remobilization and reallocation evaluated.
9. Overview and conclusions	Key results summarised and discussed and possible follow-up research work identified.

Chapter 2: Literature Review

2.1 Grassland in a global context

Grasslands cover more than one-third of the earth's surface (Shantz, 1954). This estimate includes both natural grasslands and pastures. The term natural grassland is used here to mean areas where the climax vegetation is grassland, often because of insufficient rainfall to support forest, while pasture is used to indicate grassland artificially created from forest, natural grassland, or other vegetation for agricultural purposes. Over the last three centuries, pasture land has expanded from 5 million km^2 in 1700 to 31 million km^2 in 1990, mostly at the expense of natural grasslands (Goldewijk and Ramankutty, 2004) and forests (Shantz, 1954). New Zealand is a good example of a country where pasture land expansion has occurred at the expense of forest, and similar pasture land expansion has occurred in many other temperate parts of the world (Levy, 1937; Prentice et al., 1992; Hopkins and Wilkins, 2006). Pasture land expansion has been driven by the increasing world demand for livestock products as world population has increased (Levy, 1937; Hopkins and Wilkins, 2006).

The basic function of grasslands is to provide feed for ruminant livestock. The pastures of temperate regions of the world meet the feed demand associated with production of 80% of the world's cow milk and 70% of the world's beef and veal meat (Wilkins and Humphreys, 2003). In addition to the basic function of feed supply to drive animal production, grasslands increasingly contribute to complementary agronomic and environmental objectives, such as, reduction of soil erosion by supporting slope stability, improvement of soil structure, water conservation, conservation of plant genetic resources, and provision of habitat for wildlife (Barnes and Nelson, 2003; Humphreys, 2005; Hopkins and Holz, 2006). They also contribute to social, economic and recreational activities at national, regional and catchment scales (Reid, 2005; Hopkins and Holz, 2006). As a result of a growing interest in integrating production, conservation and recreational use of grasslands around the world, a new emphasis emerging among grassland scientists at the beginning of the 21st century has been to achieve inter-disciplinary research from a multifunctional vision (Hervieu, 2002; Hopkins and Holz, 2006; Hopkins and Wilkins, 2006). This approach seeks to mitigate the negative effects of intensification of production systems through use of science to develop agricultural practices that have better

conservation outcomes (Lemaire et al., 2005). To manage grasslands in a way that provides both high productivity and sustainability of the production system, requires a solid framework of knowledge of all aspects of ecosystem functionality and keys to maintaining biodiversity (Kemp and Michalk, 2007).

A desired outcome of the emergence of the multifunctional approach to grassland management would be collaboration between neighbouring countries in establishing principles and practice. This would require development of a global perspective on geographic patterns of grassland productivity and land use, and of how these patterns are related to annual precipitation and other agro-environmental conditions. To some extent collaboration between countries is already beginning to happen. A recent article has modelled the spatial distribution of grassland productivity and land use in Europe (Smit et al., 2008) and funding for agricultural research within the European Union is now providing a vehicle for research into nutrient cycling and environmental sustainability when grasslands are seen as multifunctional systems.

2.2 The role of and need for component studies

Perhaps unexpectedly, one side effect of an emphasis on developing grassland management strategies that emphasise integration of food production, recreational, and other human needs, is the emergence of a view that production systems could be intensified on land designated to provide the food output within a multifunctional system. Such views are seen, for example, in a analysis of trends in European dairy farming systems (Lowe, 1995). This author notes the need to integrate a diversity of objectives relating to human need for food, attractive countryside, adequate services and facilities, and sources of employment. This is very much in contrast with the emphasis that has prevailed, at least in Europe and many other Western nations, over the last 20 years. While the politics were actually quite complex, one factor that has shaped current priorities is that farming subsidies aimed originally (at least in part) at ensuring security of food supply within Europe, through local production, led to over-production and food surpluses. This in turn led to a focus on researching the environmental benefits of de-intensification of production systems. For example, issues like management of biodiversity in temperate European grasslands (Pärtel et al., 2005; Hopkins and Holz, 2006) and nutrient

management in grazing systems (Oenema et al., 2007) have emerged as issues that administrators require farmers to plan for.

These changed research priorities were in turn reflected in research outputs. It is noticeable that during the last 20 years when research into de-intensification has been a greater priority, the content of grassland science journals has changed. Reflecting this, a majority of articles in '*Grass and Forage Science*' in the 1980s focused on herbage production and factors affecting production, and herbage quality, while between 2000-2005 a number of articles discussed issues other than production such as soil nutrient status and its management, the diversity and management of soil microbial populations and other issues related to biodiversity, and environmental and social benefits.

However, now it is increasingly recognised that if production elements within multifunctional grassland systems are actually to be intensified while at the same time maintaining or improving environmental sustainability of those systems, there will clearly be a need to revisit component research into aspects of ecophysiology relevant to grassland intensification. A major gap in understanding in the area of grassland ecophysiology, is the interrelationship of root and shoot systems in a broad sense and their sub-processes in a more detailed sense. For example, for New Zealand's major pasture species, perennial ryegrass, comparatively little is known about how total root growth is allocated between locations within the root system, or about the interrelationship between an adult tiller, branch shoots, and their root systems. In this context there is a need to provide scientific understanding of grass ecophysiology to underpin production intensification within future multifunctional grassland systems and at the same time improve understanding of how component processes such as leaf, tiller, and root formation are functionally integrated. A potentially useful approach is to define shoot and root dynamics in terms of the segmental morphology of the grass plant, as proposed by Matthew et al. (1998) and Yang et al. (1998). The remainder of this chapter will therefore review the current understanding of how awareness of segmental morphology of grasses can assist in understanding of leaf, shoot and root formation processes, with particular emphasis on perennial ryegrass (*Lolium perenne* L.).

2.3 Emergence of understanding of segmental morphology in grasses

A majority of historical research into the function of grassland plants has focused on processes presumed to directly contribute to sward productivity. Notable examples of topics studied include: leaf turnover and related mass flow processes e.g., Bircham and Hodgson, (1983), considerations determining light capture (e.g., Parsons et al., 1983a; Parsons et al., 1983b) and population dynamics of grass swards e.g. Kays and Harper (1974). The collective emphasis of such studies is well represented by the volume '*The Grass Crop*' (Jones and Lazenby, 1988).

By contrast, a much smaller number of studies have explored the functional significance of the segmental morphological structure of the grass tiller. Evans (1928) reported the presence of nodal structure in *Zea mays* L. after observing the vascularisation pattern. He showed that single vascular bundles seldom pass through more than two nodes without branching. Arber (1934) is another earlier example of a study which mentions the segmental architectural structure of graminaceous plants including cereals, grasses and bamboo. Evans and Grover (1940) studied the developmental morphology of the growing point of the shoot and the inflorescence of eight species of grasses including perennial ryegrass. That study noted the presence of a dome-shaped growing point (i.e., the apical meristem) and also a segmental shoot axis which unites shoot components like the leaf and tiller bud, and which may contain elongated internode units. Other classic studies of grass segmental morphology were made around this time by e.g. Sharman, (1942; 1945a; 1947). Sharman (1945b; 1947) observed a series of leaf primordia at different developmental stages while studying the stem apex of rhizomes, young aerial shoots and the main shoot of *Agropyron repens* L.

The detailed study of Etter (1951) on Kentucky blue grass (*Poa pratensis* L.) was one of the first to move beyond simple description of the segmental structure and investigate the link between segmental structure and field behaviour. This study by Etter (1951) mapped the development of individual shoots of Kentucky bluegrass in the field over three years, noting events occurring over time on each phytomer of the mapped shoots. The study observed that component parts of Kentucky bluegrass shoots, for example the leaf blade and sheath, the internode, axillary bud, and the pair of roots immediately below the leaves all represent stages of development seen on any one phytomer over time but displayed at

any one time by successive phytomers along the shoot axis. These component parts arise from the growing point in a defined sequence beginning with the formation of the leaf primordium and then the leaf blade, followed by the leaf sheath. The internode between successive leaves may elongate or remain short and finally roots are formed as a last stage in the development cycle of a single phytomer growth unit.

After this time there was widespread awareness of the segmental structure of the apical meristem and the functional significance of that for leaf production but a tendency to still view sward processes as being mass flow or population based. For example, Jewiss (1966) described the morphology and developmental stages in the growth of the leaf primordium and transformation into an emerging leaf at the phytomers below the apical meristem, and in the same article reported seasonal tiller birth and mortality patterns of two grass species under contrasting defoliation managements. However, data on tiller birth and death were not in any way linked to the description of segmental organisation of the apical meristem. This development came rather later with studies such as that of Neuteboom and Lantinga (1989); they described the pattern of increase in plant tiller number over time and associated site filling ratio, and recognised the potential contribution to tillering of the prophyll buds.

Another emphasis pursued by some researchers was to define the vascular architecture of grasses. Hitch and Shaman (1971) after studying the vascular pattern of festucoid grass axes, described the basic pattern underlying the complex structure of the nodal plexus, the details of leaf insertion at a node, and details of how the leaf trace system connects the leaf to the main axis. Bell (1976a; 1976b) made an extremely detailed study of the vascular architecture of *Lolium multiflorum* Lam., showing the interconnections between the leaf trace system of successive leaves, and the vascular details of tiller insertion and root insertion in the main tiller axis. He found that each phytomer possesses a vascular plexus. Awareness that phytomers are delineated from each other at the vascular level, and not just by the occurrence of external organs like leaves or roots, gives a hint of how fundamental this segmental structure might be to determining functional behaviour of grasses.

One of the earlier studies to recognise the potential for using knowledge of segmental morphology to understand integration of plant growth processes was that of Silsbury

(1970). This author noted that when a grass tiller is viewed as a series of phytomers, the component organs of the phytomer are the leaf, the internode, the axillary tiller and the root(s). However, despite increasing awareness of the segmental organization of grass shoots and studies such of those of Etter (1951) and Silsbury (1970), many authors continued to describe models for understanding plant function that did not acknowledge the segmental architecture of the grass root system. Various articles on seasonal root growth published between the 1960s and the 1980s considered root formation in a grass tiller to be a seasonal phenomenon that occurs annually (Jacques, 1956; Jacques and Schwass, 1956; Caradus and Evans, 1977). Both Jacques and Schwass (1956) and Caradus and Evans (1977) described a seasonal pattern where most new roots form in autumn-winter-spring, while Garwood (1968) reported that new root production in UK peaks in spring. This viewpoint was also supported by other authors, including Stuckey (1941), Troughton (1951), and Baker and Garwood (1959). A consequence of acceptance that root growth is a seasonal process was a tendency to assume root growth would not therefore be segmentally organised. Fig. 2.1 illustrates this view of grass plant architecture as it shows the segmental organization of shoot components but depiction of root organization does not show any segmental structure (from Jewiss, 1972).

From the 1980s onwards, a number of authors have attempted to represent the grass plant in computer models and simulate behaviour of field plants as a summation of segmentally organised processes as first introduced by authors like Sharman (1942), Etter (1951) and Silsbury (1970). Klepper et al. (1984) modelled the root axis of the wheat plant based on the number of nodes recorded at the tiller axis and thus developed an association between leaf sites and root initiation at the same node, with roots appearing after the senescence of the leaves. Leaf senescence interval was recorded by Vine (1983) while studying leaf life span over the changing seasons throughout the year, and the leaf senescence process was modelled by Woodword (1998) in *L. perenne*.

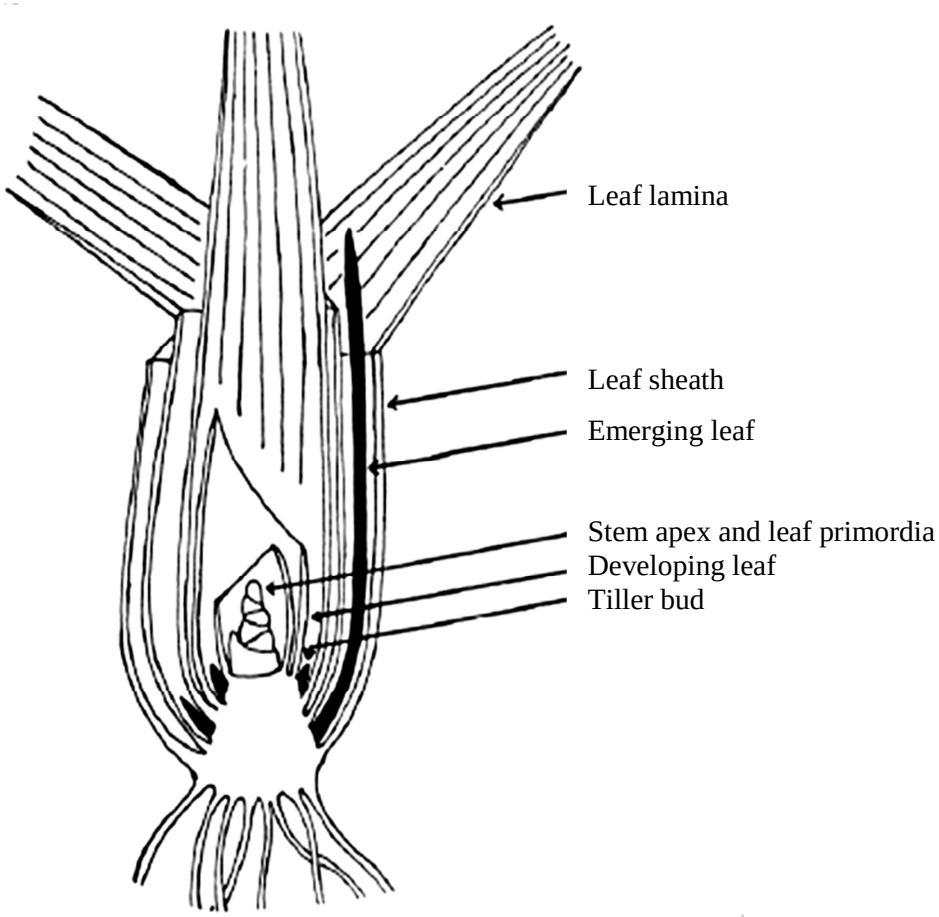


Fig. 2.1 Diagrammatic longitudinal section of the vegetative grass tiller showing position of stem apex and production of leaves and tillers from leaf primordia and buds, respectively (Jewiss, 1972). Note that in this drawing, no phytomer-related age difference between roots is indicated.

Acknowledging the segmentally organised attachment of individual roots at sites previously bearing leaves, Matthew and Kemball (1997) studied the allocation of current photosynthate down the tiller axis from younger to older nodal positions in the root system of *L. perenne*. Their results are reported in Section 2.4.2.7 below. Section 2.4 which follows, attempts to integrate the emerging knowledge that establishes the concept of segmental organization of grass tillers.

2.4 Segmental organisation as an integrating principle for component processes of grass tiller form and function

In this section a brief review of historic research on grass form and function is provided. Here, information that focuses on mass flow processes such as leaf growth and death, or on measures at a sward level such as total root mass per unit ground area at a point in time, is sequenced in such a way as to highlight current knowledge and indicate areas where the operation of the segmental morphology to influence form and function is less well understood.

2.4.1 Unit of grass growth: the phytomer

Early awareness of the segmental structure of the grass shoot has been mentioned in the previous section. The phytomer is the basic growing unit in the segmental architecture of a grass tiller (Jewiss, 1966; Silsbury, 1970). Architecturally it is a 'building block' in the grass tiller axis, comprising a node and an internode. A phytomer during its life cycle undergoes a series of developmental changes over time (Fig. 2.2 from Matthew et al., 2001). These developmental changes include the formation of a leaf, the senescence of the leaf and recycling of N and other nutrients to the rest of the plant, the possible release within a limited time window of a leaf axillary bud to form a branch shoot, possible internode elongation, and normally the formation of roots from its node over the course of its life cycle. Silsbury (1970) notes that the growth of an individual leaf, internode or root is limited but that of the axillary bud is theoretically unlimited as it bears an apical meristem which is capable of producing new phytomers. Only reproductive development at the stem apex terminates ongoing development of new phytomers (Silsbury, 1970). The production and death of phytomers which comprise the tiller axis is dynamic. In the developmental cycle of a phytomer, meristematic cells at the apical tip of a stem undergo a complex cell division pattern which eventually results in differentiated tissues forming the phytomer unit. After the developmental sequence described above, old phytomers die and decompose from the distal end together with their attached roots and thus maintain a more or less constant number of phytomers on the tiller axis at any one time, and hence a constant morphology for the tiller, despite turnover of phytomers on the axis (Matthew, 1992).

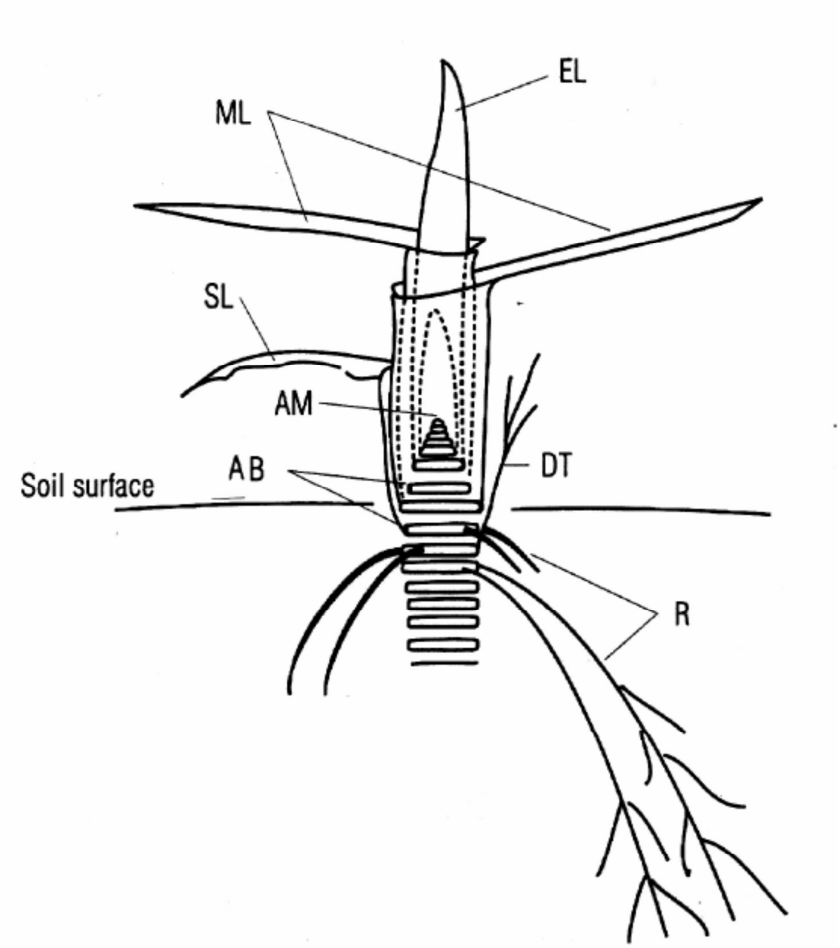


Fig. 2.2 Stylised diagram of a grass tiller showing different developmental stages of the component phytomers. AM, apical meristem; EL, elongating leaf; ML, mature leaf; SL, senescing leaf; DT, daughter tiller; AB, axillary bud; R, root. The life cycle of an individual phytomer on the true stem is indicated by the progression of morphological development from top (younger phytomers associated with leaf production) to bottom (older phytomers associated with root production) (From Matthew et al., 2001).

Given that the tiller axis comprises a series of successively more developed phytomers in a linear series below the apical meristem, as just described, it is therefore of interest to review what is known of the developmental processes at each stage in the life history of the phytomer. Section 2.4.2 below discusses the sequential developmental steps of a phytomer in detail.

2.4.2 The developmental sequence of a phytomer

2.4.2.1 Leaf growth

The visually obvious phases of leaf development relating to expansion of the leaf lamina, and increase in dry weight, surface area or specific leaf area after its appearance are in fact only a part of the overall life cycle of a leaf. The full cycle includes: i) primordia formation and development, ii) ligule formation, iii) leaf appearance, iv) leaf development, v) maturity and eventual senescence.

2.4.2.2 Apical meristem to leaf primordium

The first phase of leaf growth begins within the pseudostem cylinder, which is made up of the quasi-cylindrical sheaths of the previous leaves (Verdenal et al., 2008). The developmental anatomy and morphology of leaf primordia formation from the apical meristem in a grass tiller axis apex has been studied by many authors e.g., Sharman (1942) in *Z. mays*, Sharman (1945b) in *A. repens*, Hamilton (1948) in *Avena sativa* L., (Cooper, 1951) in *Lolium* spp, Soper and Mitchell (1956) in *L. multiflorum* and *L. perenne* and Soper (1956) in *Paspalum dilatatum* L.

Sharman (1947) classified the tiller axis apex of *L. perenne* as being of 'intermediate type' on the basis of the number of primordia on its apex (5–10 primordia). He also noted that the number of primodia on the apex varies from species to species but within a species it is usually fairly constant. For example, *L. multiforum* has a 'long type' stem apex with up to 30 primorida per apex whereas the short apex in *Oryza sativa* L. or *Z. mays* contains only 1-3 primordia. Yang et al. (1998) counted 6–7 leaf primordia on the perennial ryegrass tiller axis below the apical meristem.

At the tiller axis apex, the apical meristem undergoes successive periclinal divisions of the dermatogen and hypodermis (in this type of cell division divided cells appear in parallel with the parent cells forming a layer of cells) (Sharman, 1940) that give rise to cresentic protuberances (Sharman, 1945b; Sharman, 1947). Due to lateral spread of the divisions, the crescents later change into a collar shape (Sharman, 1947; Jewiss, 1966). The collar then grows upwards, and encloses the next younger cresent and the apex (Sharman, 1947). This cresentic structure that forms from the apical dome is termed the leaf primordium. The production of leaf primordia on the tiller axis apex is generally continuous (unless

reproductive organogenesis occurs) and the interval between the appearance of successive leaf primordia is termed the **plastochron** (Esau, 1977) (also spelled 'plastochrone', Sharman, 1942). The vascular anatomy of developing leaf primordia has been studied in order to trace plastochron differences (Hitch and Sharman, 1971; Bell, 1976a; Bell, 1976b). Soper and Mitchell (1956) and Soper (1956) compared the stem apex of *L. perenne* and *P. dilatatum* and reported that *L. perenne* bears higher numbers leaf primordia on its stem apex but although *P. dilatatum* bears fewer leaf primordia on the stem apex, that species has more leaves elongating concurrently. In a given environment the rate of development of leaf primordia, as judged by rate of leaf appearance, is fairly constant (Cooper, 1951).

For *Z. mays,* Sharman (1942) described key stages in anatomical development of the leaf primordium inside the whorl of leaf sheaths and later of the emerged leaf, using plastochrons as the time-unit. Initially at 'plastochron 0' the leaf primordium appears as a crescentic protuberance, extending in width until three-quarters of the axis is encircled by the primordium (plastochron 1), and finally the axis is completely encircled by the primordium (plastochron 2). Parts of meristems are still active although the tip ceases meristematic activity. Protophloem appears in the median strand (plastochron 3), the meristamatic activity of the tip ceases, and the first sign of the axillary bud appears as a raised structure (plastochron 4). Next, the leaf ligule is formed and the leaf tip of the emerging leaf is visible (plastochon 5), after which elongation of the emerged leaf, accompanied by maturation of the constituent tissues occurs in a comparatively short time frame (plastochron 6). Elongation of the leaf sheath (plastochron 7) and rapid extension of the internode and final maturation of epidermal and sub-epidermal tissues then occur (plastochron 8). Finally leaves senesce and roots appear at the same phytomer 10-12 plastochron intervals after the first appearance of the initial crescentic protuberance. In older plants the interval between the time when the leaf fully develops and the root appears may increase gradually. For *L. perenne*, the duration between the leaf primordium initiation and root appearance is about 10–11 pastochron intervals (Yang et al., 1998) and similar anatomical developmental stages are present as for *Z. mays*.

2.4.2.3 Leaf ligule formation

The intercalary meristem initially located at the base of the leaf primordium is responsible for ligule development (Sharman, 1942; Sharman, 1945b). Initially there is no delination

between leaf lamina and leaf sheath. Leaf ligule formation is therefore an important transitional phase which is required to delineate the meristematic activity for developing the blade and sheath. The leaf ligule forms as an outgrowth of the epidermis (Sharman, 1941). Chaffey (1985a; 1985b) studied the structure of the ligule in detail for *Lolium temulentum* L. using both light and electron microscopy. In longitudinal section, the ligule is wedge-shaped and in transverse section it is lens-shaped (Chaffey, 1985b). Soper and Mitchell (1956) also reported a wedge-shaped structure of the ligule for *L. perenne* in longitudinal section. The leaf ligule is widest in the middle, tapering at the end. In tall fescue, the ligule is observed to become visible microscopically as a small protuberance after periclinal divisions at the adaxial epidermis (Skinner and Nelson, 1994). Finally a band of parenchymatous tissue forms, which divides the intercalary meristem into two parts. Leaf lamina growth continues from the upper part (Begg and Wright, 1962) and the lower part is responsible for leaf sheath formation (Jewiss, 1966). Once the intercalary meristem is delineated by the leaf ligule, meristematic cell division starts both in the upper and lower parts of the developing lamina and thereby both the leaf blade and leaf sheath elongate simultaneously (Skinner and Nelson, 1995). Eventually, the leaf tip becomes visible above the whorl of the subtending sheath on the tiller axis.

2.4.2.4 Leaf appearance

The time interval between two successive leaf tips appearing is termed the **phyllochron** (A_{lf}). A_{lf} is commonly expressed in thermal time (growing degree days per leaf, GDD, ^{o}C d) which provides a time-scale for studying plant morphogenesis (Lemaire and Agnusdei, 2000; Bartholomew and Williams, 2005). Davies and Thomas (1983) estimated 110 ^{o}C d leaf^{-1} for *L. perenne* and Lattanzi et al. (1997) estimated 112 ^{o}C d leaf^{-1} for *L. multiflorum* using 0 ^{o}C as the base temperature. The **leaf appearance rate** (LAR), which is generally calculated as the inverse of A_{lf} (Klepper et al., 1982), is considered to be an important factor in determining shoot morphogenesis and potential tiller production (Davies, 1974). In the grass tiller axis, the production of successive leaves is generally continuous and is regulated by a number of interrelated factors, such as, production of the leaf primordium and the succeeding phytomer; leaf initiation; leaf elongation; length, architecture and dimensions of the whorl (Skinner and Nelson, 1995). There are various opinions regarding the regularity of leaf appearance in the tiller axis. Some scientists consider that the regularity in leaf appearance is associated with leaf elongation which is dependent on the rate of initiation of primordia at the apex, i.e., the same 'physiological clock' controls the

development of all leaves (Erickson and Michelini, 1957; Maksymowych, 1973; Hay and Kemp, 1990). Other scientists are in favour of a self-regulation system originally proposed by Sharman (1942) and Etter (1951). According to the 'self-regulating dynamic' hypothesis, the emergence of a particular leaf at a particular time controls leaf elongation for that leaf, and that in turn decides the timing of emergence for the successive leaf (Malvoisin, 1984; Wilson and Laidlaw, 1985; Durand et al., 1999; Durand et al., 2000; Fournier et al., 2005). In *Festuca arundinacea* Schreb., Skinner and Nelson (1994) reported that the ligule initiation at the specific phytomer n, is synchronized with the leaf initiation at a phytomer n+1, and tiller initiation at the phytomer n-1. This co-ordinated mechanism of leaf initiation at phytomer n and ligule initiation at phytomer n-1, was also found to be synchronous for cessation of sheath cell division at the phytomer n-2 (Skinner and Nelson, 1995). Sartie (2006) measured the time interval between leaf tip appearance (A_{lf}) and leaf ligule appearance (A_{lg}) of successive leaves for a mapping population of 200 plants and 2 parents (Grasslands Impact and Grasslands Samson) in *L. perenne*. In this study A_{lg} was typically greater than A_{lf} indicating leaf elongation duration was greater than the interval between two successive leaf appearance events and A_{lf} and A_{lg} were reported to have broad sense heritability values of 0.42 and 0.61, respectively (Sartie, 2006). Once a leaf tip becomes visible, the leaf primordium immediately below is triggered to follow an identical series of developmental steps (Durand et al., 2000). Developmental morphogenesis of a *F. arundinacea* leaf was conceptualized by Durand et al. (2000) as a succession of co-ordinated activities: meristematic cell division at the division zone, cell elongation at the elongation zone and cell maturation at the maturation zone. In this model the cell division activity at the division zone ceases when the elongating leaf inside the pseudostem (at node n) achieves a length equal to the sheath length of the leaf of the phytomer n-1; activity at the elongation zone continues and sheath elongation commences. This set of co-ordination rules accurately predicted the leaf formation patterns of plants studied by those authors. Some studies have indicated that the events of grass leaf elongation are related to ontogeny of the leaf growth zone (Williams, 1974; Martin, 1988; Fournier and Andrieu, 2000; Muller et al., 2001). The appearance of the leaf tip above the whorl and the emergence of the collar are the more distinctive events in leaf developmental morphogenesis as highlighted by Fournier et al. (2005). Verdenal et al. (2008) in *L. perenne* tested the self-regulation mechanism for architectural control and found that the self-regulation model could satisfactorily explain the majority of quantitative architectural traits, including: (i) the timing of leaf appearance (leaf

appearance rate, LAR), (ii) their final length (FLL), and (iii) the appearance of tillers (tiller appearance rate, TAR) with a correlation coefficient of 0.98.

2.4.2.5 Leaf elongation and extension

The elongation zone of a grass leaf blade or sheath is located at the leaf base, enclosed within the whorl of mature sheaths (Soper and Mitchell, 1956; Davidson and Milthorpe, 1966; Kemp, 1980). For *F. arundinacea* the elongation zone responsible for epidermal cell division is restricted to the basal 1.5 to 2.0 mm (MacAdam et al., 1989; Skinner and Nelson, 1994). Epidermal cells above the basal division zone then elongate only until they are displaced to a distance of 7 to 40 mm above the point of attachment to the tiller axis, but mesophyll cells adjacent to epidermal cells continue to divide for a longer period (MacAdam et al., 1989). Once the epidermal cell elongation has ceased the secondary cell wall forms (MacAdam and Nelson, 1987), cells start maturing, and also the photosynthetic apparatus is built (Skinner and Nelson, 1995). Durand *et al.* (1999) referred to the cumulative effect of leaf elongation and extension as **leaf elongation rate (LER)**. LER is generally measured as mm or cm leaf elongation per GDD. Fig. 2.3 (from Skinner and Nelson, 1995) illustrates the physiological processes involved during leaf elongation, extension and maturation. Data for chloroplast replication included in that figure were taken from Dean and Leech (1982). For *F. arundinacea*, new epidermal cell production ceases at about 65% of FLL; at this stage the elongation zone shrinks as divided cells complete the elongation process and mature, and thus the leaf attains its final length (Skinner and Nelson, 1994). In grasses, the cell production rate at the division zone, the number of cells produced per file, and the rate and duration of cell elongation at the elongation zone control LER. LER together with **leaf elongation duration** (LED) determine the size of the individual leaf and the structure of canopy at a particular time (see Fig. 2.4). For an individual leaf, FLL can be calculated as a product of LER and LED. LED of an individual leaf is not necessarily equal to A_{lf}, although closely related. As mentioned above, Sartie (2006) observed A_{lg} to be greater than A_{lf}. Similarly Robson (1967) observed that number of extending visible leaves ranged between 1.0 and 1.2. This meant that FLL increased for successive leaves, and that LED was greater than A_{lf} (Lemaire and Agnusdei, 2000). LED of an individual leaf has been shown to be proportional to the A_{lf}. A mature leaf senesces at the end of its life-span. **Leaf life span** (LLS) is the measure of duration of a leaf in days or GDD from its appearance to its senescence. In a steady-state condition, the LAR, rate of leaf death and LLS of the

individual leaves interact together to determine the maximum number of live leaves per tiller (NLL) (Lemaire and Chapman, 1996). NLL then is more or less constant for a species and genetically determined (Lemaire and Chapman, 1996). NLL can be estimated as the product of LLS and LAR, or LLS estimated as the quotient of NLL and LAR (Lemaire and Agnusdei, 2000). LLS in often calculated in thermal time. For *L. perenne* and for *F. arundinacea* with a A_{lf} of 110 ^{o}C d and 230 ^{o}C d and with NLL being 3.0 and 2.5 per tiller, respectively, the LLS would be 330 ^{o}C d and 570 ^{o}C d (Lemaire, 1988). In *L. perenne*, Davies (1977) in the UK recorded 2.55 to 2.87 live leaves per tiller. Fulkerson and Slack (1994) in Australia and Yang et al. (1998) in New Zealand recorded 3-4 live leaves per tiller.

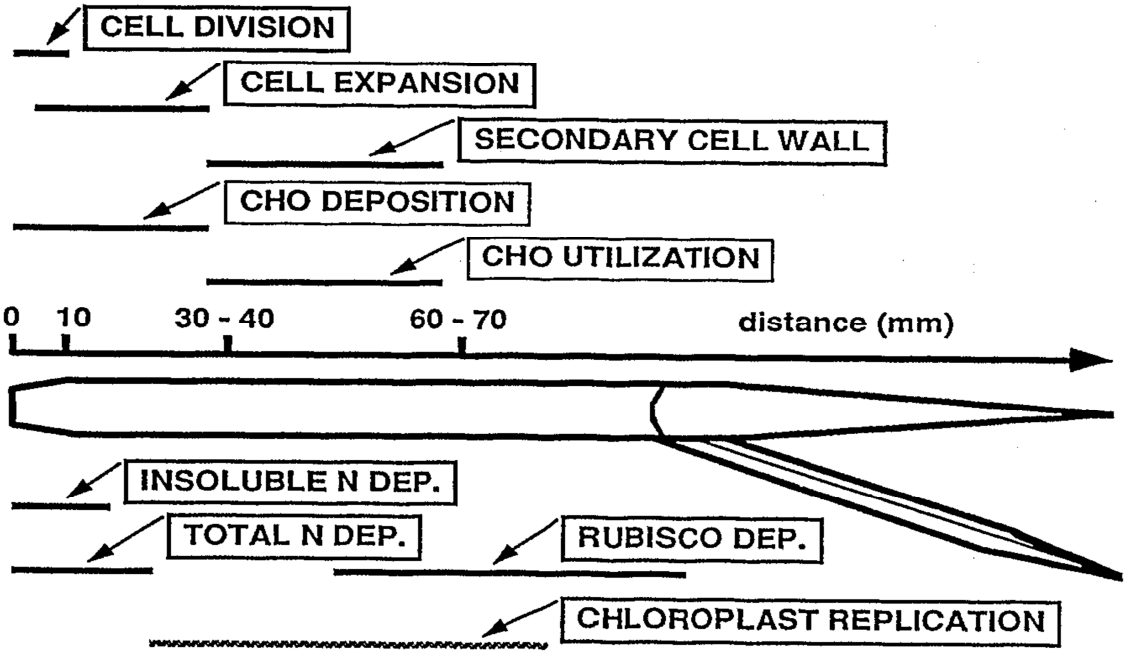


Fig. 2.3 Growth and associated physiological processes during elongation of a tall fescue leaf blade. In the figure, the ligule is located about 1 mm above the point of leaf attachment to the apex. Deposition of N-containing compounds occurs largely during cell division; deposition of carbohydrates (CHO) occurs largely during cell expansion; synthesis of Rubisco occurs during leaf maturation sometime after N deposition (From Skinner and Nelson, 1995).

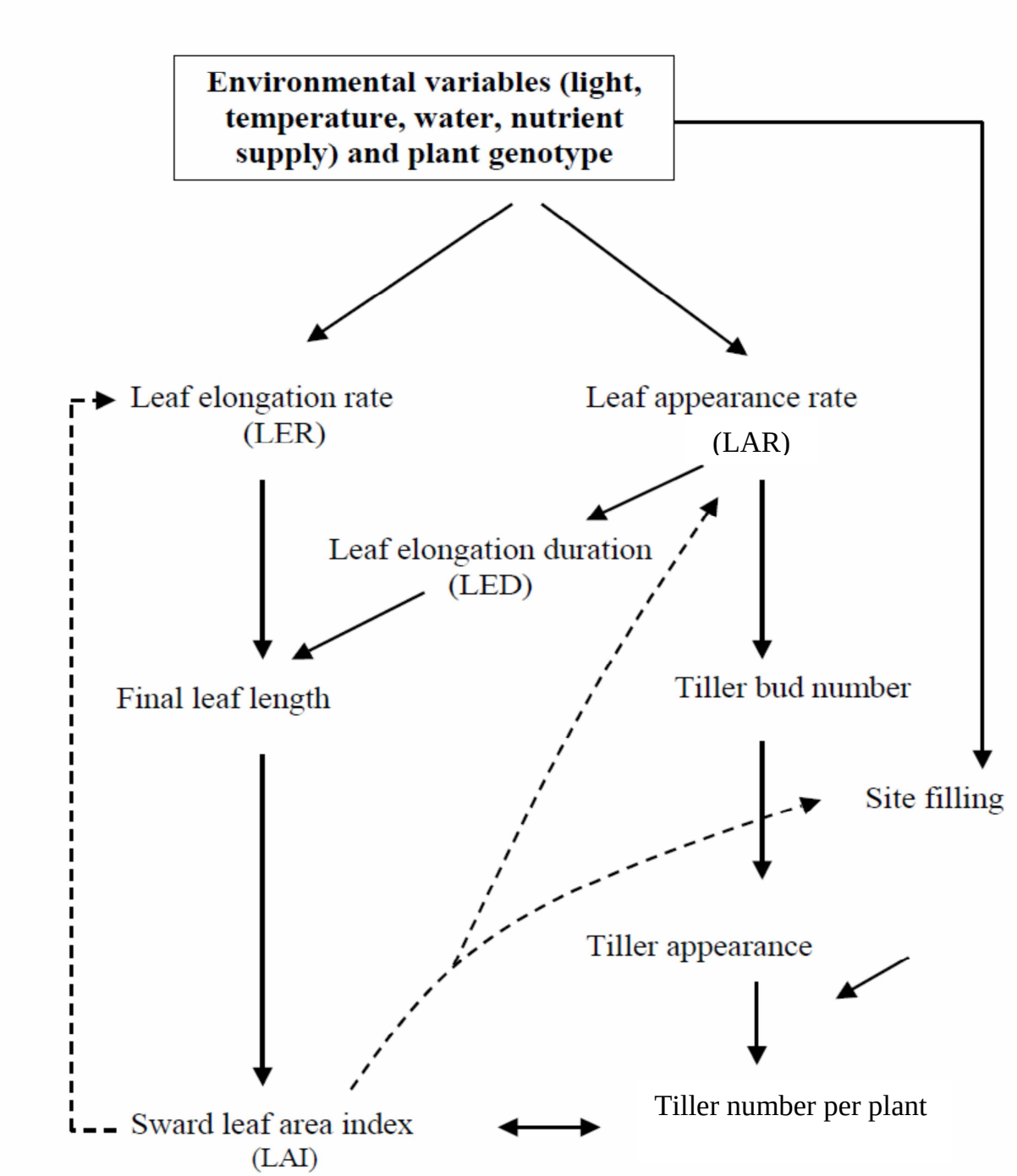


Fig. 2.4 Interrelationship between morphological and growth traits including among others leaf elongation rate (LER), leaf elongation duration (LED), leaf appearance rate (LAR), tiller appearance and leaf area index (LAI) (from Bahmani et al., 2000).

2.4.2.6 Tillering

A tiller axis which contains a series of phytomers unites shoot and root components and these together constitute a tiller (Etter, 1951; Briske, 1991; Yang et al., 1998). An individual ryegrass tiller contains 18-19 live phytomers at differential developmental stages; i.e., six to seven leaf primordia (where the oldest one elongates within the pseudostem), one elongating leaf, generally three live mature or senescing leaves and eight or more root-bearing phytomer positions (Yang et al., 1998; Matthew et al., 2001). Each phytomer produces an axillary bud in the axil of the subtending leaf and this bud has the potential to develop into a **daughter tiller** (DT), depending on growing conditions (Jewiss, 1972). The tiller on which the axillary bud producing a DT originates is often referred to as the **parent tiller or main tiller** (MT). The developmental process of the phytomers of a daughter tiller follows that of the parent tiller. Each daughter tiller again produces new phytomers at its stem apex and a daughter tiller may in turn form **secondary tillers**. The process of new axillary bud formation and initiation thus results in a hierarchical tiller organization (Langer, 1963; Neuteboom and Lantinga, 1989).

2.4.2.7 Root formation and development

At a certain phytomer developmental stage, generally near the time when leaf senescence occurs, one or more roots are initiated from the phytomer. Root initiation and development is the last morphogenetic function of a phytomer. For *L. perenne*, root initiation occurs generally at the fourth to sixth phytomer position, counting the emerging leaf as position 1 or at 10-12 plastochron intervals from leaf primordium initiation (Yang et al., 1998). As with leaf appearance on the tiller axis, initiation of roots on successive phytomers is coordinated to occur at a similar stage of phytomer development and the time interval between root initiation events at successive phytomers is termed the **rhizochron**. Klepper et al. (1984) in *Triticum aestivum* L. described a naming system for the root axes. They found that at the tiller axes, adventitious roots begin to appear approximately at the same time that the tiller attached to that phytomer appears. Matthew and Kemball (1997) differentiated the degree of root development according to phytomer position on the tiller axis. They also used radiocarbon to assess the comparative quantity of photosynthate reaching roots of various ages and found that roots located at younger phytomer positions receive a comparatively higher share of recently acquired C compared to roots at lower nodal positions further down the tiller axis. According to Matthew et al. (1998), in steady state growing conditions, the plastochron and rhizochron for a specific phytomer position

should be approximately equal as the phytomer acts as an architectural building template that initiates both the leaf primordium and the roots, though with a time delay between the two events.

Soper (1959) studied root anatomy of a group of grasses and clovers including *L. perenne*. She found that that the lateral roots of *L. perenne* maintain a vascular connection with their parental root axis which in turn originates from the pericycle of the tiller axis (Soper, 1959). Similarly, Felix et al. (2002) in a review entitled 'Maize root system and genetic analysis of its formation' reported that crown roots originate from the underground nodes of the developing stem whereas the lateral roots originate from the pericycle of differentiated roots. In *T. aestivum*, adventitious roots initiate at the base of the fourth leaf (counting downward from the emerging leaf) (Klepper et al., 1984). More developed roots are found at successively older nodes. Generally, two to four roots appear from each node (Klepper et al., 1984).

2.4.2.7.1 Root production in field swards

As mentioned in Section 2.3, a large number of earlier New Zealand and British studies found that root formation and development in a grass sward is a seasonal phenomenon (e.g.,Stuckey, 1941; Jacques and Edmond, 1952; Jacques, 1956; Jacques and Schwass, 1956; Baker and Garwood, 1959; Garwood, 1968; Caradus and Evans, 1977). Stuckey (1941) in a study in the USA classified *L. perenne* roots as being of 'annual type' meaning that roots were replaced each growing season. In New Zealand, Jacques and Schwass (1956) grew *L. perenne* plants in cylindrical pipes filled with soil and counted numbers of 'white' roots and 'other than white' roots to separate new and old roots, respectively. They counted an average of 21.8 white roots per plant at the beginning of autumn. During April and May (end of autumn) that number increased to between 40 and 50 per plant and plants maintained that figure through June with a peak in July (winter) of 64 white roots per plant. The number of white roots per plant decreased over time from winter towards the end of spring when each plant was observed to have 4 white roots only. These authors observed quite similar trends in white root formation for *L. multiflorum* and *F. arundinacea*. They also recorded the highest total number of roots per plant (approximately 400 roots) between February and March (beginning of autumn) and the lowest in September in spring (approximately 200 roots). Caradus and Evans (1977) in New Zealand reported a result consistent with results of Jacques and Schwass (1956) for

new root formation (<5 cm long with fewer than two laterals) in the top 5 cm of soil. The article reported highest new nodal root formation at the top 5 cm soil (roots m^{-2}) for *L. perenne* and *Trifolium repens* L. in autumn (>5000 m^{-2}). For *L. perenne* a second peak was recorded in mid-winter (approximately 3500 m^{-2}) and that decreased afterwards towards the end of spring (<400 new nodal roots m^{-2}). The article also reported that at various soil depths (lower than 5 cm soil depth) in general most of the new nodal roots form at the end of spring (October), followed by winter and autumn. In a *Medicago sativa* L. sward, a UK study by Baker and Garwood (1959) found the highest total root and stubble weights in a sward in December (early winter), than any other season. However, Garwood (1968) recorded highest number of new roots in April (spring with the number of new roots gradually increases between September (beginning of autumn) and April (beginning of spring) from approximately 200 new roots ft^2 to more than 500 per ft^2; a decreasing trend from April to September for *L. Perenne* in the UK. The concept of 'seasonal pattern of root production' and 'annual root replacement' has brought contradiction with some later reports on root growth based on segmental architecture of the grass tillers. Matthew et al. (1991) brought an interesting report studying root growth using a 'refilled core technique'. This study viewed root formation in a grass sward as a continuous process but indicated that root development might be modified by soil conditions at particular depths in the soil profile in particular seasons. This study reported that seasonal deposition of new root mass as measured by the refilled core technique broadly followed the seasonal curve for herbage accumulation above ground with root production being about 15% of shoot production at any one time (Matthew et al., 1991). Hence, it appeared the previously reported seasonal pattern of root production was not confirmed, but it is clear that if root production is organised on a phytomer basis like leaf production, the seasonal pattern reported by Matthew et al. (2001) is predicted, rather than the annual growth of new roots as reported by earlier authors.

2.4.2.7.2 Root production at the phytomer level

Later studies by C Matthew further reinforced the concept of continual root production at the tiller axis. For example, Yang et al. (1998) provided a map of tiller axis architecture of *L. perenne* and *F. arundinacea.* This map describes the developmental status of phytomers on the tiller axis in a sequence from the apical meristem, including leaf primordia, the emerging leaf, mature and senenscing leaves, and root formation and development at the senescence of the oldest leaf. For the eight month old tillers the authors recorded eight live

root-bearing phytomers along with four live leaves for *L. perenne* and seven live root-bearing phytomers along with five live leaves for *F. arundinacea*. That study reported only the number of roots at each phytomer with no detailed information on state of development but their result clearly indicates the fact that as the number of root-bearing phytomers is much greater than that of leaves, roots have a longer turnover cycle than leaves and that the individual roots have a life span perhaps as much as twice the life span of an individual leaf. Root data recorded by Matthew and Kemball (1997) added more information providing an indication of tissue flow at the successive root-bearing phytomers in the tiller axis as that article reported root dry weights and root length per phytomers for the tillers of approximately 3 months of age. In this study, root dry matter, main axis length and total length followed a linearly increasing trend up to the 6^{th} root-bearing phytomer (Pr6) counting from the youngest rooting phytomer and for phytomers below Pr6 did not change much. This study also reported the number of roots observed at each phytomer position as being close to two and that result is consistent with Matthew et al. (1998) and Yang et al. (1998). The authors also derived equations for estimating the rate of root dry matter accumulation (Matthew et al., 1998). The model estimates the rate of root mass accumulation for a tiller is the product of individual final root mass (RDW_i), number of roots formed per phytomer (R_p) and the rate of appearance of root-bearing phytomers on the tiller axis (PrAR). In a separate study in Germany, Lattanzi et al. (2005b) also reported the continual root growth at the tiller axis of *L. Perenne,* studying functional heterogeneity in the pattern of root growth and N accumulation capacity at different phytomers in defoliated condition compared to undefoliated condition, the results are comparable with Matthew and Kemball (1997) and Yang et al. (1998).

2.4.2.7.3 Root life span

For *L. perenne* there are a few reports which have explored root longevity. Troughton (1981) prevented the formation of new root axes by keeping the tiller base dry with a layer of sand over the soil mixture. Under no-defoliation and defoliation to a stubble height of 3 cm at 3–weekly intervals, respectively, the mean survival for *L. perenne* plants for the defoliated and un-defoliated treatments were respectively 365 days and 191 days, which was considered to be the root life span. This result indicates the maximum possible life span of newly forming root is much higher than Garwood (1967) estimated on the basis of average time for which main axis of root or lateral branches remained white, namely 61 to 188 days. Jacques and Schwass (1956) reported that in a grass sward 68% roots are

replaced annually which means the expected root life span would be more than 365 days. Watson et al. (2000) measured the root longevity of *L. perenne* and *T. repens* using a video camera and digitized images in the minirhizotron tubes at sites in UK and Italy. Images were taken at 7 day intervals for first 6 weeks and thereafter 28-42 day intervals. Root mortality was inferred from the disappearance of roots between dates or with disintegration of the cortex. At both locations, these authors reported many roots surviving less than 21 days. For *L. perenne*, greater mortality of roots was recorded in Italy, with 84% roots surviving less than 21 days, than in UK where 38% of roots survived less than 21 days. They also found that 15% of roots of *L. perenne* at the UK sites survived over 196 days but no roots at the Italian sites survived for more than 196 days. The authors hypothesized that survival differences were mainly due to temperature variation in two sites.

2.4.2.7.4 Genetic variations in root characteristics

Breeding perennial ryegrass for root characteristics has advanced only recently. Knowledge of root traits potentially offers a wider contribution to agronomic fitness and nutritional efficiency of pastures but the lag in research progress is probably due to technical difficulties inherent in research into root traits. However, J. Crush and co-authors in New Zealand have recently published a series of articles that clearly indicate scope for further genetic development of *L. perenne* cultivars for root characteristics. Crush et al. (2005) studied the root system distribution and their nitrate efficacy for eleven grass species including *L. perenne* and *L. multiflorum.* They reported wide variation between species in maximum rooting depth and distribution of root DW at various soil depths. They reported that more than the 75% of root DW of *L. perenne* was recovered from the top 30 cm soil although maximum rooting depth was about 1 m. *Cynosurus cristatus* L. was the shallowest rooted species in a comparison with 14 other grass species. For *C. cristatus* no root DW was recorded below 50 cm while *L. multiflorum* was one of the deeper rooting species with roots that penetrated to more than 1 m depth. In later studies the same research group reported genotypic variation in root distribution. Root DW was found to vary significantly even between accessions of wild type *L. perenne,* as well as between breeding lines and cultivars. This study also noted that selection for merit based on shoot performance did not necessarily result in a larger root system (Crush et al., 2009). Genotypic variation in root system distribution of 198 F_1 progeny of two parents with contrasting shoot morphology, ‘Grasslands Samson’ and ‘Grassland Impact’ for nitrate

interception and response to moisture stress was reported by Crush et al. (2007). The progenies of these two parents showed wide variation in root system distribution although both of the parents were shallow rooting (Crush et al., 2007). Some of the progeny also responded to moisture deficit by increasing root DW and root growth but for some other progeny root DW decreased on exposure to moisture deficit (Crush et al., 2007). Crush et al. (2006) reported statistically significant genotypic variations in distribution of root DW among fifteen genotypes from each of twenty half-sib families of ryegrass but found no consistent family effects. In a recent study (Crush et al., 2010b) the same research group found that the progeny of contrasting root system pattern of four *L. perenne* pools with variation in vertical distribution in root DW were true to type after one cycle of selection meaning the breeding for root system shape is possible. These pools of experimental results are helpful for *L. perenne* breeding based on root system characteristics.

2.4.2.7.5 Fine roots and root hairs

For *L. perenne* Matthew (1992) estimated mean root diameter between 0.2 and 0.3 mm, whereas the diameter of the fine laterals might be less than 0.1 mm (Evans, 1970). Care (1999) measured the root hair structure, and root hair recruitment in *L. perenne* cultivars: Grasslands Nui, Grasslands Supernui, Vigour and breeding line Ruakura, and *T. repens*. For the cultivar Grasslands Nui mean root hair length was around 500 µm, root hair diameter measured between 12-13 µm, and the number of root hairs per mm root was 1250 at high phosphorus and 1370 at low phosphorus. The diameter of the main root axis was around 0.25 mm. Root hairs have very short life span of a few days. Matthew et al. (2001) using data from Reid (1981) and Care (1999) estimated that root hairs potentially contribute approximately 90% root surface area at a cost of only 10% root volume.

2.4.3 Factors affecting patterns of growth and development

2.4.3.1 Effects on shoot growth and development

A number of environmental and physical factors influence shoot growth and development in grasses. Temperature, light intensity, day length, nutrient supply, and availability of moisture are among the most important.

2.4.3.1.1 Temperature

Temperature is a major determinant of leaf elongation and LAR. A large number of experimental results have shown that the LER is strongly affected by temperature (Silsbury, 1970; Robson, 1972; Roy, 1972; Peacock, 1975c; Peacock, 1976; Thomas and Norris, 1977; Keatinge et al., 1979; Baker and Younger, 1987). Peacock (1975b; 1976) and McMaster et al. (2003) confirmed that a change in LER is brought about by a change in the temperature of the leaf meristematic zone near the stem apex rather than through the general effect of soil or air temperature on plants. Temperature below the optimum reduces the length of epidermal cells, which results in shorter FLL although the number of epidermal cells remains similar (Cooper, 1964). For many different temperate grass species the temperature at which maximum LER occurs is between 20-25^{o}C (Mitchell, 1956; Cooper, 1964; Lemaire and Chapman, 1996). LER increases exponentially over the average daily temperature range of 0-12 ^{o}C, then increases in a linear fashion up to the optimum temperature (Lemaire and Chapman, 1996), and finally significantly decreases above the optimum temperature (Cooper, 1964; Ferris et al., 1996). An LER reduction from 30 µm min^{-1} to zero was observed in *L. temulentum* when the temperature of the expansion zone was reduced from 20 ^{o}C to 2 ^{o}C. In the field the A_{lf} is typically linearly related to thermal time up to an optimum growing temperature (e.g., Silsbury, 1970; Hay and Tunnicliffe Wilson, 1982; Klepper et al., 1982; Davies and Thomas, 1983; Frank and Bauer, 1995; Kirby, 1995; Durand et al., 1999; Porter and Gawith, 1999). Experimental evidence suggests that the leaf dry matter production by the individual vegetative tillers is largely explained by temperature (Thomas and Norris, 1977). Since temperature influences both the LAR and LER (Cooper, 1964; Williams and Biddiscombe, 1965; Robson, 1969; Peacock, 1975a) it is a vital determinant of the seasonal productivity of grass plants (Munro and Davies, 1973) as the duration of the growing season of perennial swards is predominantly determined by temperature.

2.4.3.1.2 Light

Light influences leaf growth and tiller production. In fact light intensity at the stem apex coupled with temperature plays a profound role in control on leaf growth. Kays and Harper (1974) reported that for a similar sowing density of *L. perenne* seedlings, a lower light intensity significantly reduced the mean dry weight of tillers and the number of tillers per unit area. These authors further indicated that self-shading in a dense sward increases leaf length and decreases tillering in *L. perenne* (Kays and Harper, 1974). Shade treatment

reduced LAR and TAR on the main shoot, delayed the tiller appearance at the leaf axil, reduced site filling ratio and reduced the number of tillers per plant drastically (Bahmani et al., 2000). At the individual leaf level under simulated shade treatments the LER and LED significantly increase compared to full light treatments (Bahmani et al., 2000). This difference can be attributed to increased leaf length and leaf area per tiller as a result of higher leaf elongation rate under shade treatment (Gautier et al., 1999). Allard *et al*. (1991a) showed that in *F. arundinacea* the newly developed leaf blades at low irradiance (midday PPFD of 600 µmol m^{-2} s^{-1}, wavelength 400-700 nm) were 54-65% longer and 56-77% greater in leaf area but were 12% thinner and also lower in specific leaf weight by 25% compared to the leaves formed at high irradiance (2000 µmol m^{-2} s^{-1}). Low irradiance reduced the total stomatal density by 17-24% compared to high irradiance and increased air space within the leaf blade by about 25% (Allard et al., 1991a). At low irradiance (600 µmol m^{-2} s^{-1}) CO_2 exchange rate (CER, µmol m^{-2} s^{-1}) per unit leaf area was 14-25% lower than high irradiance (2000 µmol m^{-2} s^{-1}) for the youngest fully expanded leaf blades of *F. arundinacea* (Allard et al., 1991b). High-irradiance was found to be associated with higher stomatal density (Allard et al., 1991b) and higher stomata conductance than low-irradiance leaves which are closely associated with maximum CER; whereas at low-irradiance lower CER is not only associated with lower stomatal conductance but also internal CO_2 concentrations (Allard et al., 1991a). Under low light stress plants increase specific leaf area (SLA) to maximize light interception per unit DW and change physiological processes to enhance the efficiency of C utilization (Allard et al., 1991a; Kephart et al., 1992; Sanderson et al., 1997). In *T. aestivum*, Bos and Neuteboom (1998) studied LED, LER and A_{lf} as a response to light intensity and also reported that increase in irradiance increased the ratio between LED and A_{lf}. These authors explained that an apparent delay in leaf appearance was due to the faster rate of leaf sheath elongation.

In addition to the effects of light intensity, variations in light quality also cause some remarkable responses in grass plants. For *L. perenne*, Deregibus et al. (1983) citing Deregibus and Sanchez (1981) reported that plants grown in a chamber illuminated with fluorescent light and irradiated with red at the end of daily light period recorded a higher TAR than plants irradiated with far-red. In a further study, Deregibus et al. (1983) found that far-red exposure at the end of day for 20 minutes (0.10 W m^{-2} light intensity, 700-1000 nm wavelength) decreased tillering significantly compared to far-red for 20 minutes followed by red light (3 w m^{-2}, 600-700 nm wavelength) exposure for 10 minutes at 23

days after treatment. Similarly, young *L. multiflorum* seedlings growing under white light with a higher red:far-red ratio (2.2) exhibited a significantly higher tillering rate (Deregibus et al., 1983) compared to seedlings at low red:far-red ratio (1.1). A decrease in red/far-red ratio reduced tillering and site filling but that did not change phyllocrhon as reported in a later study by Gautier et al. (1999) in *L. perenne*. Casal and Alvarez (1988) reported that blue light treatment reduces FLL by reducing leaf sheath length. In contrast Gautier et al. (1999) reported that tillering, site filling and A_{lf} were not affected by the reduction of blue light. The reduction in blue light is perceived by cryptochrome 1 and the reduction of far-red is perceived by phytochrome A (Yanovsky et al., 1995; 1998). Ballare et al. (1987) found that the reflection of far-red by green leaves lowers the red:far-red ratio of horizontally propagated light and modifies the canopy light environment. This modified ratio can change the rate of stem elongation (Ballaré et al., 1990). Mutual shading also reduces photosynthetic photon flux density (PPDF) at all wave-lengths for the leaves in lower canopy strata.

2.4.3.1.3 N Supply

N is an essential constituent of proteins, and is the next most abundant constituent of plant tissue after C/H/O. Hence plant leaf growth is systematically and greatly affected by N supply. Increased N availability generally increases both the rate of expansion of individual leaves and the rate of tillering through an increase in site filling (Wilman and Pearse, 1984; Gastal and Lemaire, 1988). Increased leaf expansion rate under high N availability in turn results in greater FLL (Gastal et al., 1992; Gastal and Nelson, 1994). High N supply has been found to increase the rate constant (length gained per unit length present, (Erickson, 1976)) for elongation by 9%, and the mean epidermal cell elongation rate by 22% (MacAdam et al., 1989). High N supply also increases cell division rate of the mesophyll cells, which constitute 42% of the cross-sectional area of the leaf blade, and the ratio between mesophyll cells and epidermal cells (Volenec and Nelson, 1984; MacAdam et al., 1989). High N supply not only increases the rate of cell production but also increases the duration of cell elongation. In *F. arundinacea*, epidermal cells of a high-LER genotype elongated for 82 h at low-N and 90 h at high N, and those of a low-LER genotype elongated for 61 h at low N and 72 h at high N (MacAdam et al., 1989). N supply stimulates cell production rate but final cell length remains less affected (MacAdam et al., 1989; Gastal and Nelson, 1994; Fricke et al., 1997). Some studies report that in response to increased N supply cell elongation rate increases but cell elongation

duration decreases (Gastal and Nelson, 1994; Fricke et al., 1997); in other studies both processes appear to be unaffected (MacAdam et al., 1989; Gastal and Nelson, 1994). N availability can decrease LAR by increasing sheath length of the successive leaf (Cruz and Boval, 2000). At lower levels of N availability the reduction in leaf expansion is often found to be associated with accumulation of non-structural carbohydrates such as fructans (Volenec and Nelson, 1984).

2.4.3.1.4 Photosynthesis in relation to leaf age

It has been known for a long time that leaf photosynthetic capacity declines with leaf age (Jewiss and Woledge, 1967; Wilhelm and Nelson, 1978). Wilhelm and Nelson (1978) found that leaves of *F. arundinacea* achieve maximum photosynthetic capacity (CER) at the time of collar formation. They also reported that CER declines approximately 15-20% per week. Similarly, Khaembah (2009) measured the net photosynthetic rate of fully expanded *L. perenne* leaves of different ages and reported that youngest fully expanded leaf had the highest net photosynthetic rate of around 20 µmol CO_2 m^{-2} s^{-1} at 11 days after leaf tip appearance, and that declined gradually to around 15 µmol CO_2 m^{-2} s^{-1} at 30 days after leaf tip appearance. The decreasing rate of photosynthetic capacity with leaf aging is probably associated with Rubisco turnover as Mae et al. (1983) showed that in the leaves of *O. Sativa* Rubisco content increases rapidly when a leaf is expanding, reaches a maximum value when the leaf is fully expanded and then declines as the leaf ages. This pattern has recently been confirmed in *L. perenne* (Khaembah, 2009).

2.4.3.2 Factors affecting root growth

2.4.3.2.1 Temperature

Temperature has a marked effect on root growth and development and ultimately overall plant productivity. The optimum temperature is that at which rate of root elongation, biomass production, branching, water and nutrient uptake characteristics and root-microbe interactions are optimum (McMichael and Burke, 2002). Clarkson et al. (1986), on the basis of root dry weight change at varying temperature, found 17^{o}C to be the optimum temperature for root growth of *L. perenne*. Brown (1939) on the basis of root biomass reported 15^{o}C as the optimum temperature for root growth of *Phleum pratensis* L. For blue grama (*Bouteloua gracilis* L.), an optimum temperature range (at which average elongation rate of adventitious root axes was 2.3 cm day^{-1}) was reported to be between 24–27 ^{o}C in field conditions (Wilson and Briske, 1979) and 25 ^{o}C in a plant growth

chamber (Briske and Wilson, 1977). For *L. perenne*, Troughton (1957) reported that maximum root production is associated with a soil temperature range fluctuating between 15 ^{o}C and 25 ^{o}C, while Hunt and Thomas (1985) in an experiment with *L. perenne* cv. Grasslands Ruanui in hydroponic culture reported no apparent optimum temperature over the range 7 ^{o}C to 33 ^{o}C. A low or high temperature rather than the optimum can vitally modify the root structure and function. Arndt (1937) reported that exposure of *Gossypium hirsutum* L. roots growing in a greenhouse to a temperature of 60^{o}C for an hour caused severe injury to the roots and markedly reduced their water absorption capacity. These heat treated roots showed limited elongation, and abnormal branching with irregular diameter. Pardales et al. (1991) found that larger exposure to 40^{o}C temperature of roots of *Sorghum bicolor* L. increased the inhibition of root growth when plants were returned to 25^{o}C. For curly parsley (*Petroselinum crispum* L.) out of five root-zone temperature treatments (18, 21, 24, 27 and 36^{o}C) maximum growth was obtained at 18^{o}C and 24^{o}C (Eidsten and Gislerød, 1986). In the same study shoot and root growth were severely retarded at 36^{o}C constant temperature in the root zone. A short time exposure of 36^{o}C temperature in the root zone for 30 minutes each day caused retarded growth (Eidsten and Gislerød, 1986).

Temperature may affect root longevity (Hendrick and Pregitzer, 1993) although this is very difficult to measure. Fitter et al. (1999) measured root longevity in minirhizotrons for a soil temperature difference of 2.8 ^{o}C at 2 cm soil depth and reported no separable effect on root longevity. Forbes et al. (1997) observed that roots of *L. perenne* under controlled conditions exhibited 30% root mortality after 35 days at 15^{o}C while those growing at 27^{o}C had 84% root mortality. Over a 35 day measurement period they observed a clear decrease in longevity with increasing temperature with always a higher root length measured at 15^{o}C than at 21^{o}C or 27^{o}C. On continuous exposure to a temperature of 27^{o}C many roots died after 14 days and only 16% roots survived for 35 days. This indicated a comparatively short life span of *L. perenne* roots at temperature over 20^{o}C. A higher death rate of older roots at higher temperature might possibly be explained by high respiration loss of substrate and C starvation as a consequence.

2.4.3.2.2 Light

In grasslands, root growth is reported to be closely associated with solar radiation rather than temperature. Fitter et al. (1998) studied root production, turnover and respiration in

two grasslands in the UK, one dominated by *Festuca ovina* L.; and the other by *Juncus squarrosus* L. and *Nardus stricta* L. Their data indicated no pattern of temperature-dependency in the rate of root respiration. The respiration rate was instead found to be correlated with the mean total solar radiation flux. This result suggests that root growth is determined more by resource availability and source-sink relationships rather than dominant environmental factors such as temperature (Fitter et al., 1998; 1999).

Light influences root growth and its architecture. Soper and Mitchell (1956) counted fewer adventitious roots with smaller diameter from plants growing in shade at 24^{o}C than those grown in full daylight at 14^{o}C. Exposure of the shoots to light influences the direction of root axis growth and gravitropic orientation of roots (Lu et al., 1996; Takano et al., 2001) and thus can change root architecture. In *O. sativa*, Morita and Yamazaki (1992) found that shoots exposed to a 14 h light period (10,000 lux) after a dark treatment led to vertical extension of dark-grown nodal roots but dark conditions in general resulted in the root axes to extending horizontally. Similar results were also reported by Takano et al. (2001) in *O. sativa,* and by Lu et al. (1996) in *Z. mays*. Under exposure to continuous red or far-red light the roots move downward. Phytochrome A is the primary photoreceptor for far-red light-regulated processes which control gravitropic responses of roots i.e., gravitropic orientation and inhibition of root elongation (Takano et al., 2001; Correll and Kiss, 2005). For red light medicated processes both Phytochrome A and Phytochrome B modulate the gravitropic responses of *Z. mays* (Lu et al., 1996). Correl and Kiss (2005) found that red light treatment of dark-grown roots decreased the rate of root elongation by 30% in Arabidopsis probably due to diversion of energy at the exposure of light for other processes like cotyledon expansion and greening.

2.4.3.2.3 Moisture status

Soil moisture status has a pronounced effect on the development, morphology and growth of roots. In the experiment of Troughton (1981) reported in the Section 2.4.2.7 where plants of *L. perenne* were prevented from forming new roots by drying the top layer of soil, the subsequent introduction of moisture in the rhizoshpere resulted in the rapid production of new root axes. Dry soil surface conditions after germination prevent adventitious root formation and a period of at least three days of continuous moist conditions is essential for adventitious root formation in *L. perenne* to recommence after the moisture deficit ends (Cornish, 1982). A comparatively high water potential is required for adventitious root

extension for both *L. perenne* and *Phalaris arundinacea* L. (Cornish, 1982); and for *T. aestivum* the threshold value is -1.5 MPa (Ferguson and Boatwright, 1968). Sharma and Ghildyal (1987) by contrast found no significant variation in root axis elongation for fluctuating soil water tension regimes between -0.03 and -0.45 MPa in *T. aestivum*. The results indicated that moisture status at the soil surface is an important factor controlling adventitious root growth.

2.4.3.3 Factors affecting root function

2.4.3.3.1 Root responses affecting nutrient uptake

Root morphological characteristics strongly influence nutrient uptake (Boot, 1989) for both mobile and immobile nutrients. In a *L. perenne* sward higher nitrate interception is associated with comparatively larger root systems than the average root system (Crush et al., 2005). Modelling for nitrate uptake in relation to root system size and distribution suggest that a deeper root system and increasing root length density deeper in the soil profile reduce nitrate-leaching (Thorup-Kristensen, 2001). High N supply has been associated with increase in root biomass and root:shoot ratio but specific root length, specific root area, mean root diameter and frequency of fine roots were unaffected (Boot and Mensink, 1990).

As for N, the increased interception of inorganic phosphorus which is a non-renewable and immobile nutrient element is associated with root architecture, frequency and length of root hairs and distribution of roots deeper to the soil (Lambers et al., 2006) and mycorrizhal symbiosis. *L. perenne* breeders aim to develop cultivars which are more efficient at acquiring inorganic phosphorus from soil and/or more efficient at using phosphorus. Some articles suggest that phosphorus-fertilization has a direct effect on biomass partitioning (De Groot et al., 2001). A low phosphorus status decreased production and export of cytokinins from roots (Kuiper et al., 1989). One theory is that change of biomass partitioning may be linked to change in cytokinin production, and possibly associated with a decreased rate of uptake and metabolism of N (Kuiper et al., 1989). Lower phosphorus status was also associated with higher total root-length production without a proportional change in root biomass (Steingrobe et al., 2001), leading to greater amounts of uptake of immobile resources, such as phosphorus. Other observations also showed that increase in specific root length is also associated with lower inorganic phosphorus-supply (Schroeder and Janos, 2005). These results suggest that

when phosphorus is limited plants produce more fine roots as an adaptation strategy for higher phosphorus-acquisition.

As nutrients are distributed in soil in a heterogeneous manner roots need to respond to local nutrient patches to enhance nutrient capture (Hodge, 2004). Grass roots show different types of plasticity responses when encountering nutrient-rich patches. The plastic changes can be limited to individual roots such as, elongation of individual roots (Bilbrough and Caldwell, 1995), or the structure of the whole root system might be changed such as, increase in total root length (Hodge et al., 2000), increase in root production (Gross et al., 1993), and increase in extent of lateral root branching (Farley and Fitter, 1999). Faster growing grass species produce significantly higher root numbers, root length density and root biomass in nutrient rich patches under heterogeneous nutrient supply compared to slow growing species (Fransen et al., 1998; Robinson and Van Vuuren, 1998). The increase of both root biomass (Robinson, 1994) and specific root length (Eissenstat and Caldwell, 1988) significantly increase root length density.

Reduction in specific root length is usually reflected in increased root diameter and vice versa but an increased tissue density can also reduce specific root length. Morphological and physiological plasticity responses of roots in a heterogeneous soil environment have been reviewed by Hodge (2004). Increased nutrient uptake rate of the grass root system is a physiological plasticity response which is influenced by uptake capacity or ion affinity of the roots. Local nitrate supply at a higher concentration (1.0 mM nitrate) yielded many lateral branches compared to the remainder of the root system in contact with lower nitrate (0.01 mM nitrate) (Drew and Saker, 1975). In fact, following a local nutrient-deposition event, physiological responses occur before morphological responses (Drew and Saker, 1978; Robinson, 1994).

2.4.4.2.2 Root responses affecting water uptake

Water uptake capacity of plants depends on root morphological characteristics and physiological properties and the plasticity of roots at different levels of soil water availability. Morphological plasticity of the root system allows plants to adapt to different soil nutrient status and moisture availability (Robinson, 1994; Hodge, 2004). Root length density was positively correlated with water uptake rate under adequate soil moisture availability, at various soil depths in an experiment of Ehlers et al. (1991). Maximum

water uptake rate from individual soil layers (normalized by evapo-transpiration rate) found linearly associated with root length (normalized by specific root length) (Ehlers et al., 1991). Ehlers et al. (1991) also reported that maximum specific uptake rate per cm root length is inversely related to the square-root of root length density and also to the square-root of specific root length. Huang and Fry (1998) reported that root morphological and physiological characters which influence water uptake may differ from genotype to genotype of a species. In *F. arundinacea*, under limited water supply, deeper rooted genotypes had increased specific root length, and lower electrolyte leakage compared to a shallow-rooted genotype. The shallow-rooted genotype also produced lower dry weight and suffered greater turgor loss (Huang and Fry, 1998). Generally, plants producing thinner roots have higher specific root length, which is considered more efficient in terms of biomass utilization required to achieve nutrient acquisition (Eissenstat, 1992). A deep rooting system has been identified as one of several traits that is important for water deficit (drought) resistance (Sheffer et al., 1987; Marcum et al., 1995; Carrow, 1996). Root branching at deeper rooting depth has been considered to be an important drought tolerance mechanism (Marcum et al., 1995). Jupp and Newman (1987) studied root morphological and physiological traits of *L. perenne* under low water potential and reported that more negative water potential promotes lateral root initiation and elongation, and increases total root length. The degree of morphological plasticity is sometimes species-specific or genotype-specific and also depends on morphological traits of the root system to some extent. Molyneux and Davies (1983) studied the capacity for deep rooting of three pasture species and observed that in water-deficit conditions, seedlings of *Dactylis glomerata* L. can penetrate to a much deeper soil depth than *L. perenne* and *P. pratense*.

2.4.4 Intra-plant competition

2.4.4.1 Root-shoot relations

The co-ordination of growth of shoot and root systems is a topic that has received much research attention. The interdependence between root system and shoot system has been explained in the literature from many different morphological and physiological perspectives related to their form and function. The functional balance between shoot system and root system defines biomass partitioning, C-N relations, and water relations. Knowledge of this balance is useful to manage environmental changes.

Wilson (1988) grouped the models controlling root:shoot balance into four categories: allometric models, functional equilibrium models, Thornley's model on C and N uptake and transport, and hormone models. The allometric models propose a fixed ratio of shoot growth rate to root growth rate. This approach assumes a linear relationship between logarithmic values of root weight and shoot weight (Troughton, 1956). The allometric model is useful to explore inter-specific differences in root-shoot partitioning in plants growing in the same environment. The slope between logarithmic root:shoot ratio (k) is generally stable but sensitive to environmental changes such as nutrient status and also developmental changes of crop plants, for example, transition from vegetative growth to flowering. Functional equilibrium models define the ratio of shoot activity to root activity (Brouwer, 1963; de Willigen and van Noordwijk, 1987). These models consider that the relationship between the shoot and root systems can be regulated by various events e.g., reduction of photosynthetic area by grazing. In a third type of model, Thornley (1972) considered the C:N ratio of plants as a controlling factor in functional equilibrium and expressed the functional equilibrium model in terms of the supply, transport and utilization of C and N. The fourth model category of Wilson (1988) is a hormonal model, the essence of which is that hormones produced in the root control shoot growth and *vice-versa*.

Hunt and Thomas (1985) studied the leaf, tiller and root appearance rate for hydroponically-grown plants of perennial ryegrass. They reported that between 7 and 20 ^{o}C, the ratio between LAR and root appearance was constant and this ratio was independent of increasing light from 0 to 250 W m^{-2}. Mitchell (1953) found that A_{lf} of two types of *L. perenne* (Aberystwyth S23, a British *L. perenne* and SR, a short rotation New Zealand selection from hybridisation between *L. perenne* and *L. multiflorum*), were constant at different light intensity and day-might temperature. For example, A_{lf} was around 6 days at 11 W m^{-2} light intensity, 13 ^{o}C day temperature and around 7 ^{o}C night temperature; and was around 9 days at 31.5 W m^{-2} light intensity and same day-night temperature.

The dry matter partitioning between roots and shoots varies depending on plant species, growth stage, environmental conditions, and time in the growing season. Parsons and Robson (1981) studied the C partitioning to roots in *L. perenne* swards in spring and autumn after radio-isotope ($^{14}CO_2$) labelling. They reported that under full light

interception, current assimilate allocation to roots was never more than 25% and for the established swards was less than 15%. For a large group of grass species Sheehy (1977) reported as overall average of 29% ^{14}C distribution below ground. Rapid leaf elongation, development and shorter turnover cycle of leaves compared to roots are likely to demand much higher C partitioning to shoots than roots. In young spaced plants where the root system is still being established the C partitioning to roots may be up to 50% (Ryle, 1970). In established swards, Parsons and Robson (1981) observed a gradual decrease in the partitioning of current assimilate to roots from the beginning to the end of autumn (14% reducing to 10%). C partitioning also gradually declined in a seedling sward from 20% to 12% from beginning to end of autumn (Parsons and Robson, 1981). The partitioning of current assimilate to roots remained lower than 10% in winter and increased to around 12% at the beginning of spring but then drastically declined to 4% at the onset of flowering (Parsons and Robson, 1981).

Plants maintain a balanced root:shoot ratio and thus the removal of part of one organ can modify the growth of another until a balance is re-established (Klepper, 1991). Danckwerts and Gordon (1987) in *L. perenne* studied long-term partitioning, storage, and re-mobilization of assimilates for defoliated and un-defoliated plants, up to 22 days after $^{14}CO_2$ labelling. They reported that defoliation virtually stopped the production of new roots at the tiller bases, because plant resources were directed towards new top growth. Assimilated C was found to be apportioned to roots, tiller bases and tops approximately equally within the first 24 hours. After 1 to 4 days the imported ^{14}C from the roots and tiller bases declined whereas the ^{14}C was found to be accumulated at the shoots continuously about 6 days after feeding (Danckwerts and Gordon, 1987). It was evident from these results that roots and tiller bases supply labile assimilate for the new top growth (Danckwerts and Gordon, 1987). A substantive amount of ^{14}C assimilated by the fed leaf (4%) remained in roots and tiller bases as re-usable reserves (Danckwerts and Gordon, 1987). Lattanzi et al. (2005b) defoliated 70% of the shoot area of a 65-day old sward and found that after 15 days the defoliated plants accumulated significantly lower dry matter at all rooting nodes. A total root dry weight of only 118 mg plant^{-1} for the defoliated plants was recorded, compared with 197 mg plant^{-1} for the un-defoliated plants. Growing roots were more affected compared to the older roots under defoliation treatment indicating greater and sustained demand of C for initital root construction (Lattanzi et al., 2005b). In *Phaseolus vulgaris* L., removing half of the seedling root system accelerated

root growth relative to shoot growth until a balance in root-shoot ratio was achieved (Brouwer and De Wit, 1969). Geisler and Ritz (1981) in *T. aestivum* as cited by Klepper (1991) found that root pruning decreased dry matter production, reduced tillering and also reduced the growth of new tillers. In *L. perenne,* James and Hutto (1972) found that plants growing in solution culture showed increased plant growth under treatments involves 'tiller separation' and 'removal of root apices' treatments. The authors believed these two treatments probably increased root branching which resulted in significant increase of net assimilation rate arising from higher production of cytokinin (James and Hutto, 1972). Another possible explanation is that tiller separation and pruning of root apices reduce the effect of the respective sinks in reducing shoot growth.

C relations between root and shoot systems have been demonstrated in a study with *Panicum maximum* L. by Carvalho et al. (2006). For the 7-week-old plants the authors labelled the MT, old primary tiller and youngest primary tiller with $^{14}CO_2$.They reported that after ^{14}C labelling, 70-85% radiocarbon was recovered from the shoots of both MT and primary tillers but only 12-16%, 6-12% and <2.5% radiocarbon was recovered respectively from the roots of MT, old primary tiller and young primary tiller of the same plant. This result indicates that for an adult tiller, 5-6 times the quantity of photoassimilate is allocated to the shoot system as to the root system. A similar result was also obtained by Clifford et al. (1973) in *L. multiflorum*. Lehmeier et al. (2008), while studying the source of energy for root and shoot respiration in *L. perenne,* found that both the shoot system and the root system have constant specific growth rates and specific respiration rates and these organs maintain a shoot:root ratio of 3.8 g C g^{-1} C during exponential growth of the *L. perenne* plants. The ratio is quite close to the proportional C partitioning observed between shoot system and root system by Carvalho et al. (2006) in *P. maximum*. Lattanzi *et al.* (2005a) reported that a majority of C used for new leaf growth is derived from new assimilates and from short-term stores. The C used in respiration for both root and shoot is supplied by the same substrate pools (Lehmeier et al., 2008).

2.4.4.2 Main tiller-daughter tiller exchange of C and N

Clifford *et al.,* (1973) used ^{14}C-urea for labelling the individual leaf lamina of either main tiller or daughter tiller of young *L. multiflorum* plants. They reported a systematic flow of the radiocarbon label from leaf lamina to root system via the shoot axis. The study found that after 24 hours of labelling the labelled individual lamina of the daughter tiller

exported 21% and 31% of radiocarbon to the shoot and roots of the MT respectively, but the leaf lamina of the MT exported only 15% of the radiocarbon to the DT, whereas 33% of radiocarbon was recovered from the roots. The authors argued that C exchange between tillers involves the shoot axis. Colvill and Marshall (1981) reported that the degree of support from the MT towards primary tillers is inversely proportional to the dry weight of the primary tillers. The older primary tiller becomes an independent assimilatory unit when it reaches a dry weight of about 25 mg in *L. perenne* even though some import of main shoot assimilate continues (Colvill and Marshall, 1981). Anderson-Taylor and Marshall (1983) fed the MT, first primary tiller and second primary tiller of *Hordeum distichum* L. with $^{14}CO_2$. Only 10% of total assimilated C was recovered from organs other than the labelled after 24 hours. They found that the MT exported assimilate equally to all other tillers and roots but the primary tillers exported the majority of the assimilate to the roots of the main tiller. In the experiment of Carvalho et al. (2006) (see previous section) when young primary tillers were labelled 7.0-7.4% of radiocarbon to total ^{14}C-photoassimilate was recovered from the roots of MT but when MT was labelled, a maximum 1.7% of radiocarbon was recovered from the roots of the DTs (young or old). The result suggests that DTs probably provide a significant share of respiratory energy to the older roots of MT.

Welker *et al.* (1987) in a study involving *Schizachyrium scoparium* (Michx.) Nash reported that N was translocated from a labelled MT to secondary and tertiary tillers within a tiller hierarchy, and had a decreasing allocation gradient towards younger tillers whose position in the hierarchy were more distantly located from the MT. That was likely because the transport of N from the MT to a tertiary tiller occurs via the primary tiller and secondary tiller. In *O. sativa*, Mimoto *et al.* (1990) labelled the roots with ^{15}N-ammonium nitrate solution for 9 days. The seedlings were a little more than a month old at labelling. The labelling process was followed by the supply of N-free solution for another 14 days of a selected primary tiller (fourth daughter tiller, DT4) from seedlings when the first leaf of DT4 emerged. The authors reported that at the 14^{th} day after labelling, the DTs of DT4 received the highest percentage of translocated ^{15}N (DT41 received 47%, DT44 received 49%; DT41 and DT44 were the DT of DT4 whereas DT41 was the elder) followed by N recovered at DT4 itself (40%), N transported to DT (14%), younger primary tillers, (DT5, 7%, DT7, 9%), elder niece tillers (daughter tillers of DT3, 2%), elder primary tiller, DT3

(2%). The results indicated that the new growth and proximal sink receive a higher share of transported N compared to older and distal sinks.

2.4.4.3 N cycling between organs

It has been reported for *L. perenne* that a significant quantity of N is recycled from older senescing leaves to the newly developing leaf through N remobilisation (Ourry et al., 1988; Millard et al., 1990; Thornton et al., 1993). The distance in terms of phytomers on the tiller axis between the senescing leaf and the newly emerging leaves in *L. perenne* is generally three phytomers and that between the senescing leaf and newly emerging root is one phytomer (Yang et al., 1998). The vascular organization as described by Bell (1976a,1976b) indicates that N moving from the senescing leaf to the tiller axis would be more readily withdrawn by the comparatively nearby sinks of the tiller axis such as newly initiating roots, than by the more distant elongating leaf.

Some studies have reported that the relative contribution of N remobilization towards new growth is greater than the supply from N uptake. Bausenwein et al. (2001) reported that remobilized N contributed respectively 70% and 82% of N contained in new growth of the above-ground components for *Festuca rubra* L. and *Agrostis capillaris* L. in early spring. The proportional contribution of remobilized N, and N uptake for new growth was shown to depend on N supply to the plant from the soil. Limited N supply from the soil accelerated the N remobilization from old leaves to new growth (Millard et al., 1990; Ourry et al., 1990; Thornton et al., 1994). Also, frequent defoliation has been reported to stimulate tillers to accelerate N uptake rate (Louahlia et al., 2008), in that case the contribution to new growth from N remobilization from the older leaves is reduced (Thornton and Millard, 1997; Lestienne et al., 2006). Ourry et al. (1988) and Millard et al. (1990) reported from ^{15}N labelling experiments that following defoliation a substantial amount of N was remobilized from the amino acids and proteins of the stubble (mainly) and roots for regrowth. Immediately following a single defoliation event, N remobilization was found to be the major source of N for regrowth (Ourry et al., 1989) as defoliation reduces N uptake by the roots (Lestienne et al., 2006). In a regularly defoliated sward, N remobilization was reported to depend on N supply and temperature. A comparatively greater N remobilization occurred when ammonium was supplied rather than nitrate. The N remobilization reported was also greater at 20 ^{o}C compared to 12 ^{o}C (Thornton et al., 1993).

2.4.4.4 C recycling within the root system

It is clearly evident from Matthew and Kemball (1997) that allocation of current photosynthate is predominantly towards younger root axes. The study reported that 70% of total radiocarbon was recovered from the five younger phytomers (Pr1- Pr5) whereas Pr6 had achieved nearly final root mass and main root axis length. The data suggested that main axis elongation is a strong sink that requires a major share of photoassimilates (Matthew and Kemball, 1997). The older roots obtained a very low share of recently assimilated C. In fact a major share of around 40% photo-assimilated C gets lost through respiration, 34% and 8.5% remains in top and stem bases, respectively and only 13.5% reaches the roots (Danckwerts and Gordon, 1987) meaning that less than 4% of total photoassimilates reaches below Pr6. C turnover in older roots is not much studied for *L. perenne* roots and data on this point could answer questions about the source of energy for respiration and maintenance of fine roots in *L. perenne* swards. Tissue turnover within the root system might be one potential source of C (Eissenstat and Yanai, 1997). In grasses the potential sources of old C are the carbohydrate stores in sheath bases and stems (Chatterton et al., 1989; Thom et al., 1989), and amino acid derived C from protein turnover. Thornton et al. (2004) reported that in *L. perenne* old C contributes 41.7% of C in root exudates and the rest of the total exudates is supplied by newly assimilated C.

2.5 Questions relevant to further component research

Present understanding of developmental morphology of a grass tiller is the synthesis of sixty years of extensive research studies in different areas, including mass flow or tissue flow in terms of growth and death of leaves and roots, segmental morphology of the above-ground and below-ground components of tillers, physiological processes and anatomical organisation of the grass shoot and root systems. Section 2.3 illustrates that even though the presence of segmental units in the grass shoot system was understood in the 1950's and 1960's, the understanding that the same organisational patterns apply to root development took another 40 years to emerge. Some authors have reviewed the ecophysiological basis for different levels of plant-plant and plant-environment interactions. For example, Robson et al. (1988) described the form and function of grass plants from seed germination, vegetative development, reproductive development and related physiological events; Chapman and Lemaire (1993) discussed morphogenetic factors that can change the course of plant growth and development, Nelson (2000)

discussed the reciprocal interactions between leaf growth and tillering, Schnyder et al. (2000) and Thornton et al. (2000) reviewed the C and N use in growth zones and Dawson et al. (2000) described ecophysiological aspects of root form and function. Nevertheless the detail surrounding segmental organisation in the grass root system and how this impinges on root-shoot relations and interactions between tillers in a hierarchical relationship remains imperfectly described. This point is highlighted by the review article '*Understanding shoot and root development*' (Matthew et al., 2001) which has traversed these points and indicated ways in which improved knowledge of the segmental architecture of in the grass shoot can potentially contribute to future improvement of plant performance.

As introduced in section 2.4.2.7, some earlier reports in the New Zealand and United Kingdom describe the annual pattern of root replacement from the grass swards (e.g., Stuckey, 1941; Troughton, 1951; Jacques and Schwass, 1956; Baker and Garwood, 1959; Caradus and Evans, 1977). Those results showed inconsistency with each other and failed to clearly explain the root growth pattern at the grass sward. Conversely, from another series of studies which emphasized the evidence of presence of segmental organisation in the grass tiller suggested that root formation and development in the grass tiller is a continuous process (Matthew et al., 1991; Matthew and Kemball, 1997; Yang et al., 1998; Matthew et al., 2001; Lattanzi et al., 2005b). Further study is therefore needed to confirm whether root initiation and root development in the tiller axis has any seasonal pattern during continuous root production at the tiller axis (Durand et al., 2000; Fournier et al., 2005; Verdenal et al., 2008).

Section 2.4.2.7 on 'Root formation and development' reviewed the existing knowledge of root development in *L. perenne*. As indicated in that section, information on perennial ryegrass root developmental processes, and seasonal variation in nodal root formation and branching pattern are also scarce in the literature. The present study will therefore try to fill the gaps in the literature to some extent. To better understand root development and turnover, a possible approach is to dissect roots from the tiller axis in order of root age, so that root development can be documented in a A_{lf} time scale, similar to Sharman's (1942) leaf turnover cycle in a plastochron time scale. A study of this kind will also describe the co-ordination between successive phytomers during root development in the same way

that progressive leaf development at successive phytomers as described by Fournier et al. (2005).

A recently developed viewpoint is root appearance on the tiller axis is governed by seasonal variation in day length and temperature that primarily modifies the leaf appearance interval over time and so timing of root appearance for that position on the tiller axis. Day length and temperature determine the time interval between the emergence of two successive leaves and thus the time interval between the events of appearing roots at the two successive root-bearing phytomers due to delay in root appearance at the same phytomer (e.g., Hunt and Thomas, 1985; Matthew et al., 1998). Seasonal variations in day length and temperature coupled with a delay of several phyllochrons between leaf and root appearance events at a phytomer mean that there is a possibility of 'desynchronisation' between shoot and root formation cycles, and hence of a morphogenetic variation in root to shoot allocation (Matthew et al., 1998).

As illustrated in Section 2.4.4.2 the work of Carvalho et al. (2006) and some other previous studies such as Clifford et al. (1973) and Colvill and Marshall (1981) revealed that DTs feed the roots of MT once they are established and they probably have a significant role in feeding older roots of MT which suffer C starvation due to reduced supply from the tiller of origin (Matthew and Kemball, 1997). The interrelations between MTs and DTs for dry matter partitioning, and C and N exchange are much less studied in *L. perenne* swards.

Considering the above information, the following broad aims were determined for the three year Ph D research programme:

1. To provide a description of root development for *L. perenne* in phyllochron time units, similar to that of Sharman (1942) for leaves and an analysis of implications for root ecophysiology;
2. To conduct a preliminary investigation of whether or not seasonal 'decoupling' (desynchronisation) of phyllochron and rhizochron influences root:shoot relations.
3. To investigate aspects of root-shoot and tiller-tiller translocation with consideration of root age and position when sampling roots for measurement.
4. To provide information on how daughter tillers might modify the pattern of root development and turnover of the parent tiller.

Chapter 3: Tiller Morphology

3.1 Introduction

Chapter 2 has included a detailed review of the morphological development of a phytomer and has indicated gaps in existing knowledge related to the fact that very few past studies have considered the influence of plant architecture as a factor determining biological behaviour of grass shoot and root systems and the link between them. A fundamental requirement in order to understand grass plant architecture is a tiller axis map defining the number of phytomers on the tiller axis, the status of phytomers at specific positions, and the time frame required for a phytomer newly formed at the tiller axis to begin leaf, and later root formation. However, as noted in section 2.3, such data are sparse in the literature and plant structure can change according to growing conditions. It was therefore decided that to assist in planning of measurements to be carried out to answer research questions raised in Section 2.5, an early harvest of a subset of plants from a larger experiment reported in Chapters 4-6 would be made to provide a map of phytomer development on the tiller axis and indicative information about size of leaves and number and size of roots at various positions on the tiller axis. This preliminary harvest involved plants of two perennial ryegrass cultivars with different breeding backgrounds; a New Zealand-bred cultivar, Alto, and a United Kingdom-bred cultivar, Aberdart.

3.2 Materials and Methods

Cores of soil approximately 8 cm in diameter and containing a healthy perennial ryegrass plant were collected in July 2008 from paddocks at Massey University No. 4 Dairy Farm, previously sown in either Alto (bred by NZ Agriseeds Ltd, Christchurch, N.Z.) cultivars of perennial ryegrass, or Aberdart (bred at the Institute for Grassland and Environmental Research, Aberystwyth, U.K.). From these cores, single adult tillers were broken out and established in a hydroponic culture system on 1 July 2008. Hydroponic culture was used for ease of access to the root system. The first two daughter tillers produced by plants in hydroponic culture were allowed to develop, and subsequent daughter tillers were removed. Plants were inspected daily and timing of each leaf appearance event noted. Approximately 85 days later, by which time phytomers which had had live leaves when established in the hydroponic culture system now had well developed roots, two randomly

selected plants of each of the two cultivars were harvested and dissected under 15x magnification using a binocular microscope. Leaves and roots of each plant removed sequentially, according to phytomer position on the tiller axis. As plants described here were chosen from among plants being grown for the experiment reported in Chapter 4, the reader is referred to Section 4.3 for further details of the hydroponic culture regime and of the measurement of individual leaves and roots. Data collected for the four test plants of the main tiller and the two daughter tillers at the destructive harvest included number and position on the tiller axis of live leaves and root-bearing phytomers, and for individual phytomer positions, leaf length, leaf dry weight, root axis length, total root length per phytomer, root dry weight per phytomer and number of roots per phytomer, as relevant to each phytomer position. Leaf area of the individual leaves was estimated using the equation 0.7 × leaf length × leaf width (C Matthew, personal communication). Total root length for each phytomer was obtained using the modified Newman Method (Tennant, 1975, see section 5.3.6). Leaf appearance rate (LAR, leaves $tiller^{-1}$ d^{-1}) at each phytomer was calculated as the inverse of leaf appearance interval (days). Root-bearing phytomer appearance rate (PrAR, Pr d^{-1}) was estimated assuming that root-bearing phytomer appearance interval would be equal to the previously observed leaf appearance interval of that phytomer. Total shoot dry weight (including pseudostem), root dry weight and number of roots per tiller were calculated by summing the data for all phytomers for the individual tiller. One-way ANOVA was carried out using Minitab 15 statistical software (Minitab Inc. State College, Pennsylvania) to compare the variation between two cultivars for each trait.

3.3 Results

3.3.1 Schematic description of the tiller axis

Plants harvested at this time carried on average 6 – 8 live leaves per main tiller and the total number of phytomer positions in the tiller axis averaged 17.5 for Alto and 19 for Aberdart (p=0.038, Fig. 3.1).

P	Alto	Pr		Aberdart
1	Leaf emergence		↑	Leaf emergence
2	Leaf elongation		↑	Leaf elongation
3			*de*	
4			↓	
5		**Pr**	↓	
6	Root initiation	1	SL-YR co-location	Root initiation
7		2		
8	Root elongation	3		Root elongation
9		4		
10		5		
11	Root branching	6		Root branching
12		7		
13		8		
14		9		
15		10		
16		11		
17		12		
18		13		
19		14		

Fig. 3.1 Schematic map for the main tiller axes of *Lolium perenne* cultivars Alto and Aberdart indicating the number, P, and developmental status of phytomers present on the tiller axis, with the emerging leaf designated P1. Pr indicates the number of root-bearing phytomers with the youngest root designated Pr1. '*de*' denotes the delay between leaf and root appearance at the same phytomer position, and was approximately 5 phyllochrons for these plants. Shaded cells indicate the presence of leaves; SL, senescing leaves; YR, young roots. (also see Robin et al., 2010).

The phytomer position P6 was the mean position at which root initiation was observed at the time of harvest. For Alto the phytomer positions P1 – P6 bore leaves with an even progression of leaf developmental stage from leaf elongation at P1 to yellowing and visible senescence at P6. Similarly, phytomer positions P6 – P17 bore roots at progressively more advanced developmental stages from short unbranched main axis initials in the region P6 – P8, to highly developed finely branched structures at the lowermost phytomers. A similar pattern was observed for Aberdart, except that in Aberdart the first 8 phytomer positions bore live leaves, meaning that at phytomer positions 6 to 8, young roots co-located on the same phytomer with mature leaves. Phytomers P6 – P19 bore roots in the 2 Aberdart tillers. The total number of root-bearing phytomers per tiller showed a statistically significant difference between the two cultivars ($p=0.038$) but the number of live leaves per tiller differed only marginally between the cultivars ($p=0.095$).

3.3.2 Leaf appearance rate

Aberdart maintained a faster rate of leaf appearance than Alto (Table 3.1). For both cultivars the leaf appearance interval was 13 days at the start of the Experiment (P11 in Alto and P13 in Aberdart, respectively) and thereafter leaves appeared at successively shorter intervals with this trend more pronounced in Aberdart, which had a leaf appearance interval at the end of the experiment of 5.0 days, compared with 7.5 days for Alto (Table 3.1). The longest leaf appearance interval of 13 days was recorded when the day length was comparatively short. Leaf appearance interval became shorter with increasing day length as the experiment progressed. The difference between the two cultivars for mean leaf appearance rate was statistically significant ($p=0.035$).

Table 3.1 Leaf appearance interval at different phytomer positions for Alto and Aberdart perennial ryegrass cultivars during a 90 d growth period in spring. The youngest leaf was used as the reference point and was designated as P1. Note that P2 is the most recent and P11 of Alto and P13 of Aberdart the first observed leaf appearance event. Appearance interval for roots on successive phytomers at positions P6-P13 is assumed to equal leaf appearance interval at those same phytomers.

Phytomer position (P)	Leaf appearance interval (d leaf^{-1})	
	Alto	Aberdart
1	-	-
2	7.5	5.0
3	7.5	5.0
4	8.0	6.5
5	8.0	6.5
6	6.5	6.5
7	7.5	6.0
8	8.5	6.5
9	9.0	7.0
10	9.5	8.0
11	13	9.0
12		9.0
13		13

3.3.3 Root development

All the root-bearing phytomers except the few lowermost were phytomers for which leaf appearance had been observed early in the experiment, and it was assumed that the age difference of roots at successive phytomers was approximately given by the leaf appearance interval observed for each phytomer. Aberdart had a higher total number of root-bearing phytomers per tiller, a higher average number of roots per phytomer, R_p ($p=0.058$) and thus a higher total number of roots per tiller compared to Alto (Table 3.2).

Table 3.2 Root-bearing phytomer appearance rate (Pr d^{-1}, assumed to be the same as for the leaf at that position) and number of roots per phytomer (R_p) at different phytomer positions for Alto and Aberdart perennial ryegrass cultivars during the 90 d growth period. The phytomer bearing the youngest root was designated as Pr1.

Root-bearing phytomer (Pr)	Appearance rate of root-bearing phytomers (Pr d^{-1})		Number of roots per phytomer R_p	
	Alto	Aberdart	Alto	Aberdart
1	0.15	0.15	1.5	1.5
2	0.13	0.17	2.0	2.0
3	0.12	0.15	1.0	2.5
4	0.11	0.14	1.0	4.0
5	0.11	0.13	1.0	1.5
6	0.08	0.11	1.5	1.5
7		0.11	1.0	1.0
8		0.08	1.5	1.5
9			1.0	1.0
10			1.5	2.0
11			2.0	1.0
12				2.0
13				2.5
Mean			1.36	1.85

3.3.4 Variation in leaf and root size at successive phytomer positions

For both cultivars the leaves at successive phytomers were progressively larger in terms of final leaf length and leaf dry weight (Fig. 3.2). The longest final leaf lengths recorded were 43 cm for the cultivar Aberdart and 37 cm for the cultivar Alto at P2 (Fig. 3.2a). However, the cultivar effect was statistically non-significant. Individual root lengths and weights were more variable than leaf length and weight so the pattern of change across successive phytomers was less well defined for root data than for leaf data. In general, the longest roots were at the oldest phytomers whereas the root weight did not appear to increase after Pr4 (Fig. 3.2). A strong positive correlation between total root length per phytomer position and root dry weight per phytomer position was observed ($p<0.001$).

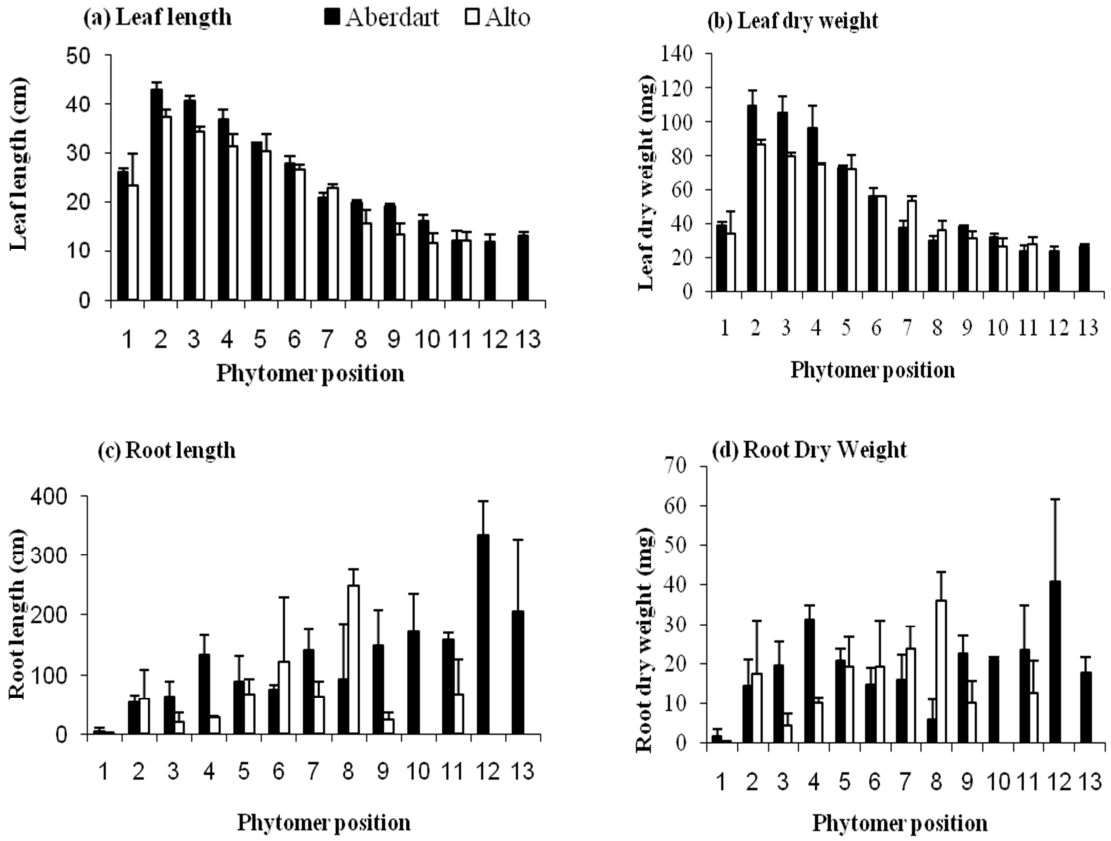


Fig. 3.2 Variation between phytomer positions for (a) leaf length, (b) leaf dry weight, (c) root length and (d) root dry weight of Alto and Aberdart – perennial ryegrass cultivars. Vertical bars indicate standard error at each phytomer position. For leaf data the emerging leaf (P1) is the reference point and for the roots the youngest root-bearing position (Pr1) is the reference point.

3.3.5 Comparison of main and daughter tillers

The DT1 is the first formed and lower on the tiller axis whereas DT2 was later formed and usually attached to the main tiller axis one phytomer above DT1. For the cultivar Alto, the two daughter tillers had generated a mean total of 16 phytomer positions per tiller (6 live leaves and 10 root-bearing phytomers). For the cultivar Aberdart, DT1 had 16 phytomer positions per tiller (6 lives leaves and 10 root-bearing phytomers) and DT2 had a mean total of 14 phytomer positionss (5 live leaves and 9 root-bearing phytomers). The data presented are the average of four plants.

Compared to the main tiller, daughter tiller phytomer positions of comparable age showed statistically significant size reduction for leaf length ($p=0.016$, Fig 3.3a), leaf dry weight

($p<0.001$, Fig 3.3b), root length ($p=0.014$, Fig 3.3c) and root dry weight ($p=0.003$, Fig 3.3d).

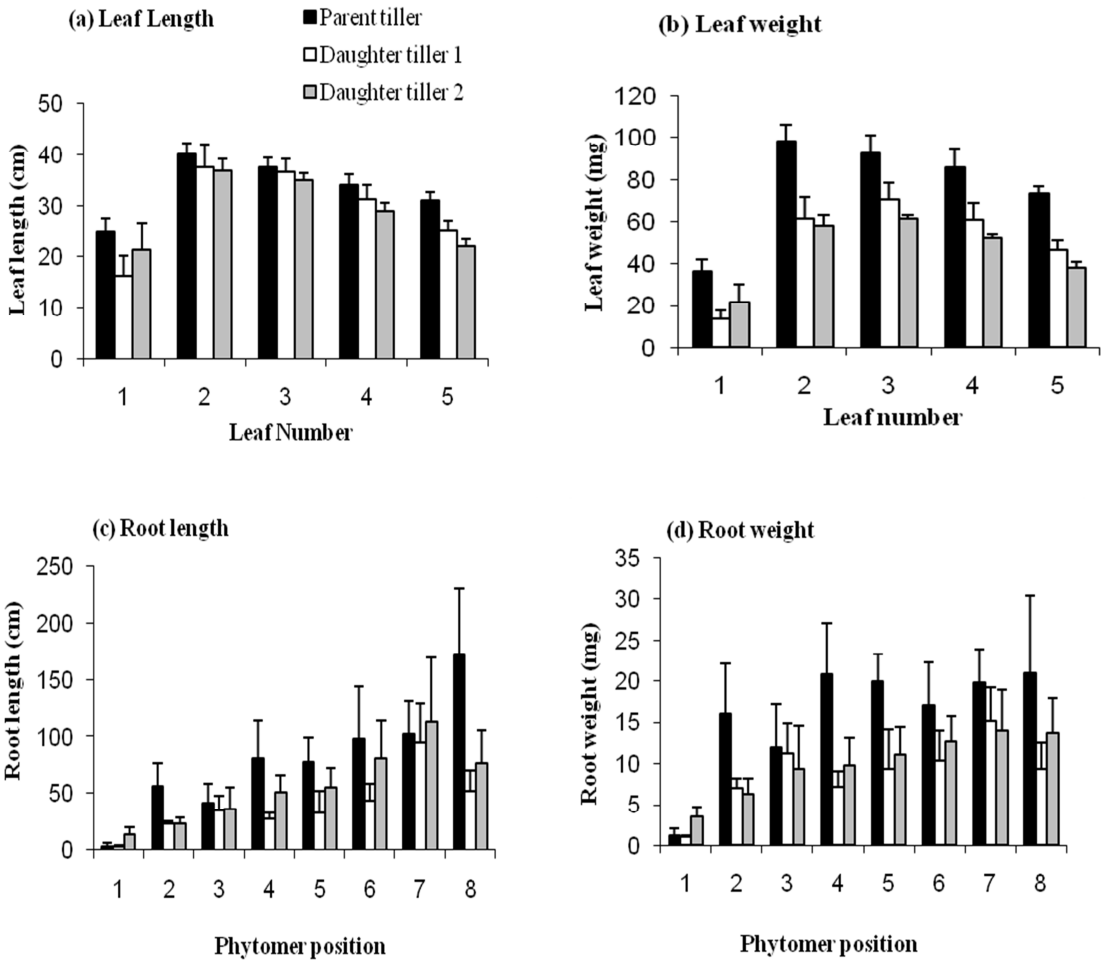


Fig. 3.3 Variation between main tiller and daughter tillers at successive phytomer positions for (a) leaf length, (b) leaf dry weight, (c) root length and (d) root dry weight averaged accross Alto and Aberdart perennial ryegrass cultivars. Vertical bars indicate standard error at each phytomer position. For leaf data the emerging leaf is the reference point (P1) and for root data the youngest root-bearing phytomer position (Pr1) is the reference point.

Consistent with differences at the phytomer level, total shoot dry weight per tiller and total root dry weight per tiller showed statistically significant difference between the main and the daughter tillers ($p=0.001$, Table 3.3). However, the variation in total number of root producing phytomers per tiller was not statistically significant between the main and the daughter tillers. Root production rate and root elongation rate were tended to be higher for the main tiller than for either of the daughter tillers ($p=0.10$, Table 3.3).

Table 3.3 Variation in shoot and root morphological traits between main tillers (MT) and daughter tillers (DT) averaged across Alto and Aberdart perennial ryegrass cultivars. DT2 is typically one phyllochron younger than DT1 and they were therefore located on adjacent phytomers, and opposite on the tiller axis. R_p, number of roots per phytomer; DW, dry weight; DM, dry matter; Pr, root-bearing phytomer positions. Data presented are the average ± SE of four tillers from two cultivars.

Tiller type	Shoot DW (mg)	Root DW (mg)	Number of root-bearing phytomers	R_p	Root DM increase mg Pr^{-1} d^{-1}	Root axis elongation rate cm Pr^{-1} d^{-1}
MT	769±54	234±36	12.25±0.70	1.59±0.13	0.57±0.18	1.38±0.32
DT 1	449±51	114±12	10.75±0.25	1.33±0.13	0.36±0.18	0.67±0.35
DT 2	380±39	104±18	10.0±0.70	1.42±0.13	0.29±0.16	0.75±0.24
p value	0.001	0.001	0.15	0.37	0.10	0.056

3.4 Discussion

Even though number of live leaves and live roots were significantly different between cultivars, a comparable sequential pattern of leaf and root development across the phytomer positions at the tiller axis for two perennial ryegrass of contrasting breeding background are evident (Fig. 3.1). The pattern is generically similar to that reported from early studies aimed at elucidating plant structure at the phytomer level (Etter, 1951; Yang et al., 1998). As discussed in Chapter 2 the pattern of leaf turnover has been studied previously but the pattern of root turnover has rarely been studied for perennial ryegrass. One important visual observation was that roots at Pr3 typically showed commencement of branching and the degree of branching increased gradually as indicated by rate of root length increase at the successively developing phytomers (Fig. 3.3). The degree of root branching, the increase in root length and other root dimensions such as increase in root surface area and volume at the successively developing phytomers should be studied further in greater detail for a larger set of plants to understand root dynamics. The co-efficient of variation for root dry matter increase at successive phytomers was very high but a comparatively rapid increase in root dry weight at successively developing phytomers for the first few younger phytomers just below the root initiation region indicated high C requirement for root construction of these younger roots (Fig. 3.3), which is likely supplied by canopy photosynthesis. Determination of the rate of photosynthesis of

the individual leaves at the different developmental stage and estimation of rate of canopy photosynthesis per tiller might assist in the understanding of the share of assimilated CO_2 being used in root construction.

The fact that successively younger phytomers exhibited increased leaf length and dry weight for both of the cultivars would likely have been caused by a combination of two factors. First, as transplanted tillers increased in size over time in the hydroponic system, to a final shoot weight of 769±54 mg (Table 3.3), much higher than values of <120 mg typically reported in field swards (Kays and Harper, 1974; Bahmani et al., 2001), an associated increase in leaf length would be expected. Secondly, in normal regrowth of a tiller following defoliation the coordination rules governing timing of leaf elongation of successive phytomers (Section 2.4.2.4) mean that each successive leaf will continue cell division and elongation for a little longer than the last (Verdenal et al., 2008). The fact that individual roots generally did not show a corresponding increase in root dimensions from the oldest to comparatively younger root-bearing phytomers could indicate that individual roots continue to grow for a much longer time than leaves although the co-efficient of variation was very high for root data and these points could usefully be clarified in a later study.

The number of live leaves per tiller in this study (6–8 leaves per tiller, Fig. 3.1) was much higher than that of field swards, which has been noted between 2.55 to 2.87 by Davies (1977), 3.5 to 4.0 by Fulkerson and Slack (1994) and Fulkerson and Donaghy (2001) and 3.0-4.0 by Yang et al. (1998). The reason for the higher than normal number of leaves per tiller is unclear and without precedent in the literature that the author is aware of, but may relate to the comparatively high level of N supply in hydroponic culture and plant manipulation (see section 3.2). It is not clear if a higher than normal number of leaves per tiller would in some way alter root dynamics and this point needs to be borne in mind when interpreting data.

Chapman and Lemaire (1993) have noted that NLL is the product of LLS and LAR. The significantly higher number of live leaves per tiller for the cultivar Aberdart than Alto is therefore consistent with the observed faster LAR of Aberdart than of Alto (Table 3.1). Variation in leaf appearance rate between cultivars in same environmental conditions has previously been reported (Hume, 1991; Brock et al., 1996; Sartie et al., 2009). It might be

worthwhile to revisit the morphological developmental sequence and photosynthetic capacity of the leaves at the successive phytomers for both of the cultivars in response to changing LAR in increasing and decreasing day length.

As the experiment progressed, the increasing day length promoted an increasingly faster rate of leaf appearance (leaves per day) for both cultivars and this was expected based on the known association between increased temperature and a decrease in the phyllochron (Peacock, 1975c; Thomas and Norris, 1977). The assumption that at a particular phytomer the rhizochron is determined by the phyllochron for the leaf at that phytomer needs confirmation but visual observation suggested an even progression in root maturity moving down the tiller axis, as reported by Matthew & Kemball (1997), meaning that this assumption would be at least approximately true (Matthew et al. 1998). In field conditions the roots usually appear at the tiller axis at the senescence of the eldest leaf, generally at 4–6 leaf appearance intervals (P4–P6) (Matthew et al., 1991; Yang et al., 1998). This study observed the co-location of older leaves and young roots at the same phytomer even though the mean position of root appearance at the tiller axis is quite consistent (5 leaf appearance intervals, Fig. 3.1) with the previous report of Yang et al. (1998). A spatial lag in leaf and root appearance at the tiller axis at the same phytomer means that in growing conditions of increasing day length a tiller will generate new leaves at a faster rate than that which applies for the appearance of new root-bearing phytomers. The phytomers initiating new leaves will have been formed in conditions of longer day length than the older phytomers initiating new roots. Conversely, in conditions of decreasing day length the leaf appearance rate will be lower than the appearance rate of root-bearing phytomers (Matthew et al. 1998; Matthew et al. 2001). If there are not compensations in terms of seasonal change in the source sink relationship between leaves and roots, then this day length effect on the number of leaf and root initiation events, and therefore on the ratio between the two, could potentially provide a seasonal change in root: shoot ratio, increasing root production in increasing day length (spring) and decreasing it in autumn. The expected variation in root: shoot ratio due to changing day length and arising from spatial separation of leaf and root appearance might be studied in a second experiment with decreasing day length to test this hypothesis (Matthew et al., 1998).

The significant reduction in size of leaves and roots at the phytomers of comparable age of daughter tiller compared to the main tillers indicated a slower growth rate of the daughter

tiller compared to the main tiller (Fig. 3.3). This assumption is supported by marginally lower main axis elongation rate and dry matter increasing rate of the daughter tillers compared to the main tiller (Table 3.3). The results suggested that daughter tillers are possibly involved in supplying C for the development of main tiller, as shown for the tropical grass *Panicum maximum* (Carvalho et al., 2006), hence limiting their own growth rate. Therefore the functional relations between main tiller and daughter tiller for C and N relations might also be studied for better understanding of main tiller and daughter tiller relations during their development.

3.5 Summary

- A general pattern of root development at successive phytomers was established for the four plants in the test harvest but further study of the root development cycle using a larger data set is desirable;
- Inference about root development rate from the comparison of root dimensions at successive phytomers may be distorted by the increase in tiller size over time after transplanting to the hydroponic system, and investigation to clarify this point would be helpful;
- Determination of photosynthesis rates of individual leaves in order to estimate the extent to which root growth may be photosynthate-limited is also desirable;
- Plants in this study have an unusually high number of leaves per tiller and it is not clear if this would affect dynamics of root production;
- The delay of around 6 leaf appearance intervals between formation of a leaf and formation of a root at the same phytomer could potentially cause a seasonal change in root:shoot ratio and a repeat experiment under contrasting day length conditions would allow this hypothesis to be tested;
- The smaller size of daughter tiller leaves and roots, compared to main tiller organs of the same age suggests daughter tiller size may be limited by export of substrates to the main tiller, and tracer studies could be used to explore this point further.

Chapter 4: Leaf Turnover and Photosynthesis

4.1 Introduction and Overview

In Chapter 3 the morphology of a vegetative grass tiller was discussed in brief. In a grass tiller, the tiller axis is composed of a series of modular units called phytomers forming a vascular connection that unites the above-ground and below-ground components (Yang et al., 1998). The growth of leaves above ground has long been known to exhibit a pattern of turnover with coordinated, simultaneous production and senescence (e.g., Silsbury, 1970; Robson, 1973; Robson et al., 1988; Pilbeam, 1992; Duru and Ducrocq, 2000; Fulkerson and Donaghy, 2001). On this basis, leaf turnover should have a co-ordination with root dynamics, a point which is often neglected in the literature. This experiment set out to define patterns of leaf and root turnover, with a sufficiently long observation period for leaf and later root production on the same phytomers to be observed. Moreover, information on the 'architectural' integration of root and shoot systems through the segmental organization of the tiller axis and tiller-to-tiller relationships can also be obtained in this type of study.

Because of the quantity of observations generated, three chapters have been allocated to discuss them. In the present chapter, the dynamics of leaf production at successive nodes in increasing day length in winter-spring and in decreasing day length in autumn are presented. The photosynthetic capacity of leaves of varying age is also considered, and the associations among the leaf morphological traits are explored. Chapter 5 presents data on root development at successive phytomers of the tiller axis. Chapter 6 discusses the implications of the data in terms of the relationship between plant architecture and plant function, especially with respect to the inter-segmental relationships between above and below ground components and tiller-tiller relations. The plant growth conditions will be described in this chapter but the materials and methods relevant to leaf, root, and tiller investigations are presented in respective chapters.

4.2 Objectives

1. To build an understanding of leaf turnover on the tiller axis of *L. perenne* by measuring morphological differences between leaves at successive phytomer positions;
2. To carry out these leaf measurements for two perennial ryegrass cultivars with contrasting breeding background (Alto and Aberdart) in contrasting growing conditions of increasing or decreasing day length in order to explore the nature of variation in leaf turnover across cultivars and seasons;
3. To determine the extent of variation in leaf morphology between genotypes within cultivars (Alto and Aberdart) compared to the variation between cultivars and seasons for leaf morphology and turnover;
4. To investigate the variation in net photosynthetic rate of leaves at different positions on the tiller axis and to determine if the pattern of variation changes with cultivar, genotype, or season.

4.3 Experimental

4.3.1 Experimental site

The plants were grown in a hydroponic culture unit (see Fig. 4.4 in section 4.3.3) in a glasshouse at the Plant Growth Unit (PGU) at Massey University, Palmerston North, New Zealand (latitude 40^{o} 19' South, longitude 174^{o} 46' East, altitude 25 m above sea level). Laboratory work was done at the Institute of Natural Resources, Massey University, Palmerston North.

4.3.2 Seasonal timing of experiments

Two separate experiments were conducted in winter-spring and autumn (Fig. 4.1). In winter-spring, plants were grown from 1 July 2008 to 28 September 2008, and in autumn from 3 March 2009 to 31 May 2009. These two Experiments were in fact Experiment 4 and Experiment 5 in the overall sequence of work for the Ph D programme, but will be referred to here as Spring and Autumn experiments, respectively. In the Spring experiment the plants experienced increasing day length and in the Autumn experiment the plants experienced decreasing day length.

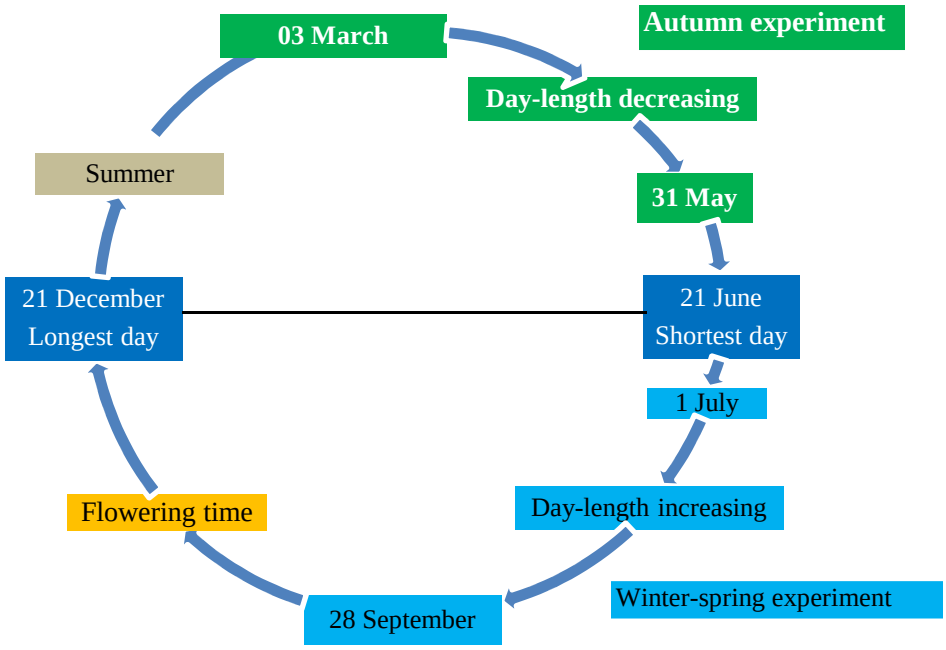


Fig. 4.1 Positioning of the Spring and Autumn experiments within the seasonal cycle of day length change.

4.3.3 Weather data

In both experiments hourly temperature data inside the glasshouse were recorded using a micrologger (Model Z, HortPlus.com). In the Autumn experiment hourly temperature data were also recorded using a data logger (Skye DataHog, Skye® Instruments Ltd). The Skye data logger also recorded light data from 4 March 2009 to 31 May 2009 in the Autumn experiment. In spring light data were collected from 1 July 2008 to 11 July 2008 while the Skye data logger was placed in another glasshouse at the Institute of Natural Resources, Massey University, about 1 km away from the Plant Growth Unit. Total sunshine hours per day were obtained from daily climatological observations recorded at the New Zealand AgResearch Ltd. Grassland Research Centre, Palmerston North, about ½ km distant from the site of the experiment.

Thermal time in growing degree days (GDD, ^{o}C d) was calculated taking 3^{o}C as the base temperature and using the equation:

$$\text{Growing degree days (GDD)} = {}^{(T_{max}+T_{min})}/_{2} - T_{base}$$

Where T_{max} and T_{min} are the maximum and minimum temperature recorded in a day; T_{base} (base temperature) is the temperature below which growth does not occur.

Average data for GDD, PAR (for the available days), temperature, total sunshine hours per day in the Spring and Autumn experiments appears in Table 4.1. The day to day variation in average daily temperature in the two experiments is shown in Fig. 4.2. The trend in GDD per day over the course of the two experiments is shown in Fig. 4.3.

Table 4.1 Mean values for variation in daily temperature and light intensity during the Spring 2008 (Experiment 4) and Autumn 2009 (Experiment 5) experiments.

Weather data	Data source	Spring 2008	Autumn 2009	SEM	p (experiment)
#Thermal time in growing degree days, GDD (^{o}C d)	Micrologger	8.6	15.4	0.38	<0.01
*PAR (MJ m^{-2} d^{-1})	Skye sensor	1.19±0.96	6.93±0.35	-	-
Sunshine hours (d^{-1})	AgResearch	3.7	4.9	0.35	0.047
Mean maximum temperature (^{o}C)	Micrologger	16.3	25.4	0.37	<0.01
Mean average temperature (^{o}C)	Micrologger	11.6	18.6	0.32	<0.01
Mean minimum temperature (^{o}C)	Micrologger	6.94	11.7	0.38	<0.01

#Daily thermal time increment; *Incomplete data

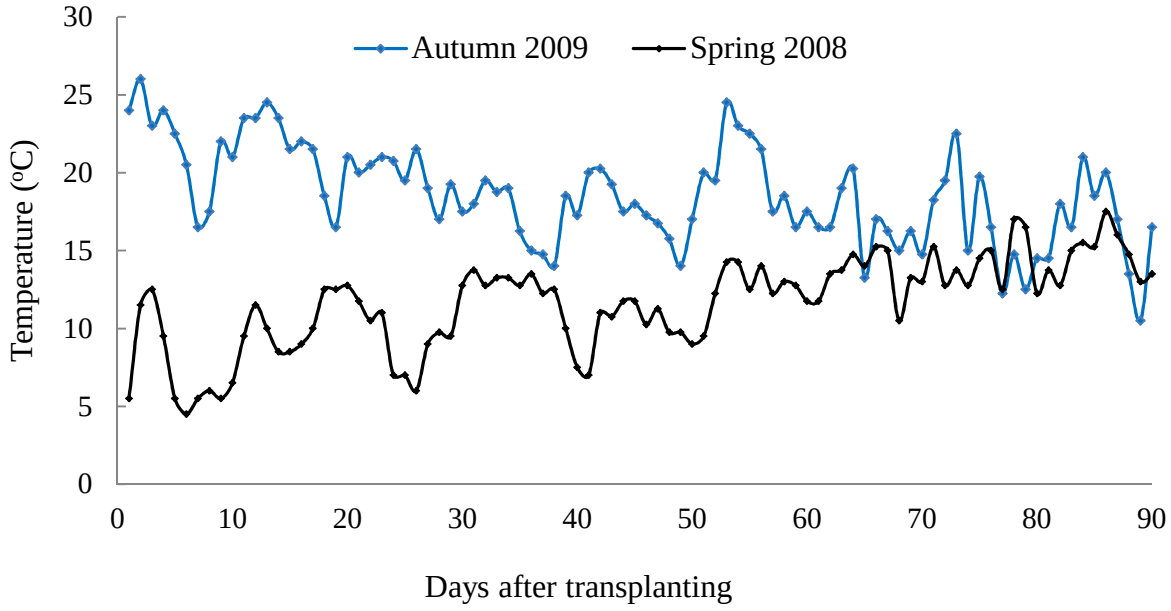


Fig. 4.2 Daily average temperature during the Spring (1 July to 28 September) and Autumn (3 March to 31 May) experiments.

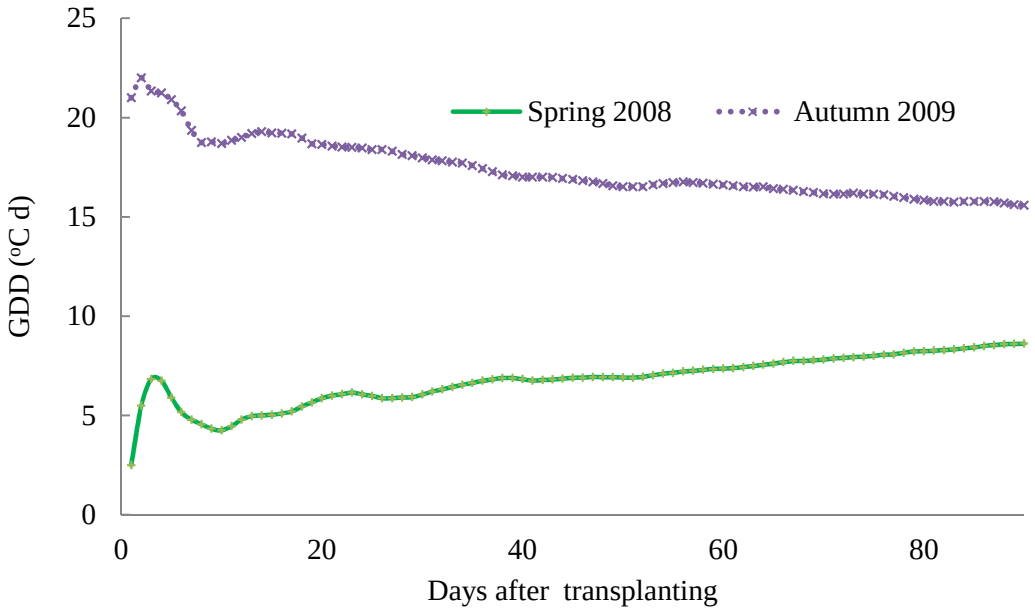


Fig. 4.3 Variation in daily thermal time (growing degree days (GDD), °C d) during the Spring 2008 (1 July to 28 September) and Autumn 2009 (3 March to 31 May) experiments.

4.3.4 Plant materials

Two commercial cultivars of *L. perenne*, Alto and Aberdart were used in these experiments. Alto is a New Zealand bred, diploid, late flowering cultivar released by New Zealand Agriseeds Limited (Anonymous, 2006). Alto is listed in sales brochures as having a flowering date "+14" (Anonymous, 2006). Flowering date is generally assessed based on 50% head emergence (Laidlaw, 2004) and the score +14 indicates a cultivar that flowers 14 days later than Grasslands Nui against which flowering dates of other cultivars are typically benchmarked by breeders. The flowering date for Grasslands Nui (0), is typically around 22 October, but this varies by 2 – 3 weeks from year to year and between geographic regions. A cold, late spring delays heading, whereas a warm spring can cause flowering to occur earlier.

The cultivar Aberdart is bred by the Institute of Grassland and Environmental Research (IGER), UK (Wilkins and Lovatt, 2004), a diploid, late flowering (+15 days), high sugar ryegrass (Downing and Gamroth, 2007; Hoekstra et al., 2007), marketed by Germinal Seeds in New Zealand. The late flowering cultivars are of interest in the industry as they have a longer vegetative growth phase in spring before the onset of reproductive growth

and associated loss of feed quality. Plants of cultivar Aberdart were endophyte free but plants of Alto were infected with AR1 fungal endophyte.

4.3.5 Experimental design

To provide a population of plants for study, in each experiment, plants of 10 genotypes from each cultivar were chosen and adult tillers divided out from each plant to provide 6 clonal replicates of each genotype. Any small daughter tillers attached to tillers at transplanting were removed. The clonal replicates were transplanted to the hydroponic unit with three clonal replicates of each genotype arranged as shown in Table 4.2 and Fig. 4.4. The length of tillers from pseudostem base to leaf tip at transplanting was 20–25 cm. Plant spacing at transplanting was 12 cm x 12 cm and thus 12 plants were accommodated in each tray of 60 cm x 42 cm (4 plants x 3 plants). The individual tillers were supported by polystyrene sheets with 2 cm diameter holes cut for plants to grow in (Fig. 4.4). In the Spring experiment, plants were held in place by foam rubber strips and in the Autumn experiment by wire paper clips. The transplanted individual tillers were allowed to grow in hydroponic solution for 84 days and 83 days, respectively, in Spring and Autumn experiments before commencing destructive harvesting. Harvesting took place over 8 consecutive days in each experiment. Hence the duration of the Spring experiment was 88±4 days and that of Autumn experiment was 87±4 days. During the growing period only the parent tiller and first two daughter tillers were allowed to develop and all other daughter tillers were removed soon after they appeared. For ANOVA purposes, trays were not represented in the statistical model because nutrient solution was cycled among all trays and positions of trays on the table of the hydroponic unit were rotated weekly. It was therefore considered that observations for individual plants within a tray-group would be uncorrelated, and that the experiment could therefore be analysed using plants within trays as the experimental units, as in a completely randomised design.

Table 4.2 Allocation of plant material to hydroponic culture trays. Each hydroponic tray contained 6 Alto and 6 Aberdart tillers as of particular genotypes as indicated. 'A' denotes cultivar Alto and 'B' denotes cultivar Aberdart. 1-10 are the individual genotypes of each cultivar. The hydroponic unit contained 10 trays in all with the same layout duplicated. Trays were moved weekly to a new position, and their orientation rotated 180^{o} at the same time.

Tray 1			Tray 2			Tray 3			Tray 4			Tray 5		
B5	B3	B1	A10	A4	A7	B7	B5	B3	A8	A3	A9	B9	B8	B4
B2	B4	B8	A5	A9	A6	B6	B10	B1	A7	A1	A2	B10	B2	B6
A5	A3	A1	B10	B4	B7	A7	A5	A3	B8	B3	B9	A9	A8	A4
A2	A4	A8	B5	B9	B6	A6	A10	A1	B7	B1	B2	A10	A2	A6

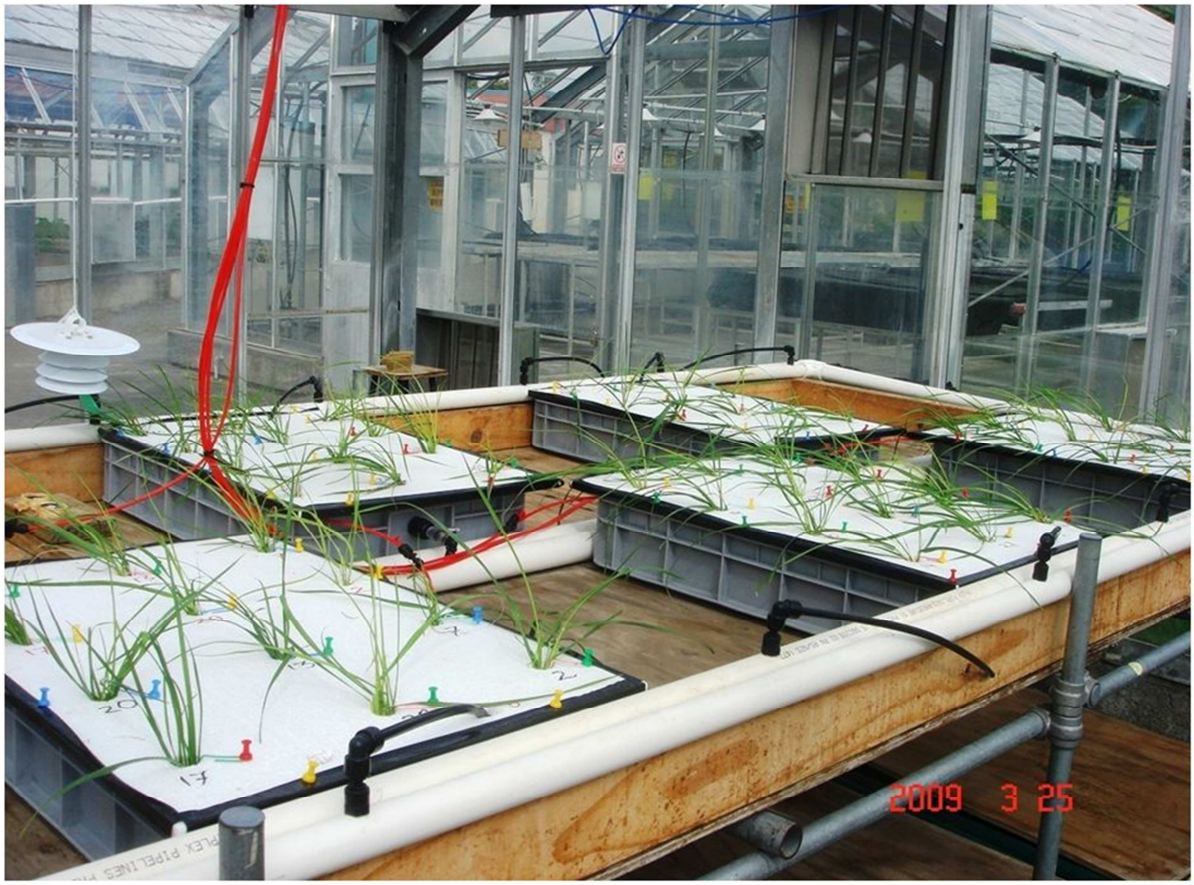


Fig. 4.4 The hydroponic plant culture unit approximately 3 weeks after establishment of perennial ryegrass plants of cultivars Alto and Aberdart in the Autumn experiment (Experiment 5). The hydroponic unit can accommodate 20 trays but in the experimental design a total of 10 trays were in operation. Each tray contained 12 plants.

4.3.6 Hydroponic culture system

The hydroponic culture system comprised two sub-systems each with its own reservoir tank of 120 L capacity with nutrient solution cycled from the reservoirs to ten 12 L trays on a bench above. Hence there were 20 trays in total and approximately 250 L nutrient solution circulating in each sub-system. In Fig. 4.4 the unit is shown with 10 trays in place. Each tank was fitted with a submersible pump delivering nutrient solution to one of two 30 mm diameter PVC perimeter pipes (see Fig. 4.4) from which small feeder tubes delivered nutrient solution to each tray at approximately 30 L h^{-1}. Solution in the trays was continually aerated from a compressed air supply, and flowed out through an overflow pipe back to the reservoir tank through a gravity-driven return system. The nutrient solution was changed weekly, with nutrients as indicated in Table 4.3 dissolved in tap water. The acidity was adjusted daily to a range pH 5.5-6.0 using 6N HCl or 2N KOH as required. A pH stabilizer (MES, 2(N-Morpholino) ethanesulfonic acid) was also added (0.2 mM, 97.6 g per 250 L). Electrical conductivity of the nutrient solution was measured periodically using a conductivity meter (Bluelab® Combo Meter) to gauge nutrient uptake by plants.

Table 4.3 Chemical composition of the nutrient solution used in the hydroponic culture system. Quantities shown in the right hand column are the amount required to make 250 L of nutrient solution.

Chemical name	Formula	Molecular mass, g	Strength	g/250L
Na Di-hydrogen Phosphate	NaH_2PO4	120	0.6 mM	18
Magnesium Chloride	$MgCl_2$	95	0.6 mM	14.25
Potassium Sulphate	K_2SO_4	174	0.3 mM	13.05
Calcium Chloride	$CaCl_2$	83	0.3 mM	6.225
Ortho-boric Acid	H_3BO_3	51	50 µM	0.638
Ferrous-EDTA	Fe-EDTA	376	90 µM	8.46
Mn Sulphate Tetra-hydrate	$MnSO_4.4H_2O$	223	9 µM	0.502
Zinc Sulphate	$ZnSO_4.7H_2O$	288	0.7 µM	0.050
Cupric Sulphate	$CuSO_4.5H_2O$	250	0.3 µM	0.019
Sodium Molybdate	$Na_2Mo4.2H_20$	242	0.1 µM	0.006
Ammonium Nitrate	NH_4NO_3	80	1 mM	20
MES		488	0.2 mM	97.6

Electrical conductivity (EC) of a newly mixed nutrient solution was typically 6 dS m^{-1} and EC when the solution was changed after one week was around 4 dS m^{-1} in the Spring experiment and 3 dS m^{-1} in the Autumn experiment at the later stage of each experiment when plants were bigger in size. The temperature of the nutrient solution was recorded intermittently.

4.3.7 Growth measurements

The tip of the leaf lamina for all leaves of each parent tiller was cut off at transplanting as a marker so that any new leaf appearance event could be easily identified. The date of leaf appearance intervals of all new leaves was recorded from transplanting to harvest for 60 plants, 30 plants from each cultivar and 3 clonal replicates from each genotype, in each experiment. Length increase in centimetres for the elongating leaves was recorded every alternate day.

4.3.8 Photosynthesis measurement

Net CO_2 exchange rate of leaf laminae was measured in both Spring and Autumn experiments using a CIRAS2 Portable Photosynthesis System. Photosynthesis measurement was carried out for the 5 youngest leaf positions from each measured tiller. In the Spring experiment on 12 September 2008, the photosynthesis rate of leaves of two clonal replicates from six genotypes of each cultivar was measured while in the Autumn experiment on 1 April 2009, the photosynthesis rate of leaves of three clonal replicates from five genotypes of each cultivar was measured. The measurements were carried out at 420 ppm CO_2 concentration, 1000 µmol m^{-2} s^{-1} PPFD and at ambient temperature. The leaves were allowed to acclimatise to the conditions in the chamber of the CIRAS2 for five minutes before recording the net CO_2 exchange rate. Leaf age in days, leaf lamina length (cm), and leaf lamina width (nearest 0.5 mm) were recorded on the day of photosynthesis measurement for the respective leaf positions. Leaf area inside the cuvette was measured using the formula: leaf lamina width at the mid-point between lamina and ligule x leaf length inside the cuvette.

4.3.9 Destructive harvest

A total of 57 plants were harvested in the Spring experiment, those being 3 clonal replicates from 9 genotypes for the cultivar Alto (plants of a 10^{th} genotype died) and 3

clonal replicates and 10 genotypes for the cultivar Aberdart. The number of live leaves, and the length and width of individual leaf laminae were recorded. Individual leaf laminae were dried in a hot air draft oven at 60 ^{o}C for 48 hours and then weighed. Similar measurements and data recording were carried out in the Autumn experiment for 32 plants: two clonal replicates of 8 genotypes for the two cultivars. The remaining genotypes and clonal replicates were not harvested due to time constraint.

4.3.10 Phytomer (P) nomenclature

At the destructive harvest the youngest visible leaf was used as a reference point and its phytomer (P) denoted number 1 (P1) (Fig. 3.1). Successively older phytomers were numbered sequentially P2, P3, P4, etc. When a tiller at destructive harvest carried *n* live leaves these were numbered P1 - P*n*.

4.3.11 Leaf data collection

Data were collected on various shoot traits as indicated below. The emerging leaf was always considered the reference point.

i) **Leaf appearance interval** (A_{lf}) – The date of appearance of successive leaves was recorded. Leaf appearance interval (phyllochron) was determined as the number of days between the appearance of two successive leaves. A_{lf} was also calculated in growing degree days (GDD, ^{o}C d). Leaf appearance rate (LAR, leaves d^{-1}) was calculated as $^{1}/A_{lf}$.

ii) **Age of phytomer positions** – Age of individual phytomers bearing a particular organ was estimated based on the A_{lf} of the corresponding leaf. The age of a phytomer was taken to be 1 d on the day when the leaf tip was first seen at the ligule of the preceding leaf.

iii) **Leaf elongation duration** (LED, d) – The length of time between the day of leaf tip appearance and day a leaf lamina ceased elongation.

iv) **Leaf lamina length** (FLL, cm) – FLL was measured as the distance from the ligule to the leaf tip.

v) **Leaf lamina width** (LW, mm) – LW was measured to the nearest 0.1 mm using a binocular microscope with an eyepiece containing an engraved scale, by adjusting the zoom until 100 scale units spanned 1 cm on a ruler. On the

leaves for which photosynthetic measurements were carried out, leaf width was measured to the nearest 0.5 mm by eye using a ruler with 1 mm gradations.

vi) **Leaf lamina dry weight** (LDW, mg) – Individual leaf laminae segments from ligule to leaf tip were dried in a hot air draft oven for 48 hours at 60^{o}C and LDW recorded to the nearest mg.

vii) **Leaf area** (LA, cm^2) – The area of individual leaf laminae was estimated using a form factor (Bryson et al., 1997) assuming LA = 0.7 x FLL x LW (C Matthew, personal communication).

viii) **Leaf life span** (LLS) – LLS was calculated as the difference in days between the date of leaf tip appearance and the date at which at least 80% of the leaf area of the same leaf had senesced visually.

ix) **Number of live leaves** (NLL) – Total number of live leaves per tiller was recorded at the destructive harvest.

x) **Photosynthetic rate** (NPR) – Net photosynthetic rate of the individual leaves was measured in µmol CO_2 m^{-2} s^{-1}. A log-normal curve was fitted for the NPR of leaves at the different leaf positions on the tiller axis following the methodology of Irving and Robinson (2006) for calculating a leaf Rubisco turnover curve. Regression equations assuming a linear decline in photosynthesis capacity over time for older leaves were obtained for each cultivar in each experiment using NPR of P3 – P5 to derive the NPR of the older leaves at P6 and later (R^2 >0.95). From summing the values so obtained for NPR of individual leaves, total photosynthesis capacity of the individual tillers was estimated. A linear regression to estimate NPR for older leaves was chosen after first calculating and comparing a log normal fit. The two estimates differed only slightly but the linear fit had the higher R^2.

The following leaf traits were derived from the measured variables:

xi) **Leaf elongation rate** (LER, cm d^{-1}) – This was calculated as LER = FLL / LED, LER was also calculated in growing degree days (i.e. mm length increase per ^{o}C GDD).

xii) **Number of elongating leaves** (NEL) – Assuming a steady-state pattern of leaf turnover, the number of elongating leaves per tiller at a particular time was calculated as the quotient of LED/A_{lf}.

xiii) **Specific leaf area** (SLA, $cm^2\ g^{-1}$) – Specific leaf area of individual leaf laminae was calculated as area per g dry weight.

4.3.12 Analysis of variance (ANOVA) for the leaf traits

There was variation between experiments and genotypes in the number of phytomers developed over the experiment duration. In the Spring experiment individual tillers developed 8 – 13 leaves and in the Autumn experiment there were 13 – 20 leaves. For a consistency in data structure the youngest 10 phytomers from the Spring experiment and the youngest 16 phytomers from the Autumn experiment were taken to conduct an ANOVA. Thus there were a total of 1082 data for the individual leaf traits: 570 from the Spring experiment and 512 from the Autumn experiment (Table 4.4).

Table 4.4 Leaf data structure for conducting analysis of variance (ANOVA)

Experiment	Cultivar	Genotypes	Clonal replicates	Phytomers per plant used in ANOVA	Actual no. of phytomers $plant^{-1}$	Actual total no. of phytomers	Total phytomers in ANOVA
Spring	Alto	9	3	10	8-11	253	270
	Aberdart	10	3	10	8-13	301	300
Autumn	Alto	8	2	16	13-20	236	256
	Aberdart	8	2	16	13-17	264	256
Total						1054	1082

The ANOVA of the individual leaf traits was performed using the GLM command of the Minitab 15 (Minitab Inc. State College, Pennsylvania) statistical software package. A nested split-plot design was followed for the analysis. Statistical variation between two experiments (Spring and Autumn), two cultivars, genotypes of each cultivar within experiment, phytomers of each cultivar within experiment and experiment x cultivar interactions were the selected sources of variation for which a user-defined statistical test was done (Appendix 4.1). The clonal replicates of each genotype for each cultivar within experiment were considered as the experimental units. To test the effects of experiments, cultivars and experiment x cultivar interactions, the mean square for genotypes of each cultivar within experiment was used as the error term. To test the effect of genotypes of each cultivar and phytomers of each cultivar within experiments the clonal replicates of each genotype for each cultivar were used as the error term in a user-defined model (Appendix 4.1).

4.3.13 Correlation analysis and principal component analysis (PCA) for the leaf traits

Pearson correlation coefficients between the measured and derived leaf traits of the individual tillers were obtained using the Minitab 15 statistical software package (Minitab Inc. State College, Pennsylvania). Mean A_{lf}, LED, LER, NEL, LW, FLL, LDW, LA, SLA, NPR, LLS of the individual leaves and NLL per tiller for 16 plants of each cultivar in each experiment were used for the correlation analysis (Appendix 4.2). Plants used in this analysis were those for which NPR data were available. Using the same data set, a PCA was carried out in Minitab 15 (Minitab Inc. State College, Pennsylvania). The principal component (PC) scores were stored and ANOVA of the PC scores was performed using the GLM procedure as above to explore the statistical significance of between experiment, cultivar, and cultivar x experiment effects.

4.4 Results

4.4.1 Phyllochron duration

Over the 90 d growing period in the Spring experiment, phyllochron duration decreased by approximately 4 d with increasing day length. For Alto the decrease was from 11.2 d to 7.1 d ($p<0.001$) and for Aberdart from 10.3 d to 6.1 d ($p<0.001$) (Fig. 4.5). Phyllochron duration measured in thermal time (3 ^{o}C base temperature) did show leaf-to-leaf variation in the Spring experiment (range 67 – 91 ^{o}C d for Aberdart and 59 – 78 ^{o}C d for Alto) ($p<0.001$ and $p=0.001$, respectively) but not systematic variation over the course of the experiment (Fig 4.6). Conversely, in the Autumn experiment the phyllochron increased with decreasing day length: from 3.5 to 8.4 d for Alto ($p<0.001$) and from 4.0 to 7.4 d for Aberdart ($p<0.001$) (Fig 4.5). In the Autumn experiment the correction for thermal time did not completely remove the tendency for phyllochron to increase with decreasing day length. The phyllochron expressed in ^{o}C d ranged from 65 to 112 ^{o}C d for Alto and 65 to 105 ^{o}C d for Aberdart ($p=0.018$ and $p=0.003$, respectively; Fig. 4.6).

The mean value of the phyllochron differed between the Spring and Autumn experiments (7.94 and 5.15 d respectively) ($p<0.001$), and the mean value for cultivars averaged over experiments also differed (Alto 6.85 d, Aberdart 6.24 d, $p=0.009$) but the experiment x cultivar interaction was not significant ($p=0.25$). Similarly, when the phyllochron was expressed in thermal time, values for the Spring and Autumn experiments differed (73.5

and 80.0 ^{o}C d, respectively, p=0.004), as did cultivars (Alto 79.8 ^{o}C d, Aberdart 73.6 ^{o}C d, p=0.007, but the experiment × cultivar interaction was non-significant, p=0.72).

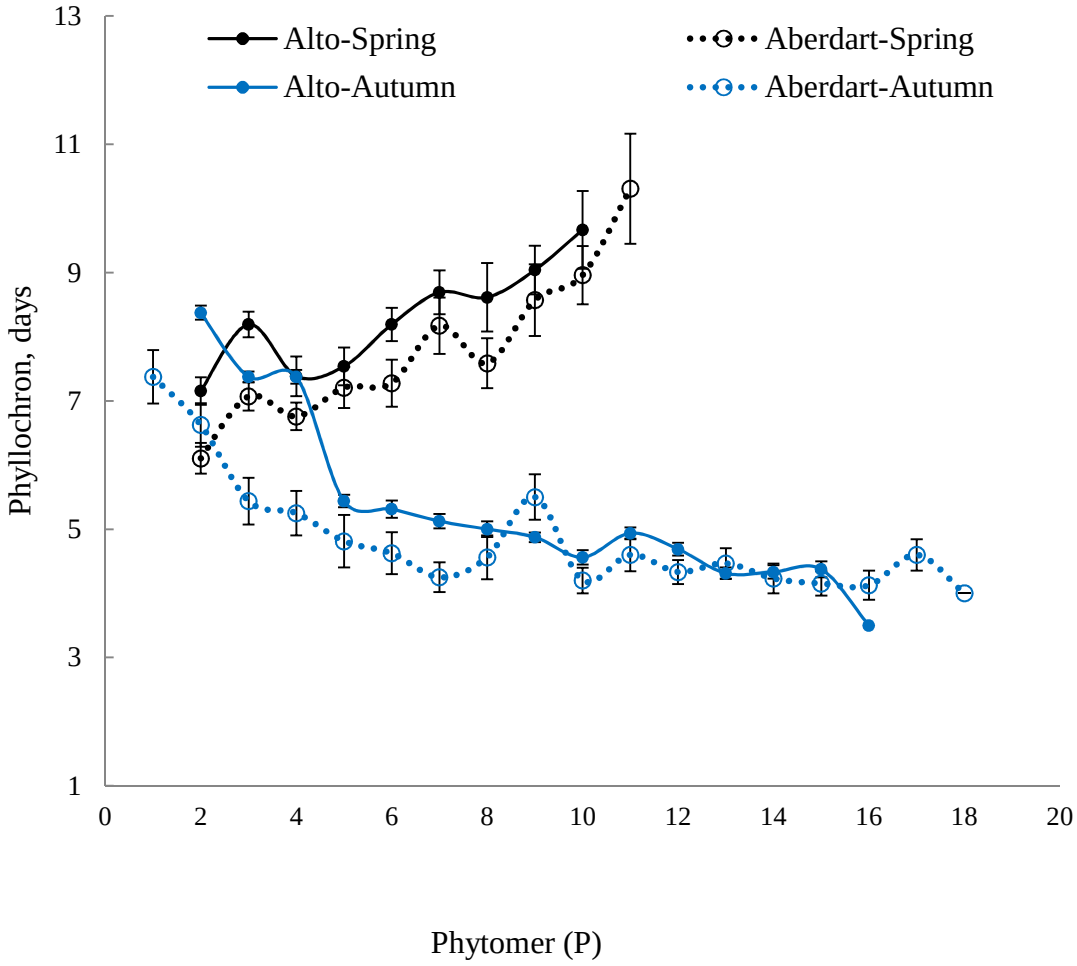


Fig. 4.5 Phyllochron expressed in days in the Spring and Autumn experiments (Experiments 4 and 5, respectively) for the two perennial ryegrass cultivars Alto and Aberdart. Phytomer position 1 is the youngest leaf. Vertical bars show standard error of means at each phytomer for each cultivar in each experiment. Note that the time sequence on the X-axis reads from right (older phytomers) to left (younger phytomers).

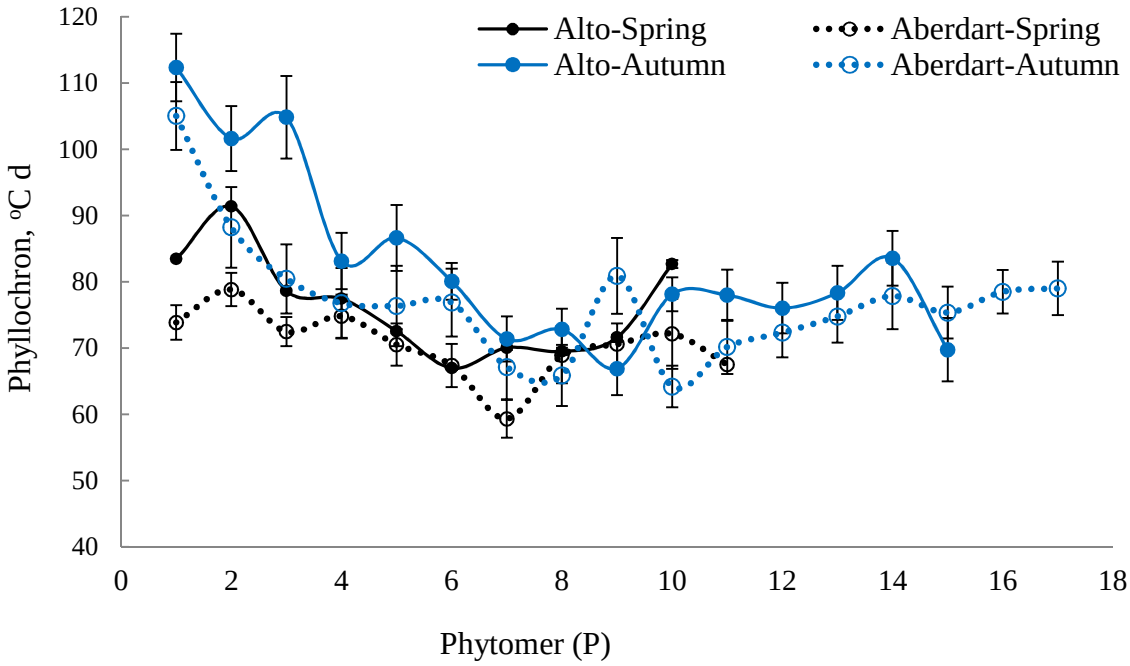


Fig. 4.6 Phyllochron expressed in thermal time (°C d) in the Spring and Autumn experiments (Experiments 4 & 5, respectively) for the two perennial ryegrass cultivars Alto and Aberdart. Phytomer position 1 is the youngest leaf. Vertical bars show standard error of means at each phytomer for each cultivar in each experiment. Note that the time sequence reads from right (older phytomers) to left (younger phytomers).

The data for phyllochron duration of successive leaves were also plotted cumulatively against time in days and against thermal time. The plot for phyllochron duration expressed in days showed the seasonal effect as a divergence of the plotted data and the cultivar effect as smaller vertical separation (Fig. 4.7). When phyllochron data were plotted cumulatively against thermal time, the seasonal effect was greatly diminished but the cultivar effect remained (Fig. 4.8).

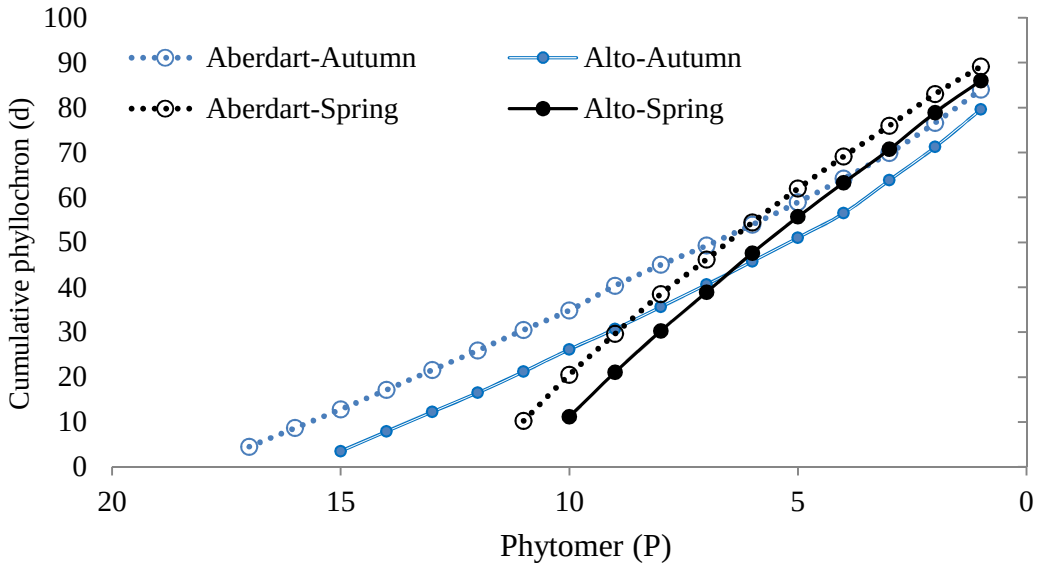


Fig. 4.7 Time course of phytomer accumulation expressed in cumulative days for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively). Phytomer position 1 is the youngest leaf.

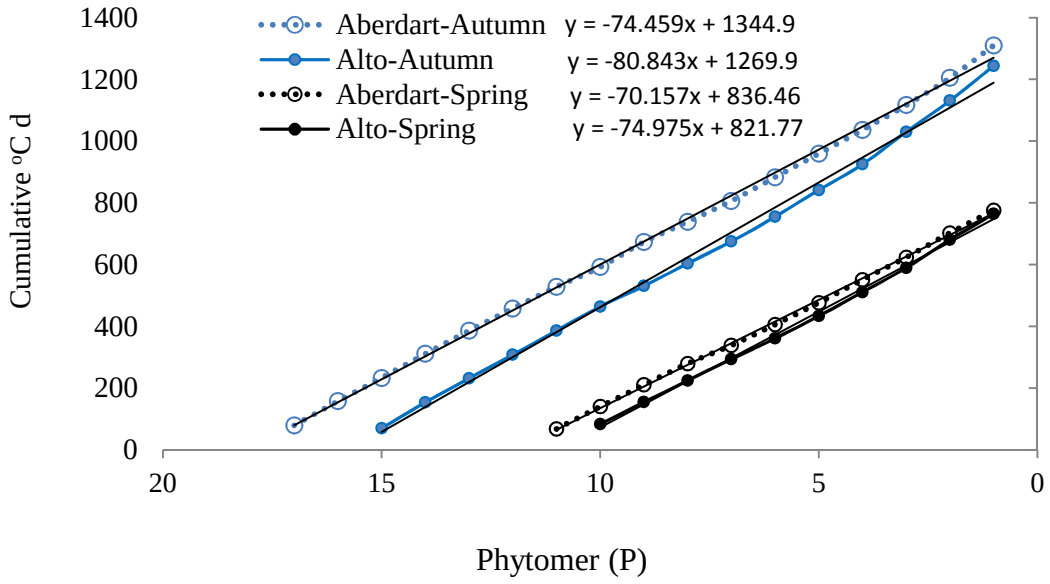


Fig. 4.8 Time course of phytomer accumulation expressed in cumulative thermal time (^{o}C d) for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively). Phytomer position 1 is the youngest leaf.

4.4.2 Age of phytomer positions

In order to provide a basis for analysing the time course of morphological events such as root development at a phytomer, each phytomer was assigned an age at harvest based on the date of appearance of the leaf located at that phytomer (Table 4.5). In parallel with season-related and cultivar-related differences in phyllochron, the leaf age at different phytomer positions showed highly significant variation between the two experiments ($p<0.001$), cultivars Alto and Aberdart ($p<0.001$), and between the phytomer positions within experiment ($p<0.001$) for each cultivar (Table 4.5). The variation between genotypes within experiments for each cultivar was significant ($p=0.008$) but the cultivar x experiment interaction was not significant ($p=0.391$).

Table 4.5 The estimated age (days from leaf appearance ±SE) at the final harvest, of the successive phytomer positions of Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments.

	Age of the phytomers, days			
Phytomers	Alto-Spring	Aberdart-Autumn	Alto-Spring	Aberdart-Autumn
1	4±0.45	3±0.45	6±0.85	5±0.84
2	10±0.53	9±0.59	14±1.04	12±0.95
3	17±0.58	16±0.66	21±1.21	19±1.17
4	25±0.69	23±0.69	28±1.21	24±1.17
5	32±0.63	30±0.79	33±1.12	29±1.23
6	40±0.83	37±0.93	38±1.10	34±1.18
7	48±0.95	45±1.04	43±1.21	39±1.25
8	56±1.32	52±1.10	48±1.31	43±1.23
9	66±1.38	61±1.25	53±1.34	48±1.50
10	77±1.63	70±1.48	58±1.36	53±1.60
11		79±1.50	63±1.30	57±1.65
12			68±1.39	62±1.73
13			72±1.24	66±1.80
14			76±1.11	70±1.67
15			80±0.72	74±1.67
16				77±1.69
17				80±1.60

4.4.3 Leaf elongation duration (LED)

LED values (Fig. 4.9) were always greater than corresponding values of A_{lf} (Fig 4.5). In the Spring experiment, LED differed between leaf positions ($p<0.001$), ranging between 10.8 d and 11.8 d for Alto and between 11.0 d and 11.9 d for Aberdart but did not show a

major trend over the duration of the experiment (Fig. 4.9). In the Autumn experiment LED increased with decreasing day length, from 5.9 d to 13 d for Alto, and from 6.5 to 13.9 d for Aberdart (Fig. 4.9).

Seasonal means for LED were 11.2 d for the Spring experiment and 8.7 d for the Autumn experiment ($p<0.001$), while cultivar means averaged over both experiments were 9.6 and 10.3 d for Alto and Aberdart, respectively ($p<0.001$). A significant ($p<0.001$) experiment x cultivar interaction was observed for LED. Mean LED values for Alto and Aberdart in the Spring experiment were 11.1 d and 11.3 d, respectively, while in the Autumn experiment the mean LED values for Alto and Aberdart were 8.1 d and 9.3 d, respectively. Significant genotype differences in LED were also observed between genotypes of each cultivar within experiments ($p<0.001$).

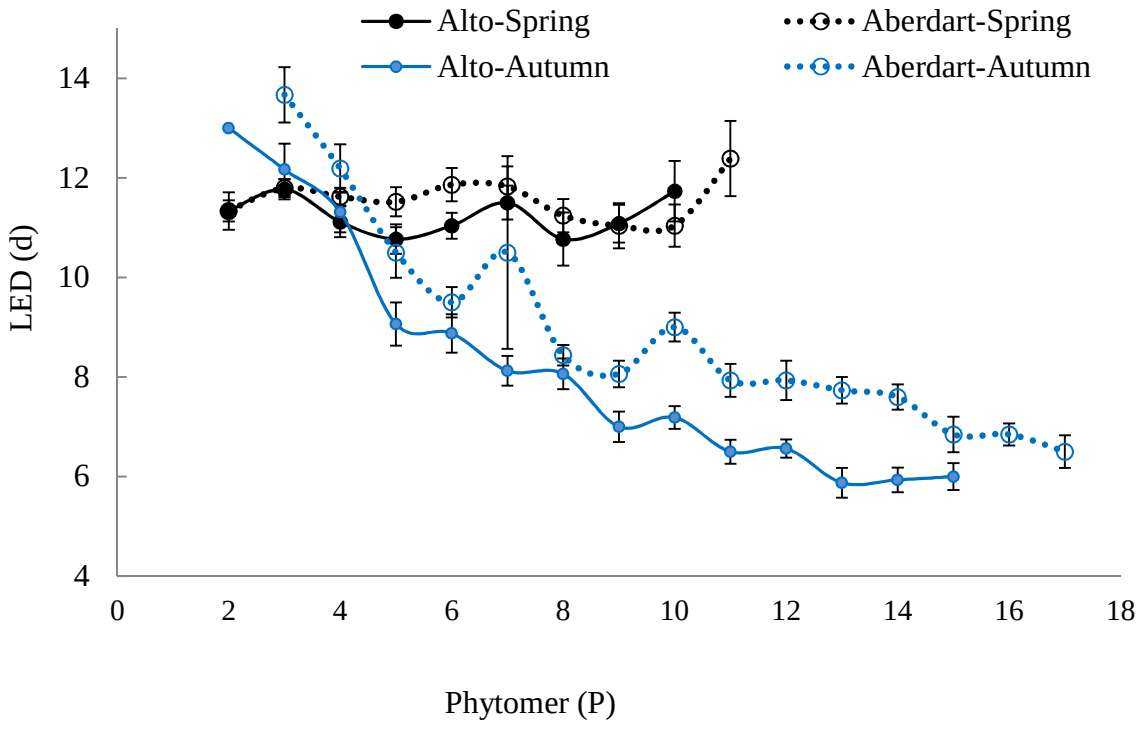


Fig. 4.9 Leaf elongation duration (LED) of Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively). Vertical bars show standard error of means at each phytomer for each cultivar in each experiment. Phytomer position 1 is the youngest leaf.

4.4.4 Leaf elongation rate (LER)

In contrast with LED, LER showed a more marked time trend in the Spring experiment than in the Autumn experiment, with LER in the Spring experiment increasing from 11 mm d^{-1} to 24.7 mm d^{-1} for Alto and from 10.1 to 29.3 mm d^{-1} for Aberdart ($p<0.001$, for both of the cultivars, Fig. 4.10). A less marked change in LER occurred in the Autumn experiment with values for Alto decreasing from 42.5 mm d^{-1} to 29.2 mm d^{-1} for Alto ($p=0.113$) and from 39.1 mm d^{-1} to 26.1 mm d^{-1} for Aberdart ($p=0.023$). The mean LER measured in the Spring experiment was 18.4 mm d^{-1} and in the Autumn experiment 36 mm d^{-1} ($p<0.001$). The mean LER for the two cultivars did not differ significantly. However, the experiment x cultivar interaction was significant ($p=0.032$). Mean LER values for Alto and Aberdart in the Spring experiment were 17.8 mm d^{-1} and 19.1 mm d^{-1}, respectively, while in the Autumn experiment mean LER was 36.8 mm d^{-1} and 35.2 mm-d^{-1} for Alto and Aberdart, respectively. LER also differed significantly between genotypes within cultivars ($p<0.001$).

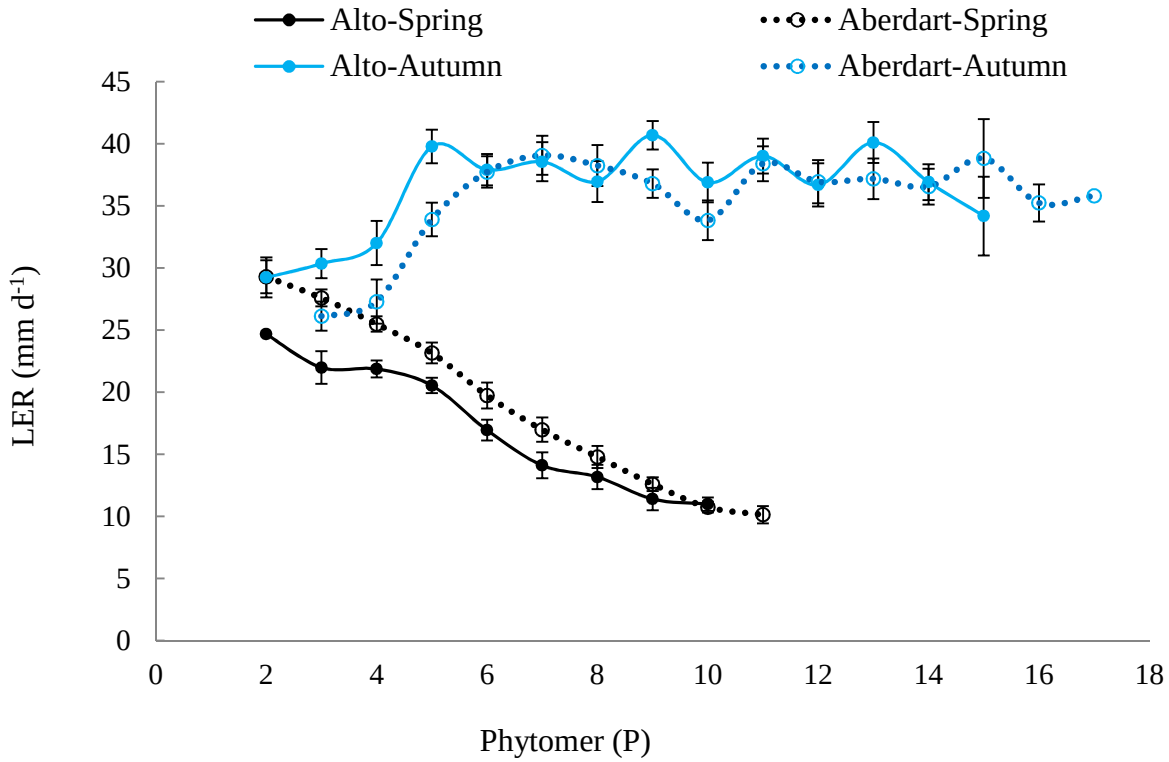


Fig. 4.10 Leaf elongation rate (LER) at different phytomer positions over a 90 d growing period for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively). Vertical bars show standard error of means at each phytomer for each cultivar in each experiment. Phytomer position 1 indicates the youngest leaf.

LER measured in mm per unit thermal time (mm $^{o}C^{-1}$ d^{-1}) showed a pattern over time in the two experiments similar to that for LER (mm d^{-1}), except that the highest observed value was 2.1 times greater than the lowest, whereas the highest value for LER (mm d^{-1}) was 4.2 times greater than the lowest (Fig. 4.11). In the Spring experiment, the LER varied from 1.49 mm $^{o}C^{-1}$ d^{-1} to 2.18 mm $^{o}C^{-1}$ d^{-1} for Alto and from 1.38 to 2.52 mm $^{o}C^{-1}$ d^{-1} for Aberdart ($p<0,001$) (Fig. 4.11). In the Autumn experiment LER varied from 1.99 mm to 2.88 mm $^{o}C^{-1}$ d^{-1} for Alto and from 1.65 mm to 2.65 mm $^{o}C^{-1}$ d^{-1} for Aberdart ($p<0.001$) (Fig. 4.11). The mean LER measured 1.93 and 2.35 mm $^{o}C^{-1}$ d^{-1} in the Spring and Autumn experiments, respectively ($p<0.001$). Mean LER for the two the cultivars was similar and the experiment x cultivar interaction was not significant. Again, significant differences between genotypes within cultivars were observed ($p<0.001$).

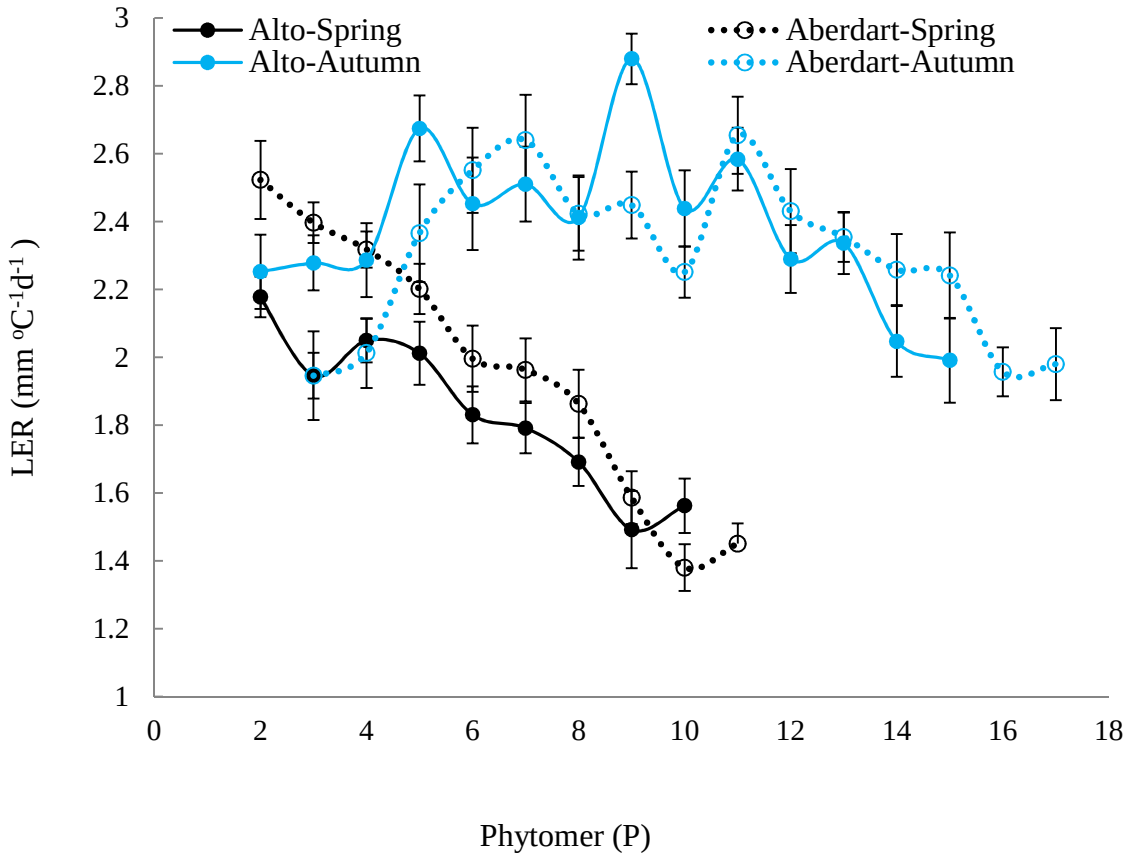


Fig. 4.11 Leaf elongation rate (LER) expressed in thermal time (mm $^{o}C^{-1}$ d^{-1}) at different phytomer positions for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively). Phytomer position 1 is the youngest leaf. Vertical bars show standard error of means at each phytomer for each cultivar in each experiment.

4.4.5 Number of elongating leaves (NEL)

NEL was always greater than 1.0 and tended to gradually increase with time after establishment of the transplanted tillers (Fig. 4.12). Values for Aberdart in the Autumn experiment appeared higher than for Alto but not in the Spring experiment, however, the cultivar x experiment interaction was non-significant (p=0.12).

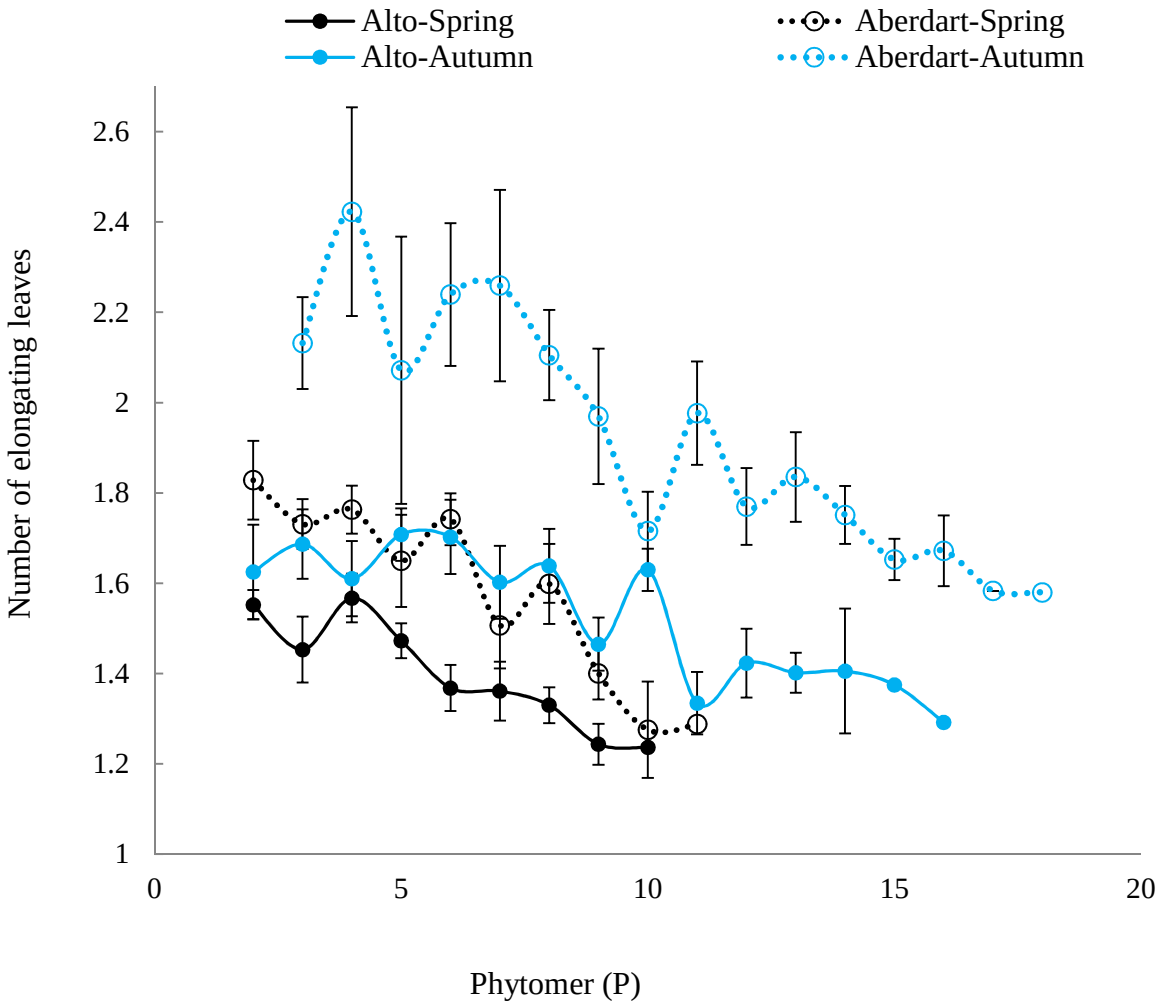


Fig. 4.12 Number of elongating leaves for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively). Phytomer position 1 is the youngest leaf. Vertical bars show standard error of means at each phytomer for each cultivar in each experiment.

4.4.6 Final leaf lamina length (FLL)

FLL increased ($p<0.001$) gradually as plant size increased (Fig 4.13). In the Spring experiment, FLL increased over time from 12.2 cm to 26.8 cm for Alto and from 12.0 cm to 32.3 cm, for Aberdart. In the Autumn experiment, the corresponding increases were from 18.7 cm to 36.3 cm for Alto and from 21.6 cm to 35.6 cm for Aberdart.

Leaves attained longer FLL (28.8 cm) in the Autumn experiment than in the Spring experiment (20.0 cm) ($p=0.005$). The two cultivars also differed significantly, Alto 23.1 cm and Aberdart 25.7 cm; ($p<0.001$) as did genotypes of each cultivar within experiments ($p<0.001$). The cultivar x experiment interaction was not significant ($p=0.58$).

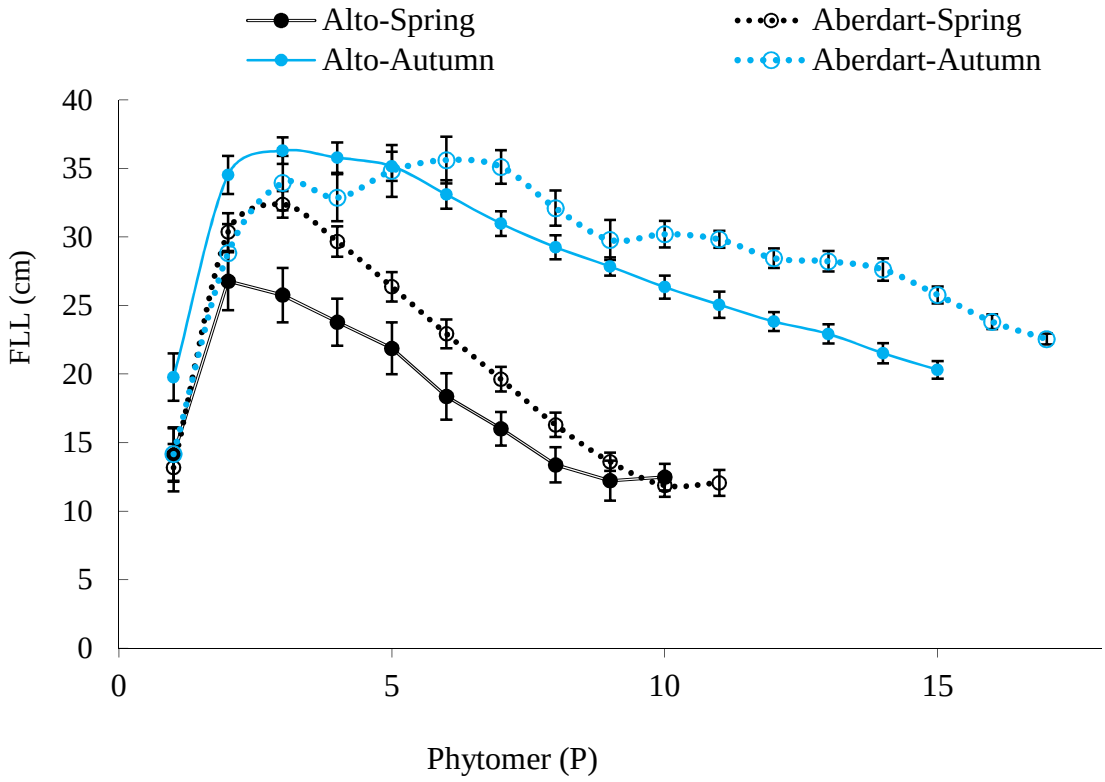


Fig. 4.13 Final leaf length (FLL) at the different phytomer positions for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively). Vertical bars show standard error of means at each phytomer for each cultivar in each experiment. Phytomer position 1 denotes the youngest leaf.

4.4.7 Leaf lamina width (LW)

LW also tended to increase from older to younger leaf positions for both cultivars in both experiments ($p<0.001$; Fig. 4.14). LW of the mature leaves increased over time from 4.88 mm to 6.97 mm for Alto and from 4.77 mm to 7.94 mm for Aberdart in the Spring experiment. In the Autumn experiment, LW increased over time from 4.62 mm to 6.62 mm for Alto and 6.12 mm to 7.25 mm for Aberdart.

LW did not differ between the two experiments ($p=0.21$), but the two cultivars Alto (6.05 mm) and Aberdart (6.77 mm) did vary significantly ($p=0.008$), as did genotypes within cultivars ($p<0.001$). The experiment x cultivar interaction for LW was not significant ($p=0.88$).

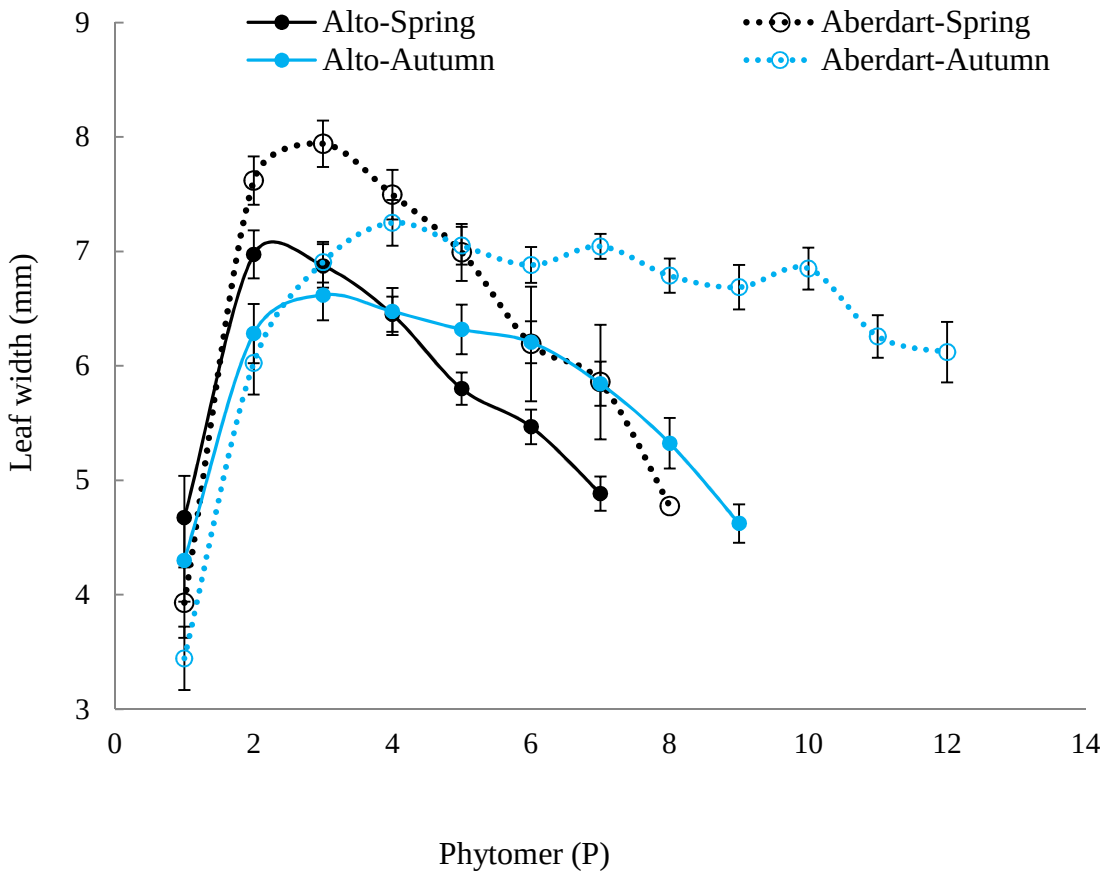


Fig. 4.14 Leaf width at different phytomer positions of Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively). Vertical bars show standard error of means at each phytomer for each cultivar in each experiment. Phytomer position 1 denotes the youngest leaf.

4.4.8 Leaf lamina dry weight (LDW)

Reflecting a dependence on FLL and LW, LDW at successive leaf positions also increased as plant size increased over the course of the experiment. The youngest 2–3 leaves were at either the elongation or maturation stage. LDW increased over successively appearing leaves from 26.3 mg to 57.3 mg for Alto and from 28.7 mg to 78.3 mg for Aberdart in the Spring experiment (Fig 4.15, $p<0.001$). In the Autumn experiment, LDW increased from 45.6 mg to 109 mg for Alto and 75.6 mg to 131 mg for Aberdart. LDW differed significantly between the Spring experiment and the Autumn experiment ($p<0.001$). The mean LDW of individual leaf laminae in the Autumn experiment (94.2 mg) was more than double the mean LDW in the Spring experiment (45.6 mg). The two cultivars Alto and Aberdart varied significantly for LDW (60.2 mg and 79.6 mg, respectively; $p<0.001$). The genotypes within cultivars again were significantly different ($p<0.001$).

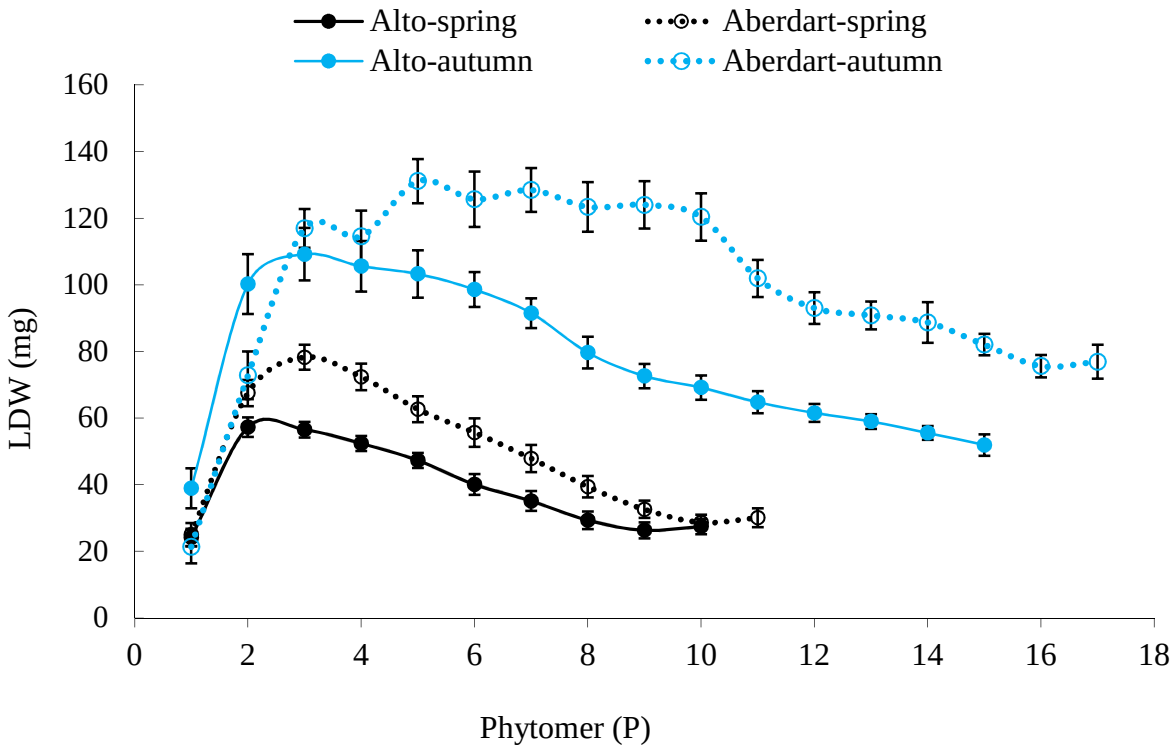


Fig. 4.15 Leaf dry weight (LDW) at different phytomer positions for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively). Vertical bars show standard error of means at each phytomer for each cultivar in each experiment. Phytomer position 1 denotes the youngest leaf.

4.4.9 Leaf area (LA)

In keeping with increase in measures of leaf size, the LA of individual leaves also increased with increasing plant size ($p<0.001$) although this trend is least pronounced for Aberdart in the Autumn experiment (Fig. 4.16). Individual leaves were larger in the Autumn experiment than those in the Spring experiment ($p=0.016$; Fig. 4.17). The cultivars Aberdart and Alto did not differ significantly in LA ($p=0.532$) and cultivar x experiment interaction was also not significant ($p=0.968$). However, genotypes of each cultivar did show highly significant ($p<0.001$) differences for LA of individual leaves.

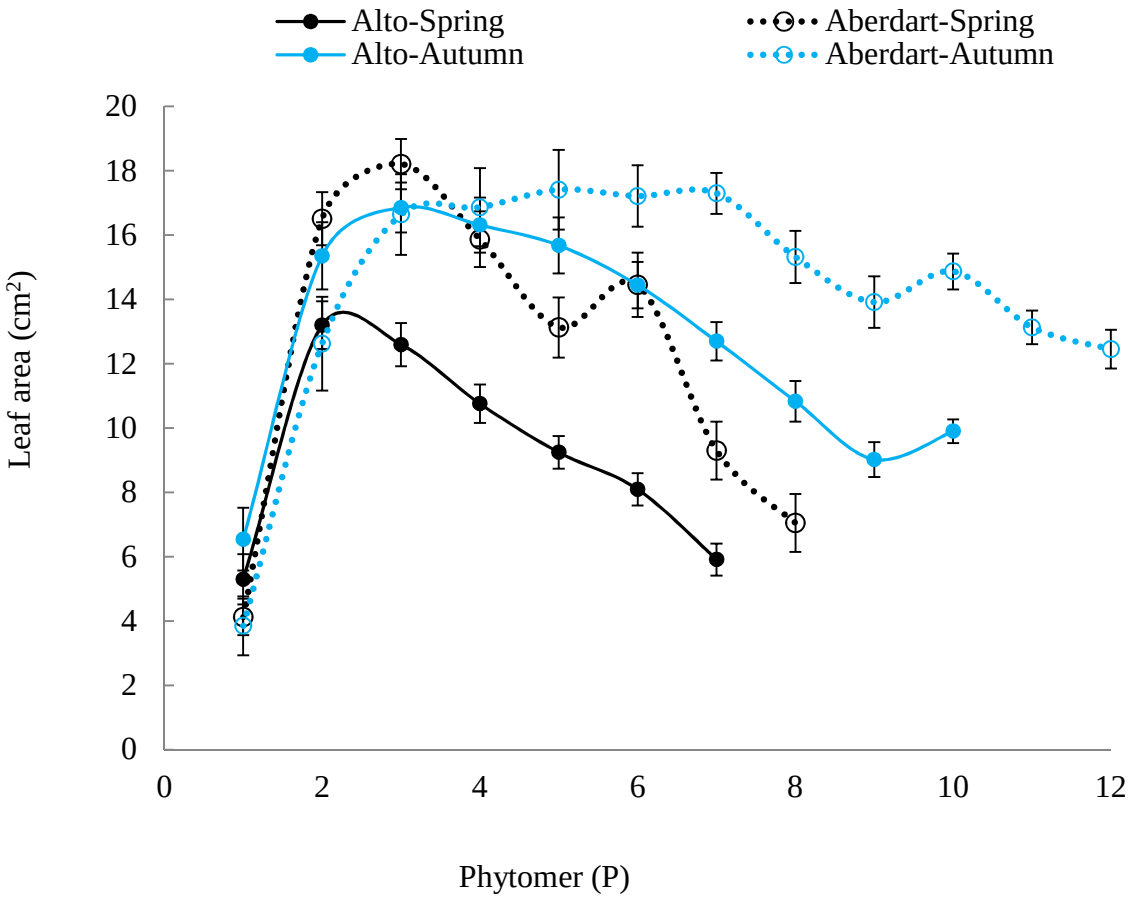


Fig. 4.16 Leaf area (cm^2) at different phytomers for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiment 4 and 5, respectively). Vertical bars show standard error of means at each phytomer for each cultivar in each experiment. Phytomer position 1 denotes the youngest leaf.

4.4.10 Specific leaf area (SLA)

Compared to FLL and LDW, SLA showed a smaller but statistically significant ($p<0.001$) increase at successively younger phytomer positions in both experiments. For Alto and Aberdart SLA varied respectively from 188 to 233 $cm^2\ g^{-1}$ and from 194 to 254 $cm^2\ g^{-1}$ in the Spring experiment. Corresponding values were from 131 to 168 $cm^2\ g^{-1}$ and from 115 to182 $cm^2\ g^{-1}$ in the Autumn experiment (Fig. 4.17).

SLA showed highly significant variation between the Spring experiment (212 $cm^2\ g^{-1}$) and the Autumn experiment (146 $cm^2\ g^{-1}$) ($p<0.001$) but the two cultivars did not differ significantly ($p=0.78$). The experiment x cultivar interaction for SLA was also non-significant ($p= 0.48$). However, the genotypes of each cultivar did differ significantly for SLA ($p<0.001$).

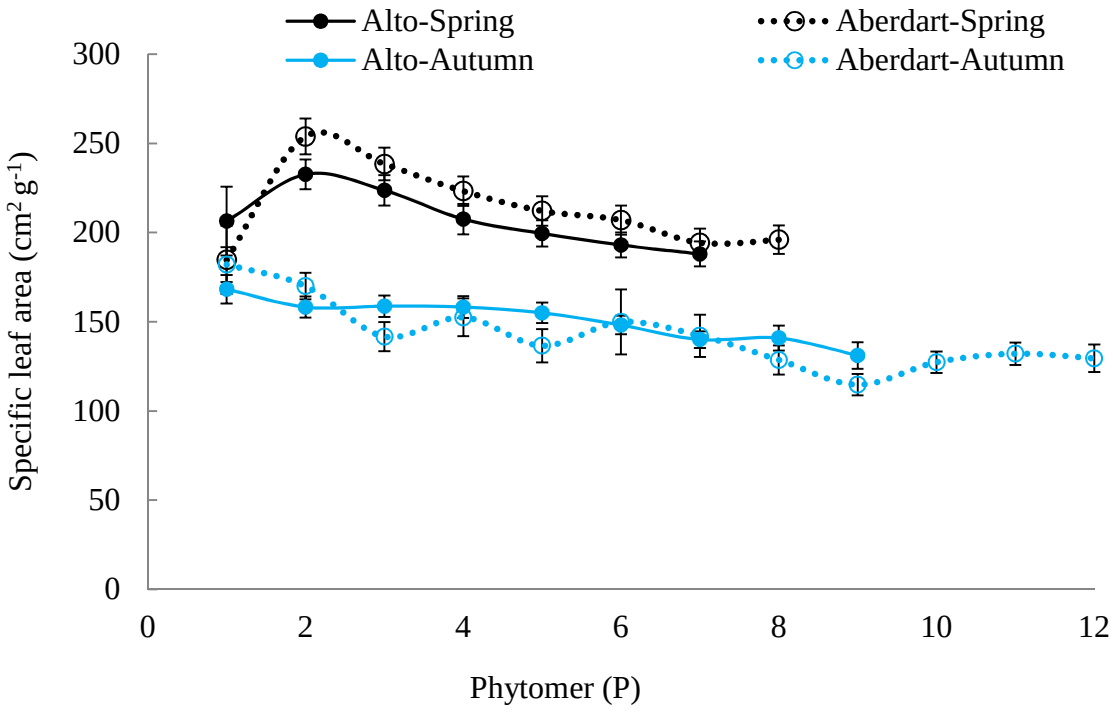


Fig. 4.17 Specific leaf area ($cm^2\ g^{-1}$) at different phytomers for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively). Vertical bars show standard error of means at each phytomer for each cultivar in each experiment. Phytomer position 1 denotes the youngest leaf.

4.4.11 Leaf life span (LLS) and number of live leaves per tiller (NLL)

The mean LLS of the recently died leaf (see Section 4.3.11) at harvest immediately below the oldest live-leaf was 61.5 d in the Spring experiment and 65.9 d Autumn experiment ($p<0.001$) and was 59.3 d for Alto and 68.1 d for Aberdart ($p<0.001$). The experiment x cultivar interaction was also significant ($p<0.001$, Fig. 4.18). NLL at the day of harvest also varied significantly ($p=0.01$) between the Spring and Autumn experiments (7.68 and 10.3, respectively), and between the cultivars (Alto = 7.6, Aberdart = 10.3, $p<0.01$) (Fig. 4.18).

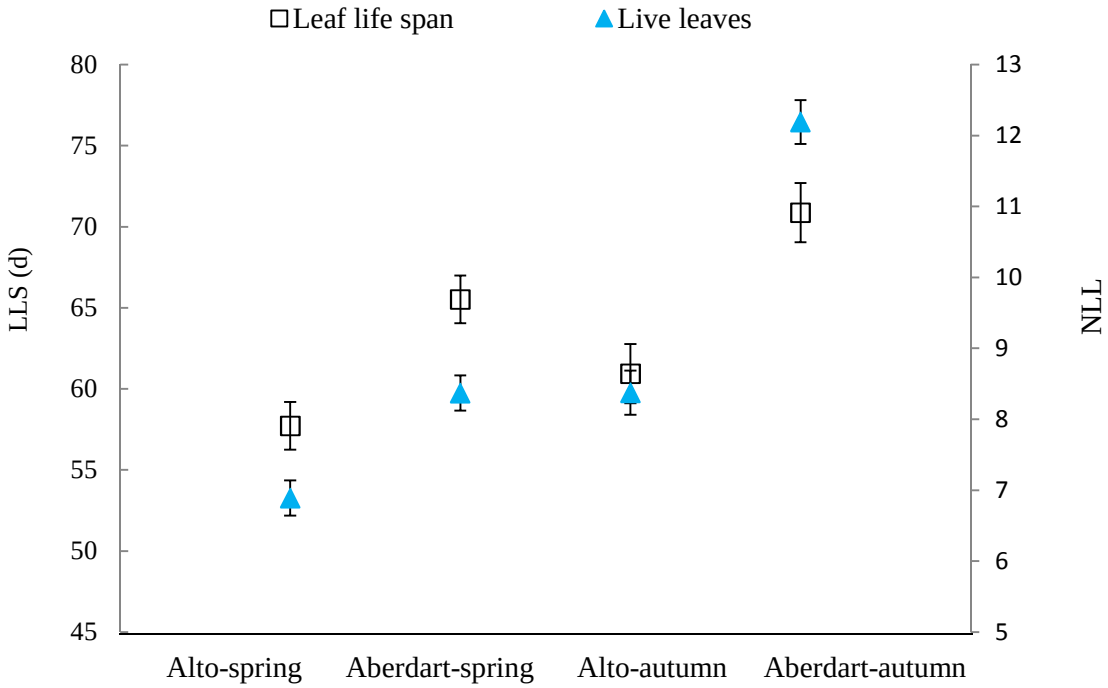


Fig. 4.18 Leaf life span (LLS) and number of live leaves per tiller (NLL) for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiment 4 & 5, respectively). Vertical bars indicate standard error of means.

4.4.12 Net photosynthetic rate

The NPR differed significantly between leaf positions ($p=0.02$) (Fig. 4.19). In the Spring experiment, NPR ranged between 14.3 and 19.2 µmol CO_2 m^{-2} s^{-1} for the cultivar Alto and between 14.4 and 17.6 µmol CO_2 m^{-2} s^{-1} for Aberdart among the youngest five live leaves (Fig. 4.19). The highest NPR recorded was at leaf position 2 for both of the cultivars, with a value for Alto 19.2 µmol m^{-2} s^{-1} (at 14.8 d) and Aberdart of 17.6 µmol m^{-2} s^{-1} (at 12.5 d)

(Fig. 4.19). The lowest NPR recorded was at leaf position 5 (the oldest leaf measured) for both of the cultivars, Alto 14.3 µmol CO_2 m^{-2} s^{-1} (at 37.3 d) and Aberdart 13.4 µmol CO_2 m^{-2} s^{-1} (at 34 d) (Fig. 4.19). In the Autumn experiment, the highest NPR recorded was at position 3 and was 17.8 µmol m^{-2} s^{-1} for cultivar Alto at 14.8 d, and 16.7 µmol m^{-2} s^{-1} at 14.1 d for cultivar Aberdart (Fig. 4.19). In the Autumn experiment, the youngest leaf position had the lowest NPR for both cultivars. The value for Alto was 13.1 µmol CO_2 m^{-2} s^{-1} (at 5.3 d) and for Aberdart 12.0 µmol CO_2 m^{-2} s^{-1} (5.3 d) (Fig. 4.19). The log normal curves fitted to the NPR (Fig 4.19). Curve parameters are presented in Table 4.6. The *d* and *g* values, which indicate, respectively, the highest NPR and the leaf age at which that was attained were similar for both of the cultivars in both experiments. The *f* value was also consistent between the cultivars for each experiment, but differed between experiments.

The mean NPR did not differ significantly between Spring and Autumn experiments. The difference between the two cultivars was marginal for statistical significance ($p=0.096$). The cultivar × experiment interaction was non-significant ($p=0.84$), and also the difference between genotypes within cultivars ($p=0.21$).

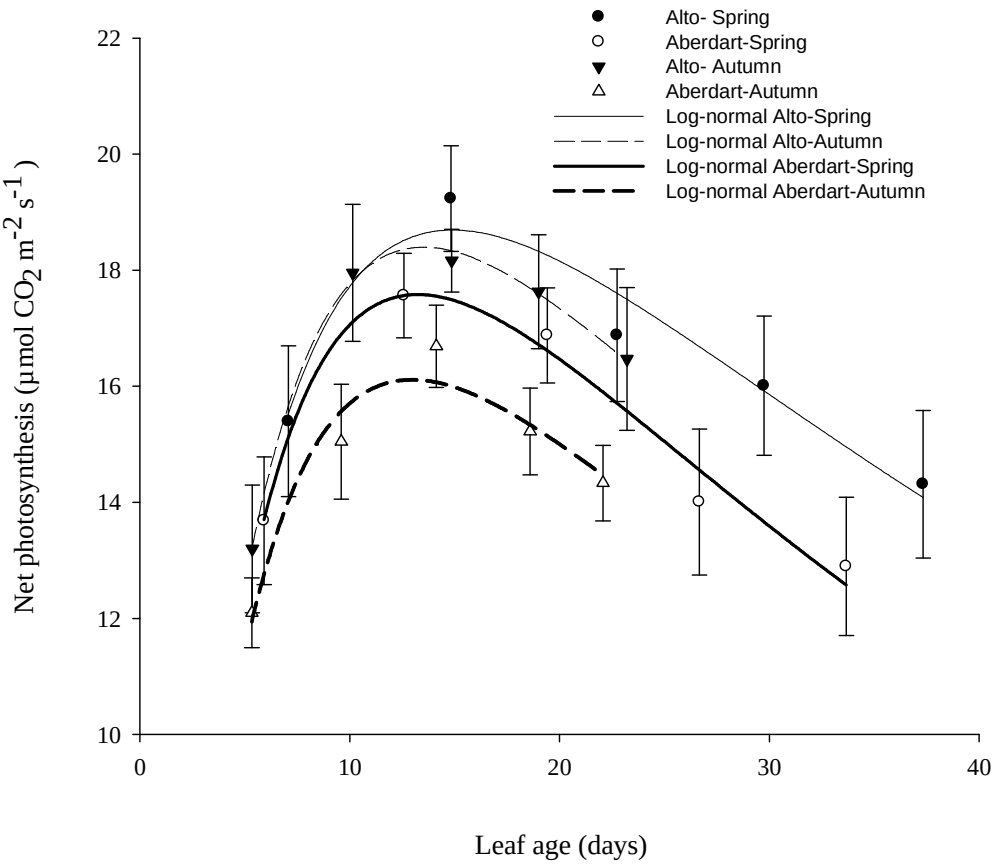


Fig. 4.19 Net photosynthetic rate (NPR) for five selected leaf positions of Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively). Vertical bars indicate the standard error at each leaf position for NPR.

Table 4.6 Curve parameters for the log-normal fit of net photosynthetic rate (NPR) and leaf age for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively). *d*: the highest NPR (µmol CO_2 m^{-2} s^{-1}); *g*: leaf age when *d* occurs (days); *f*: a measure of curve width (log days).

Curve parameters	Alto- Spring	Aberdart-Spring	Alto- Autumn	Aberdart -Autumn
d	18.6±0.89	16.8±0.81	17.6±0.67	15.8±0.47
f	1.31±0.22	1.42±0.27	1.32±0.24	1.36±0.21
g	14.2±1.42	12.3±1.41	13.3±0.67	13.3±1.44
R^2	0.15	0.12	0.16	0.21

At the individual tiller level, the two cultivars Alto and Aberdart in spring and in autumn, respectively, had photosynthetic rates (mmol CO_2 h^{-1}) of 0.37 for Alto in Spring, 0.47 for Aberdart in Spring 0.57 for Alto in Autumn and 0.52 for Aberdart in Autumn in mmol CO_2 h^{-1} for the all leaf laminae. NPR for all leaf laminae (µmol CO_2 m^{-2} s^{-1}) varied significantly between the two experiments and the two cultivars but the cultivar x experiment interaction was not significant (Table 4.7, Appendix 4.3), although for both LA per tiller and LDW per tiller, the cultivar x experiment interaction was highly significant (Table 4.7). The net photosynthetic rate per unit LA, averaged per tiller, differed between experiments ($p<0.001$), between cultivars ($p<0.001$) and for the experiment x cultivar interaction ($p<0.001$) (Table 4.7).

Table 4.7 Leaf area (LA), leaf dry weight (LDW), estimated total photosynthesis of all leaf laminae (NPR $tiller^{-1}$) and ratio between NPR and LA per tiller for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively) for 64 tillers for which photosynthesis measurements were carried out.

	LA	LDW	NPR $tiller^{-1}$	NPR/LA
	cm^2 $tiller^{-1}$	mg $tiller^{-1}$	µmol CO_2 s^{-1}	µmol CO_2 m^{-2} s^{-1}
Alto-Spring	65.0	398	1030	15.9
Aberdart-Spring	87.1	444	1230	14.1
Alto-Autumn	116	1140	1750	15.2
Aberdart-Autumn	173	1510	1960	11.4
SEM	5.89	39.7	77.1	0.205
p (Exp)	<0.001	<0.001	<0.001	<0.001
p (Cul)	<0.001	0.002	0.002	<0.001
p (Exp x Cul)	0.001	0.009	0.903	<0.001

SEM, standard error of mean; p, probability value of statistical variation.

4.4.13 Association among the leaf traits

4.4.13.1 Correlation Analysis

A_{lf} and LED were also negatively correlated with LER, NEL, FLL, LDW, LA and NLL but positively correlated with SLA (Table 4.8). The morphological traits NEL, FLL, LDW, LA, NLL and LER were positively associated between each other and all of them were negatively associated with SLA (Table 4.8). NLL showed strong-positive correlation with LLS (Table 4.8). In addition, NPR showed a weak but negative correlation with NLL and LLS (Table 4.8).

Table 4.8 Co-efficients of correlation within perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively) for 11 leaf traits assessed for 16 tillers each of Alto and Aberdart from each Experiment. $A_{lf,}$ leaf appearance interval; LED, leaf elongation duration; LER, leaf elongation rate; NLL, number of elongating leaves; NPR, net photosynthetic rate; FLL, leaf lamina length; LDW, leaf dry weight; LW, leaf lamina width; LA, leaf area; SLA, specific leaf area; LLS, leaf life span; NLL, number of live leaves. The cells contain Pearson correlation coefficients.

	A_{lf}	LED	LER	NEL	FLL	LDW	LW	LA	SLA	LLS	NLL
LED	0.87**										
LER	-0.85**	-0.88**									
NEL	-0.67**	-0.29*	0.41**								
FLL	-0.74**	-0.69**	0.94**	0.43**							
LDW	-0.76**	-0.60**	0.83**	0.62**	0.87**						
LW	0.197	0.266*	-0.106	-0.016	0.026	0.126					
LA	-0.55**	-0.48**	0.77**	0.37**	0.89**	0.84**	0.47**				
SLA	0.75**	0.69**	-0.74**	-0.45**	-0.66**	-0.77**	0.27*	-0.47**			
LLS	-0.16	<0.01	0.15	0.28*	0.24*	0.24*	0.12	0.26*	-0.08		
NLL	-0.68**	-0.36**	0.48**	0.79**	0.49**	0.64**	0.04	0.45**	-0.49**	0.62**	
NPR	0.07	-0.06	0.01	-0.16	-0.06	-0.11	-0.08	-0.09	0.04	-0.28*	-0.25*

*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively

4.4.13.2 Principal component analysis

The first three principal components from PCA explained 80% of the data variation. PC1 explained 52.8% of the data variation and indicated a contrast between LER, NEL, FLL, LDW, LA and NLL with positive coefficients and A_{lf}, LED and SLA with negative coefficients (Table 4.9). Analysis of variance for PC1 scores indicated a contrast between the experiments, with negative PC scores for the Spring experiment and positive PC scores for the Autumn experiment (Appendix 4.4). In both experiments cultivar Alto had lower PC scores than Aberdart ($p<0.001$, Table 4.10). PC1 can be explained as a 'size' PC as the plants in the Autumn experiment were larger than those of the Spring experiment and plants of the cultivar Aberdart were larger than Alto. PC2 explained 15.4% of the data variation and showed a contrast between LER and NPR with positive coefficients and LED, NEL, LW, LA, SLA, LLS and NLL with negative coefficients (Table 4.9). ANOVA of PC2 scores indicated a contrast between the two cultivars, Alto and Aberdart, with mean positive and negative scores, respectively (Table 4.10). Scores for PC2 also differed

significantly between the two experiments and an experiment × cultivar interaction was detected (Table 4.10). This PC indicated that plants with longer LED also have longer LLS, higher NLL, and comparatively greater LW but lower NPR and LER. This trait combination was seen in the cultivar Aberdart, and the opposite in Alto. PC3 explained 11.8 % of the data variation, and a notable feature of this PC was high positive coefficients for LW and LA (Table 4.9. ANOVA of PC3 scores showed no clear separation between experiments or cultivars and no significant experiment × cultivar interaction (Table 4.10) meaning that this variation in leaf morpho-physiological traits probably represents plant to plant variation (Appendix 4.4).

Table 4.9 Major principal components and their coefficients from principal component analysis of tiller morpho-physiological traits of Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiments 4 & 5, respectively) Abbreviations are: $A_{lf,}$ leaf appearance interval (d); LED, leaf elongation duration; LER, leaf elongation rate (cm d^{-1}); NEL, number of elongating leaves at a time; NPR, net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}); FLL, leaf lamina length (cm); LDW, leaf lamina dry weight (g); LW, leaf lamina width (mm); LA, leaf area (cm^2); SLA, specific leaf area (cm^2 g^{-1}); LLS, leaf life span (d); NLL, number of live leaves; PC, principal component. Coefficients of absolute value <0.15 suppressed.

Trait	PC1	PC2	PC3
A_{lf}	-0.36	–	0.17
LED	-0.31	-0.34	–
LER	0.37	0.18	–
NEL	0.26	-0.22	-0.32
FLL	0.36	–	0.23
LDW	0.37	–	–
LW	–	-0.45	0.59
LA	0.31	-0.17	0.46
SLA	-0.32	-0.22	–
LLS	–	-0.49	-0.22
NLL	0.29	-0.34	-0.33
NPR	–	0.38	0.23
%variation explained	52.8	15.4	11.8

Table 4.10 Mean principal component (PC) scores from analysis of variance (ANOVA) of the first three PCs based on the leaf data for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments (Experiment 4 & 5, respectively).

	PC1	PC2	PC3
Alto-Spring	-2.70	0.43	-0.09
Aberdart-Spring	-2.00	-0.99	0.01
Alto-Autumn	1.56	1.46	0.44
Aberdart-Autumn	3.14	-0.89	-0.36
SEM	0.11	0.16	0.21
p (Experiment)	<0.001	0.016	0.79
P (Cultivar)	<0.001	<0.001	0.24
p (Experiment x Cultivar)	0.005	0.047	0.14

SEM, Standard error of mean; p, probability of statistical significance in generalized linear model ($F_{1,30}$);

4.5 Discussion

4.5.1 Methodology

There were two reasons for the decision to work with tillers from established field plants, rather than seedlings, in these experiments. One reason was that it takes considerable time (up to 3 months) for a grass seedling to form shoots with the size and structure of those in field swards, whereas transplanted adult tillers formed an effective root system within half that time, reducing the lead time to start measurements for each experiment. A second reason is that there is emerging evidence that particular traits in plants are controlled by different genes in different environments or seasons (Sartie et al., 2010), and presumably also in plants of different ages. The decision to work with tillers transplanted from field swards did mean that it was not possible to match the endophyte status of the cultivars studied. Morphology differences between endophyte-free and endophyte-infected plants have been shown in some controlled experiments (see e.g. Eerens et al., 1998). However, morphological differences associated with endophyte-presence are not consistent between experiments and are typically quite small. Hence it was decided that the benefits of working with tillers from older field-established plants outweighed the possible confounding effects of different endophyte status of the available field material in the two cultivars studied.

The glasshouse where the hydroponic unit was situated did not have an effective means to reduce temperatures on hot days, and this was of concern towards the end of the Spring

experiment and at the start of the Autumn experiment. Peak temperature was controlled to some extent by deploying a shade cloth over the roof of the glasshouse at these times. Even so, there were occasional instances of daily maximum temperatures possibly detrimental to ryegrass growth (Fig. 4.2). It is the writer's opinion that the temperature spikes did not have a major impact on the overall pattern of the results, except possibly at the start of the Autumn, experiment as discussed in Section 5.5.2.

Plant roots growing in soil experience heterogeneous nutrient supply as soil nutrient status can vary at a microsite level in the rhizo-sphere (Hodge, 2004). Under field conditions, plant growth is also subject to limitations imposed by abiotic stresses such as nutrient deficiency or toxicity, moisture deficiency or flooding. In hydroponic culture under a balanced nutrient supply the plants in this experiment could have been expected to experience a relative advantage to their growth and development, compared to field conditions. However, plant nutrient uptake is a continuous process, and maintaining nutrient solution concentration in a steady-state in a hydroponic unit is a challenge. Uptake of the various nutrients by plants is not necessarily in proportion to concentrations in the hydroponic solution, so that accumulation or depletion of particular nutrients over time is a known phenomenon (Bugbee, 2003). In view of this consideration, the hydroponic nutrient solution was refreshed weekly, with newly mixed nutrient solution, in these experiments.

In grass plants, expression of a number of traits is modified by the light environment at the base of the plant, and this is particularly relevant where adjacent tillers form a closed canopy (Kirby and Faris, 1972). Extinction of light by the canopy reduces the release of tiller buds to form new tillers (Simon and Lemaire, 1987). Low irradiance also increases LER and results in longer FLL (Kays and Harper, 1974). The single tiller plants growing in hydroponics in these two experiments were spaced at a comparatively large distance and so would have had high light interception, and would not have experienced growth limitations relating to shading as described above. A similar plant to plant distance was maintained in both experiments.

Some existing reports suggest that DTs supply photosynthetic carbohydrate to the axis of the MT (Carvalho et al., 2006). Removal of DTs might therefore limit the growth of the MT to some extent. The removal of DTs after the first two produced on tiller main axes

was to ensure that plant structure would be simple, in order to assist dissection of roots from the individual root-bearing phytomers at harvest, but possible effects of DT amputation on MT behaviour do need to be considered when interpreting data from these experiments.

4.5.2 Phyllochron responses in changing day length

One objective for Experiments 4 and 5 was to provide an initial examination of evidence for the hypothesis (Section 2.5) that root:shoot relations would differ under increasing and decreasing day length because the delay in root and shoot formation at a given phytomer on the tiller axis implies a difference in phyllochron: rhizochron ratio. To do so, the current experiment needed to achieve change in the A_{lf} during each experiment and it is evident (Fig. 4.5) that this was achieved. Even so, the fall and rise of the A_{lf} in a *L. perenne* sward as measured by Peacock (1975c) was much higher than in the present study. Peacock (1975) measured a fall of A_{lf} from 33.6 d to 7.9 d in spring and a rise from 9.2 d to 32.7 d in autumn. The present study recorded only around 4 d fall or rise of A_{lf} in both the Spring and Autumn experiments (Fig. 4.5) as the experiments were conducted in glasshouse in controlled temperature.

Wilhelm and McMaster (1995) listed environmental factors influencing A_{lf} as: temperature, nutrient availability, water availability, salt status, CO_2 concentration and both light quality and quantity. Temperature above the optimum shortens the A_{lf}; other listed factors have a comparatively minor influence but increased CO_2 concentration and increased light intensity may decrease the A_{lf} whereas higher N availability and higher salt concentration might increase the A_{lf}. A nearly linear response of the A_{lf} to increase in thermal time (Fig. 4.6, Fig. 4.8) in this study reconfirmed that temperature is the most dominant factor affecting A_{lf}. In *Z. mays,* Birch et al. (1998) recorded that for a decrease of irradiance from 9.6 to 1.1 MJ PAR m^{-2} d^{-1} the A_{lf} increased by 2–4 ^{o}C d MJ^{-1} PAR. A significant difference between the Spring and Autumn experiments for A_{lf} duration expressed in thermal time (see e.g., slope differences for trajectories in Fig. 4.8) reconfirmed that temperature solely cannot explain the total variation in LAR although it explains vast majority of the variation. The significant variation between leaf positions for A_{lf} in thermal time further suggested that temperature *per se* is unable to explain the total variation in A_{lf}. Apart from the environmental factors, the A_{lf} at successive leaf positions

might be increased by increase in sheath length (Bos and Neuteboom, 1998; Cruz and Boval, 2000).

Similar to A_{lf}, a number of factors may influence LED at successively developing phytomers. Those includes: (i) increasing or decreasing temperature and day length and their effect on A_{lf}: when A_{lf} increases LED is also expected to be increasing, (ii) FLL at the successive phytomers: FLL should be positively correlated with LED, (iii) length of leaf sheath (i.e., pseudostem tube) controlling switching on and off cell division in the meristems: longer leaf sheath length might be associated with greater LED, and (iv) possible maturity effects meaning that successive leaves achieved their final size in terms of FLL; are among many others. FLL data (Fig. 4.13) indicated that tiller size was increasing throughout both of the experiments. In the Autumn experiment, in decreasing day length, as A_{lf} increases at the successive phytomer and plant size increases perhaps factors (i) to (iii) worked additively to each other to increase LED at the successive phytomer (Fig. 4.9). Conversely, in the Spring experiment, in increasing day length as A_{lf} decreases at the successive phytomer and plant size increases at the successive phytomer therefore effect of factor (i) perhaps reduced the effect of factors (ii) and (iii) to show nearly unchanged LED throughout the growing period (Fig. 4.9).

LER being a ratio between FLL and LED, is influenced by any factor affecting either FLL or LED. Increasing plant size means increasing FLL which again influences LED. Therefore the factors influencing FLL and LED are interrelated to some extent. The factors which increase LED are expected to decrease LER, hence the negative correlation between these two traits (Table 4.8). LER increased with successive phytomers in the Spring experiment but was approximately constant for phytomers P2 to P16 in the Autumn experiment (Fig. 4.10). The LER increase in spring was probably due to the additive effect of both increasing day length and temperature, and increasing plant size. By contrast, in the Autumn experiment, an antagonism between the effect of decreasing day length and increasing plant size would have occurred. When LER was expressed in thermal time, hence correcting for temperature effects, the LER increase with successive phytomers was seen in both seasons, but a seasonal difference in LER increment at successive phytomers remained (Fig. 4.11). It is unclear whether the slope difference between Spring and Autumn experiments for LER accumulation (Fig. 4.11) is because of a different rate of size increase or if some other factor affected plants differently in the two

seasons. Comparing proportional change of LER expressed in thermal time (Fig. 4.11) and that of FLL at the successive phytomers (Fig. 4.13) it does appear that the change in LER over time was expressed as increased FLL, and not cancelled out by an associated decrease in LED.

In both Spring and Autumn experiments, the increase in tiller size with time was associated with an increase in the ratio between LED and A_{lf} (NEL) at successive phytomers (Fig. 4.12). In the Spring experiment LED was quite consistent (Fig. 4.9) throughout the experiment and A_{lf} (Fig. 4.5) was decreasing, hence NEL increased. Meanwhile, constant LED with increasing day length resulted in an increase in FLL. Conversely, in the Autumn experiment both LED and A_{lf} were increasing at successive phytomers, but LED was increasing at a faster rate with successive phytomers than A_{lf}, so that once again an increase in NEL and FLL resulted. FLL at successive phytomer depends on length of elongation zone (Fournier et al., 2005), length of pseudostem tube, enclosing leaf sheath length (Verdenal et al., 2008) and that also depends on co-ordination of leaf growth between two successive phytomers (Skinner and Nelson, 1994; Fournier et al., 2005; Verdenal et al., 2008). Hence any of those factors affecting FLL can also mediates NEL in turn.

For *L. perenne* cultivars 'Grasslands Samson' and Grasslands Impact' Sartie et al. (2009) reported to be LED>A_{lg}>A_{lf} where A_{lg} is ligule appearance interval for the successive leaves. A consistently higher ratio of greater than 1.0 between LED and A_{lf} (Fig. 4.12) in the present study was similar that of Robson (1967) for the value of 1.2 and Sartie et al. (2009) for the value of 1.37 for 'Grasslands Samson' and 1.32 for 'Grasslands Impact'.

Assuming a constant growing environment and steady growth rate of tillers LED is expected to be proportional to A_{lf}. However, when the environmental factors, such as day length, temperature and PAR are variable over the growing period than the relations between A_{lf} and LED are quite complex.

Significantly higher NLL in the Autumn experiment than in the Spring experiment (Fig. 4.18) was achieved through both comparatively short A_{lf} (Fig. 4.5) and comparatively long LLS (Fig. 4.18) in the Autumn experiment. In steady state LLS is the product of A_{lf} and

NLL (Lemaire and Chapman, 1996). In the case of increasing or decreasing day length the LLS of the senescing leaf at the leaf position P can be modelled as:

$$LLS = x + A_{lf}\,1 + A_{lf}\,2 + A_{lf}\,3 + ... + A_{lf}\,P\text{-}1 \quad \text{.....Equation 4.1}$$

where, x is the age of the youngest leaf, P-1 is the position of the oldest and mature live leaf, $A_{lf}1$, $A_{lf}2$, $A_{lf}3$ are the leaf appearance intervals for the three youngest live leaves, respectively. For example, a tiller in spring season having five live leaves which appeared in 12, 11, 10, 9 and 8 d A_{lf} from older to younger phytomer positions will have an estimated 51 d LLS when the youngest visible leaf is 1 day old.

4.5.3 A_{lf} and LER as the determinants of plant growth in changing day length

As in results of Khaembah (2009) and Sartie (2010) A_{lf} and LED had a strong negative correlation with LER (Table 4.8) although Sartie et al. (2009) reported a non-significant correlation between A_{lf} and LER and weak-negative correlation between LED and LER. It is possible that the rate of leaf appearance event mediates the LER (Lemaire and Agnusdei, 2000). In autumn significantly higher LER probably contributed higher FLL and LA compared to spring. Clearly, with increased FLL at successive phytomers, LDW, LW and LA were also expected to show an associated increase (Table 4.8). The interaction of LER with A_{lf} changes other structural traits of the leaves at successive phytomers on other aspects of tiller development have been presented in Fig. 4.20. The leaves in spring which developed with longer LED and slower LER had lower tissue density per area (higher SLA). PC1 separated autumn tillers from those of Spring for their larger FLL, greater LDW, larger NLL as indicated by positive co-efficients and shorter A_{lf} and LED as indicated by negative co-efficients; and for the opposite direction of all of those co-efficients in Spring (Appendix 4.4, Table 4.10).

LER can be considered as a determinant of plant performance. As discussed in the previous section, mathematically A_{lf} is proportional to LED meaning that this trait in turn will be proportional to FLL and inversely proportional to LER (Lemaire and Agnusdei, 2000). A_{lf} again directly related to NLL as shorter A_{lf} increases NLL and *vice-versa* and thereby increases LAI (Lemaire and Agnusdei, 2000). A_{lf} and LER therefore can be regarded as core determinants of plant growth (Fig. 4.20). It has been also discussed in the

previous section how A_{lf} might influence NEL. A change in NEL at the successive phytomer can be regarded as an index of changing plant size over time. Hence further investigation in determining factors affecting NEL would be of merit.

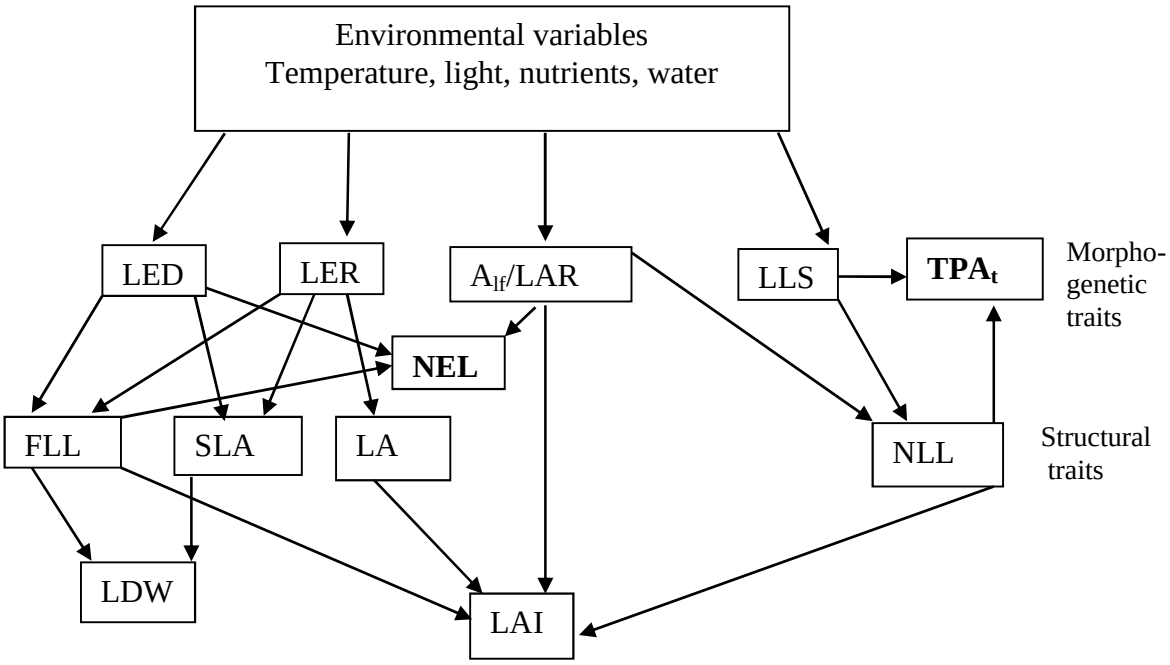


Fig. 4.20 The relationship among the main morphogenetic traits of a grass tiller. LED, leaf elongation duration; LER, leaf elongation rate; A_{lf}, leaf appearance interval; LAR, leaf appearance rate; LLS, leaf life span; TPA_t, total photosynthetic assimilation by all leaves per tiller; FLL, final leaf length; SLA, specific leaf area; LA, leaf area; NEL, number of elongating leaves; NLL, number of live leaves per tiller; LDW, leaf dry weight; LAI, leaf area index (after Lemaire and Agnusdei, 2000). The traits NEL and TPA_t in this diagram have been included using information from the present study. The arrow direction refers the influence on the trait.

4.5.4 Leaf photosynthetic capacity and its association with leaf traits

Reduction of leaf photosynthetic capacity with leaf aging is a well-known phenomenon (Jewiss and Woledge, 1967; Wilhelm and Nelson, 1978) and more recently it has emerged that leaf Rubisco concentration also declines with leaf age in an approximately log normal fashion (Irving and Robinson, 2006). The present study investigated the relation of NPR with leaf age in a way to explore the possible mathematical relation with leaf Rubisco and has found that NPR (Fig 4.19) can be seen to also fit to a log normal curve, suggesting that

the NPR might be associated with Rubisco turnover (Hudson et al., 1992; Makino et al., 1997) (Table 4.6).

A negative association between LLS and NPR indicated that leaves which live longer (autumn leaves) can exchange comparatively less CO_2 per unit time (Table 4.8). The autumn leaves formed with shorter A_{lf} and LED and had longer LLS and faster LER. Those leaves achieved a higher NPR comparatively early. The ratios between photosynthetic rate and leaf area per tiller for the two experiments and the two cultivars were found to differ significantly. The experiment x cultivar interaction was also significant (Table 4.7). These results together, suggest that spring leaves are more efficient than autumn leaves for CO_2 assimilation. Moreover, the SLA of the spring leaves was significantly higher than the autumn leaves (Fig. 4.17) suggesting the SLA is positively related to NPR (PC2 in Table 4.9). Faster LAR and higher LLS were associated with higher NLL, and were found to be negatively correlated with NPR (Table 4.8).

4.6 Summary

- The growing conditions in these two experiments did provide the variation in phyllochron duration necessary to test the hypothesis that seasonal change in day length could potentially lead to seasonal change in root:shoot ratio although the change in phyllochron duration was less marked than in some field experiments;
- Differences between Spring and Autumn experiments in plant traits such as the phyllochron duration were largely but not completely explained when traits were expressed on a thermal time basis;
- Morphogenetic changes associated with increased day length and associated decrease in the phyllochron in the Spring experiment were decreased LED and SLA, and increased LER, FLL, LDW, and LA at successive phytomers, and *vice versa* for the autumn experiment;
- FLL and LDW increased at successively developing phytomers in both experiments, confirming results of (Jewiss, Skinner and Nelson, Durand, Verdenal) and this effect reinforced the day length effect in the Spring experiment and partly negated it in the Autumn experiment;
- Phyllochron duration as reflected in traits such as A_{lf} has previously been attributed a central role in determining tiller morphogenesis (Lemaire and Agnusdei, 2000),

but the present results in changing environmental conditions indicate both A_{lf} and LER are important in tiller morphogenesis;

- NPR was highest at P2 and declined thereafter. The relationship between NPR and leaf age could be described by fitting a log-normal curve following the methodology of Irving and Robinson (2006) for modelling change in leaf Rubisco content with time, suggesting that Rubisco degradation may be the reason for declining NPR in older leaves.

Chapter 5: Tiller Axis Dynamics of Root Production

5.1 Introduction and Overview

In Chapter 4 the dynamics of the above ground components of the tiller axis have been discussed. In contrast to leaf turnover, the turnover of roots has seldom been studied and there are conflicting reports about whether the typical pattern is defined by seasonal root production events or more continuous turnover, but a preliminary data set presented in Chapter 3 suggested the latter. Root data was collected from the same plants used in the experiments for which the above ground data were presented in Chapter 4. The present chapter discusses the dynamics of the below ground components and attempts a description of characteristics of roots at successive positions on the tiller axis in order to make inference about root dynamics, in the same way that leaf dynamics were described in the previous chapter. Chapter 6 will discuss the morphological relations between root and shoot, and how seasonal changes in root and shoot growth patterns may result in morphogenetic variation of root:shoot ratio.

5.2 Objectives

The objectives of experiments reported in this chapter were:

i) To build an understanding of root turnover on the tiller axis of *L. perenne* by measuring root weight, length, surface area, volume and degree of branching at successive phytomer positions on the tiller axis;

ii) To carry out these root measurements for two *L. perenne* cultivars (Alto and Aberdart) with contrasting breeding background in contrasting growing conditions of increasing or decreasing day length, in order to explore the nature of variation in root turnover across cultivars and seasons;

iii) To determine the extent of variation in root morphology between genotypes within cultivars (Alto and Aberdart) compared to the variation between cultivars and seasons for leaf morphology and turnover;

iv) To infer from root weight and length data, and from recorded information on historical leaf appearance at successive phytomers, the quantitative distribution of root formation activity;

v) To explore methodologies for quantifying root branching and to evaluate the potential for the methodologies explored to deliver information that would be useful in plant improvement.

5.3 Materials and Methods

As described in Section 4.3.7, in the Spring and Autumn experiments (Experiments 4 & 5 respectively, see Section 4.3.2), root samples were collected at the destructive harvest from the individual tillers of the same plants.

5.3.1 Root-bearing phytomer (Pr) nomenclature

The mean distance measured as number of phytomers between the youngest leaf position, P1, (see Section 4.3.8) and first root on the tiller axis was noted for each tiller (Fig. 3.1). This time interval in phyllochrons or delay (i.e., number of phytomer positions) was denoted *de* (Fig. 3.1). The root-bearing phytomers (Pr) were numbered consecutively beginning with the phytomer with the youngest roots as a reference point (Pr1). Thus root attachment positions at successively older phytomers were numbered Pr2, Pr3, and so on.

5.3.2 Age determination for the roots at root-bearing phytomers

The age of roots at a particular Pr was estimated based on an assumption of steady state progression of phytomers on the tiller axis from leaf production to root production. That is, the root at Pr1 was assumed to be the same age as the leaf at P1 and *de* was assumed constant so that the age of roots at successive phytomers below Pr1 was estimated by adding the phyllochron for the leaf observed earlier in the experiment at the respective phytomer, to the age of the root at the phytomer above.

For older phytomers formed in the field for which there were no leaf appearance records to estimate the phyllochron, a value of 80 ^{o}C d thermal time per phytomer was assumed by extrapolating a plot of phyllochron values for the phytomers immediately above (Fig. 4.6, Appendix 5.1).

To confirm the co-ordination between leaf and root appearance, lengths in cm of all roots at each phytomer were measured during the Autumn experiment (Experiment 5) at Day 16, Day 22 and Day 27 after transplanting. This was done for 6 plants of each cultivar. From

this data root elongation rate of the main axis (cm d^{-1}) could be calculated, and the number of leaves and root-bearing phytomers at each date were also recorded (Appendix 5.2).

5.3.3 Root harvest

Roots from the individual phytomer positions on the tiller axis were excised in sequence from youngest to oldest, using a scalpel and 15x magnification with a binocular dissecting microscope. Excised roots were carefully teased apart from the other roots under water and stored as described below for later analysis. The number of root-bearing phytomers per tiller (NPr), number of roots per phytomer (R_p) and total number of roots per tiller (NR_t) were recorded at harvest for all plants, and the length of each root main axis (RAL) was measured in cm for 8 plants from 8 genotypes of each cultivar (Fig. 5.1).

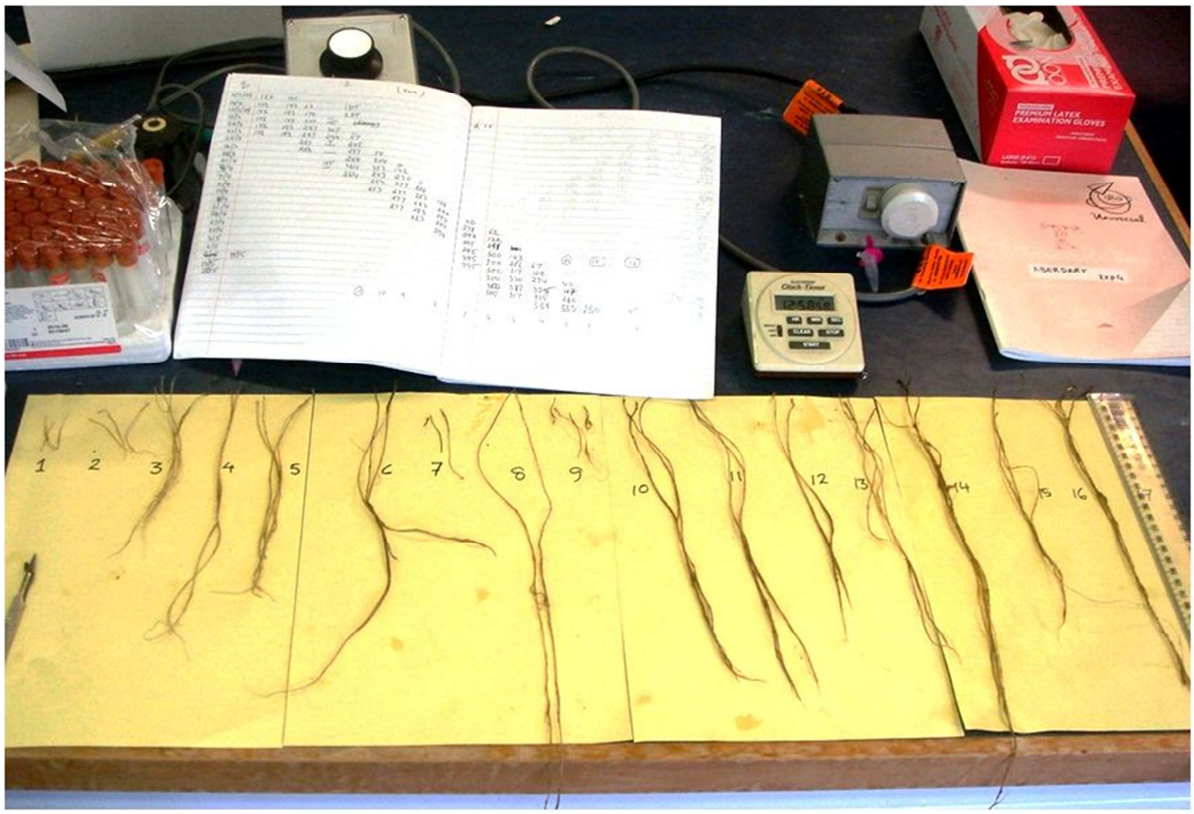


Fig. 5.1 Dissected roots from the tiller axis of a *L. perenne* plant arranged by phytomer position (Pr1-Pr16). From left to right the roots are arranged from the youngest (Pr1) to the oldest (Pr16).

5.3.4 Root measurements

A total of 2416 individual roots were isolated from 1112 phytomers of 89 tillers in the two experiments. A number of methodologies for evaluating the dimensions of individual roots were considered, ranging from determination of root length by the grid intersect method and/or root dry weight as in many past studies, to use of modern scanning technology and WinRHIZO® and VideoPro® software. The latter technologies allow collection of detailed root data such as root length (RL) by diameter category, root surface area (RSA), and root volume (RV) among others, but are very time consuming. The approach adopted after consideration was that some basic measurements (root dry weight) were made for all harvested roots, and scanned images were created for subsets of roots to enable more detailed analysis by software packages such as WinRHIZO®. The more detailed data were then used to develop calibrations to derive additional data not directly measured, from the data measured for all roots. In addition, some more detailed analyses of the scanned images of root subsets were made.

Scanning all roots for detailed root data such as root length (RL), surface area (RSA), volume (RV) for all those individual roots using WinRHIZO® was not feasible considering time constraints. Therefore roots from two randomly selected plants of two different genotypes for each cultivar in each experiment were scanned and the data analysed by WinRHIZO® and VideoPro® (see Section 5.3.7, below) to study progressive root development at the successive phytomers (see Table 5.1 in section 5.3.8). For another subset only Pr 5 & 7 from the Spring experiment and Pr 11 from the Autumn experiment were scanned (see Table 5.2 in section 5.3.8) for a comparative study on root development for roots of similar age growing in two different seasons. For another set of plants (10 plants x 2 cultivars) in the Spring experiment RL per phytomer (RL_p) was determined using the grid intersect method (Tennant, 1975) (see Section 5.3.6 below). Following RL determinations as just described, dry weight per phytomer (RDW_p) of all remaining roots was determined as described in Section 5.3.9. It was envisaged that these data would allow derivation of calibrations for estimating RL from RDW, and also allow comparison of WinRHIZO-derived data with grid intersect counts commonly reported in the literature and so provide improved understanding of previously published data collected by the grid intersect method.

Data obtained from root subsets after WinRHIZO scanning and their derived data (see Section 5.3.10) are presented in Chapter 5. A comparison for the RL data between the grid intersect method and WinRHIZO is given in Appendix 5.3.

5.3.5 Root preservation and processing

In the Autumn experiment, roots selected for RL determination by the grid intersect method or scanning and WinRHIZO® analysis were preserved in 70% alcohol. Remaining roots were dried in a forced air draft oven at 60 ^{o}C for two days, and dry weights of roots from the individual phytomers (RDW_p) were taken. All roots from the Spring experiment were preserved in 70% ethanol and stored until the samples could be processed.

5.3.6 Root length determination by the grid intersect method

As stated in section 5.3.4 to compare the RL between two methods, grid intersect and WinRHIZO, the total root lengths of all individual phytomers (RL_P) for 20 tillers (10 plants x 2 cultivars, 226 phytomers) of the Spring experiment were estimated following the modified Newman method (Tennant, 1975). Intersect counts were made using 1 cm^2 grids and the total length was estimated from the following equation:

$$\text{Root length (cm)} = {}^{\pi}/_{4} \times \text{Intersect count} \times \text{Grid size (cm)}$$

The data are presented in Appendix 5.3 as comparison between root length obtained for similar roots using the two methods – modified Newman versus WinRHIZO. RL data obtained from WinRHIZO for the scanned plants and the WinRHIZO-derived RL (see section 5.3.10) are presented in section 5.4.3.

5.3.7 Root scanning using WinRHIZO® software

Selected roots from the Spring and Autumn experiments (see section 5.3.4) were scanned at the AgResearch Ruakura campus in Hamilton, New Zealand using WinRHIZO® software (Regent Instruments Inc.) (Fig. 5.2). Individual roots were placed in the transparent Regent's water proof tray. Root branches were laid out using a needle and placed on the scanner so as to avoid overlap of adjacent branches. Water was sprayed intermittently so that root branches did not dry while being laid out. After arrangement in this way, roots were scanned in a Regent's standard size scanner, 22 x 30 cm, (STD1600+, Regent Instruments Inc.) to obtain digitized images (.TIFF files). A resolution of 210 dpi (dots per inch) was chosen as optimal for the scanned output. The TIFF files were stored

in computer memory. The .TIFF files for the scanned roots were then analysed to obtain detailed root data using 'WinRHIZO® pro' software. WinRHIZO® pro uses separate colour codes to distinguish the root branches of various diameter classes. The data obtained after analysis (ASCII format) were stored in a Microsoft Excel spreadsheet. For details about the instrument and software see:
http://www.regentinstruments.com/products/rhizo/Rhizo.html.

Fig. 5.2 Root scanner and WinRHIZO® software facilities at AgResearch Ruakura laboratories, Hamilton. The author is scanning the individual roots of different phytomers in order to estimate the rate of root development for the two perennial ryegrass cultivars Alto and Aberdart from Experiments 4 & 5.

5.3.8 Sample structure for WinRHIZO® scanning

A total of 196 individual roots were scanned. Out of those, 122 roots were scanned to define progressive root development at successive phytomers of 2 plants of differing genotypes from each of the two cultivars and from each experiment (Table 5.1). For the Spring experiment, individual roots from all Pr of the selected plants were scanned but for the Autumn experiment only individual roots from alternate root positions were scanned,

because of time constraints. This decision was made because NPr in the Autumn experiment was much higher than in the spring experiment.

Table 5.1 Sampling strategy for root scanning to obtain detailed root data using the WinRHIZO® software to study progressive root development at successive phytomer positions (Pr) of Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments. For No. of phytomers, numbers separated by "+" are for two different plants.

Experiment	Cultivar	Genotypes	No. of phytomers	No. of roots scanned
Spring	Alto	2	11+13	31
	Aberdart	2	10+12	37
Autumn	Alto	2	(8+8)*	32
	Aberdart	2	(8+8)*	22
Total			78	122#

*Even Pr numbers only
#No. roots scanned > No. phytomers because R_p often > 1.

Another 74 individual roots were scanned from particular phytomer positions of several genotypes in order to assess the possible existence of genotype differences in root morphology. Phytomer positions chosen for this assessment of genotype differences were Pr5 and Pr7 from the Spring experiment and Pr11 from the Autumn experiment (Table 5.2). Pr7 of the Spring experiment and Pr11 of Autumn experiment were of comparable age. There were a total of 81 individual roots in this data subset after adding data from 7 roots at positions Pr5, Pr7 and Pr11 of plants included in the sampling recorded in Table 5.1.

Table 5.2 Sample structure to study detailed root morphology of phytomer position (Pr) 5, and Pr7 of plants in the Spring experiment and Pr11of plants in the Autumn experiment for Alto and Aberdart perennial ryegrass cultivars.

Experiment	Cultivar	Phytomer	Genotypes	Roots
Spring	Alto	5	6	15
	Aberdart	5	6	17
	Alto	7	4	8
	Aberdart	7	4	13
Autumn	Alto	11	6	14
	Aberdart	11	4	14
Total				81

The following data were obtained using Winrhizo software for the individual roots after scanning:

i) Length of the individual roots (RL_i) in cm which includes both main axis and branches;

ii) Surface area (cm^2) of the individual roots (RSA_i);

iii) Volume (cm^3) of the individual roots (RV_i);

iv) Mean diameter (mm) of the individual roots (RD_i);

v) RL, RSA and RV of individual roots subdivided into 12 different diameter classes in 0.1 mm gradations from 0.0-1.2 mm;

vi) Total number of tips of the individual scanned roots (RT_i) was counted using Videopro 32® software.

5.3.9 Dry weight estimation for roots preserved in alcohol

In the Spring experiment, all roots were preserved in 70% ethanol after the harvest and root subsets for which RAL was measured before were then selected for scanning (section 5.3.8) and RL determination in grid intersect method (section 5.3.6).

In the Autumn experiment, the selected root subsets for which RAL was previously determined (section 5.3.8) were preserved in 70% ethanol and remaining harvested roots were dried in a herbage drying oven at 60°C for 48 hours to determine their DW.

From both experiments, the roots kept in ethanol for scanning/grid intersects counting were dried again in a herbage drying oven at 60°C for 48 hours after a thorough wash in water. Dry weights so obtained for those roots were corrected by for an assumed 22.4% weight loss of alcohol-soluble sugars during storage (Crush et al., 2010a).

5.3.10 Root data derivation

i) Root dry weight of the individual roots (RDW_i): RDW_i were recorded for the individual phytomers (RDW_P). From those data dry weight of the individual roots (RDW_i) were obtained as RDW_P divided by number of roots per phytomer (R_P), assuming that all roots at the same phytomer accumulated similar dry weight.

ii) **Dry matter deposition rate at each phytomer (DMD_p):** DMD_p (mg Pr^{-1} d^{-1}) was estimated from the difference in root dry weight of that Pr from successively older Pr, assuming the age difference of roots at the successive phytomers was equal to the phyllochron at that Pr (Appendix 5.4). The DMD_P was also expressed in terms of C cost (µmol C) considering 45 µmol C required per mg dry weight (Amthor, 1984). Total amount of net CO_2 exchange per tiller per day in Spring and Autumn experiments was calculated form the data presented in Appendix 4.3, taking leaf area of all leaves per tiller and their net photosynthetic rate (µmol CO_2 s^{-1}) into account. It was assumed that 40% of total assimilated CO_2 gets respired (Danckwerts and Gordon, 1987) and 15% of remaining CO_2 reaches to the root system (Parsons and Robson, 1981). The root construction cost for the individual Pr in mmol CO_2 Pr^{-1} d^{-1} was calculated from DMD_p assuming 45% C present in the root tissues (see Chapter 7). Respiration cost during root growth was assumed to be 17% of DMD_p (Eissenstat and Yanai, 1997). Finally the share of photo-assimilate transported to individual phytomers (Pr^{-1} d^{-1}) was calculated as a ratio between total of deposited plus respired CO_2 and total photo-assimilate from CO_2 that reached the root system. Data are presented in Table 5.15 in Section 5.5.4.

iii) **Root length, surface area, volume and number of tips per phytomer:** After scanning, RL_i, RSA_i, RV_i, RT_i were obtained for the individual roots. Root length (RL_P), surface area (RSA_P), root volume (RV_P) and number of tips (RT_P) per phytomer were obtained as a product of respective root dimension of the individual root and R_P of that particular Pr assuming that all individual roots at that Pr were of similar size.

iv) **Specific root length, surface area, and volume:** The specific root length (SRL, cm mg^{-1}), specific root surface area (SRSA, cm^2 mg^{-1}) and specific root volume (SRV, mm^3 mg^{-1}) of the scanned roots were calculated as the ratio between the respective individual root data for each trait and dry weights for those particular individual roots (RDW_i) at the different phytomer positions.

v) **RL, RSA, RV for roots other than scanned:** There were a total of 2416 individual roots dissected from 1112 individual root-bearing phytomers, from which only 196 roots were scanned (see Section 5.3.8). The RL, RSA and RV of the other harvested roots were estimated by a regression procedure that related SRL or SRSA or SRV to RDW, respectively (Appendix 5.5). Separate

regression equations were required for the roots of different ages. The RL or RSA or RV per phytomer was calculated as the product of RL, RSA or RV per root and R_P.

vi) **Tissue density (mg cm^{-3}):** Tissue density at different phytomers was calculated as RDW divided by RV.

vii) **Dimension corrected surface area:volume ratio ($RSA/RV^{2/3}$):** The ratio between square-root transformed RSA and cube-root transformed RV for the individual roots. Dimension corrected measurement of surface area and volume can be used to establish a useful geometrical relation for the roots of variable geometrical shape. The ratio between surface area and volume might be changed due to variable diameter and length, shape of the roots as the ratio varies among the objects of different geometrical shape (e.g., sphere, cylinder, and ellipse). Simple RSA:RV ratios change markedly with root diameter whereas the dimension corrected ratio are theoretically unaffected by root diameter and might possibly detect any underlying differences in root shape.

viii) **Dimension corrected root length:volume ratio ($RL/RV^{1/3}$):** The ratio between RL and cube-root transformed RV for the individual roots. Again the dimension corrected ratio was explored to try to separate effects of root shape from root size.

ix) **Predicted root development in steady-state conditions:** Assuming root growth at each phytomer was a steady-state process, the new growth from one phytomer position to the next was estimated for RDW_i, RL_i, RSA_i and RV_i by fitting a quadratic curve across the mean values for each Pr, except the youngest 2 phytomers of the Spring and the youngest 4 phytomers of the Autumn experiment. Only the younger 3-9 phytomer positions from the Spring experiment and 5-14 younger phytomer positions from the Autumn experiment which were lying above the daughter tillers (see Section 4.3.5) were included when fitting quadratic curves. For the youngest 2 phytomers of the Spring experiment and the youngest 4 phytomers of the Autumn experiment original data were used. When calculating root diameter of new roots it was assumed that all the newly added roots have the same diameter. The measure of new RDW, RL, RSA, and RV, added at each successively developed phytomer, was estimated by subtraction enumerate the difference between adjacent phytomer positions. The mean diameter of entire roots and newly formed roots at each

phytomer position was calculated from the respective RSA and RL at each phytomer position as follows:

$$\text{Mean root diameter} = \frac{\text{Root surface area}}{\pi \times \text{root length}}$$

5.3.11 Visual scoring for root branching

The order of root branching (primary, secondary, tertiary etc.) at the different phytomers in succession moving down the tiller axis was scored after visual assessment of the scanned roots (.TIFF files). To obtain a branching score-sheet for all scanned roots, phytomer positions were numbered from Pr1 to Pr 'n' (where n is the oldest phytomer) on the X-axis and the order of root branching (primary, secondary, tertiary, or quaternary) was scored on the Y-axis (see Fig. 5.14).

5.3.12 Statistical analysis

For R_p, RDW, RSA, RV an ANOVA structure like that for leaf data was used (Table 5.3, Appendix 4.1). The younger 10 phytomers from the Spring experiment and 16 phytomers from the Autumn experiment were included (Table 5.3). For the experiment × phytomer interaction, only the alternate 8 phytomers (even Pr numbers) of the Autumn experiment were analysed with the first eight phytomers of the Spring experiment (Appendix 5.6).

Table 5.3 ANOVA structure for the root data of Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments to test statistical significance of experiment, cultivar, experiment × cultivar, genotype within cultivar effects and effect of phytomer of each cultivar within experiment.

Experiment	Cultivar	Genotypes	Clonal replicates	Phytomers per tiller	Total phytomers	Phytomers in ANOVA
Spring	Alto	9	3	8-11	253	10
	Aberdart	10	3	9-13	301	10
Autumn	Alto	8	2	15-19	270	16
	Aberdart	8	2	13-21	288	16
Total					1112	1082

For SRL, SRSA, SRV, RD and RT a separate ANOVA structure was used taking data for only the scanned roots. There were a total of 78 phytomers for 2 experiments and 2 cultivars (Table 5.1, Appendix 5.7) for which ANOVA was carried out.
In addition to ANOVA, a PCA was conducted to explore the pattern of association among the various root traits for the scanned roots of similar age (Pr5 and Pr7 of the Spring experiment and Pr11 of the Autumn experiment). This data set comprised 81 roots, and could be analysed for the effect of season, cultivar and genotype for the root data structure presented in Table 5.2 (see Section 5.3.4).

ANOVA for the root traits and the PC scores of the data in Table 5.2 was carried out in SAS (SAS Institute Inc., Cary, NC, USA) using a **non-orthogonal linear contrast** (Appendix 5.8) between Pr5 and Pr7 of the Spring experiment and between Pr7 of the Spring experiment and Pr11 of the Autumn experiment, to assess root developmental differences and seasonal effects on root morphology, respectively.

5.4 Results

5.4.1 Tiller axis description

5.4.1.1 Number of leaf and root-bearing phytomers on the tiller axis

Total number of leaves produced during the growth period (NLA), number of live leaves at harvest (NLL), total number of root-bearing phytomers at harvest (NPr) and total number of phytomers (NP) produced over 88±4 d growing duration in the Spring experiment were significantly lower than those produced over the 87±4 d in the Autumn experiment (Table 5.4). Aberdart had significantly higher NLA, NLL, NPr, NP than Alto (Table 5.4). A cultivar x experiment interaction was statistically significant for NLL at harvest, NLA and NP but non-significant for NPr at harvest (Table 5.4). There was no consistent significant difference between the two experiments or two cultivars for *de* at a particular phytomer but a cultivar x experiment interaction for *de* was noted (Table 5.4).

Table 5.4 Tiller axis statistics for total number of leaf appearance events (NLA), number of live leaves (NLL) and number of live root-bearing phytomers (NPr) counted at harvest, delay between leaf and root appearance at harvest (*de*) and total number of phytomer positions developed on the tiller axis (NP) in Spring and Autumn experiments for two perennial ryegrass cultivars, Alto and Aberdart.

	NLA	NLL	*de*	NPr	NP	NPr/NLA	NPr/NLL
Alto-Spring	9.74	6.89	5.3	9.70	15.0	0.996	1.42
Aberdart- Spring	10.4	8.38	4.7	10.4	15.1	1.00	1.26
Alto-Autumn	14.8	8.38	4.8	16.8	21.6	1.14	2.02
Aberdart- Autumn	16.4	12.2	5.5	18.1	23.6	1.11	1.50
SEM	0.32	0.23	0.115	0.406	0.43	0.008	0.03
p (Experiment)	<0.001	<0.001	0.58	<0.001	<0.001	<0.001	<0.001
p (Cultivar)	0.004	<0.001	0.87	<0.001	<0.001	0.19	<0.001
p (Experiment x Cultivar)	0.042	<0.001	0.006	0.24	0.012	0.13	<0.001

SEM, standard error of mean; p, statistical probability

5.4.1.2 Seasonal variation on age of roots at successive Pr

In the Spring experiment, NLA and NPr per tiller were nearly equal (Table 5.4). Roots for Pr10 were estimated to be 89 days old for Alto in the Spring experiment (Table 5.5) but P10 was estimated to be only 77 days old (Table 4.5). For Aberdart in the same experiment the age of Pr10 was estimated to be 86 days (Table 5.5) and 79 days for P10 (Table 4.5). As expected, the data indicated that in spring leaves were forming at a faster rate compared to roots.

Table 5.5 Estimated age of roots at the different root-bearing phytomers (Pr) of successive developmental stages for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments.

	Age of root-bearing phytomers at harvest (d)			
Pr	Alto-Spring	Aberdart-Spring	Alto-Autumn	Aberdart-Autumn
1	4	3	6	5
2	12	11	11	10
3	21	19	16	14
4	29	27	21	18
5	38	34	26	24
6	46	43	31	28
7	56	53	36	33
8	67	62	40	37
9	78	74	44	41
10	89	86	48	45
11			52	48
12			57	51
13			63	54
14			69	59
15			73	65
16			78	71
17			83	75
18				80

In the Autumn experiment, a significantly higher NPr was recorded compared to leaf appearance for both cultivars (Table 5.4). Both Alto ($p<0.01$) and Aberdart ($p=0.048$) produced significantly more leaf bearing phytomers than root-bearing phytomers over the study period. The age at P15 for Alto was 81 days (Table 4.5), while that for Pr15 was 73 days (Table 5.5). The age of P17 for Aberdart was 80 days (Table 4.5) whereas that for Pr17 was 75 days (Table 5.5). Data indicated that in autumn leaves appeared at a lower rate than roots**.**

5.4.1.3 Number of roots per phytomer position (R_p) in the Spring and Autumn experiments

The mean R_p for the cultivar Alto was 1.66 in the Spring experiment and 2.39 in the Autumn experiment. R_p varied significantly between the two experiments ($p<0.01$, Fig. 5.3). The variation was non-significant between the two cultivars ($p=0.13$). Mean R_p for Aberdart was 1.9 in the Spring experiment and 2.66 in the Autumn experiment. No significant cultivar x experiment interaction was noted for R_p ($p=0.95$) but differences

between genotypes of each cultivar varied significantly (p=0.032). R_p values were not significantly different between phytomer positions within experiment and cultivar (p=0.89).

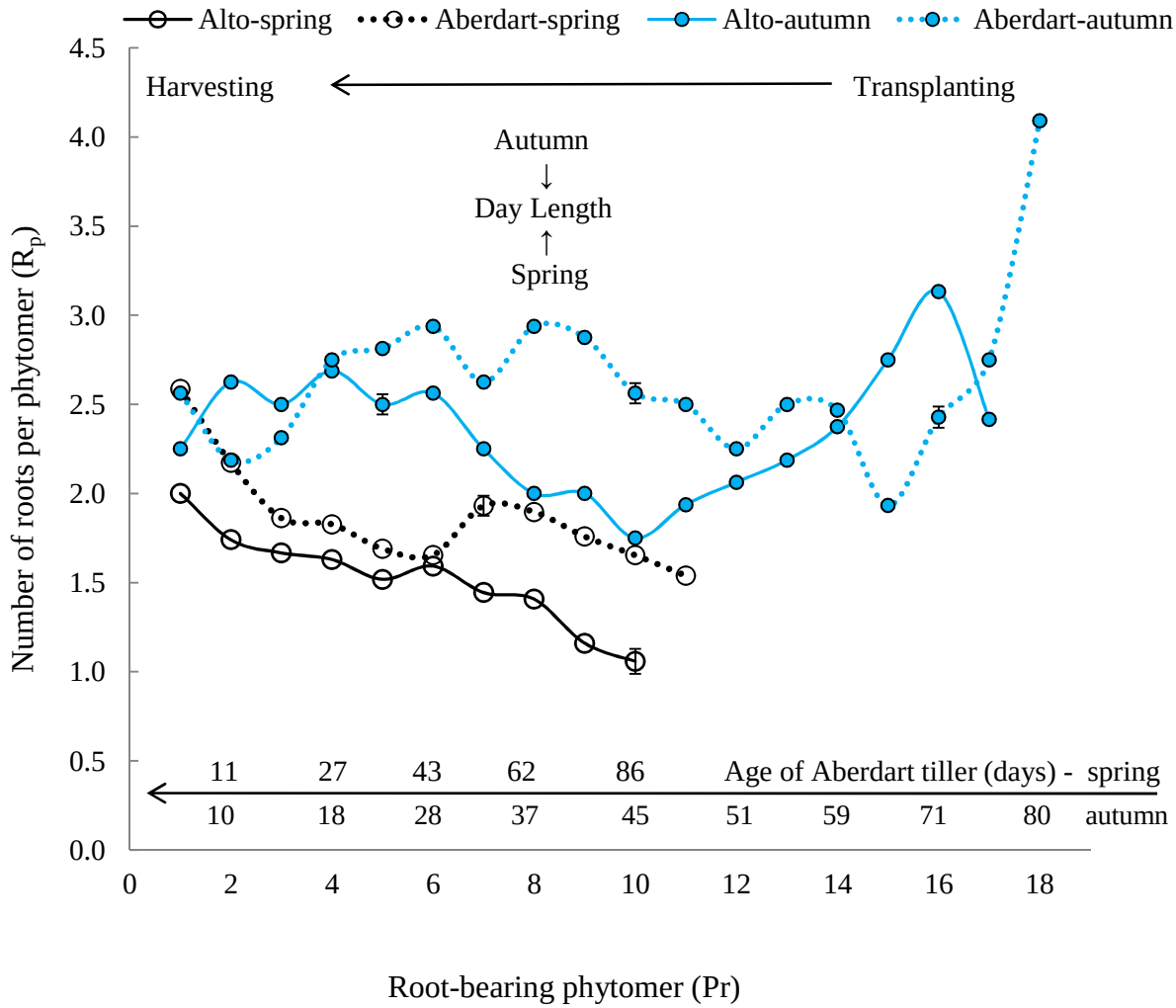


Fig. 5.3 Number of roots per phytomer for Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments. Time scale gives age of Aberdart phytomers. For Alto phytomers add approximately 10% (spring) or 5% (autumn) to the number of days on the time scale. Root-bearing phytomers are counted from the youngest phytomer with roots. Vertical bars show standard error of means for experiment x cultivar interactions.

5.4.2 Progression of root development at successive phytomers

5.4.2.1 Root dimensions at successive phytomers

RDW_i at successively developing phytomers increased linearly up to phytomer 6-7 (Fig. 5.4) for both of the cultivars, Alto and Aberdart, in both Spring and Autumn experiments. This general increasing trend up to 6-7 phytomers was also observed for RDW_p (Fig. 5.5). The DMD_p was highest at Pr1 and showed a decreasing trend from Pr1 to successively older phytomers (Fig. 5.6). For Alto in the Spring experiment DMD_p was close to zero at Pr7. For Alto in the Autumn experiment and Aberdart in both experiments DMD_p was close to zero at Pr11 (Fig. 5.6). This data indicated that DMD_p dramatically decreased at the lower phytomers after 45-50 days from root initiation. RDW_i at the phytomers with roots older than 45 days was similar (Fig. 5.4). In the Spring experiment, the value of R_p for Alto at Pr 8-10 and Aberdart at Pr 8-11 was lowest for the oldest phytomers and gradually increased for successively younger phytomers (Fig. 5.3), and RDW_p also followed a similar pattern (Fig. 5.5). The mean RDW_p and DMD_p were significantly higher in the Autumn experiment (Table 5.6) than the Spring experiment while no significant difference was noted between experiments for RDW_i (Table 5.6). The mean DMD_p for Aberdart was marginally higher than Alto (Table 5.6). No experiment x cultivar interaction for RDW_i, RDW_p or DMD_p was noted (Table 5.6). Significant differences in RDW_i and RDW_p between genotypes of each cultivar were noted (Table 5.6). DMD_p was not different between genotypes within cultivars (Table 5.6).

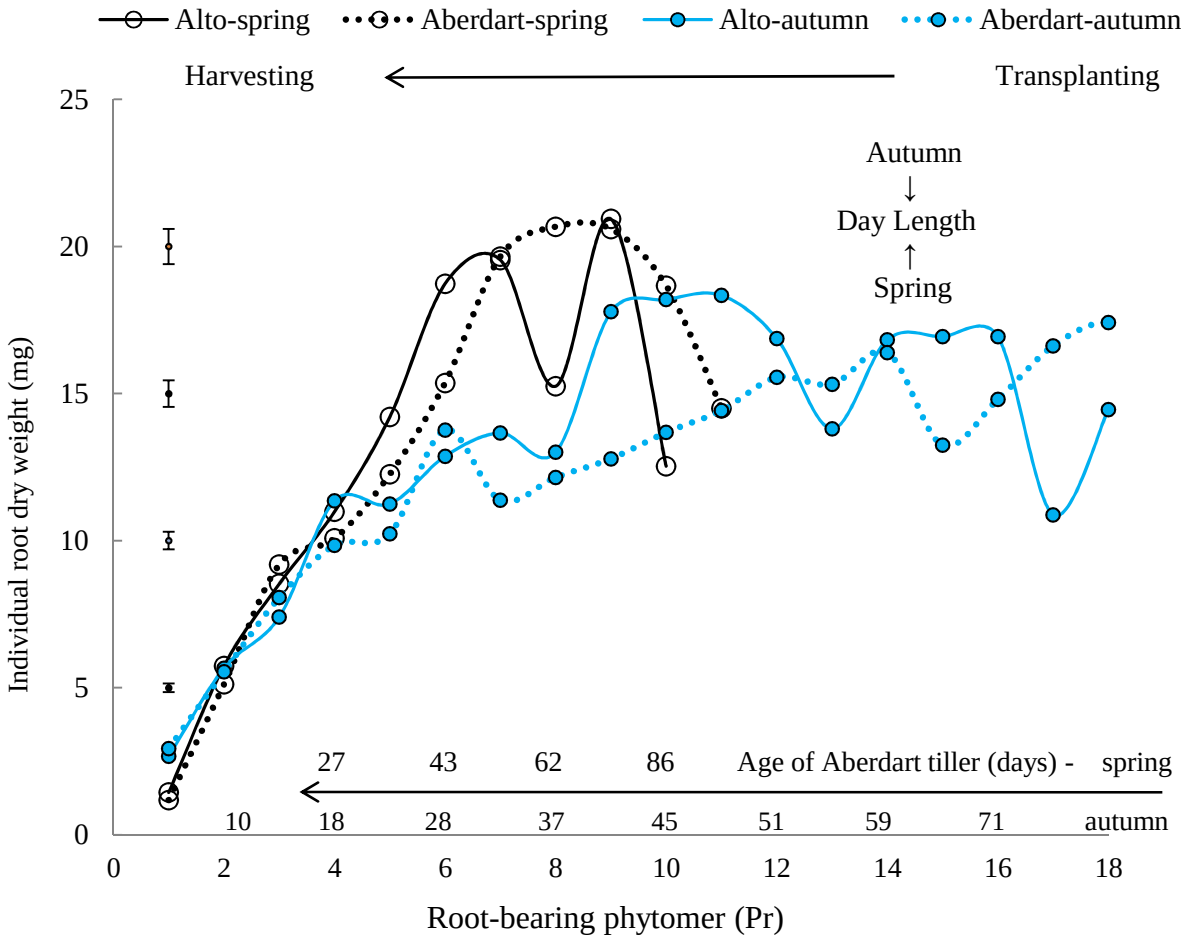


Fig. 5.4 Individual root dry weight (mg) at different phytomer positions for Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments. Time scale gives age of Aberdart phytomers. For Alto phytomers add approximately 10% (spring) or 5% (autumn) to the number of days on time scale. Root-bearing phytomers are counted from the youngest phytomer with roots. Vertical bars show standard error for the log-transformed data for experiment x cultivar means presented as % root dry weight.

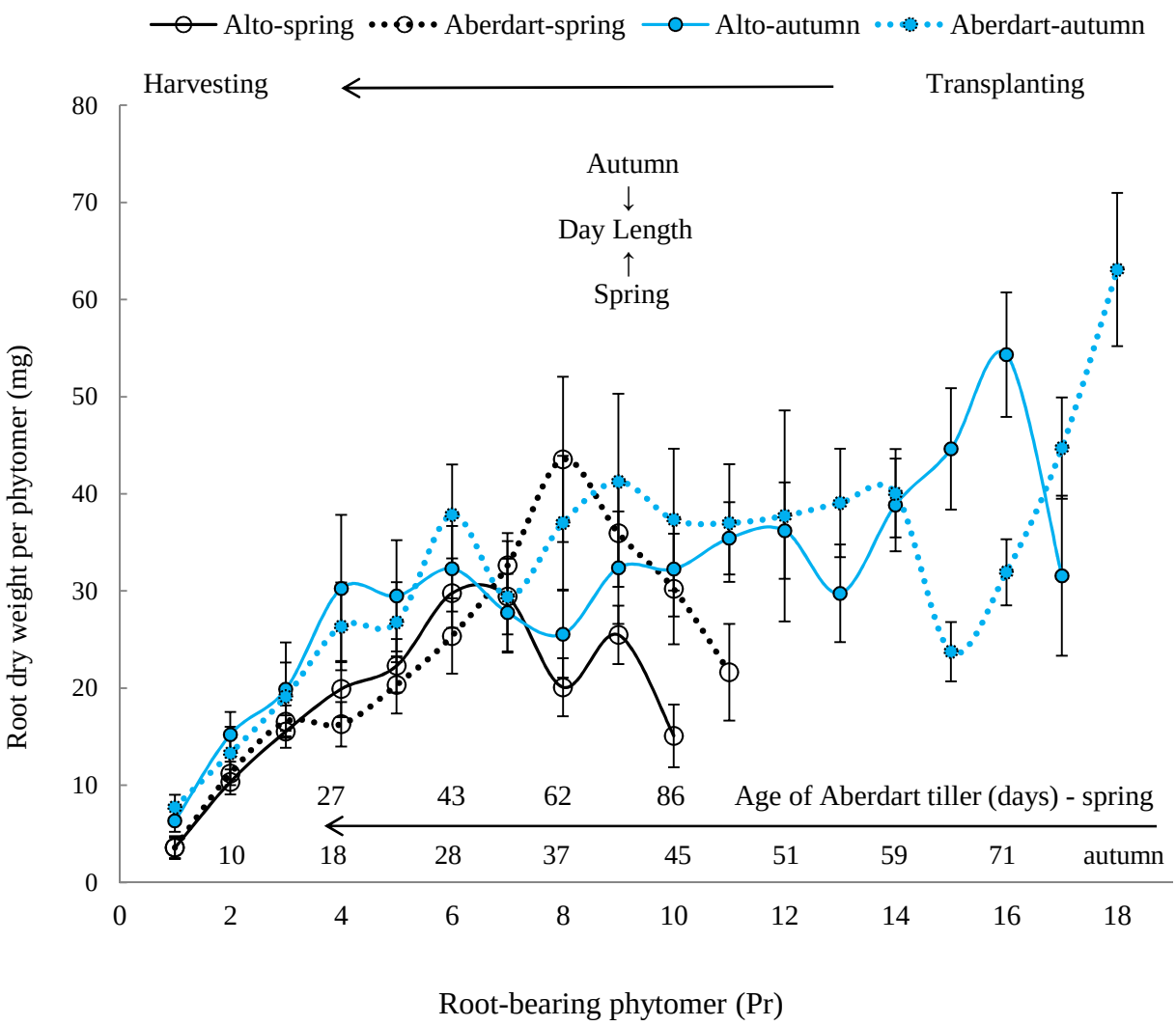


Fig. 5.5 Root dry weight per phytomer (mg) for Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments. Time scale gives age of Aberdart phytomers. For Alto phytomers add approximately 10% (spring) or 5% (autumn) to the number of days on time scale. Root-bearing phytomers are counted from the youngest phytomer with roots. Vertical bars show standard error of means at each phytomer for each cultivar in each experiment.

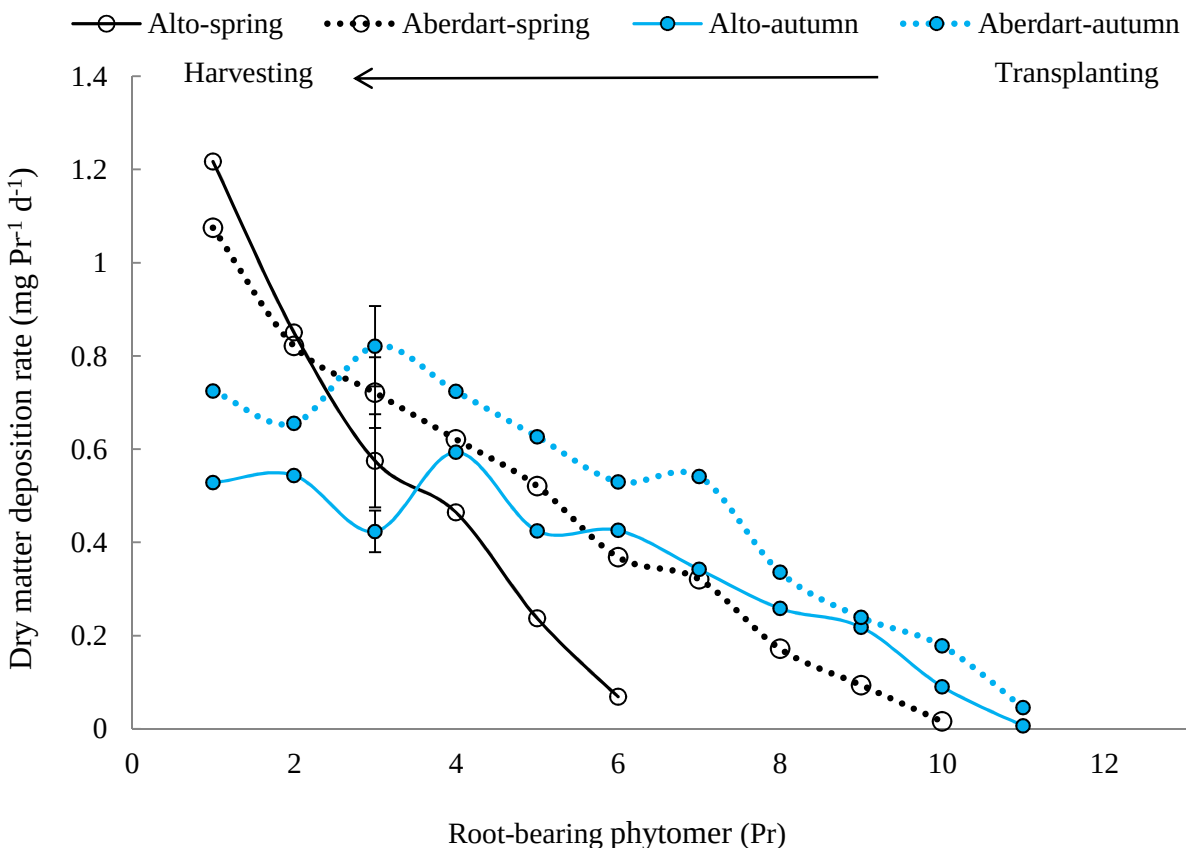


Fig. 5.6 Root dry matter deposition rate (mg Pr^{-1} d^{-1}) for Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments (Experiment 4 & 5, respectively). Root-bearing phytomers are counted from the youngest phytomer with roots. Vertical bars show back-transformed standard error of means in % of the log-transformed data for cultivar x experiment.

Table 5.6 Statistical probability values for effect of experiment, cultivar, experiment x cultivar interaction, genotype within cultivar, phytomer of each cultivar and experiment x phytomer interaction on various root dimensions of Alto and Aberdart perennial ryegrass cultivars for the Spring and Autumn experiments.

Variables	Experiment	Cultivar	Experiment x cultivar	Genotype Within Experiment cultivar	Phytomers Within Experiment cultivar	Experiment x phytomer
RDW_p	0.007	0.66	0.71	0.01	<0.001	0.73
RDW_i	0.15	0.23	0.97	0.007	<0.001	0.037
DMD_p	0.012	0.085	0.92	0.79	<0.001	0.01
RAL	<0.001	0.14	-	-	<0.001	0.76
RL_p	0.117	0.95	0.07	0.03	<0.001	0.35
RL_i	0.004	0.156	0.224	0.007	<0.001	0.116
SRL	0.844	0.536	0.649	-	0.001	-
TD	0.197	0.952	0.04	-	0.535	-
RD_i	0.22	0.32	0.116	-	<0.001	-
RSA_p	0.18	0.93	0.026	0.044	<0.001	0.07
RSA_i	0.004	0.26	0.074	0.009	<0.001	0.003
SRSA	0.384	0.891	0.352	-	0.003	-
RV_p	0.01	0.35	0.002	0.055	<0.001	<0.001
RV_i	0.15	0.046	0.004	0.01	<0.001	<0.001
SRV	0.36	0.90	0.05	-	0.26	-
RT_p	0.047	0.97	0.53	-	0.006	-
RT_i	0.93	0.50	0.33	-	0.123	-
RL_i/RT_i	0.22	0.315	0.494	-	0.016	-

The RL_i increased up to phytomer 9 for Alto in the Spring experiment (Pr8 was the exception) and up to Pr8 in Aberdart (Fig. 5.7). In the Autumn experiment, RL_i increased up to Pr15 for Alto and up to Pr11 for Aberdart (Fig. 5.7). In the Autumn experiment, Aberdart exhibited lower RL_i at Pr13 – Pr15 (20–30 days after transplanting) (Fig. 5.7) when highest temperatures were recorded (28–33^{o}C; Fig. 4.2). RAL increased up to Pr6-Pr7 in both cultivars and experiments (Fig. 5.8). Specific root length (SRL) also increased up to Pr6–Pr7 in both cultivars and experiments (Fig. 5.9). A pattern of increase in RL_i (Fig 5.7) and SRL (Fig 5.9) at older phytomers together with a decrease in mean root

diameters with increasing phytomer age (Fig. 5.10) was noted and likely indicated the addition of comparatively finer root branches at older phytomers. Consistent with this, % distribution of RL, RSA and RV among different diameter classes indicated that as the root growth progressed the proportion of coarse roots decreased and that of fine roots increased gradually (Table 5.7).

No significant differences were noted in RL_p and RD_i between the Spring and Autumn experiments (Table 5.6). RL_i and RAL were significantly higher in Spring than Autumn. RL_i, RL_p, RAL, SRL and RD_i were not significantly different between the two cultivars (Table 5.6). A marginally significant experiment x cultivar interaction was noted in RL_p (Table 5.6). RL_i and RL_p differed significantly among genotypes of each cultivar within experiment. Differences in RL_i, RL_p, RAL, SRL and RD_i were significant among different phytomers within experiment and cultivar and indicated the progression of root development at the successive phytomers (Table 5.6).

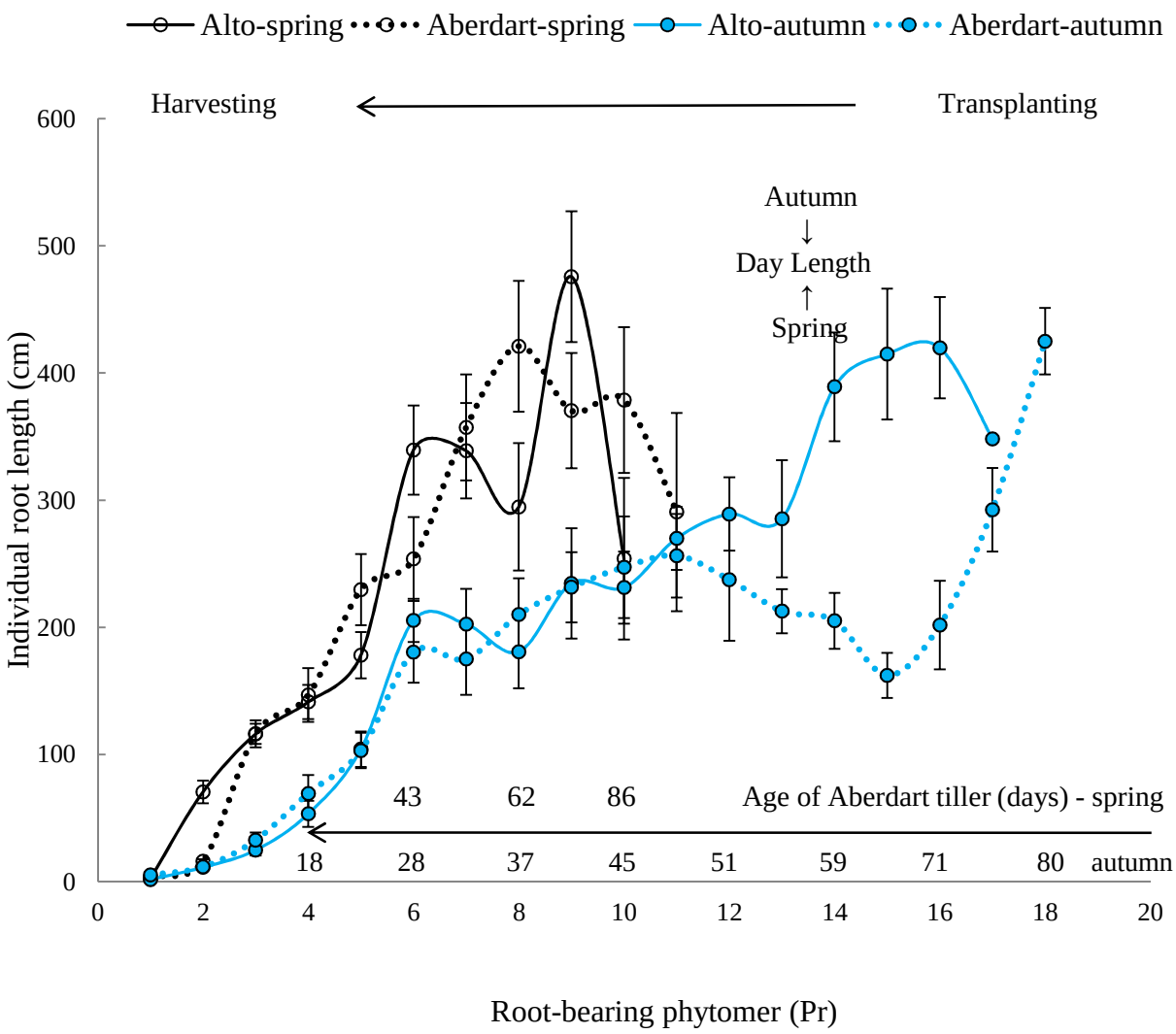


Fig. 5.7 Individual root length (cm) at different phytomers for Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments. Time scale gives age of Aberdart phytomers. For Alto phytomers add approximately 10% (spring) or 5% (autumn) to the number of days on time scale. Root-bearing phytomers are counted from the youngest phytomer with roots. Vertical bars show standard error of means at each phytomer for each cultivar in each experiment.

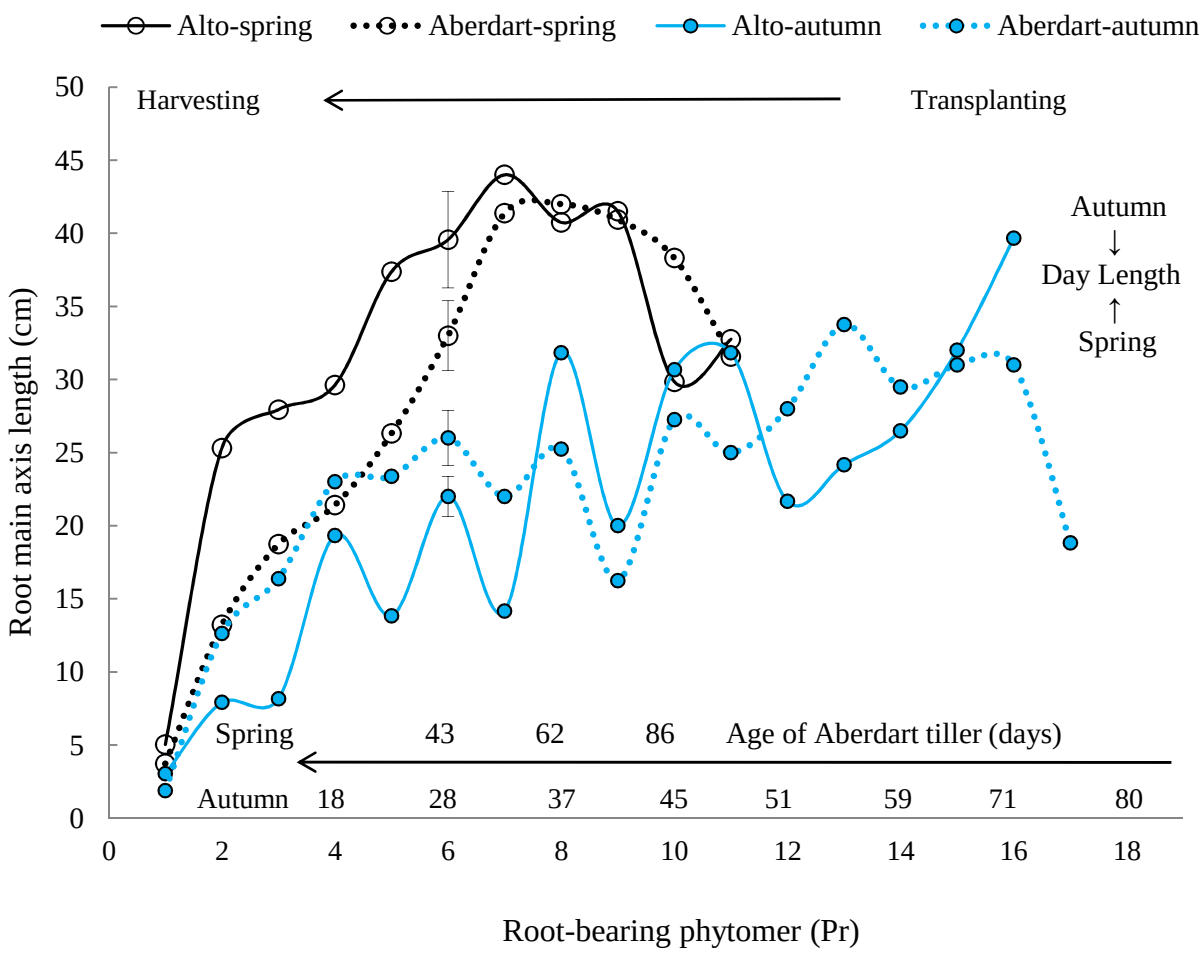


Fig. 5.8 Root main axis length at different phytomer positions for Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments. Time scale gives age of Aberdart phytomers. For Alto phytomers add approximately 10% (spring) or 5% (autumn) to the number of days on time scale. Root-bearing phytomers are counted from the youngest phytomer with roots. The standard errors are derived from those of the log transformed data in for the experiment x cultivar effect and are presented as a % for the untransformed data.

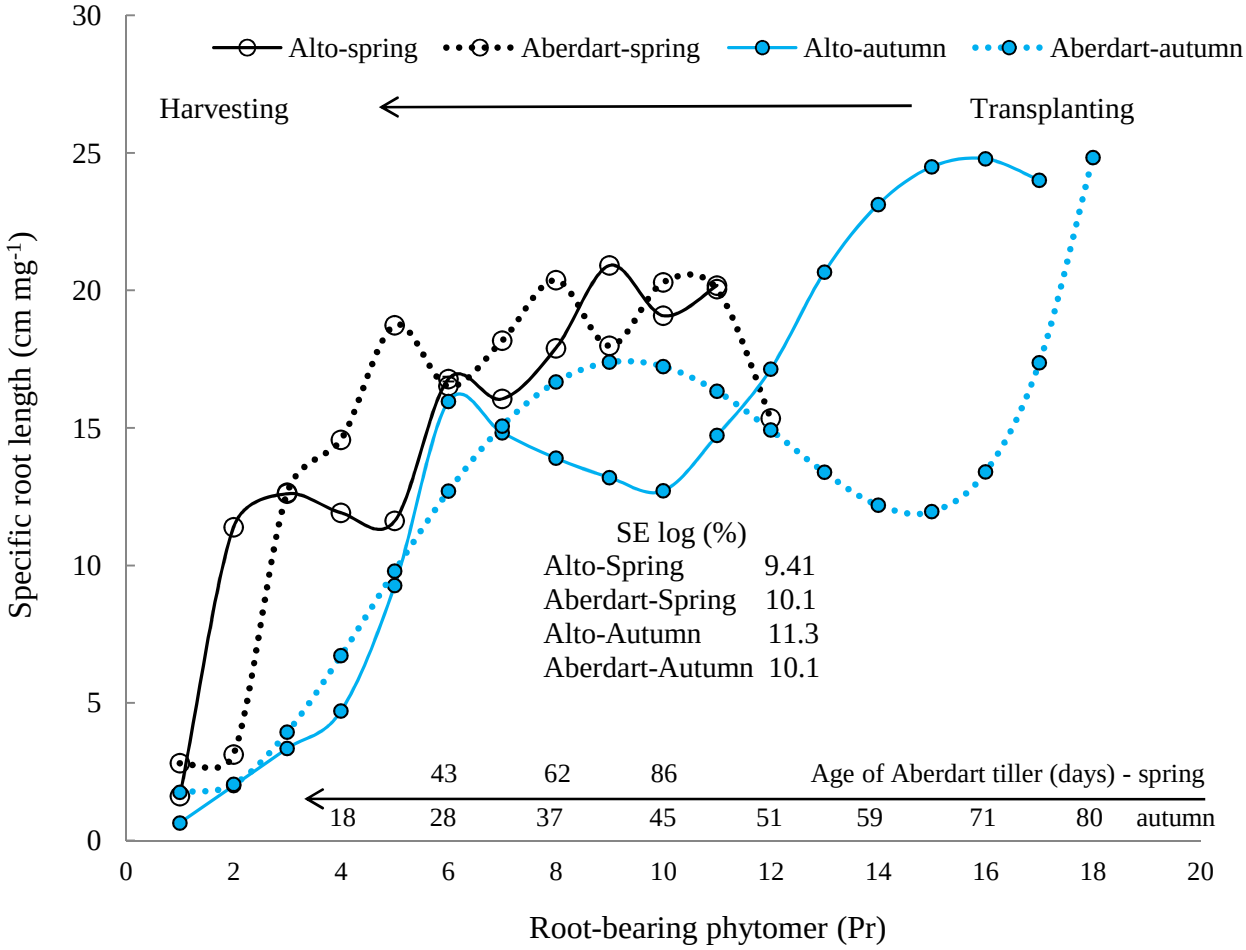


Fig. 5.9 Specific root length (cm mg^{-1}) of Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments. Time scale gives age of Aberdart phytomers. For Alto phytomers add approximately 10% (spring) or 5% (autumn) to the number of days on the time scale. Root-bearing phytomers are counted from the youngest phytomer with roots. The standard errors are derived from those of the log transformed data in for the experiment x cultivar effect and are presented as a % for the untransformed data.

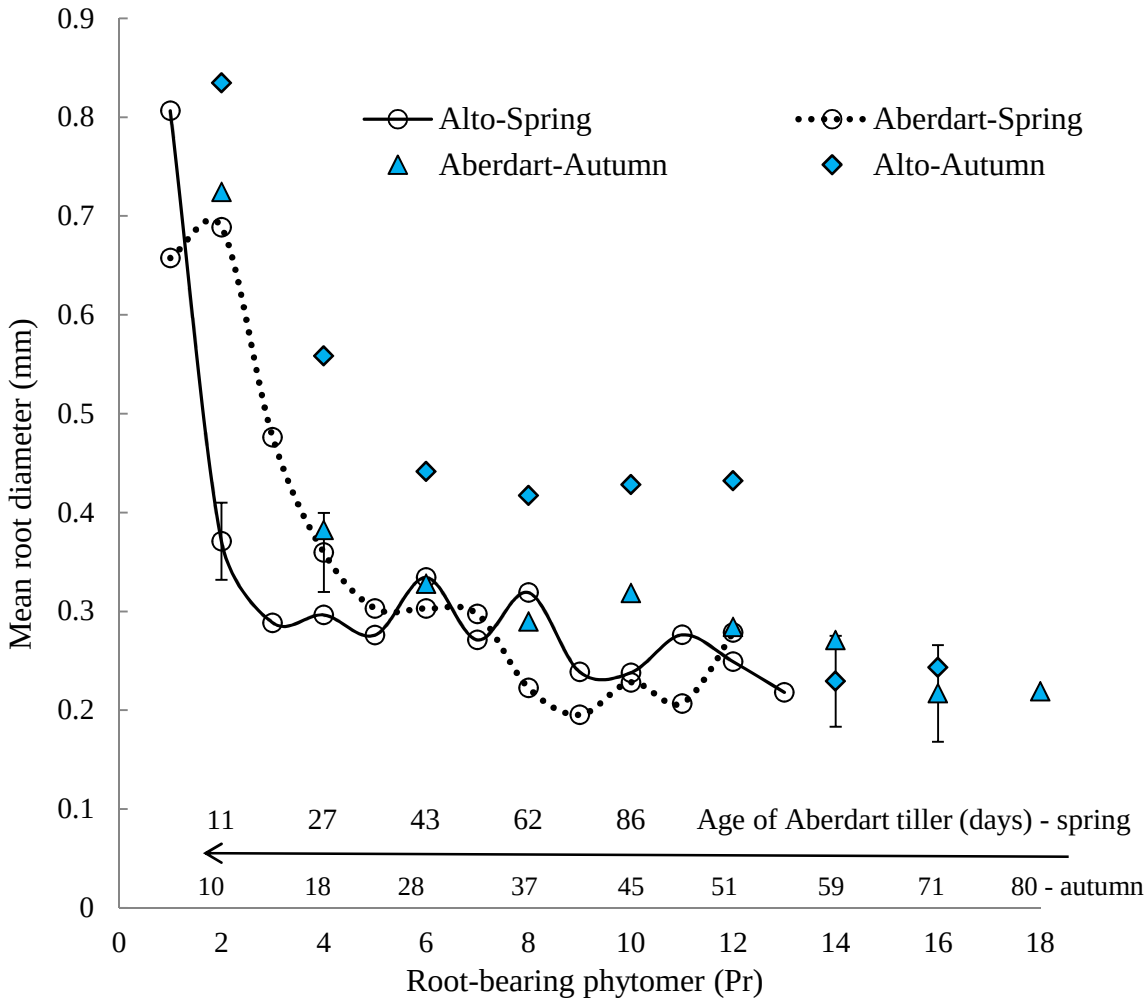


Fig. 5.10 Mean root diameter of the individual roots at different phytomer positions of Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments. Time scale gives the age of Aberdart phytomers. For Alto phytomers add approximately 10% (spring) or 5% (autumn) to the number of days on time scale. Root-bearing phytomers are counted from the youngest phytomer with roots. Vertical bars show back-transformed standard error of means for the experiment x cultivar interactions, presented as % of the untransformed data.

Table 5.7 Mean root diameter (mm) distribution among four diameter classes for (RD) at different phytomer positions (Pr) and % of total root length (RL), root surface area (RSA) and root volume (RV). Each data point is the average of two perennial ryegrass cultivars Alto and Aberdart from two different experiments, the Spring and Autumn experiments.

Pr	RD (mm)	% RL distribution				% RSA distribution				% RV distribution			
		<0.1	0.1-0.2	0.2-0.5	>0.5	<0.1	0.1-0.2	0.2-0.5	>0.5	<0.1	0.1-0.2	0.2-0.5	>0.5
1	0.73	11.9	10.6	27.9	49.6	1.7	3.8	22.2	72.3	0.0	1.0	13.5	85.4
2	0.65	9.9	7.5	30.7	52.0	1.7	3.4	27.1	67.8	0.3	0.9	19.3	79.5
3	0.38	34.5	24.6	34.7	6.3	11.1	16.8	51.1	21.0	2.6	7.3	47.0	43.1
4	0.40	39.9	20.3	26.0	13.9	15.1	14.3	34.2	36.4	3.2	6.3	30.2	60.3
5	0.29	48.9	24.3	21.3	5.5	20.5	21.0	35.5	23.0	5.1	10.4	34.0	50.5
10	0.30	52.0	24.9	16.9	6.1	24.0	22.5	30.7	22.8	7.2	11.1	33.5	48.2

As with RDW, RSA_i and RV_i of the individual roots increased up to Pr6 for both cultivars in both experiments (Fig. 5.11, Fig. 5.12). RSA_i followed a similar pattern to RDW_i for the older phytomers but the RV_i showed a greater decrease at the older phytomers compared to RSA_i (Fig. 5.11, Fig. 5.12). For Alto in the Spring experiment, SRSA greatly increased at Pr2, indicating rapid root branching (Table 5.8). For other experiment x cultivar combinations SRSA gradually increased up to Pr5 or Pr6 (Table 5.8). For both cultivars in the Autumn experiment, SRV was higher at Pr6 compared to any older Pr (Table 5.9) and the regression between Pr and SRV for the Pr1-Pr6 was significant ($p<0.001$ for Alto and $p=0.014$ for Aberdart) indicating that SRV is associated with root age. For Alto in the Autumn experiment, the decreasing pattern of SRV between Pr6–Pr17, with increasing root age was also significant ($p=0.034$). For Aberdart in the Spring experiment SRV was higher at Pr4 than older phytomers (Table 5.9). RSA_i and RV_p were higher in spring than autumn (Table 5.6). RSA_p and RV_i were not significantly different between the two experiments (Table 5.6). RV_i was significantly higher in Alto than Aberdart (Table 5.6). RSA_p, RSA_i, SRSA, RV_p and SRV were not significantly different between cultivars (Table 5.6). The experiment x cultivar interaction was significant for RSA_p, RSA_i, RV_p and RV_i (Table 5.6). No significant experiment x cultivar interaction was noted for SRSA, but for SRV and tissue density (TD) this interaction was significant

(Table 5.6). TD was 97.4±9.6 mg DW cm^{-3} for Alto in autumn followed by Aberdert in spring (105±8.6 mg cm^{-3}), Aberdart in autumn (111±11.1 mg cm^{-3}) and Alto in spring (105±8.2 mg cm^{-3}). Significant differences were noted between genotypes of each cultivar for RSA_i, RSA_p, RV_i and RV_p (Table 5.6) reflecting variation in root branching traits among genotypes. The variation between phytomers within each experiment and cultivar was significant for RSA_i, RSA_p, SRSA, RV_p and RV_i (Table 5.6) indicated that rate of development of roots at different phytomers for the two cultivars in the two experiments was diffferent.

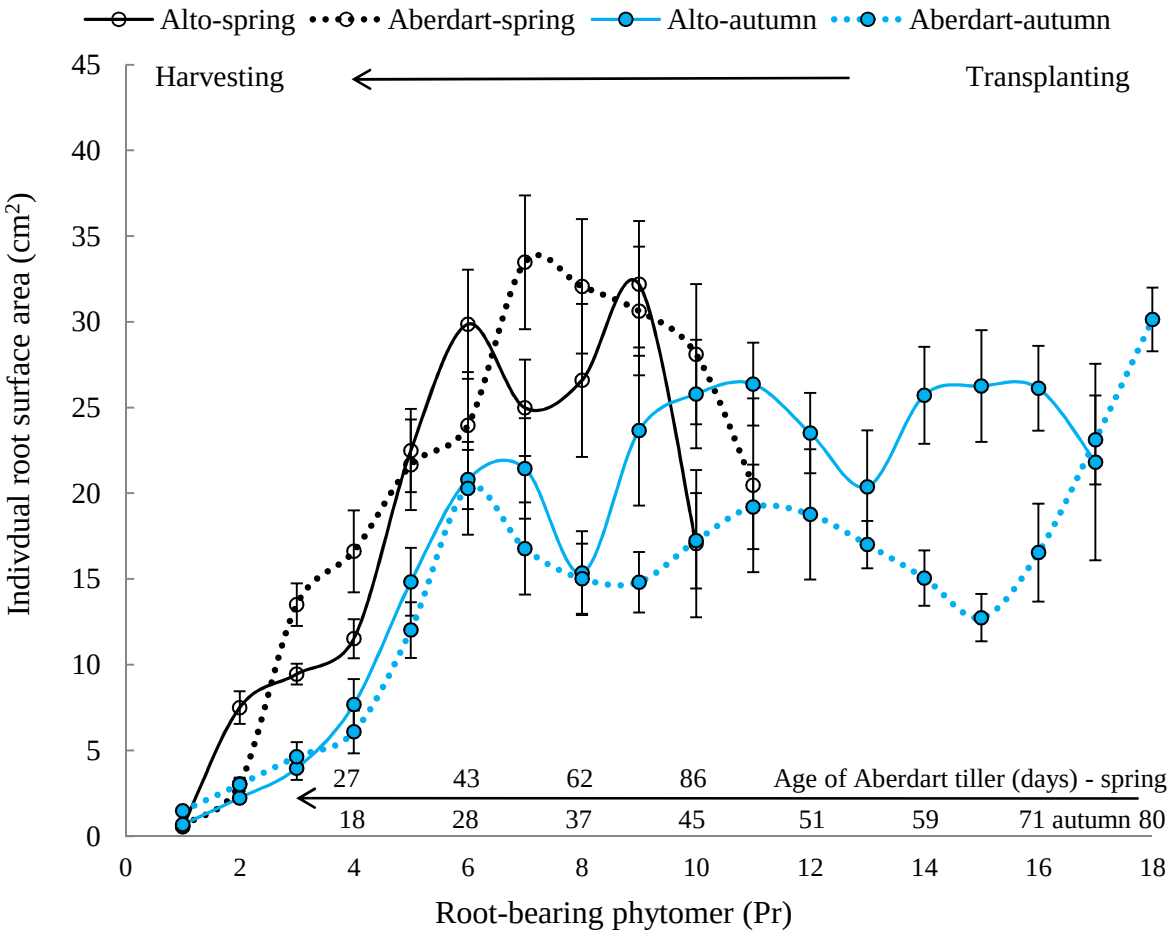


Fig. 5.11 Individual root surface area at different phytomers (cm^2) for Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments. Time scale gives age of Aberdart phytomers. For Alto phytomers add approximately 10% (spring) or 5% (autumn) to the number of days on the time scale. Root-bearing phytomers are counted from the youngest phytomer with roots. Vertical bars show standard error of the means at each phytomer for each cultivar in each experiment.

Table 5.8 Specific root surface area (cm^2 mg^{-1}) at different phytomer positions of Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn. SE(%), standard error back-transformed from logarithmic data for the experiment x cultivar interaction and presented as % of the untransformed data.

Pr	Specific root surface area (cm^2 mg^{-1})			
	Alto-Spring	Aberdart-Spring	Alto-Autumn	Aberdart-Autumn
1	0.4	0.5	0.3	0.5
2	1.2	0.6	0.4	0.5
3	1.0	1.5	0.5	0.6
4	1.0	1.6	0.7	0.6
5	1.5	1.8	1.3	1.1
6	1.5	1.6	1.6	1.4
7	1.2	1.7	1.6	1.4
8	1.6	1.6	1.2	1.2
9	1.4	1.5	1.3	1.1
10	1.3	1.4	1.4	1.2
11		1.3	1.4	1.2
12			1.4	1.2
13			1.5	1.1
14			1.5	0.9
15			1.6	0.9
16			1.5	1.1
17			1.5	1.4
18				1.8
SE (%)	8.55	8.98	10.1	10.8

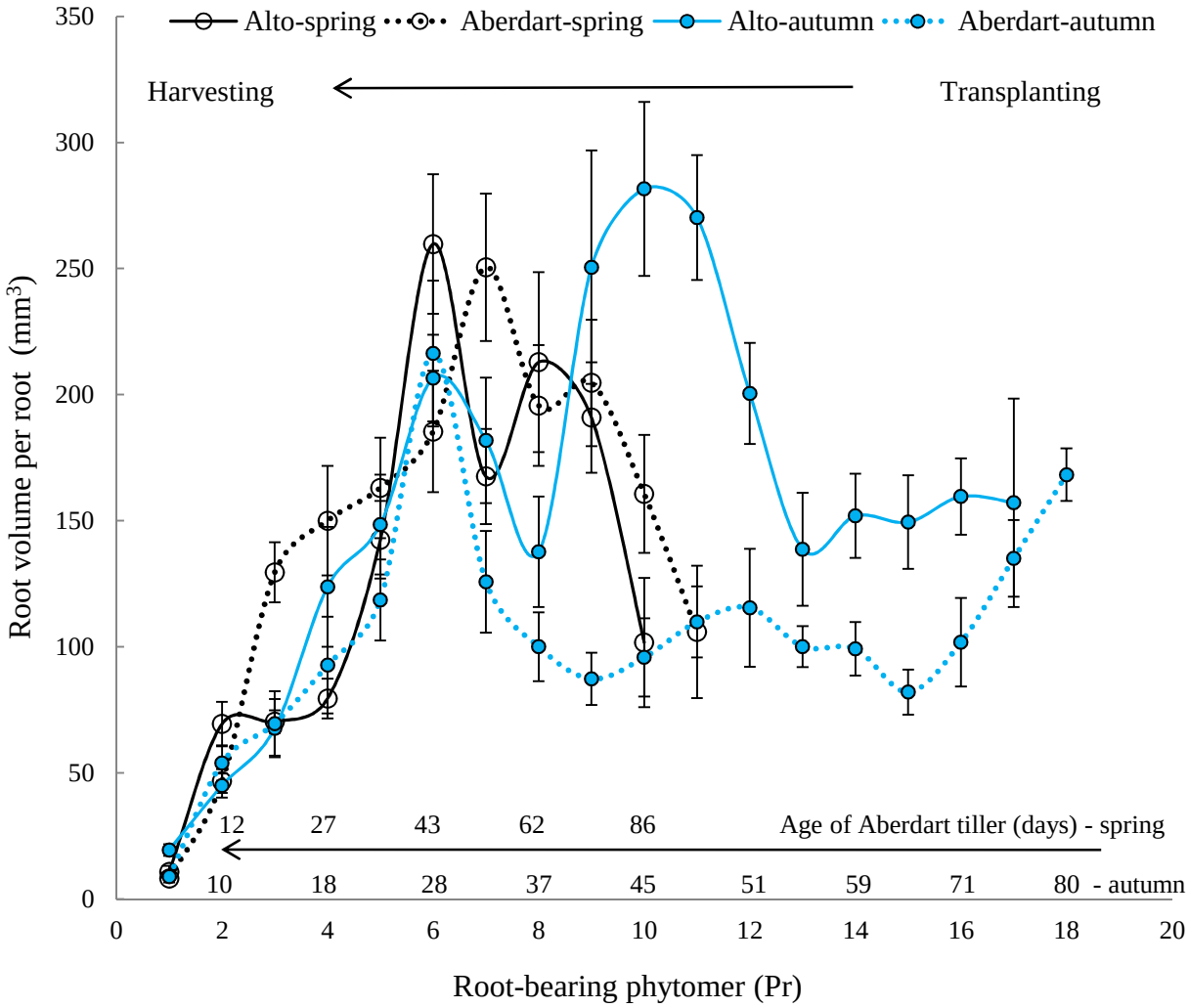


Fig. 5.12 Root volume per root (mm^3) at different phytomers for Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments. Time scale gives age of Aberdart phytomers. For Alto phytomers add approximately 10% (spring) or 5% (autumn) to the number of days on time scale. Root-bearing phytomers are counted from the youngest phytomer with roots. Vertical bars show standard error of the means at each phytomer for each cultivar in each experiment.

Table 5.9 Specific root volume (mm^3 mg^{-1}) at different phytomer positions of Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments. SE(%), standard error back-transformed from logarithmic data for the experiment x cultivar interaction and presented as % of the untransformed data.

Pr	Specific root volume (mm^3 mg^{-1})			
	Alto-Spring	Aberdart-Spring	Alto-Autumn	Aberdart-Autumn
1	6.97	6.92	7.28	3.00
2	11.2	9.15	7.94	9.43
3	7.63	14.1	9.15	8.37
4	6.70	14.9	10.9	8.98
5	9.29	13.3	13.2	11.3
6	12.8	12.1	16.1	15.2
7	7.94	12.7	13.3	10.8
8	12.9	9.47	10.6	7.94
9	8.39	9.94	14.1	6.56
10	7.64	8.28	15.5	6.68
11	9.13	6.74	14.7	7.00
12		9.37	11.9	7.26
13			10.1	6.30
14			9.03	5.90
15			8.82	6.05
16			9.42	6.76
17			10.8	8.02
18				9.84
SE (%)	8.98	9.42	10.6	11.4

Number of root tips per root (RT_i) followed a comparable increasing pattern to RL_i. In the Spring experiment, RT_i increased up to Pr5 for Alto and up to Pr8 for Aberdart. In Autumn, RT_i increased for Alto up to Pr16 and increased for Aberdart up to Pr6. Pr2 had the highest RL:RT ratio for both of the cultivars in spring and for Aberdart in autumn when the main axis elongates rapidly. No significant difference was noted in the RL:RT ratio between spring and autumn roots (Table 5.6) but this ratio was different between phytomers within experiement ($p=0.016$, Table 5.10). RT_p was significantly higher in autumn (275±20.3 tips) than spring (167±17.1 tips) (p value in Table 5.6). The variation among phytomers for RT_p was significant (Table 5.6).

Table 5.10 Number of root tips (RT_i) per root and ratio of root length (RL, cm):RT per root tip at different phytomer positions (Pr) of Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments. SE(%), standard error back-transformed from logarithmic data for the experiment x cultivar interaction and presented as % of the untransformed data.

Pr	RT_i				RL:RT			
	Alto-Spring	Aberdart-Spring	Alto-Autumn	Aberdart-Autumn	Alto-Spring	Aberdart-Spring	Alto-Autumn	Aberdart-Autumn
1	1.33	3.00			0.90	0.89		
2	40.7	2.17	3.50	3.00	4.16	6.59	1.91	5.05
3	97.5	55.8			3.15	3.37		
4	73.3	163	34.5	64.5	1.63	2.07	1.46	1.53
5	212	116			2.29	2.76		
6	123	140	80.3	265	2.36	2.27	1.93	1.53
7	214	136			1.70	3.09		
8	141	208	113	184	2.67	2.69	1.82	1.33
9	162	133			2.30	2.74		
10	177	120	125	189	2.89	2.30	1.52	1.43
11		180				3.09		
12			124	135			2.39	1.78
14			152	171			2.30	2.00
16			162	120			2.55	2.26
18				146				2.91
SE (%)	11.6	12.2	13.8	14.8	22.1	23.3	24.6	28.4

5.4.2.2 Root branching orders and root branching pattern

A total of 4 branching orders (primary, secondary, tertiary and quaternary) were observed after visual scoring (Figs. 5.13, 5.14 and 5.15). In the Spring experiment, Alto exhibited earlier root branching at Pr2 compared to Aberdart, in which root branching started at Pr3 (Fig. 5.14). Alto in spring had the longest phyllochron than any other cultivar × season combination (Fig. 4.5). Root branching at the successive phytomers was continuous and there was overlapping between the order of branching. However, in Fig. 5.15 an approximate Pr-interval for the commencement of each branching order is given. The approximate timing for commencing each phase and estimated C cost at each order of branching are given below:

Branching Phase 0: Only main axis elongation occurs. This phase is limited to the Pr1-Pr2 positions in general. The C expenditure to develop the main axis is much higher than for later phases of root development and ranges between 24 – 55 µmol C Pr^{-1} d^{-1}. This phase is limited to the first 10 days of root development after root initiation.

Branching Phase 1: First order of lateral root branching occurs at the main axis. Main axis elongation is still occurring. Primary root branching typically commenced at Pr2-Pr4, when the individual roots were between 10-20 days of age. The C energy required to develop the primary branches of unit length along with the main axis is comparatively lower than phase 0 but still much higher than that of the higher order branching. The C requirement at this phase ranges between 20 – 40 µmol C Pr^{-1} d^{-1}.

Branching Phase 2: Secondary root branching occurs. Main axis elongation and primary root branching still remain in progress. In the Spring experiment this phase commenced between Pr2 – Pr4 but in the Autumn experiment this phase commenced between Pr4 – Pr6. For both of the experiments phase 2 started at the stage when age of the individual roots was between 25-30 days. C required for this phase ranged between 18-36 µmol C Pr^{-1} d^{-1}.

Branching Phase 3: Tertiary root branching phase. This phase began between Pr5 – Pr7 in the Spring experiment and at Pr8 in the Autumn experiment. Tertiary root branching commenced when individual roots were more than 40 days of age. In this phase main axis elongation had ceased. The C requirement at this phase is very low; between 8 – 24 µmol C Pr^{-1} d^{-1}.

Branching Phase 4: Quaternary root branching phase. This phase commenced when individual roots were approximately 50 days old, and after Pr10. The C requirement for this phase is <8.0 µmol C Pr^{-1} d^{-1}.

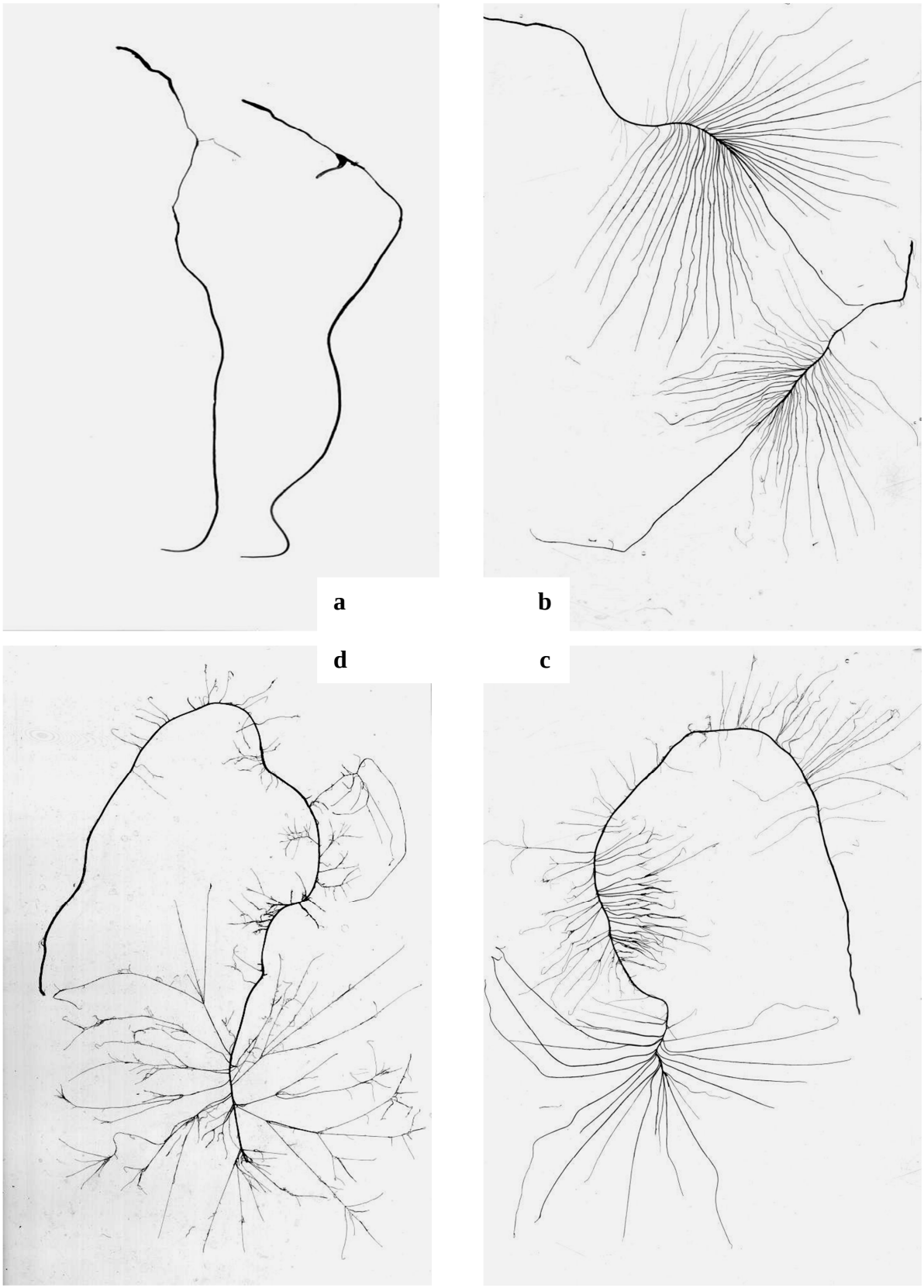


Fig. 5.13 Root branching orders at different phytomer positions of same genotype (a) main axis elongation at phytomer 2, (b) primary root branching at phytomer 3, (c) secondary branching at phytomer 5, (d) tertiary branching at phytomer 11.

Fig. 5.14 Visual scores for individual scanned roots at different order of root branching for different phytomer positions of Alto (A) and Aberdart (B) perennial ryegrass cultivars in Spring and Autumn experiments. To obtain the score-sheet root-bearing phytomers are numbered in X-axis and the orders of root branching are scored on Y-axis.

Spring experiment

0	AAA BB	BBB										
1		B	B		BBBBBBBB		B					
2		AAA	A B	AAA BB	AAAAAAAA BBBBBBBBBB	A BBB	A BBBBBB	AAA BBBB	A BBB	A BB	AA B	A B
3					AAAAAA	AAAA	AAA BBBB	AAA B	A	A B	A	
4											A	
Branching order	Pr1	Pr2	Pr3	Pr4	Pr5	Pr6	Pr7	Pr8	Pr9	Pr10	Pr11	Pr12

Autumn experiment

0	AAAA BB										
1		AAAA B	A	A							
2		A	AAAA B	A B	AAA BBB	AAAAA BBBBB	B		AA BB		
3		B		A BB	AA BBB	AAAA BBBBBBB	AA BB	AA BB	A	B	
4						A		B			
Branching order	Pr2	Pr4	Pr6	Pr8	Pr10	Pr11	Pr12	Pr14	Pr16	Pr18	

There were wide variations in root branching patterns between cultivars and genotypes of each cultivar (Fig. 5.16). Fig 5.16a and Fig. 5.16b illustrate variation between two genotypes of cultivar Alto at Pr5. Fig. 5.16b and Fig. 5.16c illustrate variation between two genotypes of cultivar Alto and Aberdart respectively at Pr5. Fig 5.16c and Fig. 5.16d illustrate variation between Pr5 and Pr8 of Aberdart cultivar in the Spring experiment and in the Autumn experiment respectively. Pr5 in spring and Pr8 in autumn were of comparable age. These variations were separated by positive and negative PC scores in each case for the genotypic difference (Fig. 5.16).

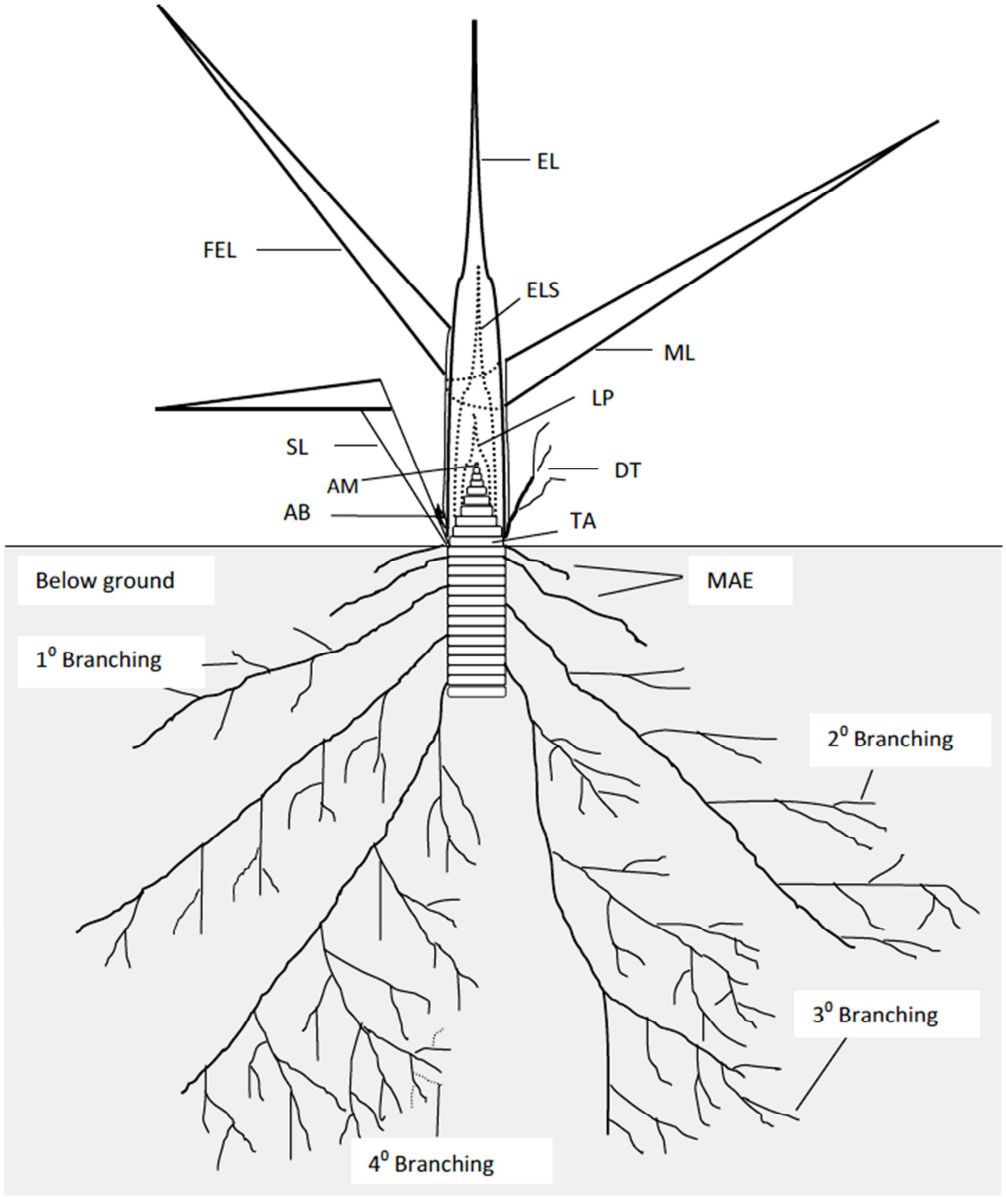


Fig. 5.15 Developmental stages of phytomers in a vegetative grass tiller from the apical meristem to quaternary level of root branching in a stylised diagram. AM, apical meristem; LP, leaf primordium; ELS, elongating leaf inside the pseudostem; EL, elongating visible leaf; FEL, fully elongated leaf; ML, mature leaf; SL, senescing leaf; AB, axillary bud; DT, daughter tiller; TA, tiller axis; MAE, main root axis elongation for the youngest adventitious roots; 1°, 2°, 3° and 4° branching: primary, secondary, tertiary and quaternary level of root branching. For the root branching phases only the alternate root-bearing phytomers are partly drawn. Leaf developmental stages have been drawn taking data from Yang *et al.* (1998). The diagram has been drawn following Matthew et al. (2001).

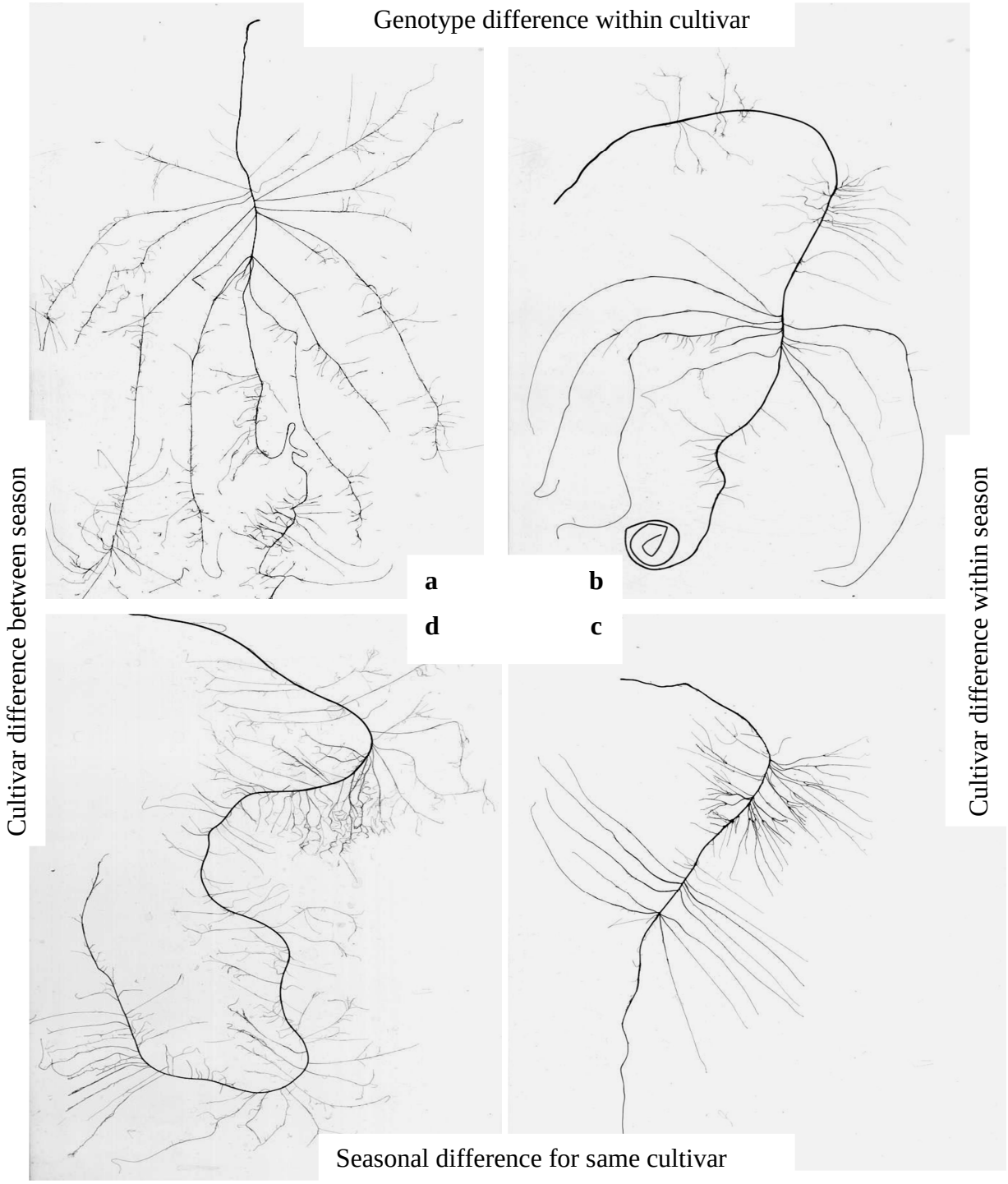


Fig. 5.16 Variation in root branching pattern between genotypes (a, Pr5 versus b, Pr 5 of cultivar Alto in the Spring experiment), between cultivars (b, Pr5 of Alto versus c, Pr 5 Aberdart in the Spring experiment) and between phytomers of similar age in different experiments (c, Pr5 of Aberdart in the Spring experiment versus d, Pr8 of Aberdart in the Autumn experiment). (a) had positive scores for PC1 and negative scores for PC2; (b) had negative scores for PC1 and positive scores for PC2; (c) had negative scores for PC1 and PC2; (d) had positive scores for PC1 and PC2.

5.4.2.3 Root development at successive phytomers

To gain insight into the process of root development over time (and as elsewhere in this chapter using as a proxy for time the status of successive phytomers at the destructive harvest), fitted values of RDW_i, RL_i, RSA_i, and RV_i obtained by quadratic regression were plotted on common axes (Fig. 5.17, Appendix 5.9). Fig. 5.17 is based on data for cultivar Alto in the Autumn experiment and detailed derived data are presented in Appendix 5.9. RL_i continued to increase and RV_i decreased after Pr9 (Fig. 5.17).

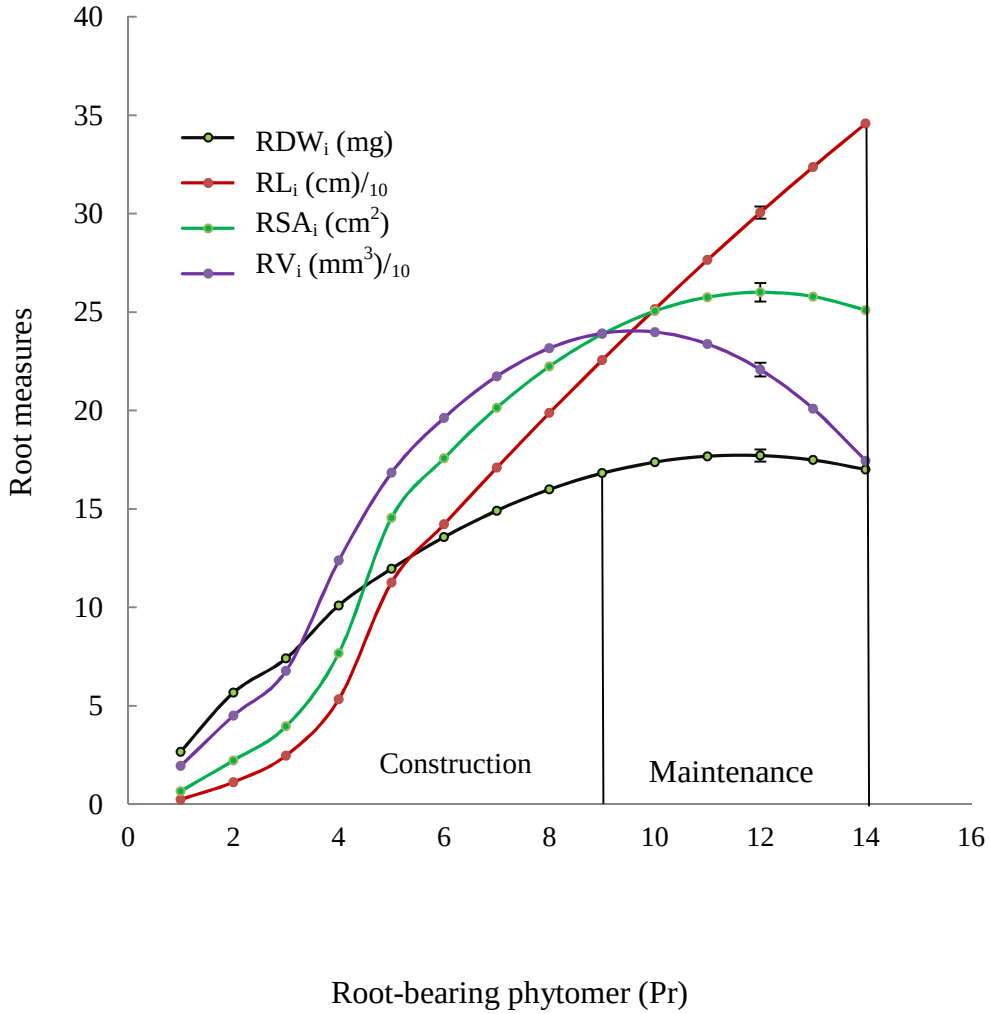


Fig. 5.17 Root measures for the individual roots at different phytomer positions for the Alto perennial ryegrass cultivar in the Autumn experiment. The trend lines between Pr4 and Pr14 were derived from quadratic polynomial equations. RDW_i, root dry weight of the individual roots; RL_i, root length of the individual roots; RSA_i, root surface area of the individual roots; RV_i, root volume of the individual roots.

5.4.3 Detailed examination of selected root-bearing phytomers

Fig. 5.6 illustrated that DMD_p reduces to zero at approximately Pr7 for cultivar Alto in the Spring experiment and Pr11 in the Autumn experiment. In this section these two phytomers are studied in detail. In addition, Pr5 from the Spring experiment is compared with Pr7 of same experiment to study root morphological variations between two phytomers with a maturity difference.

5.4.3.1 Measured variables

Roots of similar age, Pr7 of the Spring experiment and Pr11 of the Autumn experiment, varied significantly between experiments for RAL, RL_i, RSA_i (Table 5.11). Significantly higher values for Pr7 of spring compared to Pr11 of autumn indicated that seasonal variables affected branching pattern and main axis elongation. The Pr7 values were significantly higher for RDW_i, RAL, RL_i, RSA_i, RV_i and RT_i and lower for RD_i than Pr5 of the Spring experiment, reflected the increased development of roots located at the older Pr (Table 5.11). Cultivar Alto had marginally higher RDW_i and RAL and lower R_p at all three phytomers compared to Aberdart (Table 5.11). Genotypes within cultivar and phytomer varied significantly for all of the above measured root variables (Table 5.11).

Table 5.11 Whole root statistics for roots of Pr5 and Pr7 in the Spring experiment and Pr11 in the Autumn experiment. The aim was to compare immature and mature roots in the Spring experiment and roots of a similar developmental stage in the Autumn experiment and in the spring experiment. Pr, root-bearing phytomers; R_p, number of roots per phytomer; RDW_i, individual root dry weight (mg); RAL, root main axis length (cm); RL_i, root length (cm); RSA_i, root surface area (cm^2); RV_i, root volume (cm^3); RD_i, root diameter (mm), and RT_i, number of tips per root for Alto and Aberdart perennial ryegrass cultivars at the Pr5 and Pr7 in the Spring experiment and Pr11 in the Autumn experiment.

Experiment	Pr	Cultivar	Age	R_p	RDW_i	RAL	RL_i	RSA_i	RV_i	RD_i	RT_i
Spring	5	Alto	38	1.61	22.7	43.6	283	29.4	0.26	0.37	137
	5	Aberdart	34	1.74	16.1	18.9	213	21.9	0.18	0.34	96.6
	7	Alto	56	1.00	28.1	47.4	496	45.3	0.33	0.29	228
	7	Aberdart	53	1.67	19.1	45.9	410	35.9	0.26	0.29	141
Autumn	11	Alto	52	2.08	24.4	39.0	395	35.1	0.26	0.30	230
	11	Aberdart	48	3.63	14.2	34.7	289	27.6	0.22	0.31	170
SEM				0.11	1.06	1.37	20.1	1.70	0.01	0.01	9.56
p value											
Pr5 versus Pr7				0.39	0.09	<0.01	<0.01	<0.01	0.04	<0.01	0.009
Pr7 versus Pr11				<0.01	0.20	0.005	0.02	0.05	0.20	0.23	0.22
Cultivar (Pr)				0.056	0.086	0.075	0.50	0.46	0.40	0.79	0.98
Genotype (Pr cultivar)				<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

p, probability of statistical variation

5.4.3.2 Derived variables

SRL, RL/RV, $RL/RV^{1/3}$, RSA/RV and $RSA/RV^{2/3}$ were all significantly higher at Pr5 than at Pr7, reflecting the effect on those traits of the finer mean diameter of older roots (Table 5.12). Values for SRL, SRSA and SRV were significantly lower at Pr7 of the Spring experiment and higher at Pr11 of the Autumn experiment (Table 5.12). The $RL/RV^{1/3}$ and $RSA/RV^{2/3}$ were significant between Pr7 and Pr11 where Pr11 measured lower values. SRV was significantly higher for cultivar Aberdart than Alto (Table 5.12). Significant differences between genotypes within cultivar for each phytomer existed for for all derived root traits except SRV (Table 5.12).

Table 5.12 Derived measures for comparing root morphology: Pr, root-bearing phytomers; SRL, specific root length (cm mg^{-1}); SRSA, specific root surface area (cm^2 mg^{-1}); SRV, Specific root volume (mm^3 mg^{-1} DW); TD, tissue density (mg cm^{-3}); RL/RV, root length per unit root volume (cm cm^{-3}) and $RL/RV^{1/3}$, dimension corrected root length per unit root volume$^{1/3}$; RSA/RV, surface area per unit volume (cm^2cm^{-3}); $RSA/RV^{2/3}$, dimension corrected surface area/volume$^{2/3}$ for Alto and Aberdart perennial ryegrass cultivars at the Pr5 and Pr7 in the Spring experiment and Pr11 in the Autumn experiment.

Exp	Pr	Cultivar	SRL	SRSA	SRV	TD	RL /RV	$RL/RV^{1/3}$	RSA /RV	$RSA/RV^{2/3}$
Spring	5	Alto	13.0	1.31	11.6	91.4	1216	448	118	72.4
	5	Aberdart	13.2	1.36	11.4	109	1187	364	121	66.0
	7	Alto	17.1	1.58	11.5	93.3	1550	704	139	92.5
	7	Aberdart	22.6	2.01	14.8	80.2	1671	646	143	88.9
Autumn	11	Alto	31.1	2.27	10.7	102	1535	608	137	85.4
	11	Aberdart	27.3	2.65	16.6	68.0	1450	488	133	77.9
			1.63	0.14	0.51	4.20	63.0	25.8	2.88	2.03
p value										
Pr5 versus Pr7			0.08	0.20	0.26	0.43	0.007	<0.01	0.002	<0.01
Pr7 versus Pr11			0.006	<0.01	<0.01	0.77	0.24	0.23	0.22	0.05
Cultivar (Pr)			0.86	0.94	0.011	0.046	0.98	0.63	0.97	0.73
Genotype (Pr cultivar)					0.401	0.075	<0.01	<0.01	<0.01	<0.01

p, probability of statistical variation

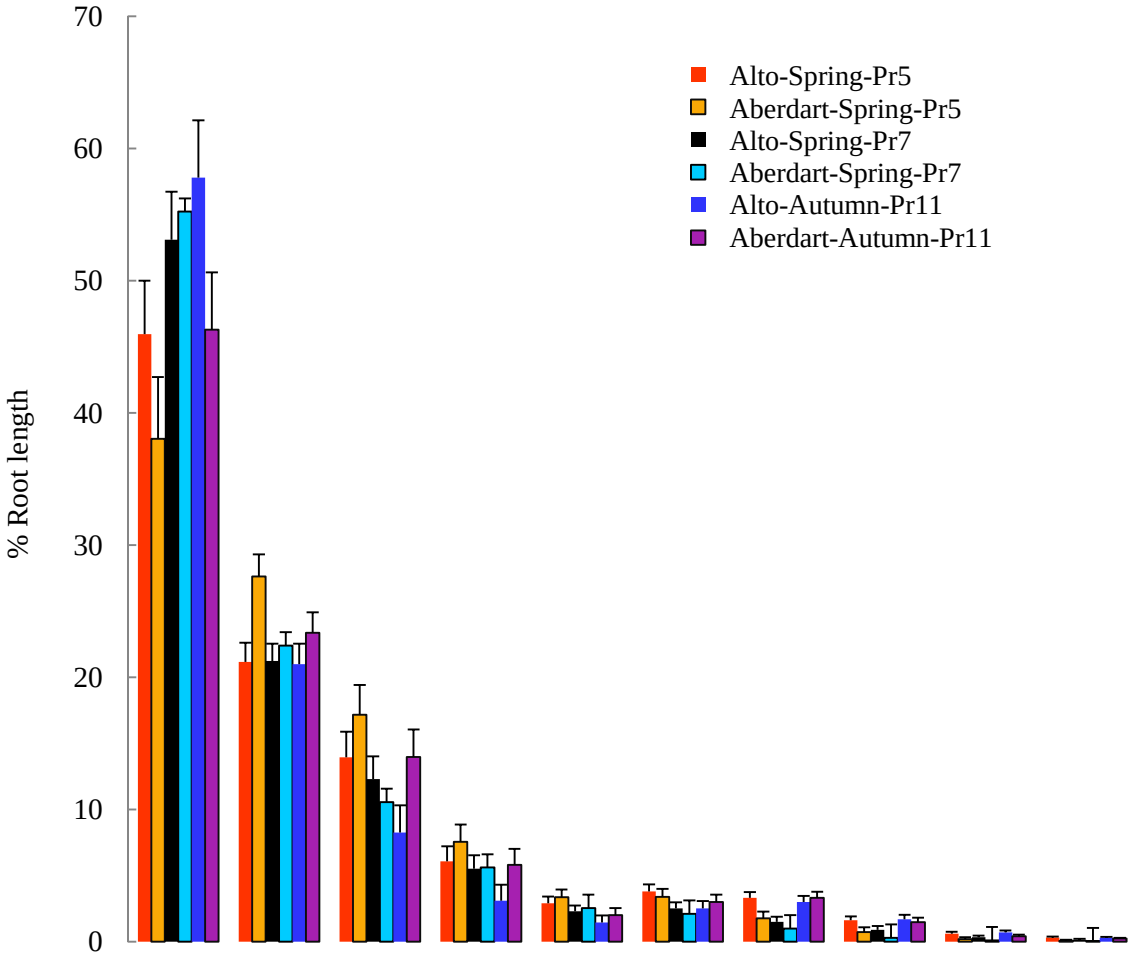
5.4.3.3 Distribution of total root length, surface area and volume among root diameter classes

For Pr5 in the Spring experiment, the finest diameter class of <0.1 mm comprised around 3% of root volume and contributed around 40% of total length and 15% of surface area, while for more mature roots at Pr7 in the Spring experiment and Pr11 in the Autumn experiment the corresponding figures were 5% of RV, 50% of RL, and 20% of RSA for both cultivars, and in both experiments (Fig. 5.18-5.20). The increase in RL and RSA of fine roots between Pr5 and Pr7 in the Spring experiment was significant, but the cultivar and experiment variations were not significant for these traits.

Roots of the diameter class 0.1-0.2 mm comprised around 23% RL, 20% RSA and 10% RV (Fig. 5.18-5.20). Two cultivars showed highly significant difference for % RL, RSA and RV at this diameter class. The cultivar Aberdart at all three phytomers had higher RL, RSA and RV compared to Alto. The differnce in RV between Pr5 and Pr7 and that between Pr7 of the Spring experiment with Pr11 of the Autumn experiment were significant (Fig. 5.20).

Around 13% RL, 20% RSA and 13% RV of the individual roots were contributed by the diameter class of 0.2-0.3 mm roots (Fig. 5.18-5.20). The first three diameter classes between 0.0-0.3 mm diameter ranges showed inversely proportional relations between RL and RV indicated that the finer roots measure higher RL but lower RV. The root surface area of the finer roots measured very high as these three diameter classes cumulatively estimated around 60% of the RSA of the individual roots.

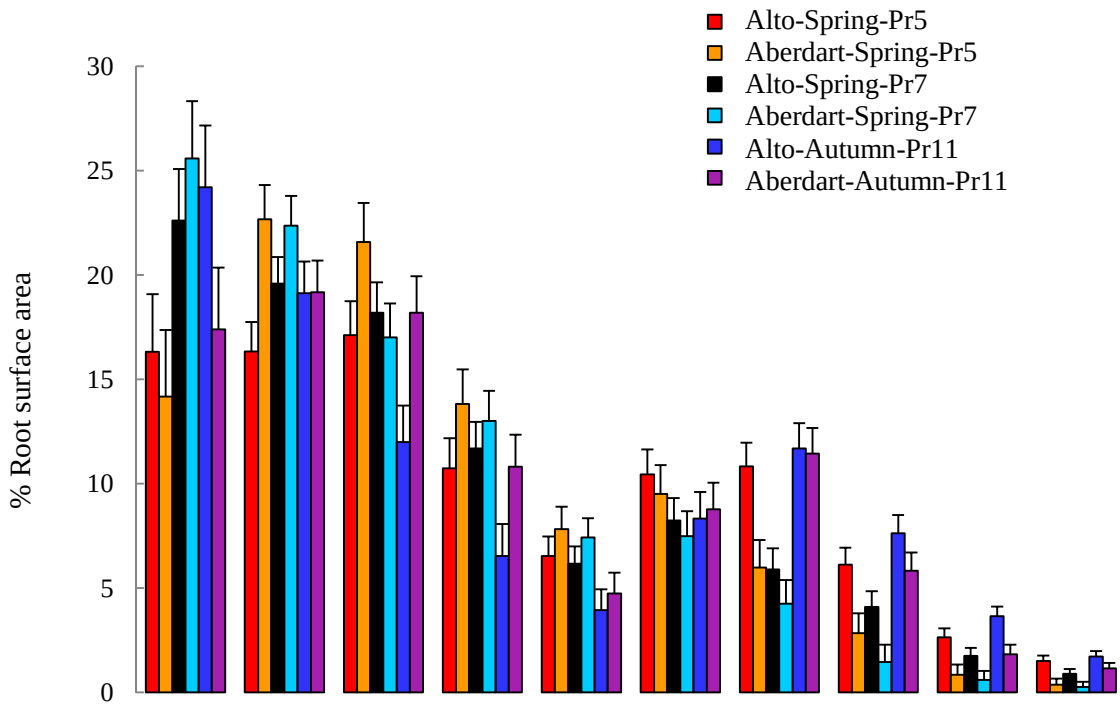
The root diameter classes between 0.3-0.6 mm contributed around 3-6% to RL, 6-11% to RSA and 9-15% to RV (Fig. 5.18-5.20).



Diameter classes	<0.1	0.1-0.2	0.2-0.3	0.3-0.4	0.4-0.5	0.5-0.6	0.6-0.7	0.7-0.8	0.8-0.9	0.9-1.0
Cultivar	0.26	0.001	0.24	0.51	0.33	0.61	0.003	<0.001	<0.001	0.004
Pr (5-7)	<0.001	0.09	0.015	0.23	0.032	0.001	0.001	0.011	0.019	0.063
Pr (7-11)	0.83	0.93	0.66	0.10	0.11	0.22	<0.001	0.007	0.018	0.008

Root length (%) and p value at each diamater class

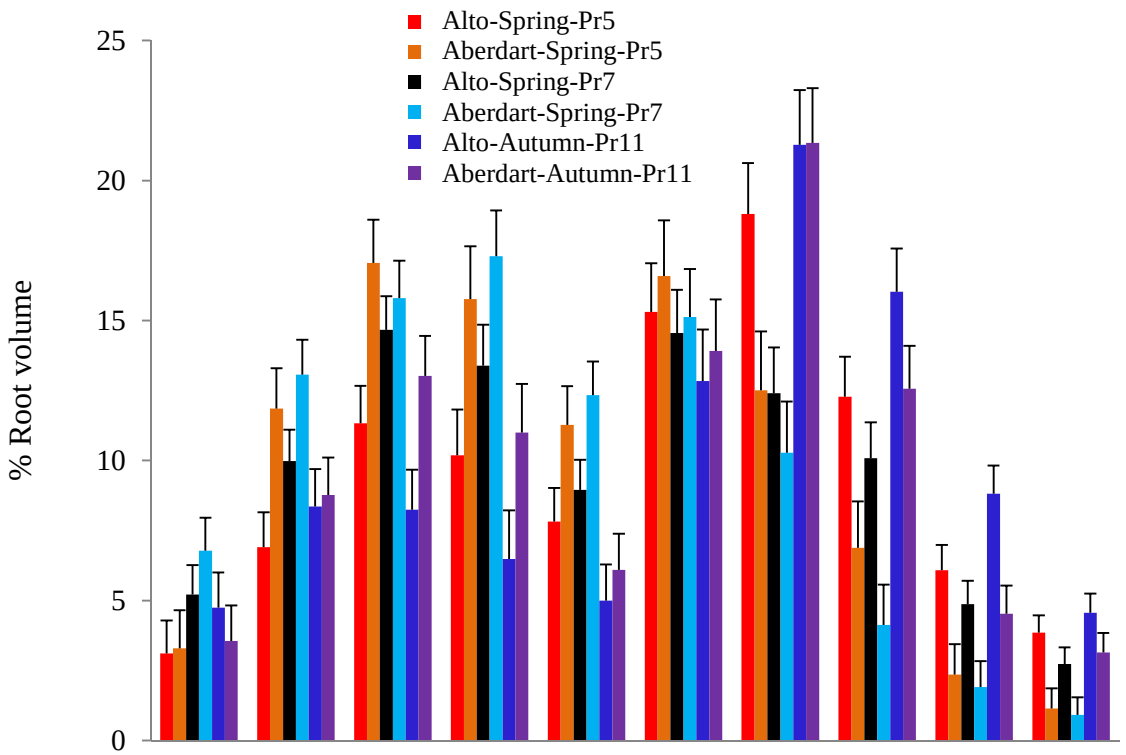
Fig. 5.18 Percentage distribution of root length among different root diameter class (mm) for roots of Alto and Aberdart perennial ryegrass cultivars at phytomer (Pr) 5 and Pr7 in the Spring experiment and Pr11 in the Autumn experiment.



Diameter classes	<0.1	0.1-0.2	0.2-0.3	0.3-0.4	0.4-0.5	0.5-0.6	0.6-0.7	0.7-0.8	0.8-0.9	0.9-1.0
Cultivar	0.46	0.001	0.038	0.06	0.06	0.97	0.007	<0.001	<0.001	0.003
Pr (5-7)	0.001	0.26	0.49	0.78	0.39	0.021	0.006	0.037	0.098	0.195
Pr (7-11)	0.25	0.13	0.07	0.005	0.024	0.14	<0.001	<0.001	0.002	0.008

Root surface area (%) and p value at each diameter class (mm)

Fig. 5.19 Percentage distribution of root surface area among different root diameter class (mm) for roots of Alto and Aberdart perennial ryegrass cultivars at phytomer (Pr) 5 and Pr7 in the Spring experiment and Pr11 in the Autumn experiment.



Diameter classes	<0.1	0.1-0.2	0.2-0.3	0.3-0.4	0.4-0.5	0.5-0.6	0.6-0.7	0.7-0.8	0.8-0.9	0.9-1.0
Cultivar	0.73	0.008	0.004	0.005	0.004	0.15	0.09	<0.001	<0.001	0.005
Pr (5-7)	0.01	0.044	0.16	0.086	0.64	0.19	0.022	0.097	0.29	0.36
Pr (7-11)	0.035	0.002	<0.001	<0.001	<0.001	0.61	<0.001	<0.001	0.003	0.018

Root volume (%) and p values at each diameter class (mm)

Fig. 5.20 Percentage distribution of root volume among different root diameter class (mm) for roots of Alto and Aberdart perennial ryegrass cultivars at phytomer (Pr) 5 and Pr7 in the Spring experiment and Pr11 in the Autumn experiment.

The root classes ranging between 0.6–1.0 mm diameter were largely axial roots (see Fig. 5.10) as root diameter data indicated that at first Pr generally bear no branches which measure RD above 0.6 mm. The diameter classes ranging 0.6–1.0 mm contributed around 3% to the RL, 15% to the RSA and 35% to the RV (Fig. 5.18–5.20).

These diameter classes exhibited significant differences between the two experiments (Pr7 of the Spring experiment and Pr11 of the Autumn experiment) and two cultivars for RL, RSA and RV for all of the diameter classes except diameter class 0.6–0.7 mm for RV. The phytomers within each experiment also showed significant differences for RL of the

diameter classes between 0.6–0.9 mm, for RSA of the diameter classes between 0.6–0.8 mm and for the RV of the diameter class 0.6–0.7mm.

5.4.3.4 Principal component analysis

When the various root data for Pr5 and Pr7 of the Spring experiment and Pr11 of the Autumn experiment were analysed together by PCA as described in Section 5.3.13, the first four principal components accounted for more than 90% of the total variation in the data, and these are shown in Table 5.13. The first principal component (PC1) explained 45% of the data variation with strong positive coefficients for RL, RT and RDW among others, a contrasting negative coefficient for RD, and a highly significant difference between scores for roots at Pr5 and Pr7 in the Spring experiment (Table 5.13, Appendix 5.10). These points indicate this to be a PC describing root maturity status.

PC2 explained 21% variation of the data with strong negative coefficients for RSA, RV and RD among others with the contrasting positive coefficients for TD (Table 5.13). This PC mainly explained genotypic variation within phytomers and cultivars based on root size (Table 5.13, Appendix 5.10). PC3 explained about 13% variation of the data with strong positive coefficients for RDW and with contrasting negative coefficients for R_p, SRL, SRSA and SRV (Table 5.13). PC3 scores showed a significant difference between phytomers within experiment with negative PC scores for the Spring experiment and positive PC scores for the Autumn experiment (Table 5.13, Appendix 5.10). PC4 explained 9.2% variation of the data with positive coefficients for R_p and SRV, and contrasting positive coefficients for RD, SRL, TD and SRSA among others. This PC showed a significant variation between Pr7 of spring and Pr11 of autumn for positive and negative PC scores, respectively (Table 5.13, Appendix 5.10). Genotypes with Pr and cultivar were also exhibited significant variations for PC4 scores (Table 5.13, Appendix 5.10).

Table 5.13 PCA of root morphological traits for Alto and Aberdart perennial ryegrass cultivars of Pr5 and Pr7 in the Spring experiment and Pr11 in the Autumn experiment. R_p, number of roots at the phytomer position; RDW, dry weight of the individual root; RAL, length of the main root axis; RL, root length; RSA, root surface area; RD, root diameter; RV, root volume; RT, number of tips per root; SRL, specific root length, SRSA, specific root surface area; SRV, specific root volume; TD, tissue density; RL/RV, root length per root volume; $DRL/RV^{1/3}$, dimension corrected total root length per unit root volume; RSA/RV, surface area per unit root volume; $DSA/RV^{2/3}$, dimension corrected surface area per unit root volume; %variation, percentage variation explained; p, probability of statistical variation in non-orthogonal contrast ($F_{1,81}$); p (Pr5 v Pr7) and p (Pr7 v Pr11) respectively denote probability of statistical variation between Pr5 versus Pr7 of the Spring experiment and Pr7 of the Spring experiment versus Pr11 of the Autumn experiment; PC, principal component, SE, standard error. Coefficients of absolute value <0.15 suppressed.

	Root traits	PC1	PC2	PC3	PC4
	R_p	-	-	-0.341	0.163
	RDW	0.266	-0.181	0.271	-0.351
	RAL	0.220	-0.182	-	-
	RL	0.358	-	-	-
Measured	RSA	0.316	-0.278	-	-
variables	RD	-0.255	-0.350	-	-0.200
	RV	0.225	-0.417	-	-
	RT	0.317	-	-	-
	SRL	0.171	-	-0.474	-0.401
	SRSA	-	-	-0.515	-0.472
	SRV	-	-0.328	-0.405	0.376
Derived	TD	-	0.344	0.348	-0.377
variables	RL/RV	0.238	-0.385	-	0.210
	$DRL/RV^{1/3}$	0.367	-	-	-
	RSA/RV	0.249	0.380	-	0.209
	$DSA/RV^{2/3}$	0.368	-	-	-
	% Variation	44.6	20.8	12.8	9.2
PC score	Spring-Pr5	-1.41±0.42	-0.10±0.33	0.65±0.21	-0.06±0.21
± SE	Spring-Pr7	1.57±0.52	-0.11±0.40	0.45±0.26	0.45±0.26
	Autumn-Pr11	0.44±0.45	0.19±0.35	-1.07±0.23	-0.27±0.23
P value[1]	Pr5 v Pr7	<0.001	0.83	0.48	0.095
	Pr7 v Pr11	0.097	0.57	<0.001	0.039
	Cultivar (Pr)	0.44	0.55	0.026	0.402
	Genotypes (Pr cultivar)	<0.001	0.004	0.002	<0.001

[1]Brackets indicating nesting of effects within the ANOVA model

5.5 Discussion

5.5.1 Co-ordination between phyllochron and rhizochron

Appearance of a new root on the tiller axis depends on site (phytomer) availability for initiation. As described in section 2.4.2.7 a phytomer on the tiller axis becomes available for root formation after a chain of sequential events. A phytomer initiates root growth 10-12 plastochrons after it is initiated from the apical meristem (Sharman, 1942; Yang et al., 1998). In a phyllochron time-scale, a phytomer becomes available for root appearance on average after a 5 and 6 phyllochron interval for *L. perenne* and *F. arundinacea,* respectively (Yang et al., 1998). The rate of change in phytomer availability for root appearance depends on the rate of leaf appearance and the factors influencing leaf appearance. Section 4.4.1 described the probable determinants of phyllochron. Perennial ryegrass plants growing at a constant temperature, light and day length are expected to maintain the same phyllochron (Mitchell, 1953) and rhizochron interval, unless any severe stress such as moisture stress, nutritional stress or severe grazing imposed.

A significantly lower NLA over the approximately 90 days growing period in autumn compared to the NPr isolated at destructive harvest suggested that a more rapid phyllochron during late summer and early autumn left more phytomers available for root production in the late-autumn when leaf appearance rate gradually decreased (Table 5.4). A highly significant difference between phyllochron of P1-P5 and rhizochron of Pr1-Pr11 ($p<0.001$) was probably associated with decreasing day length and temperature in autumn (Table 5.5). An equal NLA and NPr isolated at the destructive harvest, and significantly higher rhizochron of Pr1-Pr5 than phyllochron of P1-P5 ($p=0.027$) suggested a lower rate of root formation during winter at those phytomers which were formed in late autumn and early winter when phyllochron was longest (Table 5.5).

For a constant phyllochron and rhizochron at a particular time the ratio between NPr and NLA expected to be always 1. Significant difference in leaf and root appearance interval between two experiments resulted in highly significant variation in NPr/NLA ($P<0.001$), NPr/NLL ($p<0.001$) (Table 5.4). NPr is dependent on rhizochron which is in turn regulated by phyllochron. A significant increase in NPr/NLA in autumn suggests a higher PrAR than LAR and *vice-versa* in spring. An NPr/NLA ratio lower than 1 is possible in

spring as LAR increases during spring when PrAR is comparatively slower that early-spring or winter formed phytomers at the phytomer, P-*de.*

NLL is directly proportional to LAR and LLS (Lemaire and Chapman, 1996). Significantly higher LLS in autumn should lead to an increase in NLL and therefore a lower NPr/NLL ratio. Assuming LLS in both experiments was more or less similar and no root death occurs during experiment, then the NPr/NLL ratio would depend on PrAR and LAR at a particular time. A ratio higher than 1.0 between NPr and NLL in autumn (Table 5.4) is possibly achieved through a higher PrAR than LAR. These results suggest that in late-autumn when LAR becomes slower at the successive phytomer, the root appears at a faster rate at those phytomers which formed early-autumn in a comparatively longer day. Conversely, in winter-spring a lower ratio of NPr/NLL compared to autumn is possible when the leaf appears at a faster rate in spring whereas at the same time roots appear at a slower rate at the lower phytomers.

5.5.2 R_p and its association with other root physical traits

Variation in R_p in different seasons has also been previously reported. Matthew and Kemball (1997) for their study of autumn grown perennial ryegrass plants in the United Kingdom, reported R_p values of 2.67 and 2.33 at Pr9 and Pr10 (those were formed after transplanting in September) and 1.67 and 2.25 at the youngest two Pr at a destructive harvest in December. The R_p reported by Yang et al. (1998) varied between 1.2-1.8 for both tall fescue and perennial ryegrass. In their study, tillers were transplanted in the middle of autumn and harvested at the end of spring. The R_p values from Yang et al. (1998) are similar to the spring plants in this study. Matthew and Kemball (1997) reported R_p over 2 for the majority of the phytomers for the plants growing between September and December in UK, with a maximum of 2.75 which is similar to the R_p value recorded in the Autumn experiment of the present study. R_p differed significantly between genotypes of each cultivar within season was similar with Matthew et al. (1998) as they reported R_p values of 2.17-2.27 and 1.6-1.83 for New Zealand Agriseeds unnamed cultivars A1 and A3, respectively.

A moderately strong and positive correlation ($r=0.586$, $p<0.001$) between R_p and RDW_p for Pr6 and older was noted, indicated that factors influencing R_p essentially brings higher

RDW_p as no significant differences in RDW_i between developed phytomers (Pr6 and older) were noted (Fig. 5.4). It is also worthwhile to note that increasing plant size in autumn as indicated by leaf size (Fig. 4.13 and Fig. 4.15) did not show corresponding increase in RDW_i at the older Pr in autumn. The highly significant differences between genotypes of each cultivar suggested that the genotypic variation is a vital factor in determining RDW_i between seasons and among phytomers. Genotypic variation of RDW per tiller was evident in earlier studies, e.g. Crush et al. (2006; 2007).

One important point to note that, a comparatively lower mean value for RDW_i at the Pr12 for Alto and at Pr14 for Aberdart compared to the RDW_i at adjacent phytomers (Fig. 5.4) reflects the effect of two highest temperature regimes between 1 and 18 days after transplanting in autumn (Fig. 4.2) before covering the glasshouse with shade-cloth (see section 4.5.1).

5.5.3 Root development progression at successively developing phytomers

5.5.3.1 Main axis development

RAL did not increase after Pr6 or Pr7 and was constant until decomposition led to tip-first shortening of roots. This is presumably due to reduced photosynthetic C supply to older Pr (Matthew and Kemball, 1997). At Pr7-Pr8, plants produce 4.4 times more root branches other than main axes, although the root diameter decreased by 60% (Table 5.14). For example if the diameter of the main axis is 0.75 mm and the mean root diameter at Pr7-Pr8 is 0.3 mm this means that the root diameter of the root branches other than main axis is 0.19 mm. The main axis elongation rate is approximately 2.0 cm per day at the youngest two phytomers in the absence of any lateral branching (Fig. 5.10, Appendix 5.2). The C cost of the root system is generally considered as the combined construction and maintenance costs (Eissenstat and Yanai, 1997). The addition of 0.38 mg C Pr^{-1} d^{-1} (i.e. 50 µmol CO_2 Pr^{-1} d^{-1} assuming 45% C assimilated in the root tissues) constructs 47.1 mm^2 RSA. The rate of C deposition decreases to half at Pr3-Pr4 when branching commences and further decreases to one-quarter at the Pr6-Pr7.

The maximum RAL measured in both experiments was <50 cm whereas in some other studies it is evident that ryegrass roots reach more than 1 m in length (Jacques, 1943) although Matthew and Kemball (1997) measured a maximum RAL at Pr6 of only 36 cm

for plants growing in sand. The reason for these disparities in RAL is unknown but the presence/absence of daughter tillers may be important as previous studies have demonstrated that daughter tillers can contribute significantly to the root C budget (Carvalho et al. 2006).

Table 5.14 Main root axis length (RAL), RL (total root length): RAL ratio and % diameter reduction at the first eight root-bearing phytomers (Pr) of progressive development.

Pr	RAL (cm)	RL:RAL (times)	Diameter reduction (%)
1	5	1.0	0
2	17	1.1	11
3	24	1.8	48
4	30	2.7	45
5	36	3.7	60
6	43	5.3	52
7	46	5.2	62
8	44	5.6	58

5.5.3.2 Root development curve

In both experiments for both cultivars, the linear increase in root dimensions up to the Pr6 for the RDW_i, RAL, RL_i, RSA_i and RV_i suggests the progressive increase in root development at the successive phytomers. This result is similar to data in Matthew and Kemball (1997), which also indicates a linear growth curve for RDW_i, RAL, and RL_i up to Pr6. These results indicate that major root construction costs are incurred up to a certain stage (40-45 days) of root development. Table 5.14 indicates that during the linear growth phase the ratio between RAL and RL also increases linearly. This suggests that the branching of roots increases the RL at a much faster rate than RAL. The rate of increase in RL compared to RAL was more rapid in Matthew and Kemball (1997) than in this study, but the trend is similar. Root branching produces sequentially finer roots, which greatly reduces C cost.

The initial construction cost for newly formed roots is the highest (approximately 48 mmol C g^{-1} RDW) compared to roots at any other older phytomers. Peng et al. (1993) calculated that the construction cost of new roots of Volkamer lemon both inoculated with *Glomus intraraidices* and non-inoculated ranges between 42-49 mmol C g^{-1} RDW d^{-1} under high or low phosphorus supply. Amthor et al. (1994) also estimated a similar

construction cost for soybean roots. Additionally, plants require 2 mmol C g^{-1} RDW d^{-1} for maintenance respiration (Amthor, 1984; Peng et al., 1993). A linear decrease in dry matter deposition rate at the older phytomers is probably associated with reduced C supply to these roots as reported by Matthew and Kemball (1997). Branching of roots therefore is one of the adopted strategy for the plants to reduce construction cost per unit soil explored.

The increasing trend of RL of the individual roots at the successively developing Pr suggests the continuity of root growth and which may also be an indicator for root survival. RSA_i does not exactly agree with the RL for few higher phytomer positions. As RL increases it would be expected that the RSA_i would increase in a similar manner. However, this relationship does not hold for autumn roots particularly between Pr11-Pr17 for Alto and between Pr6-Pr12 for Aberdart.

The RV_i also did not show any clear pattern that is comparable with RL_i (Fig. 5.7 compared with Fig. 5.12). Therefore $RL/RV^{1/3}$ (Fig. 5.21) and $RSA/RV^{2/3}$ (Fig. 5.22) was calculated. $RL/RV^{1/3}$ indicates linear increases up to Pr8 as root develops at successive phytomers. This ratio indicates that RSA increases exponentially when branching commences at the phase 1. The rate of increase decreases in phase 2 and phase 3. In phase 4, in the Autumn experiment the ratio was found to follow a decreasing trend for both $RL/RV^{1/3}$ and $RSA/RV^{2/3}$.

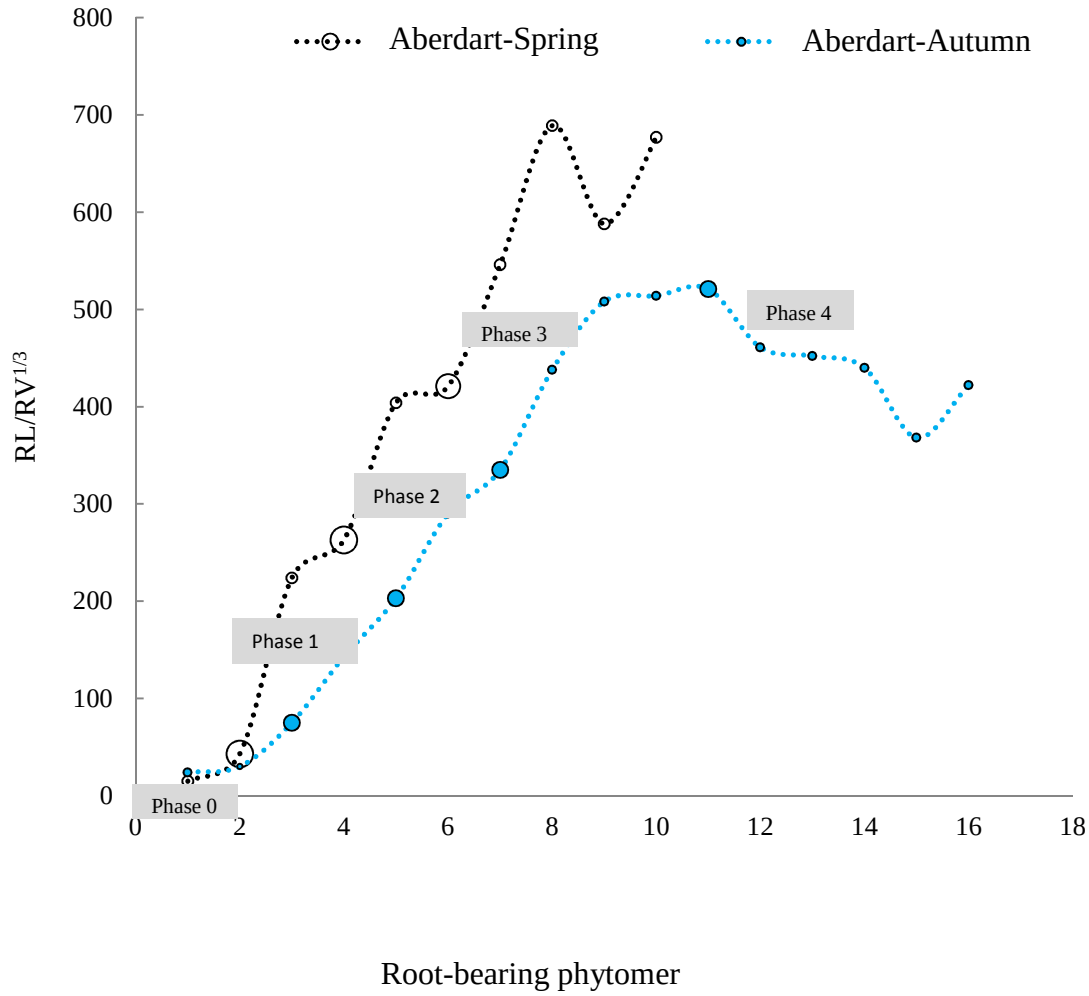


Fig. 5.21 Dimension corrected total root length/root volume ($RL/RV^{1/3}$) of Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments. Bold data points indicate the commencement of each phase; Phase 0, no root branching; Phase 1, primary branching; Phase 2, secondary branching; Phase 3, tertiary branching; Phase 4, quaternary branching.

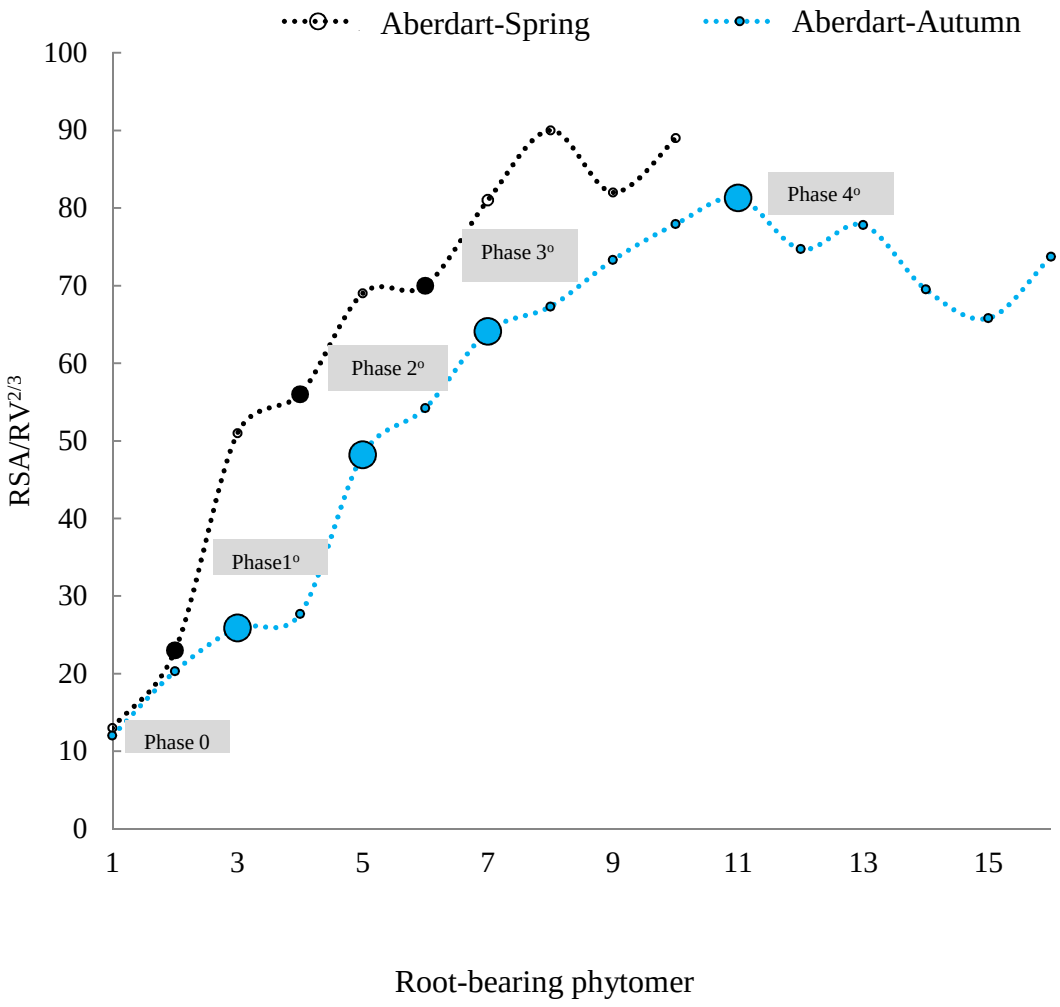


Fig. 5.22 Dimension corrected root surface area/root volume ($RSA/RV^{2/3}$) of Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments. Bold data points indicate the commencement of a branching phase; Phase 0, no root branching; Phase 1, primary branching; Phase 2, secondary branching; Phase 3, tertiary branching; Phase 4, quaternary branching.

5.5.4 C expenditure and dynamics of root branching

The root branching process is continuous and the commencement of one branching order does not require the termination of another branching order. For example, when second order of root branching starts, primary branching is still remain in progress near the root tip. This relations also applies to other orders of root branching.

The main axis elongation phase, which is a C expensive phase can facilitate approximately 2 cm RL and 0.3 cm^2 RSA per day. As the root diameter at this phase ranges between 0.6 to 0.9 mm the roots of even shorter length can add proportionately higher RV.

Table 5.15 indicates that when main axis elongation progresses at the Pr1-Pr3, these phytomers receive the highest share of photo-assimilated C. Supply of photosynthetic substrates possibly decreases gradually at the older phytomers and at around Pr10 that gets down close to zero.

Table 5.15 Estimated proportion of photosynthetic C (%) distributed at different phytomer positions (Pr). The proportions are the share of the total photosynthate distributed to the roots (15% of total photo-assimilation, see Section 5.3.10) for root construction (i.e., DMD_p d^{-1}) for Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments. The proportions in % were calculated for estimated CO_2 deposited per Pr for root DM construction (mmol CO_2 Pr^{-1} d^{-1}): total photo-assimilated CO_2 supplied to the root system (mmol CO_2 $tiller^{-1}$ d^{-1}).

Pr	Alto-Spring	Aberdart-Spring	Alto-Autumn	Aberdart-Autumn
1	39.3	26.2	6.49	8.21
2	27.4	20.0	6.68	7.42
3	18.6	17.6	5.20	9.30
4	15.0	15.1	7.29	8.20
5	7.67	12.7	5.22	7.10
6	2.22	8.98	5.23	6.00
7	<1.0	7.82	4.20	6.13
8	<1.0	4.18	3.17	3.81
9	<1.0	2.29	2.68	2.71
10	<1.0	0.39	1.11	2.01
11	-	<0.01	0.08	0.51
12-rest			<0.07	<0.4
Total	94.95	99.39	40.83	52.94

Possible reasons for the maintenance of C-expensive main axis elongation could be i) roots with comparatively greater diameter can provide better mechanical strength to travel to the deeper soil depth. Quantitative Trait Loci (QTLs) studies confirm main loci are common between root thickness and maximum root length (Yadav et al., 1997). ii) Roots with greater main axis diameter are likely to accommodate more primary branches with comparatively greater diameter to facilitate further branching. iii) Possibly thick main

axis diameter can provide better resistance to the plants against uprooting by grazing animals. iv) A greater main axis diameter can possibly facilitate a more efficient vascular distribution network with laterals.

Next to main axis elongation, the primary root branching is another highly C expensive process. Root diameters of the primary branches were 0.5-0.33 times those of the main axis in this study (Fig. 5.10 and Fig. 5.13). At the commencement of first order of root branching root surface area and root volume exhibited exponential increases.

Theoretically, a root axis of 'd_o' diameter and 'h' length can accommodate $4.d_o.h/d_1^2$ root branches of 'd_1' diameter on its surface. If the mean root diameter of the branches declines the number of branches of smaller diameter that can be accommodated per unit length of axis for a reduction 'x' where 'x' is a fraction of d_1, is $(^1/_x)^2$ larger (i.e. when root diameter is reduced by a factor of 0.5, 4 times more roots could be accommodated). Hence the reduction of diameter of the laterals allows more branches to be accommodated on the axes. A similar calculation can be made for root hairs at any order of root branching.

Reduction in root diameter of the branches advantages the plant by reducing the construction cost. It is known that dry matter supply to the older phytomers reduces gradually (Matthew and Kemball, 1997). A ryegrass primary root branch with $^1/_x$ of the root diameter of its main axis only requires $^1/_x^2$ times the dry matter to build a unit RL and therefore can produce x^2 fold of RL using same amount of construction cost. On this basis, a 5-10 times higher RL production would be calculated for first order branches, compared to the main axis, for the same DW deposition.

The primary branches accommodate spaces for secondary branches to grow from. The amount of root surface area at this phase is greatly increased which is significant in terms of nutrient and water uptake (Boot, 1989).

The secondary root branches generally originate from the surface of the primary branches, and sometimes they also originate by forming forks at the tips of the primary branches (Fig. 5.13). The diameter reduction at this phase was estimated to be approximately $^3/_4$ of the primary branches' meaning that the C requirement would be reduced to approximately

$^{9}/_{16}$ of the primary branches requirement for the same RL increase. This would equate to a 33% increase in RSA per unit C.

Tertiary root branches also originate in a similar fashion to secondary root branches. At this level of root branching, the root diameter reduces by up to 6-8 times compared to the main axis and nearly 2 times compared to the phase 2. RL addition per unit C at this phase is 3 times greater than branching phase 0. Given a similar rate of photosynthetic carbohydrate supply as phase 0, 36-64 times higher RL production is theoretically possible. The greater reduction from expected RL suggests massive reduction of C expenses for the root construction at this phase. The estimated RSA and RV at this phase are also in agreement with reduction of C supply to the roots at phase 3. This result supports the view that in absence of daughter tillers at the parent tiller's axis, the lower phytomer positions begin to show the C starvation effect.

Quaternary branching was rarely seen in the Spring experiment, while in the Autumn experiment this level of branching was observed below Pr10 (Fig. 5.14). The roots of quaternary branches are <0.1 mm diameter in general. The addition of RL at each Pr at the beginning of this phase increases slowly, reaches steady state and then decreases, probably due to the death of some roots. Decreases in RV at the beginning of this phase while RL and RSA increase suggests the death of larger roots while some fine roots may be added. A negative estimated-value of root diameter for the newly formed roots supports the assumption that some coarse roots supply C for the construction and maintenance of fine roots and perhaps these two events occur simultaneously. The dry matter addition at this phase is estimated to be close to zero. It has been observed that the root branches at this phase lose some cortical tissue from the main axis, which partly disintegrates (Henry and Deacon, 1981). If C is transported from the old tissues to generate new finer branches and root hairs, then uptake activity of such roots might be continued.

5.5.5 Evidence of root death

An increase in RL with decreasing RV for the older phytomers (Fig. 5.17) suggested the maintenance respiration of the older roots is supplied by C possibly from the old tissues of the same roots. Some previous studies also reported increasing TD as an indicator of root decomposition that perhaps occurs for the supplying construction cost to the new fine

roots and maintenance respiration (Eissenstat, 1992; Peng et al., 1993; Eissenstat and Yanai, 1997). There is no direct evidence in the literature of the renewal of old C at the older roots for grasses, but the data of Thornton et al. (2004) indicated that root exudates are supplied by old C. It can be speculated that construction cost of fine roots at the older phytomers might be supplied by the old C of the same root.

Table 5.16 compares Alto Pr12 with another older Aberdart Pr in Autumn for RDW, RSA and RV and also compares the RL distribution into four diameter classes. The data for Alto suggests that the RL of roots <0.2 mm diameter markedly increases while the RL of roots of >0.4 mm diameter decreases. Assuming that older roots when C starved disintegrate some tissues from coarse branches to build some finer roots, the following mathematical deductions are possible. As in Table 5.16, if 6.1 cm roots of 0.75 mm diameter and 2 cm roots of 0.55 mm after their death construct 81 cm roots of 0.07 mm diameter then that process will cause the net reduction of 0.1 cm^2 RSA and 19.2 mm^3 RV, and net reduction of -0.005 mm RD. From this it can be mathematically argued that old C is being used for construction of new fine roots at the same root.

For Aberdart (Table 5.16), if 6.4 cm (nearly 32%) coarse roots of 0.4-0.7 mm diameter disintegrates, then 24 cm finer RL of <0.4 mm might be constructed. This relation is mathematically possible if 6.4 cm roots of 0.55 mm mean root diameter dies and 17 cm roots of 0.063 mm and 7 cm roots of 0.3 mm forms. In this process the net RSA reduces by -2.3 cm^2, net root diameter reduces by -0.36 mm and net root volume reduces by -23 mm^3. In a practical situation, the equation for C supplying to new fine roots from old tissues is perhaps more complex as reflected by the reduction in greater RV than estimated (Table 5.16). Many different combinations and recombination can be mathematically drawn to estimate the construction cost of the fine roots. The old C might be translocated from any part of the roots or might be from adjacent roots. These mathematical deductions have been conducted to illustrate the theme to show a balance but these results deserve further research involving labelling some targeted root positions followed by a series of later harvests for isotopic mass ratio detection of the labelled roots.

Table 5.16 Mean root dry weight (RDW), surface area (RSA), volume (RV) at the Pr12 and Pr14 for Alto and Pr12 and Pr16 for Aberdart perennial ryegrass cultivar in autumn, and the root length (RL) at those phytomers distributed along four different diameter classes.

Cultivar	Pr	RDW mg	RSA cm^2	RV mm^3	RL cm	RL (cm) distributed at the diameter classes (mm)			
						<0.2	0.2-0.4	0.4-0.7	>0.7
Alto	12	16.9	25.8	201	220	196	28.6	12.1	8.91
	14	16.8	25.7	152	246	277	28.3	10.1	2.84
Aberdart	12	15.6	18.8	116	246	206	19.6	20.1	1.18
	16	14.8	16.5	102	346	223	26.6	13.7	3.52

5.5.6 Seasonal variation in root dimensions

Previous investigations have reported seasonal variation in root production in New Zealand and UK grass swards. Both Jacques and Schwass (1956) and Caradus and Evans (1977) recorded higher new adventitious root formation in Autumn than late winter and spring in New Zealand, with Jacques and Schwass (1956) recording peak in early winter. The much higher number of new adventitious roots per tiller in their study is likely associated with both faster PrAR in autumn than late winter and spring as reflected by both shorter phyllochron in autumn than spring (Fig. 4.5) and significantly higher R_p in autumn (Fig. 5.3). Hence the higher root DM production in autumn is associated with faster PrAR coupled with higher R_p values in autumn than winter-spring which is in turn related to greater C partitioning to roots in autumn than winter-spring (Parsons and Robson, 1981).

Seasonal variation in DM allocation (Fig. 5.6, Table 5.6) probably affects the diameter of the main axis. In Fig. 5.18 and Fig. 5.19 roots of more than 0.6 mm diameter was in autumn than spring although the RAL of the developed roots of similar age was shorter in autumn (Table 5.11).

Matthew et al. (1991) recorded the highest mean root diameter in perennial ryegrass of greater than 0.6 mm in winter (in August) and lowest of around 0.4 mm in summer (in December). Those diameters were higher than in the present study, which estimated mean root diameter of the developed roots at less than 0.3 mm (Fig. 5.10). This disparity in results could arise from loss of fine roots during washing of soil in the field studies or

from the choice of method used for root length estimation. This study used WinRHIZO software which may have provided a better estimate of fine root length, accounting for the lower mean root diameter. This assumption is supported by the ratio between SRL obtained from WinRHIZO scanned roots and that obtained from the modified Newman method. The ratio for SRL between WinRHIZO: Newman was respectively 2.28 and 2.22 for Alto and Aberdart in the Spring experiment suggesting that previous studies using the modified Newman method may have underestimated the total RL (Appendix 5.3), unless the present WinRHIZO study had overestimated total RL, which seems less likely.

In this study, there was no significant difference in RL or RSA for root diameter classes less than 0.6 mm when the roots of similar age of two growing seasons was compared (Fig. 5.18, Fig 5.19). The significantly higher SRL, SRSA and SRV for the autumn roots suggested that the plants utilized C more efficiently for constructing lateral branches at the older phytomers in autumn-tillers compared to higher order phytomers of similar age in spring-tillers (Table 5.12).

5.5.7 Significance of studying root turnover: Increase in nutrient and water absorption area

Root branching in the first place increases root surface area. More surface area means more root-soil contact. The added root surface area at the root branches facilitates the root hairs to be accommodated with. Reid (1981) reported 88 root hairs mm^{-1} root having average length of 1120 µm and average diameter of 10 µm in *L. perenne* (cv. Aberyestwyth S24). Matthew et al. (2001) from Care (1999) reported a root hair density of 1369 root hairs mm^{-1} of 145 µm length and 12.3 µm diameter under low phosphorus conditions and 1250 root hairs mm^{-1} of 132 µm length and 12.7 µm diameter under high phosphorus conditions in *L. perenne* (cv. Grasslands Nui). In that study the ratio between the diameter of the root hairs and the axis (~0.25 mm) on which the root hairs develop was close to 20. Grassland Nui roots only use 20% of the surface area of the root branches to generate root hairs, leaving the remaining surface area vacant (derived from Matthew et al., 2001). Calculations based on data of Reid (1981) and Care (1999) and reported by Matthew et al., (2001) indicate that surface area of root hairs potentially contributes 89-92% of the total RSA. The root branches thus provide a skeleton for root hair growth.

Root branching has a widely accepted role in nutrient acquisition. Increasing root size is an index of C translocation from shoot to root and so changes in root mass allocation as plants can allocate up to 50% of total net photoassimilate to belowground production (Liljeroth et al., 1994; Swinnen, 1994). Root branching changes the root diameter of the root system and provides a vascular network of fine roots to the soil.

PCA showing genotypic difference in root diameter could be a useful single criterion of genotype selection but that involves sophisticated technical expertise. Root diameter defines the volume of the roots in the soil. Production of the finer roots involves less photosynthate per unit length / surface area (Atkinson, 1991) but these roots are potential to explore larger soil volume per unit root surface area.

The higher heritability measures for some root traits, e.g., 0.83 for total RL of oats (Barbour and Murphy, 1984), 0.35 for RL, 0.61-0.81 for root thickness, 0.56-0.80 for RDW, 0.44-0.77 for root length density of rice (Ekanayake et al., 1985) 0.54 for root diameter of white clover (Woodfield and Caradus, 1990), 0.33-0.44 for root hairs of white clover (Caradus, 1979), suggest the importance of studying root branching and root morphology in ryegrass breeding. Intra-specific variability in root hairs (Caradus, 1979), and evidence of Mendelian inheritance in root hair traits suggest that breeding ryegrass genotypes for desired root hair type is possible. The present study has created wider awareness on root turnover pattern and also seasonal and genotypic variation in root growth in spring and autumn and at the same time left a number of questions for further exploration.

5.6 Summary

- Progression in root development at successive phytomers was studied in the Spring and Autumn experiments using Alto and Aberdart perennial ryegrass cultivars.
- Over a growing period of approximately 90 days, just over 10 root-bearing phytomers and an approximately equal number of leaves were developed per tiller in the Spring experiment. In the Autumn experiment, for the similar growing duration the number of root-bearing phytomers per tiller (17.5) was significantly higher than number of leaves produced (15.6).

- On average, 1.78 and 2.53 roots were produced per phytomer in the Spring and Autumn experiments, respectively, suggesting that the variation is associated with either plant size or variation in photosynthate supply.
- Root dry weight of the individual roots increased linearly over the first six root-bearing phytomers. The dry matter deposition rate per phytomer (mg Pr^{-1} d^{-1}) decreased gradually from younger to older roots. Data indicated higher photo-assimilate allocation towards younger roots.
- Similar to individual root dry weight, other root dimensions such as root axial length, individual root surface area, and root volume, increased linearly with the progression of root development at successive phytomers.
- Root branching decreases mean root diameter, specific root length, specific root surface area and specific root volume and increases root length and number of root tips at successive phytomers.
- Visual scoring of WinRHIZO scanned roots provided a total of four root branching orders. Primary root branching commences on the second and third root-bearing phytomers, secondary root branching commences between Pr3-Pr5, tertiary root branching commences between Pr5-Pr8 and quaternary root branching commences later than Pr8.
- There was significant variation among genotypes of each cultivar for roots of similar age.
- Root volume of the individual roots was decreasing rapidly after Pr10 in the Autumn experiment, when root length was increasing and dry weight and surface area were steady, suggesting the start of root death in some branches.
- Finer roots less than <0.2 mm diameter that comprised approximately 63% root length and 35% surface area contributed only 13% volume. Conversely coarse roots of >0.6 mm diameter comprised approximately 3% root length, 15% surface area and contributed 35% root volume.
- PC1 separated comparatively more mature roots from the younger roots for their dry weight, main axis length, total length, surface area, volume, number of tips and diameter. PC2 separated roots of different size for dry weight, main axis length, surface area, volume and diameter. PC3 separated roots of higher and lower SRL, SRSA and SRV related to R_p between two experiments. These data suggest that selection of genotypes based on root traits for different seasons would be possible.

Chapter 6: Root-shoot interrelations and seasonal morphogenetic variations

6.1 Introduction and Overview

Chapter 4 and Chapter 5 discussed, respectively, the detailed leaf and root turnover pattern of perennial ryegrass cultivars Alto and Aberdart growing in two different seasons, spring and autumn. As expected, plants growing in spring in increasing day length increased leaf appearance rate (LAR) at successive phytomers while plants growing in autumn in decreasing day length exhibited the reverse phenomenon, and this formed a background context against which to study the interaction of plant morphology traits. There were significant variations for leaf morphological traits in the two different seasons. Seasonal variation in day length was linked to changes in total NLA, total NPr, and R_p between spring and autumn plants. There were also significant variations in root dimensions of the spring and autumn grown plants. The first part of Chapter 6 integrates data previously presented on a per phytomer basis to the whole tiller level in order to discuss the seasonal variation in root:shoot ratio and how root:shoot ratio might be changed in increasing and decreasing day length as phyllochron (in days) decreases in increasing day length and conversely increases in decreasing day length at successively developing phytomers (see Section 4.4.1).

Another facet of plant behaviour explored in this chapter is the interaction between parent and daughter tillers. In Chapter 3 it had been reported that daughter tillers of approximately similar age had significantly smaller size and greatly-reduced growth rate compared to their parents (Table 3.3). There is evidence that photo-assimilates may travel in either direction between the main tiller and daughter tiller (see e.g. Clifford et al., 1973; Carvalho et al., 2006). The last section of this chapter will examine data from these experiments which allow exploration of how the presence or absence of daughter tillers might influence the root growth of the main tiller.

6.2 Objectives

As indicated by the above background, objectives for Chapter 6 were:

1. To investigate root:shoot relations at the whole tiller level for the data collected in Experiments 4 and 5.
2. To test the hypothesis of Matthew *et al.* (1998) that plant architecture may provide a signal that spontaneously increases root:shoot ratio in spring and decreases it in autumn.
3. To investigate the effect of daughter tiller removal on shoot and root dry matter production at individual phytomers of the main tiller for the perennial ryegrass cultivars Alto and Aberdart in the Spring and Autumn experiments.

6.3 Methodology

6.3.1 Investigation of root-shoot relations

To study root-shoot relations at the individual tiller level, data for leaf lamina dry weight per tiller (LDW_t), leaf sheath dry weight per tiller (SDW_t), dry weight of the tiller axis (TADW), leaf area per tiller (LA_t), root dry weight per tiller (RDW_t), root length per tiller (RL_t), root surface area per tiller (RSA_t), number of live leaves per tiller (NLL), number of root-bearing phytomers per tiller (NPr) and number of roots per tiller (NR_t) were collated for three clonal replicates of 9 and 10 genotypes of Alto and Aberdart respectively (57 plants) in Spring and two clonal replicates of 8 genotypes of each cultivar in Autumn (32 plants) at the destructive harvest of the plants after a 90 d growing period. These data collated at the tiller level to study root-shoot relations were from the same plants which are described at the individual phytomer level in Chapter 4 for the leaf traits and in Chapter 5 for the root traits. After collation in this way, three separate ratios were then calculated to explore root:shoot relations: RDW_t/LDW_t, RSA_t/LA_t and NR_t/NLL.

6.3.2 Effect of increasing or decreasing day length on root:shoot ratio

For the same plants for which morphology data were collated at the tiller level (Section 6.3.1), the ratio of root dry weight per phytomer to leaf dry weight per tiller (RDW_p/LDW_t; mg g^{-1}) was used to evaluate the root:shoot ratio at the individual phytomer level. This ratio was selected as a measure of the allocation to root production at each phytomer because in the Autumn experiment plant size was much larger than in the Spring experiment and so direct comparison of the size of single roots across experiments was not

a good indication of change in DW allocation to roots. For each of the spring plants 10 phytomers, and each of the autumn plants 16 phytomers were selected for statistical analysis. Thus there were a total 570 phytomers from the 57 spring plants and 512 phytomers from the 32 autumn plants for which RDW_p/LDW_t were calculated.

6.3.3 Effect of daughter tiller removal on main tiller morphology

As stated in section 4.3.5, in both experiments the first two DTs which appeared after transplanting were allowed to grow but any DT appearing later was removed. In the Spring experiment, the first two DTs had appeared within 10-15 d after transplanting and in the Autumn experiment they had appeared within 5-10 d after transplanting (Fig. 6.1). The secondary tillers appearing at the leaf axils of the two retained DTs were also removed. For the DTs, a set of morphological data including LL, LDW, LW, NLL, NPr, R_p and RDW_p was collected at the destructive harvest.

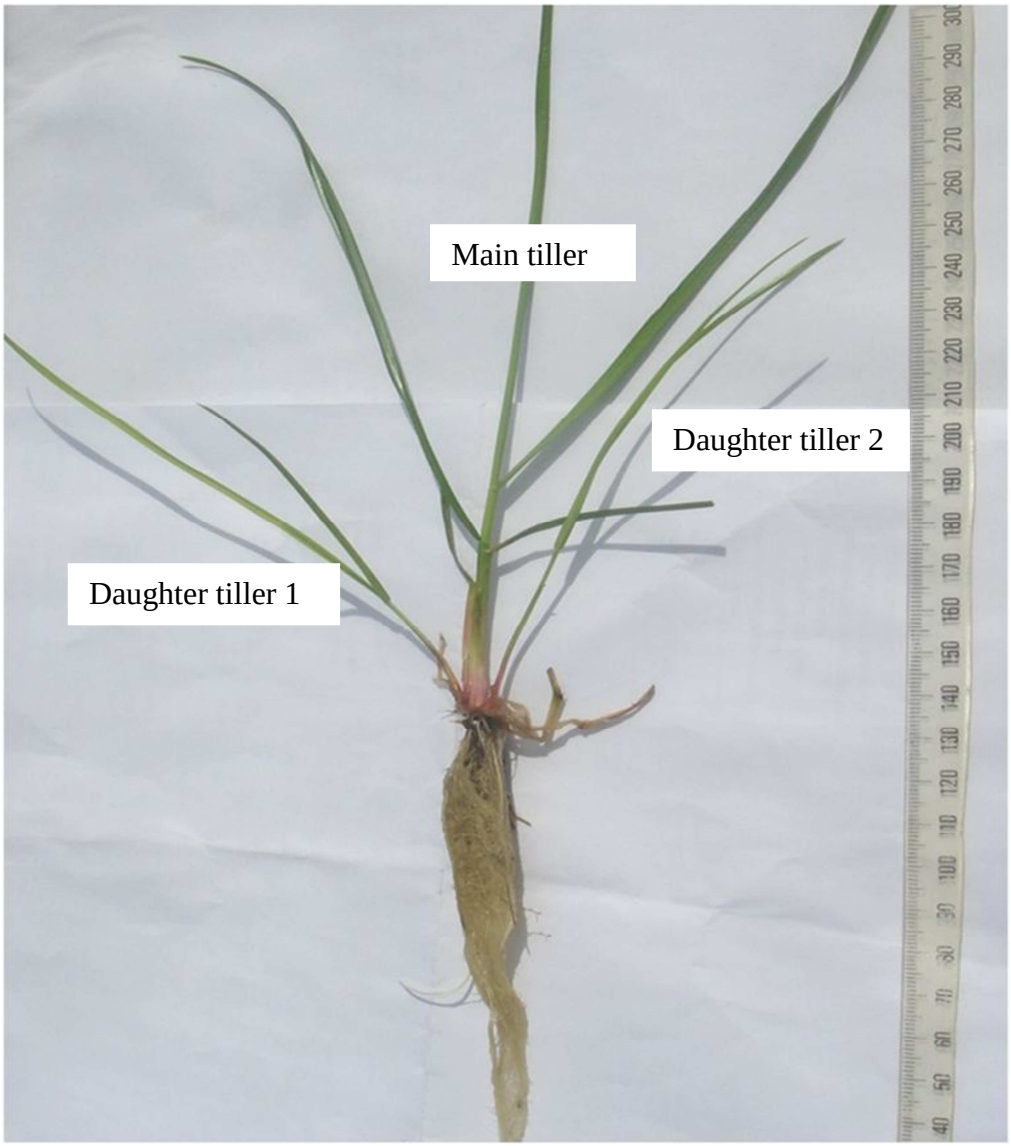


Fig. 6.1 A perennial ryegrass plant with a main tiller and two daughter tillers at 10 days after transplanting in the Autumn experiment

In both Autumn and Spring experiments, separate investigations using additional plants were conducted to explore the interrelations between MTs and DTs. In the Spring experiment, two existing DTs from 98 d old plants were removed (DT-) and a destructive harvest made on average 16 d later, in which morphology of these plants was compared with Control plants from which two daughter tillers had not been removed (DT+). In the Autumn experiment, some plants were maintained without daughter tillers (DT-) throughout the 93 d growing period for a comparison with the plants which had two daughter tillers (DT+). Hence, the DT excision experiment in Spring tested the short term effects of removal of adult DTs; while the DT excision experiment in Autumn tested the longer term effects of removal of juvenile DTs.

6.3.3.1 Short term effects of adult daughter tiller removal

In the Spring experiment, 18 "spare" plants that had been grown in the same hydroponic system with the plants destructively harvested to obtain data reported in Chapters 4 & 5 were selected (9 genotypes of each cultivar, Alto and Aberdart) and both daughter tillers were excised on 6 October 2008 at 98 d after transplanting. Another set of 18 plants (again 9 genotypes of each cultivar) were maintained with the two DTs intact. The MTs of all 36 plants were destructively harvested as described in Section 4.3.7 and 5.3.3 starting from 12 d after DT excision. The destructive harvest of all 36 tillers took 9 d, ending 20 d after DT excision. During the destructive harvest period, 4 plants were harvested daily: one DT- and one DT+ plant of each cultivar. To assess the effect of DT excision on leaf and root dynamics for the MTs, data for A_{lf}, FLL, LW, NLL, R_p and RDW_p were collected. SLA was calculated as area (cm^2) per g dry weight (see Section 4.3.11). NR_t and RDW_t represent the total number of roots of all phytomers and root dry weight of all roots per tiller. RDW values of Pr 1-8 and the oldest five phytomers were summed and statistical difference between DT+ and DT- were tested.

6.3.3.2 Longer term effects of juvenile daughter tiller removal

In the Autumn experiment, to study the effect of long-term absence of DTs on the MT a set of 8 "spare" plants (4 tillers of each cultivar) was maintained in the hydroponic system without any DTs (DT-) for the 93 d along with other plants with the two first-formed DTs retained (DT+). These 8 plants were destructively harvested as described in Section 4.3.7 and Section 5.3.3 so that leaf and root development at particular phytomers on DT- plants could be compared with the development of MTs of the DT+ plants. Measurements included LL, LW, LDW, NLL, R_p and RDW_p. LA and SLA were calculated as described in Section 4.3.11. RDW_i was calculated as in Chapter 5, assuming all roots at the same phytomer to have the same DW. In addition, the total number of roots from all Pr of a tiller (NR_t) and the total DW of those roots (RDW_t) were calculated as measures of the root system morphology for comparison with leaf traits. The ratios RDW_p/LDW_t, and NR_t/NLL were calculated to explore the effect of DT excision on root morphology. For a comparison between Pr5 – Pr10 of DT+ and DT- plants for RDW_p/LDW_t either a quadratic or a cubic polynomial curve was fitted in a Microsoft Excel spread-sheet.

6.3.4 Statistical analysis

There were a total of 89 tillers from two cultivars in two experiments for which data on individual tillers were collated (Section 6.3.1). ANOVA was conducted using the GLM procedure in the MINITAB 15 statistical software package (Minitab Inc. State College, Pennsylvania) (The model used is reported in Appendix 6.1). Pearson correlation analysis was conducted for the variables LDW_t, LA_t, NLL, NPr, NR_t and RDW_t which were measured independently, to assess correlations between root and shoot traits in particular. Finally a PCA was conducted using the PCA command of Minitab 15 and data for LDW_t, LA_t, NLL, NPr, NR_t, RDW_t, RL_t and RSA_t.

RDW_p/LDW_t data as described in section 6.3.2 were analysed to test the statistical significance for the effects of experiment, cultivars, genotype within cultivar and phytomer position within experiment and cultivar. The ANOVA structure was similar to that given in Appendix 4.1 since this ratio was calculated for the same plants described in Chapter 4.

To ascertain the statistical significance of differences between the two cultivars, between DT- and DT+ treatments, and to test the treatment x cultivar interaction for parent-daughter tiller effects described for the Spring experiment in Section 6.3.3.1, ANOVA was conducted using Minitab 15 statistical software package (Minitab Inc. State College, Pennsylvania) and following the given model: MTB> GLM SLA = cultivar | treatment; SUBC> means cultivar | treatment. Variables analysed were SLA, NR_t and RDW_t/LDW_t, ratio between root dry weight and leaf dry weight $tiller^{-1}$ ($mg\ mg^{-1}$).

A separate ANOVA as described above was conducted to test the treatment x cultivar interaction for mean FLL_i, LW_i, LDW_i, SLA, LA_i, R_p, RDW_i, RDW_p/LDW_t and total LDW, LA, NLL, NPr, NR_t, RDW and NR_t/NLL for the individual tillers of the experimental plants referred to in section 6.3.3.2 to explore the effects of the long-term absence of DTs in the Autumn experiment. Phytomer level data for SLA, Pr5 – Pr10 and the individual older Pr positions for RDW_p/LDW_t of DT- and those of DT+ were compared in GLM procedure using a user-defined model and using the sum of squares for genotypes within cultivar and treatment as the error term (Appendix 5.7). To calculate the standard deviation of the residuals of the RDW_p/LDW_t ratio, the residuals were regressed

against two predictors one of which was phytomer position and the other being the square phytomer position. DT+ plants used here for comparison with DT- plants were the 16 Alto and 16 Aberdart plants of the Autumn experiment mentioned in Table 4.4.

6.4 Results

6.4.1 Root: shoot morphogenetic relations

Size of individual tillers in the Autumn experiment was significantly greater than in the Spring experiment as reflected by LDW_t, SDW_t, TADW, LA_t, RDW_t, RL_t, RSA_t, NLL, NPr and NR_t (Table 6.1). One notable variation between the two experiments was that LDW_t was much greater in the Autumn experiment than the Spring experiment (2.5 – 2.9 times) compared to RDW_t (2.0 – 2.4 times), although autumn plants had significantly higher number of roots per tiller than the spring plants (Table 6.1). The autumn plants had more roots per tiller to feed with comparatively smaller number of live leaves compared to the spring plants as reflected by NR_t/NLL ratio (Table 6.1). Despite a highly significant variation for NR_t/NLL between the two experiments, the difference for RDW_t: LDW_t ratio between the Spring experiment and the Autumn experiment was marginal (Table 6.1). Another notable variation between the Spring and Autumn experiments was significantly higher total NPr in Autumn than Spring which is directly associated with NR_t (Table 6.1). Genotypes for each cultivar varied significantly for both RDW_t:LDW_t and NR_t:NLL in addition to LDW_t, LA_t, RDW_t, RL_t, RSA_t, NLL, NPr, NR_t (Table 6.1). The experiment x cultivar interaction showed significant variation for the traits LDW_t, LA_t, RL_t, NLL and NR_t:NLL (Table 6.1).

Table 6.1 Root: shoot morphogenetic relations for two perennial ryegrass cultivars Alto and Aberdart in Spring and Autumn experiments (Experiment 4 & 5, respectively) at whole tiller level. LDW_t, leaf lamina dry weight $tiller^{-1}$ (mg); SDW_t, leaf sheath and pseudo-stem dry weight $tiller^{-1}$ (mg); TADW, tiller axis dry weight (mg); LA_t, leaf area (cm^2 $tiller^{-1}$); RDW_t, root dry weight $tiller^{-1}$ (mg); RL_t, root length $tiller^{-1}$ (m); RSA_t, root surface area $tiller^{-1}$ (cm^2); NLL, number of live leaves $tiller^{-1}$; NPr, number of root-bearing phytomers $tiller^{-1}$; NR_t, total number of live roots $tiller^{-1}$; RDW_t/LDW_t, ratio between root dry weight and leaf dry weight (mg mg^{-1}); RSA_t/LA_t, ratio between leaf area and root surface area $tiller^{-1}$; NR_t/NLL, total number of live roots against number of live leaves; SEM, standard error of mean; p, probability value; Exp, experiment; Cul, cultivar; Geno, genotype.

	Alto-Spring	Aberdart-Spring	Alto-Autumn	Aberdart-Autumn	SEM	p (Exp)	p (Cul)	p (Exp x Cul)	p (Geno (Cul))
LDW_t	305	457	778	1310	20.0	<0.001	<0.001	<0.001	<0.001
SDW_t	120	157	226	286	9.77	<0.001	0.059	0.643	-
TADW	54.0	83.8	160	168	7.62	<0.001	0.089	0.330	-
LA_t	62.6	94.4	116	174	6.78	<0.001	0.001	0.084	<0.001
RDW_t	201	234	487	478	13.1	<0.001	0.715	0.516	0.004
RL_t	3.07	3.98	7.64	6.24	0.28	<0.001	0.61	0.02	0.008
RSA_t	261	353	644	516	14.1	<0.001	0.635	0.007	0.015
NLL	6.92	8.27	8.37	12.2	1.48	<0.001	<0.001	0.002	0.001
NPr	9.70	10.4	16.8	18.1	0.18	<0.001	0.007	0.38	<0.001
NR_t	15.9	18.9	37.2	40.1	3.46	<0.001	0.081	0.976	0.034
RDW_t/LDW_t	0.70	0.56	0.64	0.38	0.03	0.085	0.004	0.365	0.031
RSA_t/LA_t	4.35	3.80	5.69	2.39	0.25	0.053	<0.001	0.005	0.135
NR_t/NLL	2.33	2.36	4.52	3.38	0.12	<0.001	0.023	0.055	0.003

6.4.1.1 Correlation analysis

Given the inherent variation in tiller size between experiments, cultivars, and genotypes, significant correlations between measures of root or shoot morphology were expected. The points of note in the correlations between plant morphology measures (Table 6.2) are the lower correlations between RDW_t and leaf traits than within the root or shoot traits and the comparative independence of NLL indicated by lower correlations between NLL and other traits of root and shoot (Table 6.2). Only independently measured traits, and not

traits with values derived from the measured traits, were included in the correlation analysis.

Table 6.2 Coefficients of correlation within perennial ryegrass cultivars in Spring and Autumn experiments and across combined data of independently measured leaf and root traits assessed in 89 tillers of Alto and Aberdart. LDW_t, leaf lamina dry weight $tiller^{-1}$; LA_t, leaf area per tiller; RDW_t, root dry weight; NLL, number of live leaves $tiller^{-1}$; NPr, number of live root-bearing phytomers $tiller^{-1}$; NR_t, total number of live roots $tiller^{-1}$. A single cell contains the Pearson correlation coefficient.

	LDW_t	LA_t	NLL	NPr	NR_t
LA_t	0.92				
NLL	0.83	0.77			
NPr	0.85	0.75	0.70		
NR_t	0.77	0.71	0.54	0.82	
RDW_t	0.66	0.69	0.41	0.70	0.87

All correlations are significant at the 0.1% level

6.4.1.2 Principal component analysis (PCA)

The first two principal components (PCs) explained 91.4% of the data variation. PC1 (74.2% of data variation) had positive coefficients for all root and shoot morphological traits (Table 6.3), so effectively distinguished between smaller and larger plants from the Spring and Autumn experiment, respectively. ANOVA of PC scores indicated that PC1 included experiment ($p<0.001$), cultivar ($p<0.001$), and genotype within cultivar effects ($p=0.04$) (Table 6.4). The majority of the PC scores for the plants in the Spring experiment had negative values, whereas in the Autumn experiment, they had positive values (Appendix 6.2). Thus, PC1 can be considered as a 'size' PC.

Table 6.3 Principal component analysis of tiller root and shoot morphological traits of Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments. LDW_t, leaf lamina dry weight $tiller^{-1}$ (mg); LA_t, leaf area $tiller^{-1}$ (cm^2); NLL, number of live leaves $tiller^{-1}$; NPr, number of live root-bearing phytomers $tiller^{-1}$; NR_t, total number of live roots $tiller^{-1}$, RDW_t, root dry weight $tiller^{-1}$ (mg); RL_t, total root length $tiller^{-1}$ (m); NR_t, total number of live roots $tiller^{-1}$; RSA_t, root surface area $tiller^{-1}$ (cm^2). PC, principal component.

Trait	PC1	PC2
LDW_t	0.362	-0.355
LA_t	0.359	-0.267
NLL	0.282	-0.550
NPr	0.361	-0.189
NR_t	0.378	0.086
RDW_t	0.377	0.313
RL_t	0.352	0.419
RSA_t	0.349	0.428
% variation explained	74.2	17.2

Table 6.4 Analysis of variance (ANOVA) of scores for the first two principal components (PCs) based on the shoot and root data of Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments. SE, standard error of mean; p, probability value ($F_{1,89}$); Exp, experiment; Cul, cultivar; Geno, genotype.

	PC1	PC2
Alto-Spring	-2.16	0.22
Aberdart-Spring	-1.05	-0.06
Alto-Autumn	2.20	1.05
Aberdart-Autumn	3.41	-1.31
SE	0.26	0.12
p (Exp)	<0.001	0.46
p (Cul)	<0.001	<0.001
p (Exp x Cul)	0.87	0.001
p (Geno (Cul))	0.04	<0.001

PC2 explained 17.2% of the data variation and reflected a contrasting contribution to PC scores of the leaf traits LDW_t, LA_t and NLL with negative coefficients, and the root traits RDW_t, RL_t and RSA_t (Table 6.3) with positive coefficient values. The ANOVA of the PC2 scores showed highly significant variation between the two cultivars, an experiment x cultivar interaction and variation between genotypes of each cultivar (Table 6.4). The PC2 scores showed a significant contrast between the two cultivars, with a strong contrast in autumn and a weak contrast in spring (Table 6.4, Appendix 6.2). Considering both the PC

coefficients in Table 6.3 and the means of PC scores in Table 6.4, it would appear that the cultivar Alto favoured root production over leaf production in the Autumn experiment, in a way that cultivar Aberdart did not.

6.4.2 'Architectural Signal' determining seasonal change in root:shoot ratio hypothesized by Matthew et al. (1998)

The phytomer root weight: tiller leaf dry weight ratio (RDW_p/LDW_t) of these plants after approximately 90 d growth confirmed the ongoing increase in root size from Pr1 up to Pr6 in both experiments ($p<0.001$, Fig. 6.2) (c.f. Section 5.4.3). However, the spring tillers had a strikingly higher ($p<0.001$) RDW_p/LDW_t at each phytomer position than the autumn tillers (Fig. 6.2). The variation between the two cultivars Alto and Aberdart for RDW_p/LDW_t ratio was also highly significant ($p<0.001$) with cultivar Alto having higher root:shoot ratio in both experiments than cultivar Aberdart. The cultivar Alto in the Spring experiment recorded the highest phytomer root weight:shoot dry weight ratios followed by cultivar Aberdart in the same experiment (Fig. 6.2). The highest RDW_p/LDW_t of 113 mg g^{-1} was at Pr6 for cultivar Alto in the Spring experiment (Fig. 6.2). For Aberdart in the Spring experiment, the highest phytomer root weight:shoot dry weight ratio of 93 mg g^{-1} was at Pr9. In the Autumn experiment, the ratio was much lower compared to the Spring experiment, with the highest values for each cultivar being 60 mg g^{-1} for Alto at Pr15 and 35 mg g^{-1} for Aberdart at Pr17 (Fig. 6.2). The genotypes of each cultivar also differed significantly ($p=0.003$) for RDW_p/LDW_t ratio.

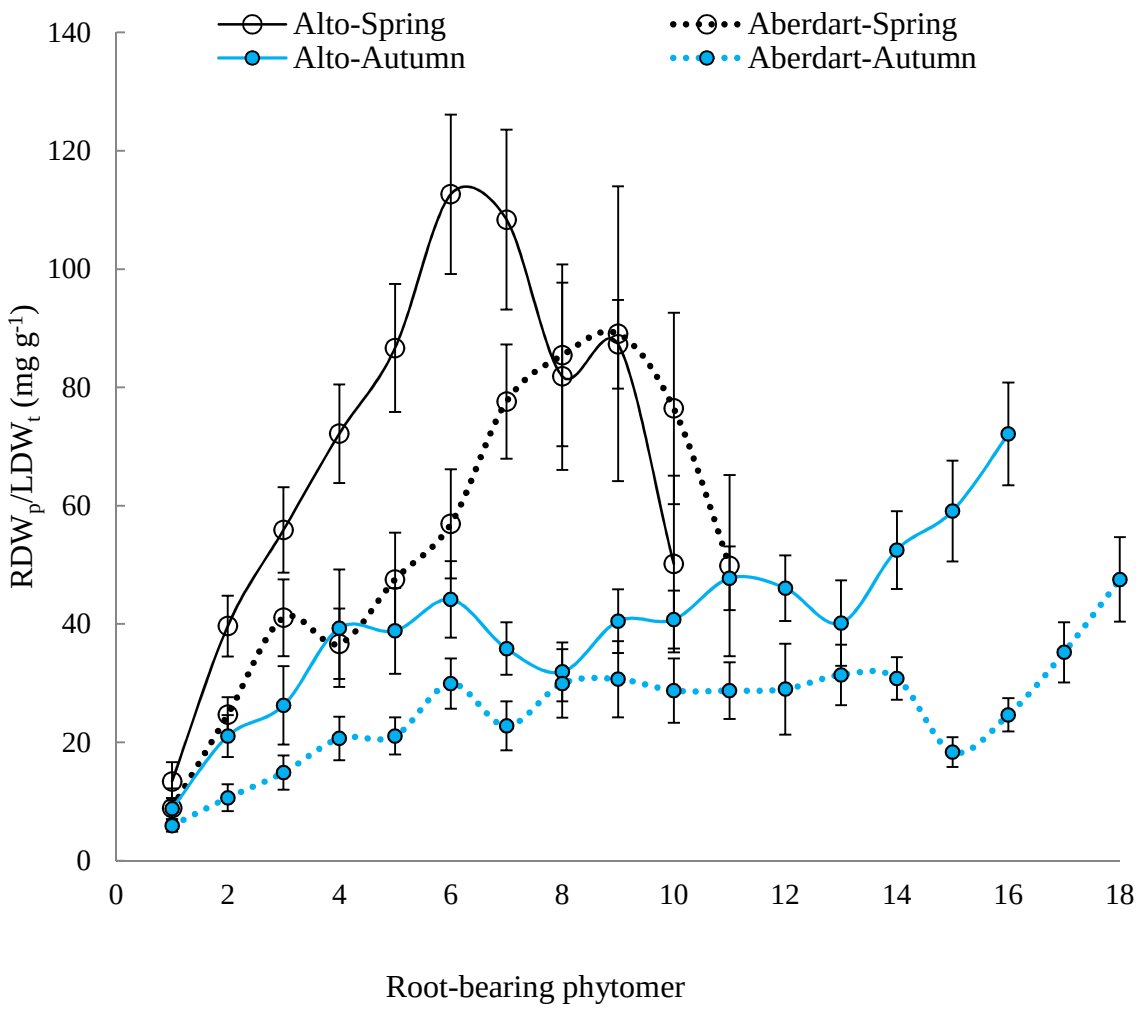


Fig. 6.2 Ratio between root dry weight per phytomer (RDW_p) and leaf dry weight per tiller (LDW_t) at different root-bearing phytomers (Pr) for Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments (Experiment 4 & 5, respectively). The duration of each experiment was approximately 90 d. Vertical bars indicate standard error at each phytomer position for each cultivar in each experiment. Pr1 is the youngest root-bearing phytomer.

6.4.3 Main tiller-daughter tiller morphological relations

6.4.3.1 Effects on the main tiller of the excision of adult daughter tillers

A detailed description of the MT and two DTs at the destructive harvest before the excision treatement are given in Appendices 6.3a and 6.3b for Alto and Aberdart, respectively. A brief comparison between the MT and the two DTs for LDW, LA, SLA, NLL, NP_r, R_p, RDW_t, R/S and DT/MT at the time of excision treatment is presented in Appendix 6.4. The excision of adult DTs in the spring experiment resulted in significantly lower (p=0.002) NPr for both Alto and Aberdart, and it would appear that that DT excision slowed new phytomer formation (Fig. 6.3) by approximately one phytomer within the 12 – 20 d period between excision and destructive harvest. In contrast with NPr, NLL for the DT- plants was increased by DT excision for Aberdart and slightly decreased for Alto with a significant treatment × cultivar interaction (Table 6.5). DT excision marginally increased the number of leaf appearance events compared to root appearance for the DT- plants compared to DT+ plants for both cultivars as reflected by NLL/NPr ratio (Table 6.5). Comparative details at the phytomer level between the DT+ plants and DT- plants are given in Appendices 6.5a and 6.5b for Alto and Aberdart, respectively, for the FLL, LDW, LA, SLA, R_p, RDW_i and net RDW increase at each phytomer. The RDW_t of the DT- plants was much lower than that of DT+ plants for Alto (p=0.011) and that reduction was localised to older roots at Pr8 and below (Fig.6.4, Appendix 6.5). DT excision decreased RDW_t but LDW_t was unaffected (Table 6.5).

Table 6.5 Leaf and root parameters of individual tillers for plants with two daughter tillers (DT+) compared to plants with the daughter tillers excised (DT-). NLL, number of live leaves per tiller; SLA, specific leaf area (cm^2 g^{-1}); NLL/NPr, ratio between number of live leaves and number of root-bearing phytomers; LDW_t, leaf dry weight per tiller (mg); NR_t, number of roots $tiller^{-1}$; RDW_t, root dry weight $tiller^{-1}$ (mg); RDW_t/LDW_t, the ratio between RDW_t and LDW_t; SEM, standard error of mean; p, statistical significance.

	NLL	SLA	NLL/NPr	LDW_t	NR_t	RDW_t	RDW_t/LDW_t
Alto- DT+	8.12	208	0.58	613	16.4	310	0.54
Alto- DT-	7.43	222	0.61	612	15.4	235	0.44
Aberdart- DT+	9.22	230	0.67	844	19.6	360	0.42
Aberdart- DT-	10.0	242	0.78	927	19.4	336	0.36
SEM	0.25	11.5	0.022	42.3	0.64	34.1	0.025
P (Cultivar)	<0.01	0.21	0.002	0.001	0.002	0.05	0.06
P (Treatment)	0.91	0.44	0.08	0.57	0.49	0.19	0.108
p (Treatment x Cultivar)	0.055	0.96	0.29	0.57	0.56	0.48	0.67

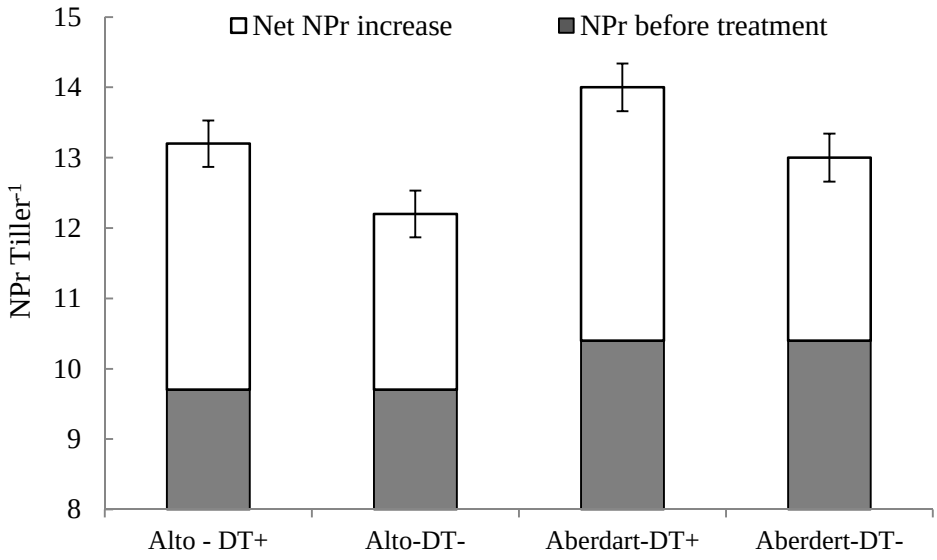


Fig. 6.3 Number of phytomers per tiller (NPr) at the destructive harvest and net NPr increase per tiller at the destructive harvest 12 – 20 d after removal of two daughter tillers for Alto and Aberdart perennial ryegrass cultivars in the Spring experiment. DT+, plants with two daughter tillers; DT-, plants with daughter tillers excised. Vertical bars indicate standard error of mean for each cultivar and treatment.

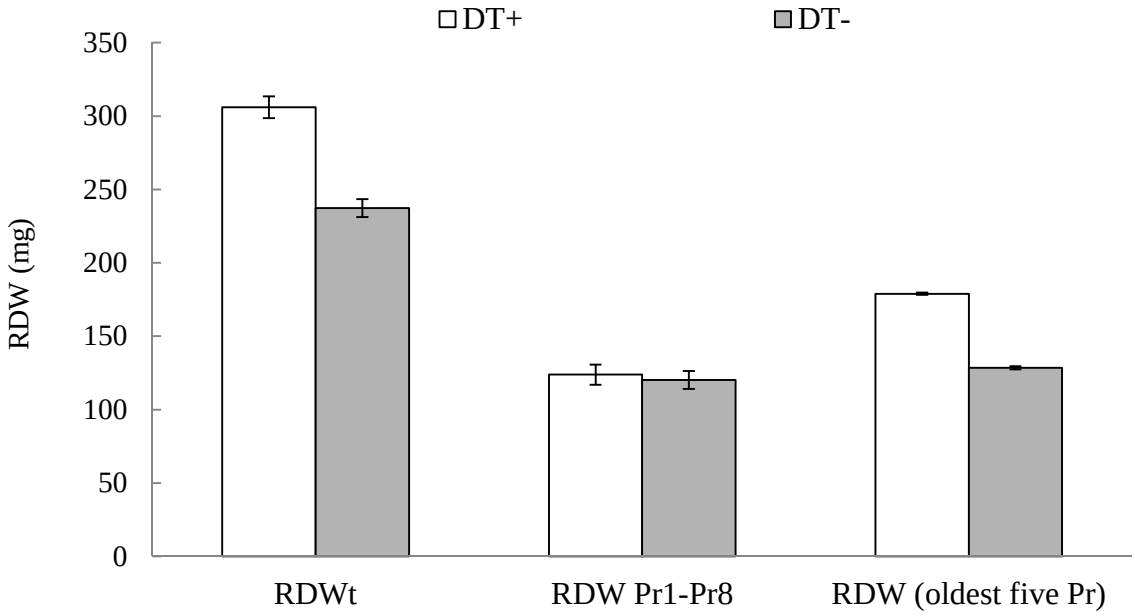


Fig. 6.4 Total root dry weight per tiller (RDW_t), RDW of the first eight phytomers (Pr1–Pr8), and RDW of the oldest five phytomers for perennial ryegrass cultivar Alto in the Spring experiment. Vertical bars indicate standard error of mean for each treatment. Data are for plants with two daughter tillers (DT+) and plants with daughter tillers excised (DT-) harvested 12 – 20 d after DT excision.

6.4.3.2 Effects of preventing daughter tiller formation

6.4.3.2.1 Effect on tiller morphological traits

Although mean FLL and LA of the individual leaves were marginally higher for the plants with two daughter tillers (DT+) than the plants without any daughter tillers (DT-) (p=0.09 in both cases, Table 6.6), LDW_t was significantly higher in DT- plants (Table 6.7) and this was contributed by significantly higher NLL of DT- plants (Table 6.7). SLA was consistently higher for DT+ plants than for DT- plants, both when averaged for the whole tiller (Table 6.6) and at individual phytomer positions (Fig. 6.5) which also probably contributed to higher LDW_t for DT- plants. The decreased SLA of the DT- compared to DT+ plants was evident for both cultivars (Fig. 6.5). The DT- plants had marginally higher R_p (p= 0.08, Table 6.6) and significantly higher NPr (p=0.013, Table 6.7). Thus, the NR_t was significantly higher in DT- plants (Table 6.7). The DT- plants had 39% and 43% higher RDW_p for Alto and Aberdart cultivar respectively than DT+ plants and the variation was statistically significant (Table 6.6). DT- plants also had significantly higher

RDW_p/LDW_t (Table 6.6). For the phytomer level data of DT- plants for both Alto and Aberdart see Appendix 6.6.

Table 6.6 Comparison of shoot and root morphological traits of individual roots and leaves of main tillers of Alto and Aberdart perennial ryegrass cultivars for plants with two daughter tillers (DT+) and plants without daughter tillers (DT-) after 93 days of growth in the Autumn experiment (Experiment 5). FLL_i, final leaf length per leaf (cm); LW_i, mean leaf width (mm); LDW_i, leaf dry weight per leaf (mg); LA_i, leaf area per leaf (cm^2); SLA, specific leaf area ($cm^2\ g^{-1}$); R_p, mean number of roots per phytomer; RDW_p, root dry weight per phytomer (mg); RDW_i, mean root dry weight of the individual roots at each phytomer (mg); RDW_p/LDW_t (mg g^{-1}), ratio of root dry weight per phytomer:leaf dry weight per tiller; SE, standard error of mean; p, statistical significance; Treat, difference between DT+ and DT-; Cul, cultivar.

	FLL_i	LW_i	LDW_i	LA_i	SLA	R_p	RDW_p	RDW_i	RDW_p/LDW_t
Alto- DT+	30.4	5.78	86.7	12.6	148	2.35	30.7	13.3	40.3
Alto- DT-	28.8	5.33	102	11.0	111	2.67	50.2	21.9	46.2
Aberdart- DT+	30.3	6.42	103	14.0	144	2.54	30.5	11.9	23.7
Aberdart- DT-	26.8	6.10	112	11.9	107	3.30	53.2	17.0	33.0
SE	0.86	1.09	6.22	2.21	6.19	1.15	5.58	3.19	5.25
p (Treat)	0.09	0.21	0.18	0.09	0.001	0.08	0.001	0.001	0.07
p (Cul)	0.47	0.03	0.17	0.30	0.69	0.12	0.92	0.08	0.001
p (Treat x Cul)	0.53	0.84	0.75	0.77	0.97	0.48	0.94	0.32	0.67

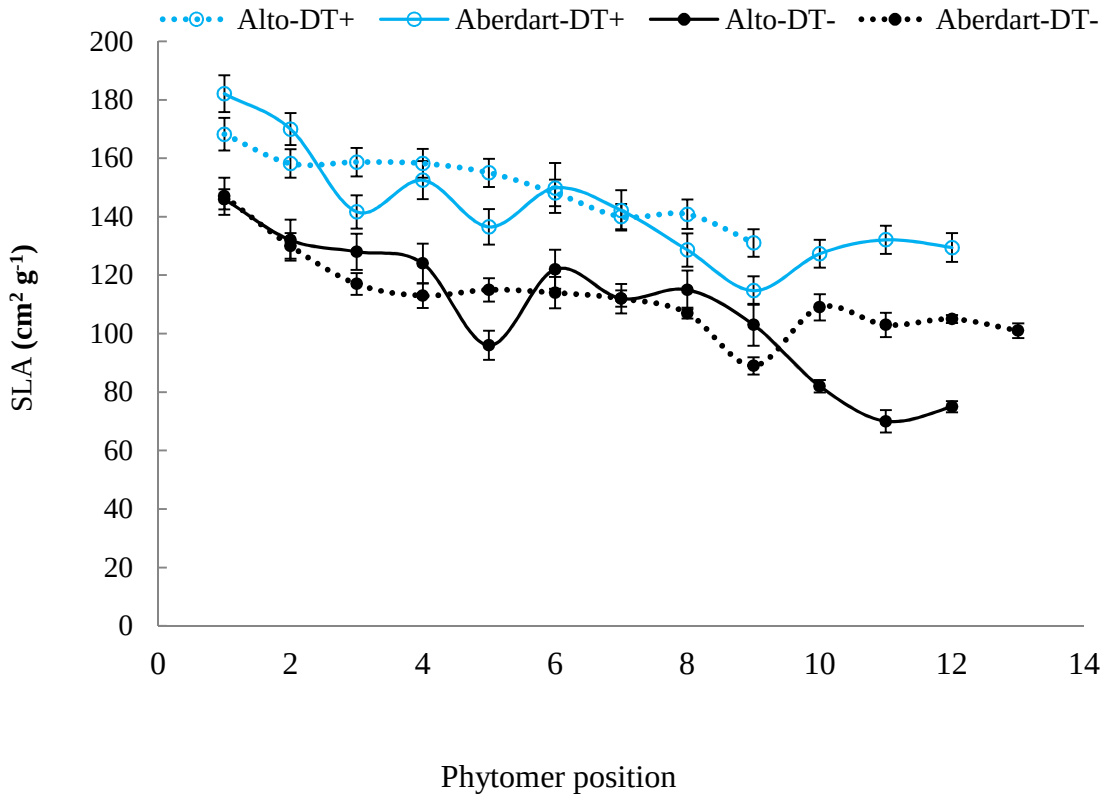


Fig. 6.5 Specific leaf area (SLA) at the different leaf positions of Alto and Aberdart perennial ryegrass cultivar for the plants with two daughter tillers (DT+) and plants without daughter tillers (DT-) for the 93 d growth period. Vertical bars show standard error at each phytomer position. Phytomer position 1 denotes the emerging leaf.

Table 6.7 Comparison of shoot and root morphological traits for individual main tillers of Alto and Aberdart perennial ryegrass plants with two daughter tillers (DT+) and plants without daughter tillers (DT-) for a 93 d growing period in autumn. LDW_t, leaf dry weight per tiller (mg); LA_t, leaf area per tiller (cm^2); NLL, number of live leaves per plant; NPr, number of root-bearing phytomers; NR_t, total number of roots per tiller; RDW_t, root dry weight per tiller (mg); NR_t/NLL, ratio between total number of roots and total number of live leaves per tiller; LDW_{DT}, total leaf dry weight of two DTs (mg); RDW_{DT}, total root dry weight of two DTs (mg). SE, standard error of mean; p, statistical significance; Treat, treatment difference between DT+ and DT-; Cul, cultivar.

	LDW_t	LA_t	NLL	NPr	NR_t	RDW_t	NR_t/NLL	LDW_{DT}	RDW_{DT}
Alto- DT+	778	116	8.38	16.8	37.2	467	6.27	1477	806
Alto- DT-	1110	125	10.8	17.3	46.0	888	4.51	-	-
Aberdart- DT+	1310	174	12.2	18.1	40.1	478	9.72	2027	1020
Aberdart- DT-	1630	176	14.3	19.2	64.5	1080	4.55	-	-
SE	64.7	6.03	0.41	0.22	2.11	43.8	0.21		
p (Treat)	0.023	0.554	0.008	0.013	0.004	<0.001	0.325		
p (Cul)	0.001	<0.001	<0.001	0.072	0.049	0.317	0.348		
p (Treat x Cul)	0.96	0.73	0.84	0.072	0.014	0.272	0.321		

6.4.3.2.2 Root development in plants with or without daughter tillers

The age of the two daughter tillers of DT+ plants at harvest was between 80-85 days in the Autumn experiment. The ratio between RDW_p/LDW_t at the destructive harvest for the younger 12 phytomers, which were located above the DTs at the tiller axis, of DT+ and DT- plants are presented in Fig. 6.6 for Alto and in Fig. 6.7 for Aberdart. The ratio differed significantly between the DT+ and DT- plants for phytomers 5 to 10 (p=0.053). The two cultivars Alto and Aberdart varied significantly (p=0.049) for that ratio at phytomers 5-10. The cultivar x treatment interaction was non-significant (p=0.61). The variation between phytomer positions for each cultivar was also non-significant for RDW_p/LDW_t (p= 1.00).

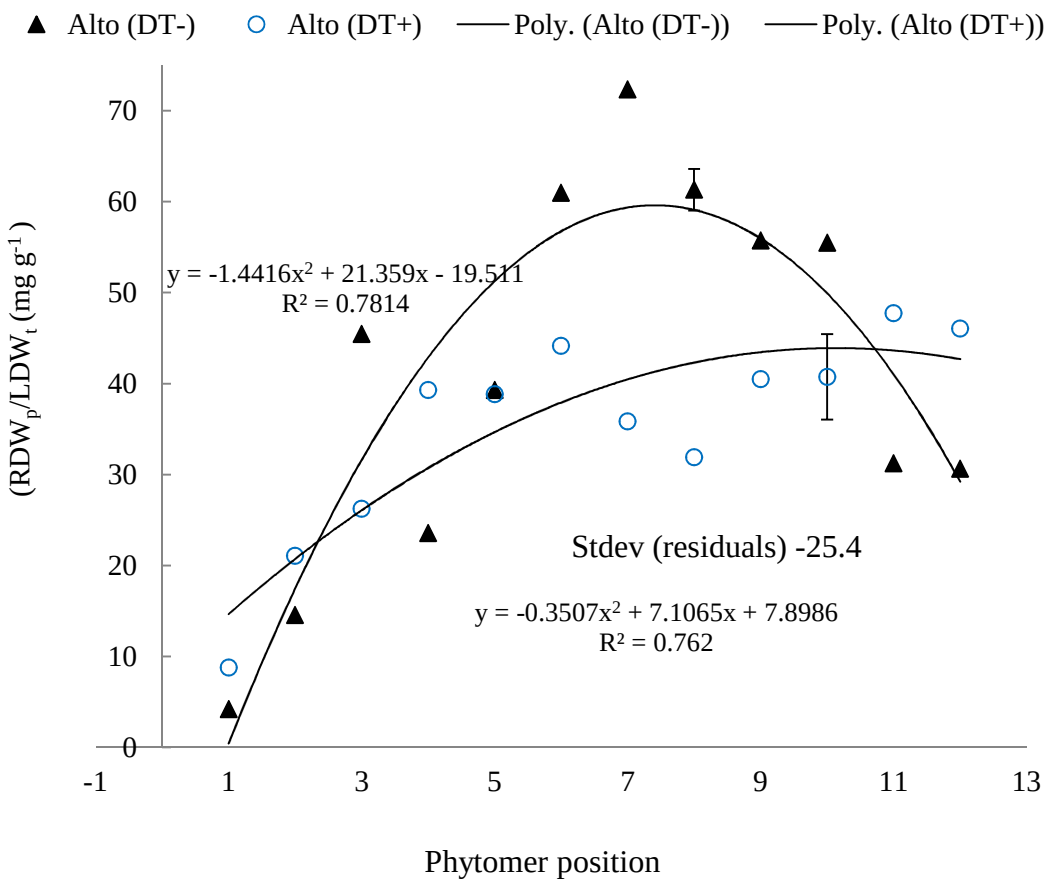


Fig. 6.6 Ratio of root dry weight per phytomer position (RDW_p): leaf lamina dry weight per tiller (LDW_t) for perennial ryegrass cultivar Alto in the Autumn experiment for DT+ and DT- plants. Vertical bars indicate standard error of mean for all data for each treartment. The trend line is represented by a quadratic curve. Stdev, standard deviation.

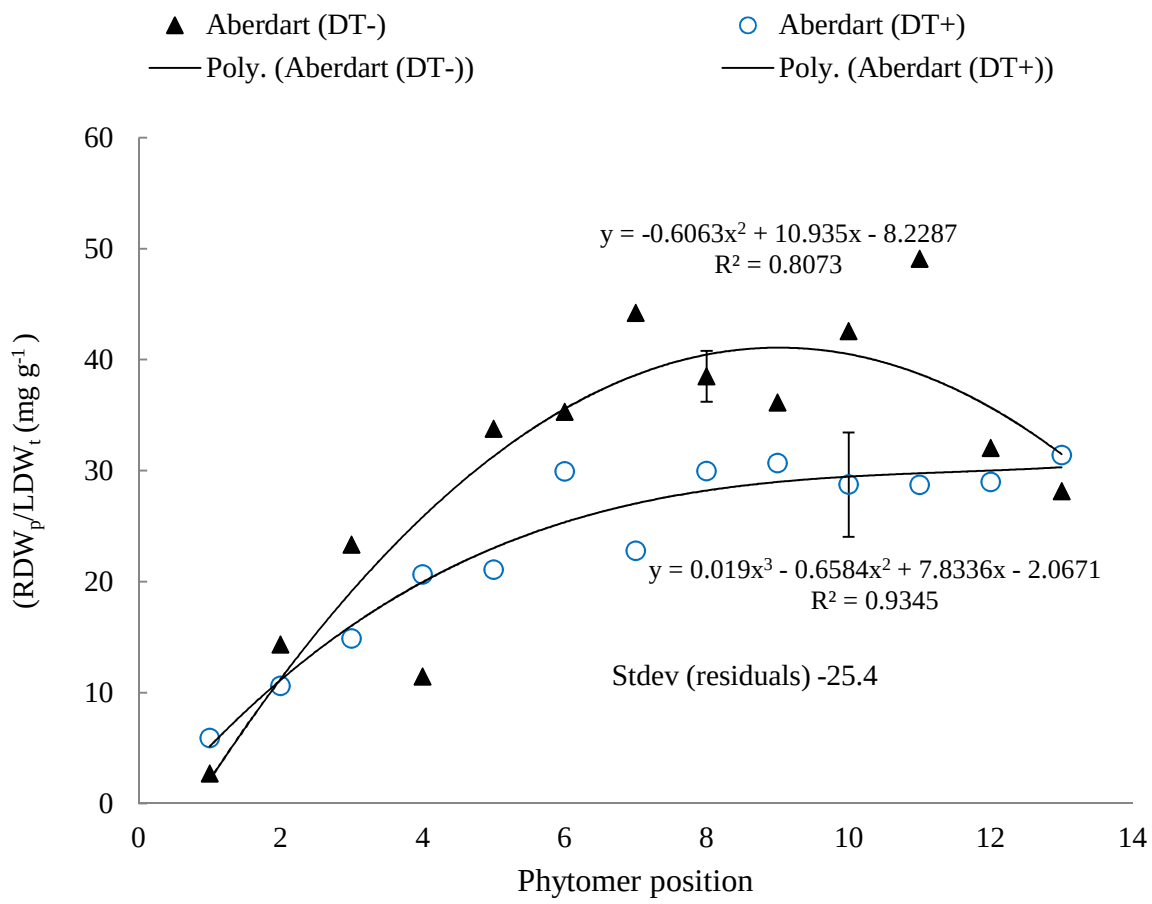


Fig. 6.7 Ratio of root dry weight per phytomer position (RDW_p): leaf lamina dry weight per tiller (LDW_t) for Aberdart perennial ryegrass in the Autumn experiment for plants with (DT+) and without daughter tillers (DT-). Vertical bars indicate standard error of mean for all data for each treartment. The trend line is represented by a quadratic curve for DT- plants and a cubic curve for DT+ plants. Stdev, standard deviation.

6.4.3.2.3 Effect of daughter tiller removal on adjacent root-bearing phytomers

The mean NPr was 17 for both DT+ and DT- plants for the cultivar Alto. The approximate mean locations of the two DTs at the MT for DT+ plants were at Pr15 and Pr16. There was a statistically significant difference (p=0.027) for the ratio RDW_p/LDW_t at Pr14, Pr15 and Pr16 for Alto (Fig. 6.8).

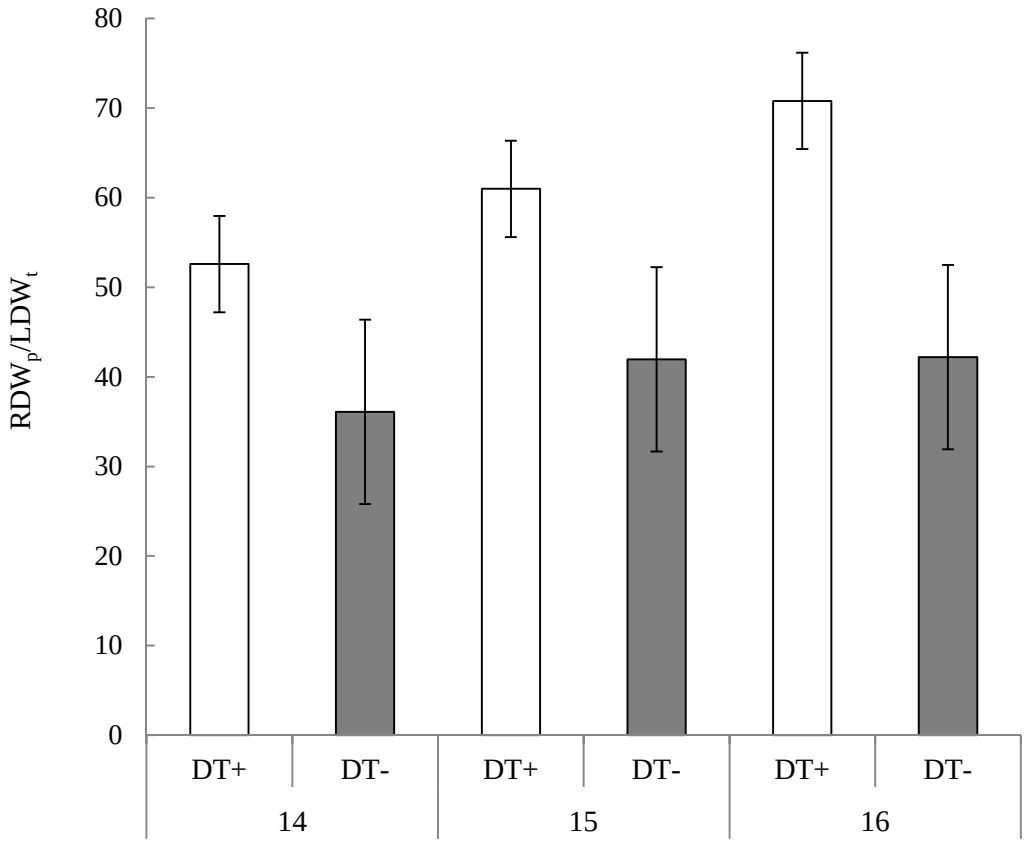


Fig. 6.8 Root:shoot ratio expressed as root dry weight per phytomer position (RDW_p) divided by total leaf dry weight (LDW_t) per tiller for perennial ryegrass cultivar Alto in the Autumn experiment (Experiment 5) at the oldest three phytomer positions in DT+ and DT- Plants. Vertical bars indicate standard error of mean for each treatment.

6.5 Discussion

6.5.1 Seasonal variation in root:shoot ratio

Plant size difference between experiments conducted in spring and autumn was a significant factor to consider when comparing root:shoot ratio. Tillers in autumn were on average more than double the DW of spring tillers and this difference was clearly reflected by PC1 scores (Table 6.4). For the autumn-grown tillers a much higher number of roots per tiller were fed by comparatively fewer leaves (Table 6.1) even though NLL, LDW_t and LA_t were higher in the autumn than in the Spring experiment. PC1 scores also separated the two cultivars and indicated that Alto was smaller in size than Aberdart in both experiments. The correlation analysis was carried out to explore how tightly the various measures of root and shoot morphology interrelate or vary independently across genotypes for the plants of different size growing in increasing and decreasing day length. These data suggested that greater leaf area coupled with higher NLL resulted in higher RDWt (Table 6.2).

In autumn, a significantly larger number of roots was fed by a comparatively smaller number of live leaves and in spring, there were comparatively fewer roots to be fed by the higher number of leaves (Table 6.1). For both of the cultivars proportionately greater leaf areas were involved in feeding a single root in spring than in autumn (Table 6.1) and this area difference did not appear to be offset by a major difference in mean photosynthesis rate (Fig. 4.19). These variations probably contributed to the smaller root:shoot ratio (RDW_p/LDW_t) at the individual phytomers (Fig. 6.2) which was as hypothesized by Matthew et al. (1998). That means seasonal variation in LAR (Fig. 4.5) which is in turn associated PrAR (see Section 5.5.1), and a lag in synchrony between these two mostly modified these two, ratios although leaf area of the individual leaves and the photosynthetic capacity of the unit leaf area should be also considered. In the Autumn experiment, the ratio between Alto:Aberdart for NR_t:NLL was 1.34 meaning that Alto had more roots to feed by less leaves compared to Aberdart, but the RDW_t/LDW_t ratio of 1.68 between Alto:Aberdart indicated that Alto was able to accumulate more RDW even when smaller number of live leaves and/or smaller leaf area was allocated for feeding each root. Therefore it can be argued that Alto plants in Autumn were more efficient in root DM production from fewer leaves than Aberdart plants and this argument is supported by Table 4.7 which suggests that Alto leaves were more photo-efficient than Aberdart leaves.

PC2 scores also separated these two cultivars for comparatively higher RDW_t of Alto than Aberdart against proportionately lower LDW_t and LA_t, with the effect being stronger in the Autumn experiment (Table 6.3).

The root:shoot ratios in the two seasons (range 0.70 to 0.38, Table 6.1) were close to those recorded by Crush et al. (2005). They reported root: shoot ratios of 0.63 and 0.57 for *L. perenne* (cv. Grasslands Samson) and *L. multiflorum* (cv. Grasslands Tama), respectively.

6.5.2 Contribution of daughter tillers to main tiller development

6.5.2.1 Adult daughter tillers fed older roots of the main tiller

One common observation from the two experiments involving a daughter tiller excision treatment is that adult daughter tillers appear to be involved in feeding older roots of the main tiller. In the absence of adult daughter tillers in the Spring experiment, roots of the oldest 5 phytomers of DT- plants of Alto had lower RDW than the DW of the oldest five phytomers of the DT+ plants, while RDW of the younger 8 phytomers did not differ between the treatments (Fig. 6.4). Similarly, in the Autumn experiment, significantly higher RDW_p/LDW_t of the older roots of Alto DT+ plants was observed in the presence of two adult daughter tillers (Fig. 6.8). Since in both Spring and Autumn experiments the increased RDW at older phytomers was limited to cultivar Alto, further investigation is needed both to confirm the link between the presence of a DT and increased RDW of MT phytomers near the DT, and to establish why the effect in cultivar Aberdart was non-significant for both of the experiments.

6.5.2.2 DT's shoot has a homeostatic relation with MT's root

Removal of adult daughter tillers in the spring experiment resulted in significantly lower NPr (Fig. 6.3). Existing literature suggests that removal of shoot parts accelerates shoot growth, and removal of root parts accelerates root growth until a balance is established between the root and shoot system (Klepper, 1991). A marginal increase in NLL/NPr and a marginal decrease in RDW_t/LDW_t for the DT- plants compared to the DT+ plants indicated that after DT removal, the rate of leaf appearance was faster than the rate of root-bearing phytomer appearance (Table 6.5). For the cultivar Alto, 12 – 20 d after the excision treatment was imposed, NLL had decreased only slightly (0.67 phyllochrons) whereas NPr decreased by 1.74 phyllochrons. As Aberdart was a faster growing cultivar

than Alto (Table 5.4), NLL of DT- plants after DT excision exceeded even that of DT+ plants (Table 6.5). The plants behaved so as to maintain a homeostatic relationship between DT's shoot and MT's root is further supported by significant reduction of RDW_t for the DT- plants of Alto compared to DT+ plants (Fig. 6.4) even though LDW_t at the destructive harvest was similar (Table 6.5). The faster growth rate of Aberdart cultivar may have masked the variation in RDW_t within the 12 – 20 day period under study.

6.5.2.3 Effects of preventing any daughter tiller formation for longer duration

As with Aberdart in the Spring experiment, absence of DTs throughout the growing period increased NLL for the DT- plants compared to the DT+ plants for both of the cultivars (Table 6.7) in the Autumn experiment. Significantly higher NLL for the DT- plants compared to the DT+ plants in the Autumn experiment suggested that maintaining the DT- plants without DTs resulted in higher NLL at harvest by increasing LAR. In field swards, cutting or grazing usually increases tillering and leaf appearance rate, unless a heavy cutting treatment is imposed (Van Loo, 1993). The assumed faster LAR after DT excision in this experiment might be associated with either hormonal influence at the growing point or higher substrate allocation to new shoot growth.

In the absence of any DTs, DT- plants produced marginally shorter FLL_i and smaller LA_i than DT+ plants (Table 6.6), but had a much higher NLL (Table 6.7) and lower SLA (Fig. 6.5) resulted in significantly greater LDW_t for the DT- plants compared to DT+ plants (Table 6.7). A greater value of NPr in the Autumn experiment for the DT- plants compared to the DT+ plants (Table 6.7) was probably associated with the faster LAR as no root death was noted in either treatment and as it was therefore assumed that the higher NPr was achieved from faster LAR rather than reduced root death at the base of the tiller axis.

An increase in NLL may have supplied more photo-assimilate for the root formation at the youngest Pr and thus resulted in marginally higher R_p for the DT- plants compared to DT+ plants (Table 6.6). Significantly higher NPr coupled with marginally higher R_p at each Pr for the DT- plants could explain the significantly higher NR_t compared to the DT+ plants (Table 6.7). One interesting observation was that RDW_i for the DT- plants were significantly higher compared to the DT+ plants (Table 6.6). In Chapter 5 (see Fig. 5.4) it was reported that the Spring and Autumn experiments did not produce any significant

variation in RDW_i. This strikingly different observation of smaller RDW_i for the DT+ plants was likely to be associated with lower deposition of photo-assimilated C when two DTs of the DT+ plants were involved in withdrawing C for their development and could explain the significantly lower RDW_i of the developing roots. The presence of these two DTs may also have had a role in reducing NLL (Table 6.7), and increasing SLA (Fig. 6.5) and ultimately decreasing LDW_t of the MT of DT+ plants (Table 6.7) due to size-compensation of the MT by the DTs. Significantly higher RDW_i along with a marginally higher R_p value resulted in significantly higher RDW_p for the DT- plants, compared to the DT+ plants (Table 6.7). Eventually the DT- plants had higher RDW_t compared to DT+ plants.

6.5.2.4 Effect of DT removal on root:shoot ratio in the Autumn experiment

Significantly greater RDW_p/LDW_t at Pr 5 – Pr10 for the DT- plants compared to the DT+ plants for both of the cultivars in the Autumn experiment (Fig. 6.6, 6.7) suggested higher C availability at those Pr positions. As discussed in the previous section, significantly higher RDW_p for the DT- plants compared to the DT+ plants was associated with marginally higher R_p and significantly higher RDW_i. The significantly lower RDW_p/LDW_t at Pr5–Pr10 (Fig. 6.6, 6.7) of the DT- plants was more likely to be associated with significantly lower RDW_i at those Pr. A significantly smaller NLL of the DT+ plants (8–11 and 12–14 for the DT+ and DT- plants, respectively) compared to the DT- plants would have resulted in a lower potential for photo-assimilation (Table 6.7). Besides a lower C assimilation potential, two extra DTs of the DT+ plants perhaps withdrew a share of photo-assimilate when roots at Pr5–Pr10 were developing.

6.6 Summary

- When shoot and root data of the individual tillers (Chapter 4 and 5, respectively) were pooled in a PCA, PC1 reflected the larger size of plants in autumn than in spring. PC1 scores also indicated that Alto plants were comparatively smaller than Aberdart plants in both experiments. PC2 explained 17.2% of data variation and reflected a higher root:shoot ratio of cultivar Alto than Aberdart in the Autumn experiment (i.e. cultivar Alto had proportionately less leaf area to feed a comparatively larger mass of roots than Aberdart.

- Autumn tillers growing in decreasing day length had significantly lower RDW_p/LDW_t at each phytomer than spring tillers growing in increasing day length (Fig. 6.2). This result appears to support the hypothesis (see Chapter 3) that seasonal variation in phyllochron and the delay between leaf and root appearance at a particular phytomer changes root:shoot ratio.
- In the Spring experiment, the short term (16 d on average) results of removal of adult DTs included significantly reduced NPr at the axis of MT. As NLL did not differ significantly between treatments, the NLL/NPr ratio was increased marginally by the DT excision treatment suggesting that removal of shoot area increases shoot growth rate and decreases root growth rate until and a balance in root:shoot ratio is achieved (Table 6.5).
- In the Spring experiment, the excision of DTs resulted in significantly decreased RDW_p at the oldest five phytomers of the MT at the destructive harvest 12–20 d later. In the Autumn experiment, the lack of DTs for the whole 93 d growing period significantly decreased RDW_p/LDW_t at the phytomers below the DTs but RDW_p/LDW_t for the younger Pr5-Pr10 above the DTs was increased. These results suggested that DTs possibly feed the older roots below their position and in absence of DT older roots suffer from C starvation and start decomposition. These relationships were statistically significant only for the cultivar Alto.
- A significant decrease in RDW_p/LDW_t at the Pr5–Pr10 for both of the cultivars in the Autumn experiment for the DT+ plants suggested that DTs during their establishment might extract C from the neighbouring young roots of MT, but the DT+ plants also had less number of live leaves for C assimilation. A further study is therefore needed to explain this complex situation.
- DT excision in the Autumn experiment significantly increased NLL, an effect associated with higher NPr, NR_t, R_p, RDW_i, RDW_p and thus RDW_t of the DT- plants compared to the DT+ plants.

Chapter 7: Functional Implications of Segmental Organisation

7.1 Introduction and Overview

In Chapter 6 the root-shoot relations of two *L. perenne* cultivars, Alto and Aberdart in increasing and decreasing day length of spring and autumn, respectively, were discussed. Chapter 6 also discussed the effect of removal of daughter tillers on dry matter deposition at phytomers elsewhere on the tiller axis. For some grass species there is evidence that photo-assimilates derived from daughter tillers are translocated to the main tiller. As reported in Chapter 2, Clifford et al. (1973) studied the C exchange pattern between the main tiller (MT) and daughter tiller (DT) of young seedlings of *L. multiflorum*, Carvalho et al. (2006) studied the C exchange between MTs and DTs of different age for *P. maximum* and found that young primary tillers translocated a larger share of photoassimilates to the roots of MT than older primary tillers. These results were in agreement with Colvill and Marshall (1981) who suggested that with increasing age of DTs they become an independent assimilatory unit. In the present chapter we therefore explore the C and N exchange patterns between MT and adult primary tillers which appeared 1-2 weeks later after transplanting. The approach used for this research was that of stable isotope labelling with non-natural ratios of ^{12}C:^{13}C and ^{14}N:^{15}N. In addition, during sample analysis it was discovered that there were definite patterns of C and N isotope fractionation within shoot and root systems, and this isotope fractionation phenomenon was further explored.

7.2 Objectives

1. To study the exchange of C and N between the MTs and the attached mature DTs using stable isotope tracer methodology.
2. To report incidental findings of C and N isotope fractionation in root and shoot segments.

7.3 Materials and Methods

7.3.1 Plant material

To explore the C and N exchange pattern between the MTs and the adult DTs a separate experiment, Experiment 6, using some plants from the same population in hydroponic culture that was used in Experiment 5, was conducted during autumn of 2009 (see Section 4.3.5). The C labelling experiment was carried out twice (Experiment 6.1 for the cultivar Alto and Experiment 6.2 for the cultivar Aberdart) and in Experiment 6.2 the individual tillers were dual-labelled with both C and N isotopes. In Experiment 6.1 plants were labelled during day light hours for 5 d from 9 May 2009 until 13 May 2009, 67 d after transplanting into the hydroponic system. The age of the DTs was around 60 d during labelling. In Experiment 6.2, the individual tillers were again labelled for 5 d commencing 18 May 2009 when DTs were around 70 d old.

A total of 8 plants of cultivar Alto were selected in Experiment 6.1 for C labelling. The MTs of four plants and the DTs of another four plants of cultivar Alto were labelled. Two control plants were kept to compare the isotopic ratio of unlabelled plants with that of labelled plants. In Experiment 6.2, a similar experimental structure was followed. Out of two DTs present (Fig. 6.1), one randomly chosen DT was excised before labelling.

7.3.2 C labelling system

For C labelling it was decided that a stable C isotope would be used rather than radiocarbon. For that it was necessary to find a CO_2 source that has a different ^{13}C:^{12}C isotopic ratio from the atmosphere. A labelling system was developed for feeding the leaves of individual tillers ^{12}C-enriched CO_2, recovered from a commercial brewing operation, as described below.

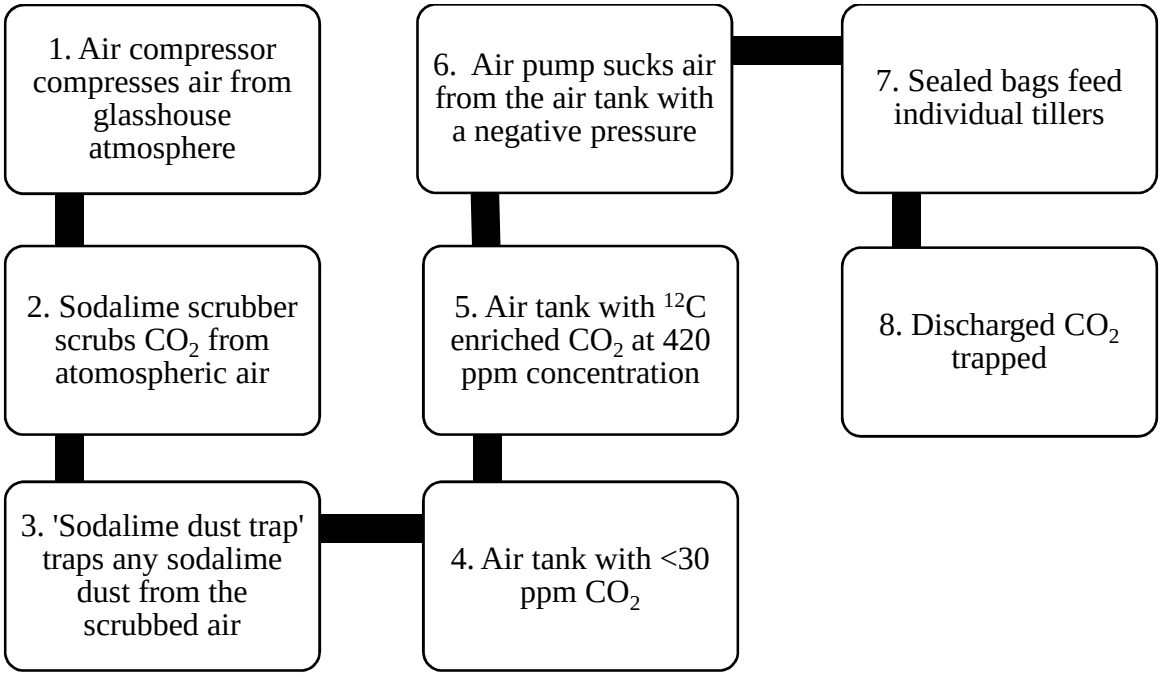


Fig. 7.1 Flow chart showing key components of the system set up for feeding individual tillers with ^{12}C-enriched CO_2.

An air tank comprising a mylar bladder of approximately 3.5 m^3 was constructed to prepare an air mixture with a $^{13}CO_2$:$^{12}CO_2$ isotopic ratio different from atmospheric (Fig. 7.3). The air tank was initially filled with air from a compressor passed through a soda-lime scrubber to remove atmospheric CO_2, then bubbled through water to remove soda-lime dust. In this way air with a very low CO_2 concentration (20 – 30 ppm CO_2) was provided to the tank (Fig. 7.1, Fig. 7.2, Appendix 7.1). A converted photosynthesis meter (LICOR 6200) was used to monitor CO_2 concentration at various points in the system (Fig. 7.4). CO_2 captured from a brewery fermentation vat was supplied by Lion Nathan brewery (Auckland) with $\delta^{13}C$ of -28.7 per mil (‰) (Appendix 7.2). This 'brewery' CO_2 was added by injection through a rubber septum to bring the CO_2 concentration to 420 ppm in Experiment 6.1 and to 430 ppm in Experiment 6.2. Approximately 1400 mL of brewery source CO_2 was required to raise the internal CO_2 concentration of the air tank by 400 ppm (Fig. 7.5, Appendix 7.3). An 8 mm diameter plastic tube led from the bladder to a PVC manifold from which eight individual aquarium pumps, each of approximately 1 L min^{-1} capacity, were connected to draw air from the manifold and pump the air to bags enclosing tillers to be labelled (Fig. 7.1, Fig. 7.6). The tillers to be labelled were placed in a sealed bag containing an inlet, which was supplied the prepared gas mixture, and an outlet that discharged the gas mixture continuously to allow the exchange of fresh air

inside the bag (Fig. 7.7). The discharged CO_2 from each sealed bag was trapped in a small soda-lime scrubber (Fig. 7.8) and the continuity of air flow was monitored visually by bubbling the outlet through a beaker of water. Periodically during each tiller labelling run CO_2 concentration in and out of the tiller enclosure bag was monitored using the LICOR 6200, and the flow rate through each bag was measured by trapping bubbles in an inverted measuring cylinder and timing the collection of a known volume of gas. When air was trapped inside the cylinder to measure flow in this way, the measuring cylinder was held so that water inside and outside the inverted cylinder remained at the same level, to avoid any pressure variation. Labelled tillers were fed ^{12}C enriched CO_2 in this way during day light hours for 5 d, a time period calculated (based on published CO_2 uptake and retention rates per unit lea area) to build up detectable levels of ^{12}C in plant organs receiving current photosynthate at the time of labelling.

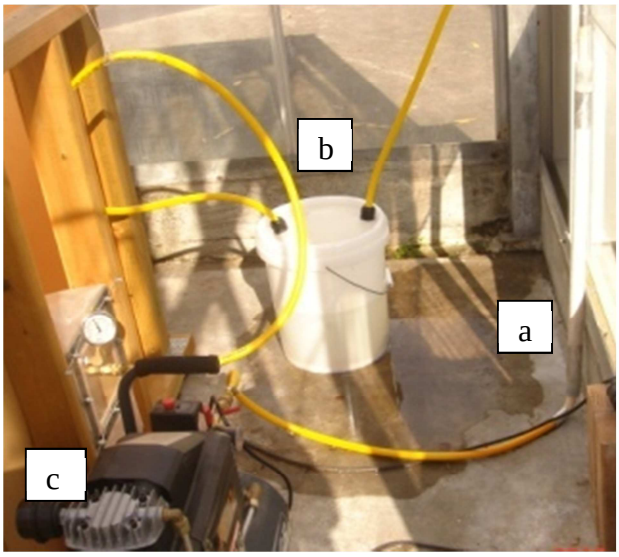


Fig. 7.2 Soda-lime CO_2 scrubber (a) and soda-lime dust trap (b) to remove atmospheric CO_2 supplied from the air compressor (c)

Fig. 7.3 The mylar air tank (>3m^3). Tank contains an internal mixing fan. Black tube supplies air to pump manifold. Taps allow Licor 6200 connection to monitor CO_2 concentration in the air tank

Fig. 7.4 Reading CO_2 concentration with an adapted Licor 6200

Fig. 7.5 Injecting ^{12}C enriched CO_2 (delta ^{13}C -28.7 per mil) collected from a fermentation vat at Lion Nathan brewery

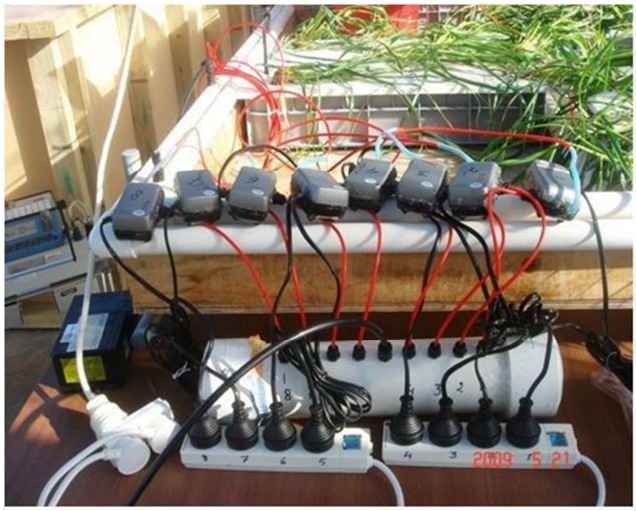


Fig. 7.6 Aquarium pumps, capacity 1 L min^{-1} supplying CO_2-air mixture to the individual plants

Fig. 7.7 Sealed bags feeding CO_2 to individual tillers. The mouth of each bag was sealed around the tiller pseudostem using a plastic jointing compound ('bluetack')

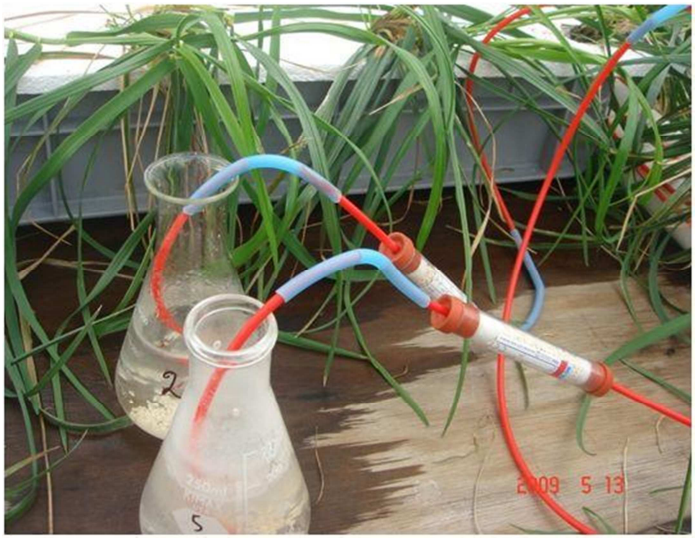


Fig. 7.8 Small soda lime scrubbers and water beakers to trap discharged CO_2 at the outlet and provide a visual confirmation of gas flow through the system

7.3.3 Calibration and testing of the C labelling system

7.3.3.1 Estimating C required per day

Assuming 4.0 cm LER per leaf d^{-1} (see Fig. 4.10 in Section 4.4.3) and that 2 leaves were elongating at a time (see Section 4.4.4) it was estimated that 64 cm leaf elongation occurs d^{-1} for the 8 individual tillers (4.0 cm x 2 leaves x 8 tillers). Assuming 1.0 cm leaf elongation accumulates 2.5 mg dry mass (Fig. 4.13 & 4.15), 160 mg DM is required d^{-1} for 8 plants for only leaf growth (10 mg DM $plant^{-1}$). Further assuming a C content per unit DM of 45%, approximately 72 mg C is required d^{-1} for shoot development of 8 plants. Again, assuming 40% of total photosynthetic C is lost by respiration (56 mg) and 15% of

remaining photosynthates are allocated to roots (13 mg), the requirement of C for 8 plants d^{-1} is approximately 141 mg (Danckwerts and Gordon, 1987). 141 mg C is equivalent to 517 mg CO_2 (141mg x 44/12). Further assuming that plants can extract an average of 150 ppm CO_2 when the supplied air contains more than 400 ppm CO_2 with an air density of 1.225 kg m^{-3}, an air volume of 2.81 m^3 (517 mg/ (150 mg/kg) x (1.225 kg/m^3)) was calculated as needed to feed the 8 plants each day.

7.3.3.2 Checking the air tank for leaks and diffusion gains

After filling the air tank with <30 ppm CO_2 concentration, the internal CO_2 concentration was monitored for the next 24 hours to check for the possibility of leaks or diffusion through the plastic. It was found that the CO_2 concentration inside the bladder remained quite stable or increased slowly at a rate of <2.0 ppm per hour (on 15 May 2009, at 07:00 am CO_2 concentration was 12 ppm and at 01:00 pm 21 ppm CO_2 was recorded inside the bladder).

7.3.3.3 Modifying and calibrating the air pumps

The aquarium pumps as purchased had no air intake, but were designed to allow air entry through screw holes and seams in the case. Each was sealed with silicone to prevent air intake through the case, and an inlet tube placed by drilling a hole in the case and screwing a fitting to fasten a plastic tube. A screw valve was fitted in the air line downstream of each pump. The flow rate delivered by each pump was measured from time to time and pumps were found to deliver in the range of 100 - 800 mL min^{-1} depending on the setting of the screw valve. A selection of pump flow data is presented in Appendix 7.4. Variation in pump flow was not felt to be of concern as the objective was merely supply labelled C in order to compare the final distribution of the labelled C between plant organs.

7.3.3.4 Calculating the CO_2 dilution due to leakage

The portion of the system between the bladder and the aquarium pumps was in negative pressure, creating a possibility of leakage into the system of atmospheric CO_2. The possible leakage points were at the junction of the bladder and the supply tube, at the junction of the supply tube and the PVC manifold, at the manifold outlet to each pump, and at the inlet junction of each pump. To check for such leakage air depleted in CO_2 was pumped through the system and the CO_2 concentration at the outlet of each pump was

recorded by LICOR (Appendix 7.4), and compared with the CO_2 concentration inside the bladder. Air ingress at either end of the pipe between the bladder and the manifold or manifold and air pumps would result in outlet CO_2 concentration being higher than bladder CO_2 concentration. With an assumed bladder CO_2 concentration of 26 ppm, the air output of the various pumps ranged from 32 – 124 ppm (Appendix 7.5a). At this level of leakage, the plants should still have received CO_2 of δ^{13C} in the range -22 to -25‰ (Appendix 7.5a, b).

7.3.3.5 Measuring flow rate and collecting gas samples

When the system was in operation, gas flow was regulated using the needle valves to provide a CO_2 concentration of typically 200 – 300 ppm at the outlet, after uptake by the tiller being labelled. The flow rate and CO_2 concentration upstream and downstream of the labelled tiller were measured periodically during operation (Appendix 7.4) to allow an estimate of photosynthesis rate. The flow rate at the outlet was also measured to be sure that gas mixture was being supplied at the expected flow rate and not lost by any undetected leakage. At the fastest rate of 800 mL min^{-1} gas flow, the 3.5 m^3 bladder contained sufficient air to supply the 8 labelled tillers for approximately 8 hours; hence the bladder was refilled daily.

Five samples of the gas mixture in the bladder were taken every day in 10 mL 'vacutainers'[1]. The average delta value measured was estimated at -28.7 $\delta^{13}C$ (‰) (Appendix 7.2), although the gas samples may have been contaminated in transit.

7.3.4 C and N labelling in Experiment 6.2

In Experiment 6.2, the roots of individual tillers were simultaneously labelled with 1 atom% $(^{15}NH_4)_2SO_4$ by isolating the roots of one particular tiller while leaves of the same tiller were simultaneously fed with ^{12}C-enriched CO_2 as described above for delivery of 1 atom% ^{15}N to plants (see calculation in Appendix 7.6). The isolated roots of the tiller being labelled were placed in a 1.2 L glass jar containing the 1 atom% $^{15}(NH_4)_2SO_4$, and sitting inside a 6.0 L capacity bucket containing nutrient solution. In this way one tiller was fed 1 atom% $^{15}(NH_4)_2SO_4$, while the roots of remaining tillers were fed the standard nutrient solution (Fig 7.9).

[1] Trade name for an evacuated glass tube with rubber septum normally used for blood sample collection.

Fig. 7.9 Dual labelling process: Feeding leaves of the selected tillers simultaneously with ^{12}C enriched CO_2 while the roots of the same tillers (inside the glass jar) received ^{15}N-labelled $(NH_4)_2SO_4$.

7.3.5 Analysis of isotope ratios for C and N

For the purpose of determining the distribution of isotope label within the plant, each labelled plant in Experiment 6.1 was dissected into 39 categories when one of the DTs was labelled and was dissected into 28 categories when the MT was labelled. In the case of MT labelling the categories denoted 18–28 were combined for both of the DTs (see Table 7.1). In Experiment 6.2 each plant was dissected into 30 categories (Table 7.1).

Table 7.1 Dissection categories for isotopically labelled root and shoot of Alto (Experiment 6.1) and Aberdart (Experiment 6.2) perennial ryegrass cultivars, in order to determine isotope distribution within the plant. EL, elongating leaf; Pr, root-bearing phytomer.

Experiment 6.1	Experiment 6.2
Main tiller	Main tiller
1. Basal 75 mm of EL	1. Base EL 1-2
2. Remainder of the EL	2. Tip EL 1-2
3. Other leaves	3. Other leaves
4. Pseudostem	4. Sheaths and pseudostem
5. Tiller axis	5. Tiller axis
6. Root tips Pr 1-2	6. Root tips Pr (1-3)
7. Root axis Pr 1-2	7. Root axis Pr (1-3)
8. Root tips Pr 3-4	8. Root tips Pr (4-6)
9. Root axis Pr 3-4	9. Root axis Pr (4-6)
10. Root tips Pr 5-6	10. Root tips Pr (7-9)
11. Root axis Pr 5-6	11. Root axis Pr (7-9)
12. Root tips Pr 7-8	12. Root tips Pr (9-12)
13. Root axis Pr 7-8	13. Root axis Pr (9-12)
14. Root tips Pr 9-12	14. Root tips Pr (13-15)
15. Root axis Pr 9-12	15. Root axis Pr (13-15)
16. Root tips Pr 13-rest	16. Root tips (Pr 16-rest)
17. Root axis Pr 13-rest	17. Root axis (Pr 16-rest)
Each daughter tiller *	Daughter tiller
18. Base EL	18. Base EL
19. Tip EL	19. Tip EL
20. Other leaf	20. Other leaf
21. Pseudostem	21. Pseudostem
22. Tiller axis	22. Tiller axis
23. Root tips Pr1–5	23. Root tips upper Pr1–5
24. Root axis upper Pr1–5	24. Root axis upper Pr1–5
25. Root tips middle Pr6–10	25. Root tips middle Pr6–10
26. Root axis middle Pr6–10	26. Root axis middle Pr6–10
27. Root tips lower Pr11-rest	27. Root tips lower Pr11-rest
28. Root axis Pr11-rest	28. Root axis Pr11-rest
*In the case of a labelled daughter tiller the two daughter tillers were sampled separately but when the main tiller was labelled, segments of both daughter tillers were combined.	N labelled roots for either main/daughter tiller 29. Tips 30. Axis

The individual plant parts (dissection categories) were oven dried for 48 hours and dry weights of all categories were recorded so that the total dry weight of the individual tillers could be determined by adding weights of the parts. The individual dissection components were ground in a ball mill (model MM200, Retsch®) after 48 hours of drying and stored in Eppendorf tubes to carry from New Zealand to Germany. The samples were analyzed in the isotope ratio mass spectrometer at the Lehrstuhl für Grünlandlehre, Technische

Universität München (TUM), D-85350 Freising-Weihenstephan, Germany. C and N content and ^{13}C:^{12}C and ^{15}N:^{14}N isotope ratios were determined on 1.0 mg dry matter aliquots using a CHN elemental analyzer (NA1500; Carlo Erba Strumentazione, Milan) interfaced to a continuous-flow isotope mass ratio spectrometer (Delta plus; Finnigan MAT, Bremen, Germany) as described by Lattanzi *et al.* (2005a). The isotope ratio analysis produced the following data for each plant sample analysed: %C, %N, C/N ratio, $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰).

7.3.6 Estimation of new N received by the individual plant parts

The natural occurrence of ^{15}N isotope in the samples of 0.3665 atom% was estimated from a standard curve plotting atomic % ^{15}N on the X-axis and $\delta^{15}N$ (‰) on the Y-axis (Appendix 7.7). The percent new N received by individual plant parts was then calculated using the following equation:

$$\%\ \text{New N} = \frac{(\text{atomic}\ \%\ 15\text{N in sample} - \text{natural abundance}) \times 100}{(1 - \text{natural abundance})}$$

……. *Equation* 7.1 (personal communication Dr. Rudi Schäufele).

Total new N recovered from the individual tillers was then calculated taking the dry weight (mg) of each plant part into account. Total new N recovered from each tiller was derived by summing the N recovered from all parts of the tiller. The new N recovered from each plant part or each tiller was determined as mg g^{-1} tiller DW.

7.3.7 Statistical analysis

Given Objective 1 (Section 7.2) to examine the exchange of C and N between adult MT and mature attached DT, the 8 labelled tillers and their attached tillers (see Section 7.3.1 above) were classified into 4 groups for statistical analysis purposes, ignoring possible covariance between attached tillers:

Group 1: MTL-MT, a MT tiller to which isotope label was applied;

Group 2: MTL-DT, a DT attached to a labelled MT and receiving label by translocation from the labelled MT;

Group 3: DTL-MT, a MT attached to a labelled DT and receiving label by translocation from the labelled DT;

Group 4: DTL-DT, a DT to which isotope label was applied.

To test the statistical significance of differences in distribution, the three statistical degrees of freedom for Groups were partitioned into a series of orthogonal contrasts: labelled tillers v. unlabelled tillers (Groups 1 & 4 v. 2 & 3), unlabelled MTs v. unlabelled DTs (Group 3 v. Group 2), and labelled MTs v. labelled DTs (Group 1 v. Group 4) (ANOVA model a, Appendix 7.8). Data presented in Table 7.2, Fig. 7.10, and Fig. 7.11 – 7.13 were statistically tested using orthogonal contrasts. Both original and log-transformed data for ^{15}N labelling experiment were tested using the orthogonal contrast model.

To test variation among shoot, root and tiller axes (three different compartments) for C and N isotope distribution traits and for new N uptake, the sum of squares for plants within treatment (MTL or DTL) was used as an error term (ANOVA model b, Appendix 7.8). For new N there were three compartments (shoot, root and tiller axis) for each tiller which made a total of 48 observations (8 plants x 2 tillers x 3 compartments) for MTs and DTs of the 8 plants. Data presented in Table 7.4 and Fig. 7.14, were tested using this model.

A total of 28 root and shoot dissection categories of each plant were tested to explore variation among them for C and N traits. A nested ANOVA was conducted using a GLM procedure. There were a total of 560 observations (28 dissection components x 10 plants, 8 labelled and 2 control plants x 2 cultivars) for %C, %N, C:N ratio, $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰). For new N received by each dissection category a total of 224 observations (8 plants x 28 dissection categories) were used in ANOVA (ANOVA model c, Appendix 7.8). Data are presented in Figs. 7.15 & 7.16 and Appendices 7.9 & 7.10.

To examine the data from a multivariate perspective, a PCA including the measures %C, %N, C:N ratio, $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) was conducted using 28 dissection components of all 10 plants from each experiments (8 labelled plants and 2 control plants) (see Table 7.1). There were thus a total of 560 data for each C and N trait. A nested ANOVA model was conducted to explore variation of the PC scores among compartments (ANOVA model d, Appendix 7.8) and dissection categories of each tiller (MT or DT) (ANOVA model e, Appendix 7.8) using the Minitab 15 statistical software package (Minitab Inc. State College, Pennsylvania) where the sum of squares for plants within tillers was used as an error term. PCA is presented in Table 7.5 and ANOVA for the PC scores are presented in Table 7.6.

A separate ANOVA was also conducted to test variation between three treatments: MTL-MT, DTL-DT and control. To do so, $\delta^{13}C$ (‰) values of all dissection categories of each treatment were taken to carry out a one-way ANOVA using Minitab 15. The results are presented in Section 7.4.1.

7.4 Results

This section presents data in the following sequence:

i) C exchange between MT and DT (Section 7.4.1);

ii) N exchange pattern between MT and DT (Section 7.4.2);

iii) N exchange pattern between shoot and root dissection categories of MT and DT (Sections 7.4.3 and 7.4.4 respectively);

iv) Inter-segmental C and N relations between shoot, roots and tiller axis and between different dissection categories of shoot and root in Section 7.4.5.

7.4.1 C exchange between main tiller and daughter tiller

In Experiment 6.1 the three different treatments: MTL-MT ($\delta^{13}C$ -27.26), DTL-DT ($\delta^{13}C$ -26.85) and Control ($\delta^{13}C$ -26.36) showed a subtle but statistically significant difference ($p<0.01$, SEM=0.06) for $\delta^{13}C$ (‰) discrimination indicating that plants received only a very weak ^{12}C enrichment signal during labelling. In testing the presence of labelled C in individual plant parts, there was a tendency for samples of the DTL-MT group to have a more negative delta ^{13}C indicating transfer from the labelled DT, and this trend attained significance for samples from the axes of young roots at Pr 1-5, middle aged (Pr 5-10) root tips and axes of unlabelled DT of MTL while by contrast the MTL-DT group had the least negative delta ^{13}C values (Table 7.2).

Table 7.2 Mean C isotope ratio ($\delta^{13}C$ (‰)) of different plant parts for different tiller categories of Alto perennial ryegrass in Experiment 6.1 for MTL-MT, main tillers of main tiller labelled plants; MTL-DT, daughter tillers of main tiller labelled plants; DTL-MT, main tillers of daughter tiller labelled plants; and DTL-DT; daughter tillers of daughter tiller labelled plants.

Tiller category	Base of elongating leaf	Other leaves	Leaf sheaths	Tiller axis	Young root tips, Pr 1-5	Young root axes, Pr 1-5	Middle aged root tips Pr6-10	Middle aged root axes Pr6-10	Lower root tips Pr11-rest	Lower root axes Pr11-rest
MTL-MT	-27.4	-27.7	-27.5	-27.4	-27.1	-27.1	-26.9	-26.9	-26.8	-27.2
MTL-DT	-27.4	-27.6	-27.3	-27.1	-26.8	-26.5	-26.2	-26.3	-26.1	-26.7
DTL-MT	-27.9	-28.1	-27.8	-27.5	-27.1	-27.2	-27.0	-27.1	-26.7	-27.0
DTL-DT	-27.4	-27.6	-27.3	-27.0	-26.8	-26.9	-26.4	-26.9	-26.7	-27.1
SE	0.19	0.15	0.16	0.21	0.16	0.15	0.13	0.15	0.12	0.12
*P value	0.37	0.26	0.24	0.62	0.52	0.09	0.024	0.05	0.14	0.30

*P value indicates statistical probability of difference between means for MTL-DT and DTL-MT as assessed by orthogonal linear contrast (see ANOVA model a, Appendix 7.8). The other orthogonal contrasts were not statistically significant for any dissection component.

In Experiment 6.2, C labelling did not produce any statistical difference between the three treatments: MTL-MT, DTL-DT and Control plants. In contrast to Experiment 6.1, in Experiment 6.2 MTL-DT had more negative delta values than DTL-MT. Orthogonal linear contrasts identified a marginally significant contrast between MTL-DT vs DTL-MT, only for leaf sheaths (p=0.07, Fig. 7.10). Contrasts for labelled vs unlabelled tillers, and for labelled MT vs labelled DT for all dissection categories were not significant (Fig. 7.10).

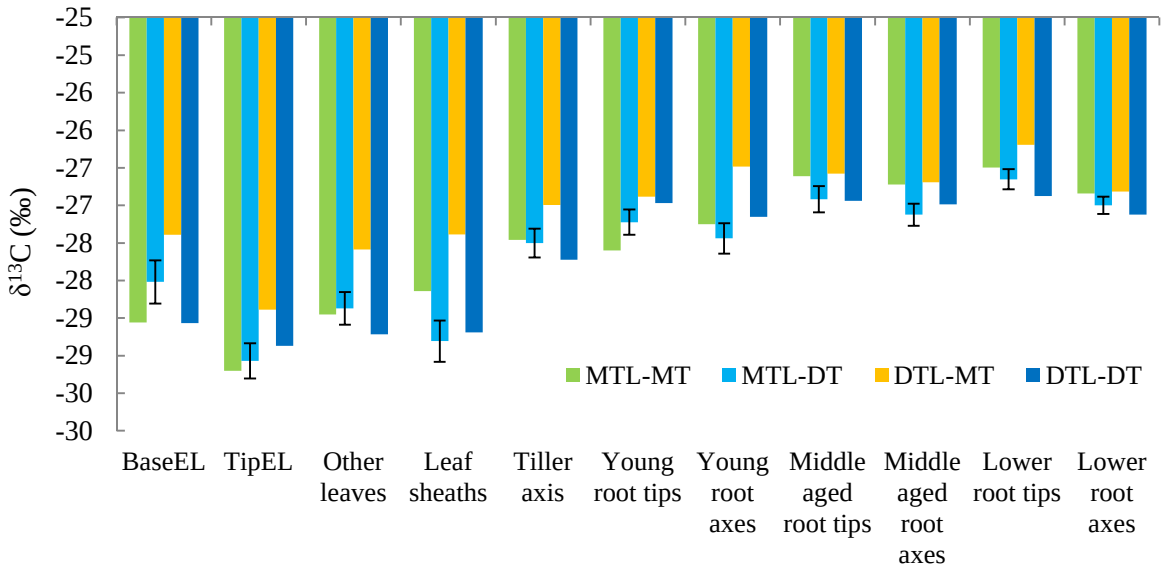


Fig. 7.10 C isotope ratio ($\delta^{13}C$ (‰)) in different dissection components of Aberdart perennial ryegrass for either main tiller labelling (MTL) or daughter tiller labelling (DTL) in Experiment 6.2. BaseEL, lower 75 mm of the elongating leaf inside the pseudostem; TipEL, tip of the elongation leaf. Vertical bars indicate standard error of mean for each dissection category.

7.4.2 Differential new N acquisition by the main tiller and the daughter tiller labelled plants and by individual tillers

The total new N taken up by the individual plants (MT plus DT) for either MT or DT labelling with $(^{15}NH_4)_2SO_4$ for 5 d was 0.93 mg N g^{-1} DW and in both cases with non-significant variation. The new N acquired by the labelled main tiller (MTL-MT) and the labelled daughter tiller (DTL-DT) was 1.53 and 1.48 mg N g^{-1} DW, respectively. The daughter tillers of the main tiller labelled plants (MTL-DT) acquired 0.424 mg N g^{-1} DW and the main tillers of the daughter tiller labelled plants (DTL-MT) received 0.142 mg N g^{-1} DW ($p=0.03$ in orthogonal linear contrast, Fig. 7.11, Table 7.3). As expected, the difference in N uptake by the labelled and unlabelled tillers was also significant when tested by orthogonal contrast (Table 7.3).

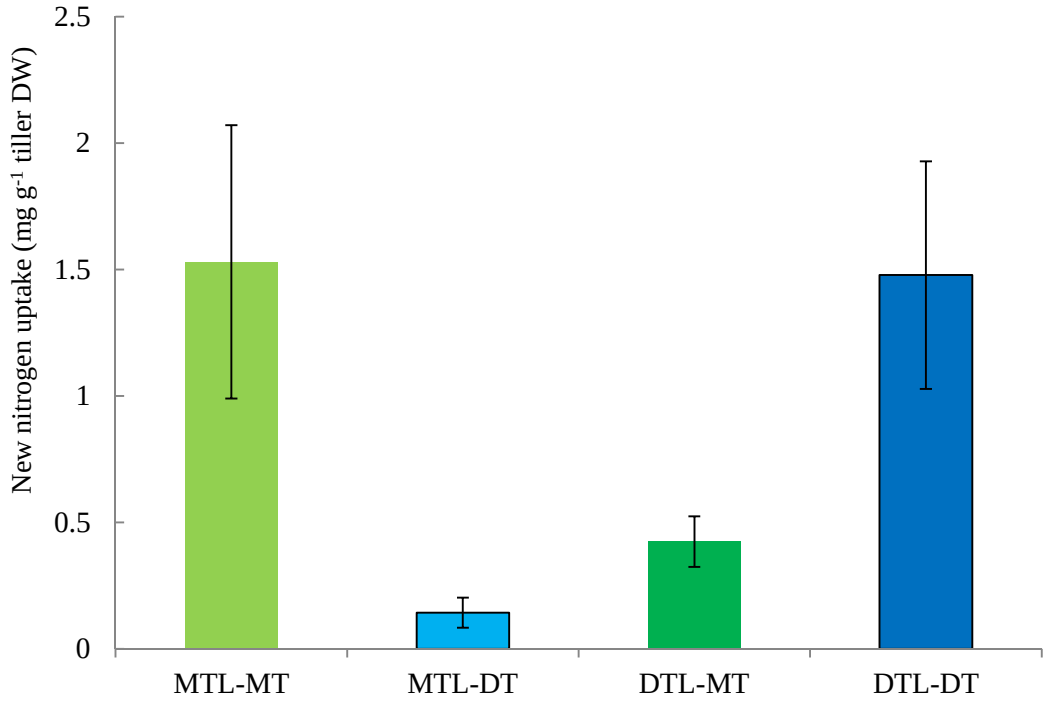


Fig. 7.11 New N uptake (mg g^{-1} tiller) for individual tillers of Aberdart perennial ryegrass after 5 d labelling with $^{15}(NH_4)_2SO_4$ in Experiment 6.2. MTL-MT, main tiller of main tiller labelled plants; MTL-DT, the daughter tillers of the main tiller labelled plants; DTL-MT, main tillers of daughter tiller labelled plants; DTL-DT, daughter tiller of daughter tiller labelled plants. Vertical bars indicate standard error of the mean for each tiller.

7.4.3 Differential new N acquisition by the shoot components of main tiller and daughter tiller labelled plants

The shoot dissection categories: The base of the elongating leaf, the tip of the elongating leaf, mature leaves, sheaths and tiller axis for labelled versus unlabelled tiller had highly significant differences in acquired ^{15}N between labelled and unlabelled tillers for both untransformed (Fig. 7.12, Appendix 7.9) and log-transformed data (Table 7.3). The unlabelled tiller groups MTL-DT and DTL-MT differences were statistically significant after log transformation (Fig. 7.12, Table 7.3).

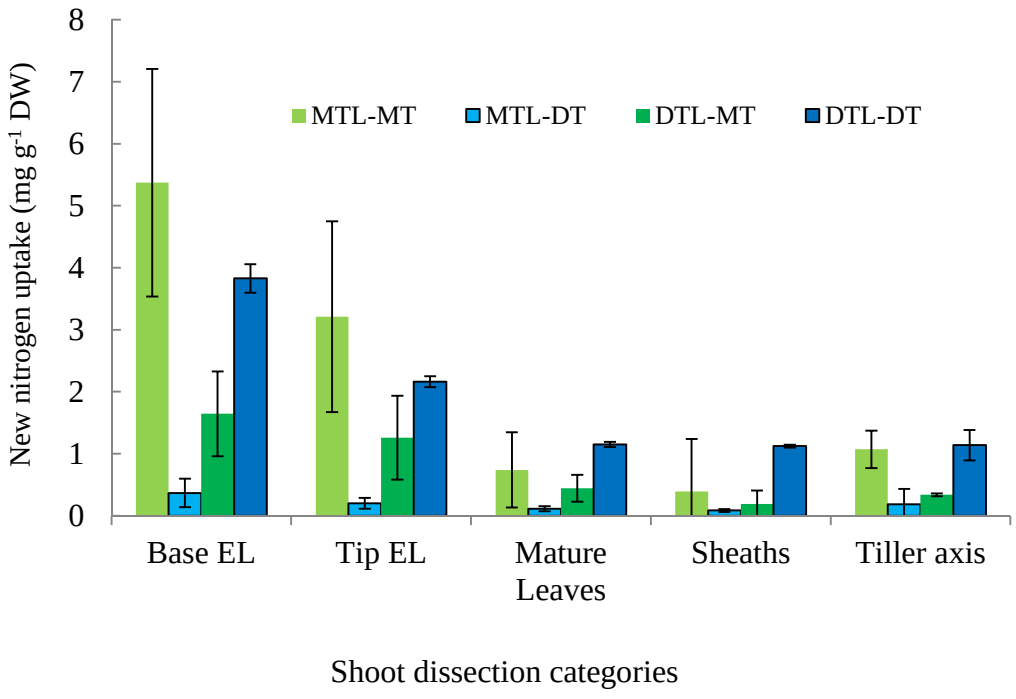


Fig. 7.12 The share of new N (mg g^{-1} DW) received by individual dissection categories for the shoot of Aberdart perennial ryegrass after 5 d labelling with $(^{15}NH_4)_2SO_4$. The roots of either main tiller or daughter tillers were supplied with ^{15}N labelled. EL, elongating leaf; MTL- MT, main tiller of main tiller labelled plants; MTL-DT, the daughter tillers of the main tiller labelled plants; DTL-MT, main tillers of daughter tiller labelled plants; DTL-DT, daughter tiller of daughter tiller labelled plants. Vertical bars indicate standard error of mean for each tiller for each of the dissection category.

7.4.4 Differential new N acquired by roots of varying ages of the labelled tillers and their attached tillers

Unlike shoot, root dissection categories displayed a non-significant difference between labelled and unlabelled tillers in ^{15}N acquisition (Table 7.3, Fig. 7.13, Appendix 7.9). Untransformed data for MTL-DT and DTL-MT yielded no significant differences for any root dissection component but log-transformation revealed a strong treatment effect in data for the young roots, diminishing with distance down the tiller axis and undetectable in roots of Pr11 and older phytomers (Table 7.3, Fig. 7.13, Appendix 7.9).

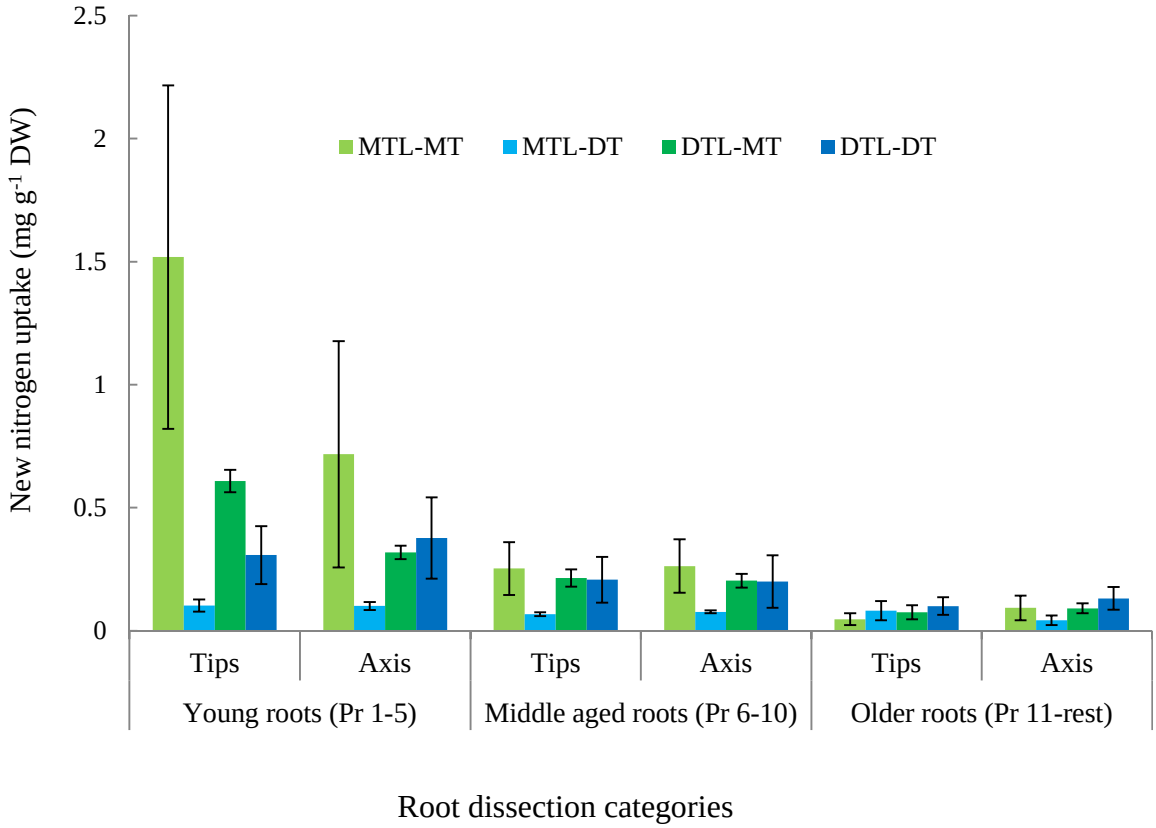


Fig. 7.13 The share of new N (mg g^{-1} segment) received by the individual root dissection categories of Aberdart perennial ryegrass after 5 d labelling with $^{15}(NH_4)_2SO_4$. MTL-MT, main tiller of main tiller labelled plant; MTL-DT, daughter tiller of the main tiller labelled plant; DTL-MT, the main tiller of a daughter tiller labelled plant, DTL-DT, daughter tiller of the daughter tiller labelled plant. Vertical bars indicate standard error of mean for each tiller for each of the root dissection category.

Table 7.3 Statistical significance of difference in ^{15}N uptake (mg g^{-1} tiller DW) for the various tiller dissection categories for Aberdart perennial ryegrass in Experiment 6.2, as tested by orthogonal linear contrast between labelled and unlabelled tillers (MTL-MT and DTL-DT versus MTL-DT and DTL-MT); unlabelled tillers (MTL-DT versus DTL-MT) and labelled tillers (MTL-MT versus DTL-DT). The data for which statistical information is presented here are reported in Fig. 7.11 to 7.13 above.

	Labelled vs unlabelled tillers		Unlabelled MTL-DT vs DTL-MT		Labelled MTL-MT vs DTL-DT	
	Original	Log-transformed	Original	Log-transformed	Original	Log-transformed
Individual tiller	0.008	0.001	0.61	0.03	0.92	0.95
Base of elongating leaf	0.016	0.002	0.49	0.018	0.40	0.71
Tip of elongating leaf	0.08	0.007	0.48	0.024	0.48	0.69
Mature leaves	0.06	0.004	0.44	0.015	0.90	0.79
Leaf sheathes	0.099	0.003	0.57	0.015	0.60	0.74
Tiller axis	0.003	0.003	0.64	0.05	0.84	0.87
Young root tips (Pr 1-5)	0.12	0.034	0.28	0.003	0.03	0.01
Young root axes (Pr 1-5)	0.22	0.11	0.57	0.095	0.35	0.10
Middle aged root tips (Pr 6-10)	0.26	0.47	0.20	0.08	0.68	0.53
Middle aged root axes (Pr 6-10)	0.28	0.35	0.29	0.08	0.59	0.53
Lower root tips (Pr 11 and older)	0.98	0.98	0.76	0.67	0.33	0.28
Lower root axes (Pr 11- and older)	0.26	0.43	0.50	0.42	0.94	0.46

7.4.5 Inter-segmental C-N relations

7.4.5.1 C-N relations among shoot, tiller axis and roots

The shoot, tiller axis and the roots of the two perennial ryegrass cultivars showed significant differences for %C and %N. The higher C:N ratio in roots and lower in shoots was associated with a significantly lower concentration of N and a higher concentration of C in roots compared to shoots (Table 7.4). The tiller axis had the lowest % of N among the compartments (shoot, axis and roots) for Alto, and was intermediate between shoot and root for Aberdart (Table 7.4). In the Experiment 6.2, the Aberdart plants were labelled with $^{15}(NH_4)_2SO_4$, therefore to make a comparatively reasonable comparison with cultivar Alto, only data from the unlabelled control plants for cultivar Aberdart were used to present $\delta^{15}N$ (‰) values in Table 7.4. It is also important to note that the cultivars Alto and Aberdart were harvested at different time points. For all other variables except $\delta^{15}N$ (‰) the data presented in Table 7.4 represents all experimental plants used in Experiments 6.1 and 6.2. The C:N ratio for the cultivar Alto differed significantly and that of Aberdart did not differ significantly among the three plant compartments (Table 7.4). For the unlabelled plants there was also evidence of isotopic fractionation of N and C between the compartments (Table 7.4). The tiller axis showed a trend towards depletion in ^{15}N with the shoot enriched and the root intermediate, but this trend was not significant for Alto and only marginally significant for Aberdart (Table 7.4). The isotopic ratio of C indicated an increasing proportion of ^{13}C from the shoot to the tiller axis and then to the roots (Table 7.4). The detailed data for different dissection categories for %C, %N and C:N ratio are in Appendix 7.10a and for $\delta^{15}N$ and $\delta^{13}C$ (‰) are in Appendix 7.10b.

Table 7.4 %C, %N and C:N ratio for shoot, tiller axis and roots of Alto and Aberdart perennial ryegrass cultivars in Experiment 6.

Compartments	% C		% N		C:N ratio		δ^{15}N (‰)		δ^{13}C (‰)	
	Alto	Aberdart	Alto	Aberdart	Alto	Aberdart	Alto	Aberdart	Alto	Aberdart
Shoot	40.0±0.38	41.5±0.16	4.39±0.11	3.84±0.11	10.3±0.54	12.40±0.60	1.31±0.13	1.60±0.40	-27.4±0.05	-28.4±0.07
Tiller axis	44.3±0.88	44.2±0.32	2.86±0.25	3.05±0.22	15.8±1.25	14.57±1.20	-0.34±0.29	-0.23±0.77	-27.1±0.11	-27.4±0.13
Root	45.5±0.27	43.3±0.11	3.00±0.08	2.80±0.81	16.6±0.38	16.39±0.43	0.17±0.09	0.91±0.27	-26.5±0.04	-26.9±0.05
P value	0.009	<0.001	0.001	0.018	0.013	0.21	0.247	0.09	0.059	0.062

The shoot, the tiller axis and the roots showed significant differences (p=0.044) for the uptake of new N (mg g^{-1} DW) (Fig 7.14).

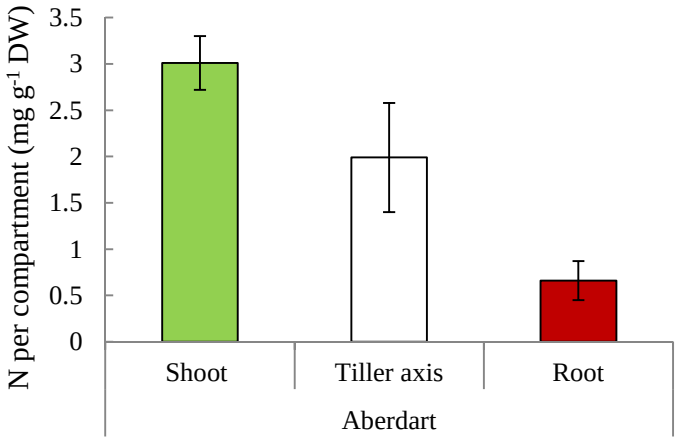


Fig. 7.14 New N concentration in the shoot, the tiller axis and the roots (compartments) of Aberdart perennial ryegrass after ^{15}N labelling for 5 d. Vertical bars indicate standard error at each compartment.

7.4.5.2 N isotope ratio in shoot and root dissection categories

The youngest shoot and root dissection categories showed similar N isotopic ratios ($\delta^{15}N$ (‰)) (Fig. 7.15). The $\delta^{15}N$ (‰) was progressively more positive from younger to older shoot dissection categories and more negative from the younger to the older roots (Fig 7.15), ($p<0.01$, see Appendix 7.10b), reflecting accumulation and depletion of the heavier ^{15}N isotope, respectively.

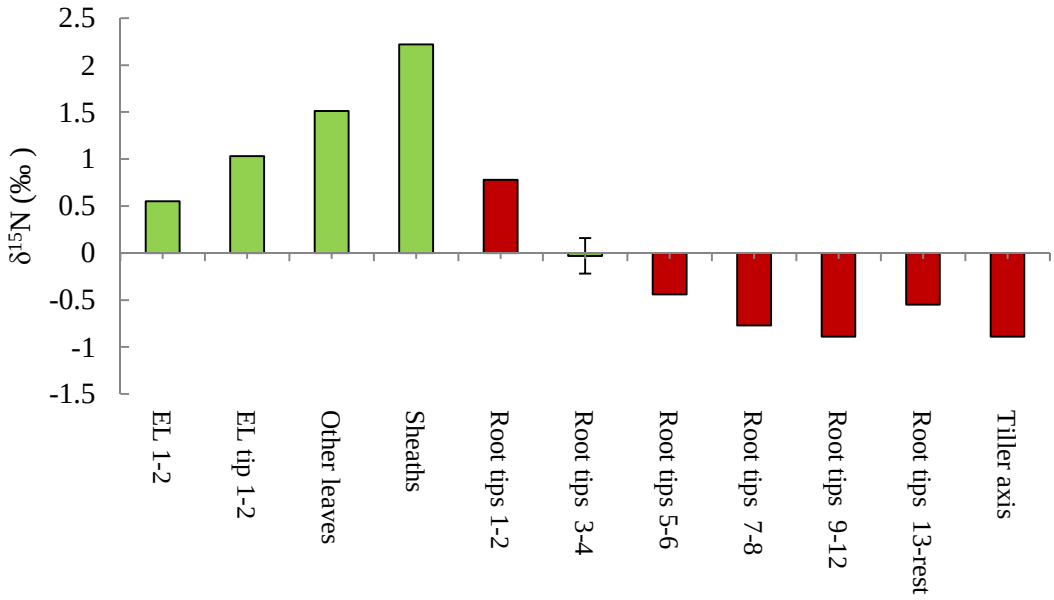


Fig. 7.15 $\delta^{15}N$ (‰) values in different shoot and root components of perennial ryegrass cultivar Alto in Experiment 6.1 for unlabelled plants. Vertical bar indicates the standard error of mean of all dissection categories. EL 1-2, elongating leaves; root 1, 2, 3 indicates root-bearing phytomer positions counting from the youngest root-bearing phytomer as a reference point.

7.4.5.3 C isotope ratio in the shoot and the roots

$\delta^{13}C$ (‰) was the most negative in mature leaves where the concentration of recent photosynthates was the greatest (Fig. 7.16). $\delta^{13}C$ was comparatively less negative in the youngest leaves and older leaf sheaths where less photosynthesis occurs (Fig. 7.16). Overall, the shoot components had more negative $\delta^{13}C$ (‰) than the roots and there was a trend of an increase in $\delta^{13}C$ (‰) ($p<0.01$) from the younger to the older roots (Fig. 7.16, Appendix 7.10b).

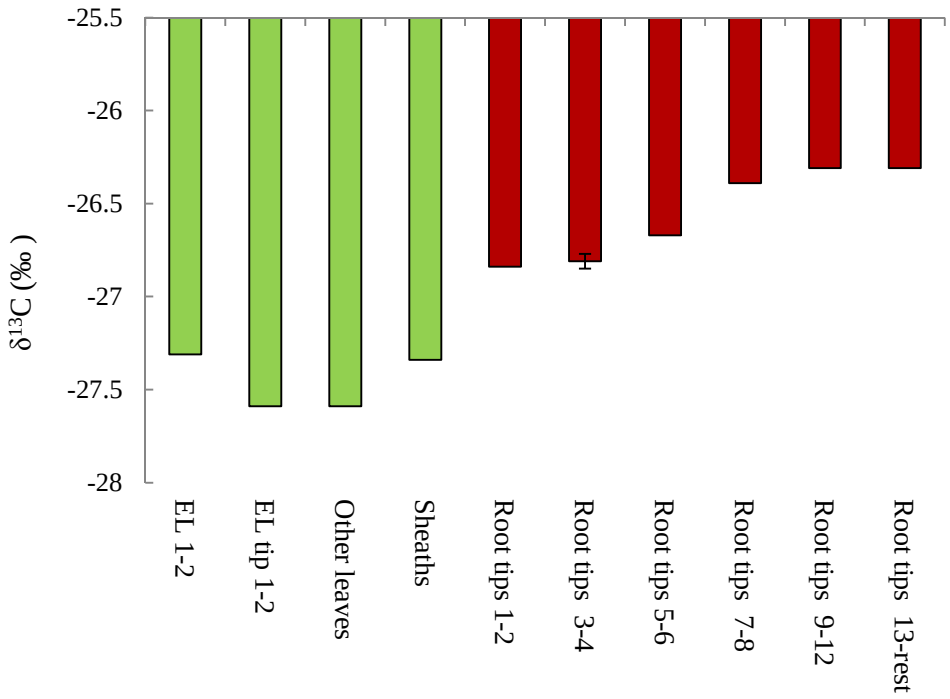


Fig. 7.16 Carbon isotope ratio ($\delta^{13}C$, ‰) of different shoot and root dissection components of Alto perennial ryegrass in Experiment 6.1 and 6.2 for unlabeled plants. Vertical bar indicates the standard error of mean. EL 1-2, elongating leaves; root 1, 2, 3 indicates root-bearing phytomer positions taking the youngest root-bearing phytomer as the reference point.

7.4.5.4 PCA for the inter-segmental C-N relations

The first three principal components (PCs) explained 85% of the data variation for C and N traits of dissection components for Alto and Aberdart perennial ryegrass cultivars. PC1 explained 46.7% of the data variation and reflected the differing C:N ratios of root and shoot components, as well as the gradation down the tiller axis from comparative abundance of ^{12}C and ^{15}N in shoots to depletion of those same isotopes in older roots (Table 7.5). The mean PC scores for PC1 were highly significant between compartments

(roots, tiller axis and shoots) and also for the dissection categories of each compartment (Table 7.6 and Fig. 7.17). PC2 explained 22.8% of the data variation and reflected a contrast between %C, C:N ratio and $\delta^{15}N$ (‰) for positive coefficients with %N and $\delta^{13}C$ (‰) for the negative coefficients (Table 7.5). PC2 was also highly significant for dissection categories within tiller (Table 7.6). ANOVA of the PC scores showed a contrast between shoot and tiller axis with roots (Table 7.6 and Fig. 7.17). Considering the tiller axis as a compartment of the shoot, this PC showed a marginally significant difference (p=0.056) between the root and the shoot systems. PC2 thus explained the difference between 'N isotopic ratio versus C isotopic ratio' in root and shoot dissection categories. PC3 explained 15.5% variation in the data for higher %C and %N in the root and tiller axis compared to shoot components which remained unexplained in PC1 and PC2 (Table 7.5, Table 7.6). PC3 scores also had significant difference in dissection categories within tiller (Table 7.6) indicating a variation in %C and %N in the old and new tissues (Fig. 7.17).

Table 7.5 Principal component analysis (PCA) coefficients for C and N traits of shoot, tiller axis and root dissection categories of Alto and Aberdart perennial ryegrass cultivars. C:N, carbon:nitrogen ratio; $\delta^{15}N$, isotopic mass ratio between ^{15}N and ^{14}N; $\delta^{13}C$, isotopic mass ratio between ^{13}C and ^{12}C.

Traits	PC1	PC2	PC3
%N	-0.57	-0.23	0.41
%C	0.39	0.13	0.89
C/N	0.59	0.30	-0.19
$\delta^{15}N$	-0.24	0.71	-0.09
$\delta^{13}C$	0.33	-0.58	-0.05
% variation explained	46.7	22.8	15.5

Table 7.6 Mean PC scores of PCA in Table 7.5 for C and N traits of shoot, tiller axis and root dissection categories of Alto and Aberdart perennial ryegrass cultivars and statistical significance determined by ANOVA. PC, principal component; SE, standard error of mean; p, statistical probability.

Compartment	PC1	PC2	PC3
Shoot	-1.49	0.24	-0.29
Tiller axis	0.19	0.31	0.12
Root	0.75	-0.13	0.11
SE	0.066	0.046	0.038
p (compartments)	0.001	0.14	<0.001
p (dissection categories within tiller)	<0.001	0.002	<0.001
p (Tiller)	0.762	0.765	0.152

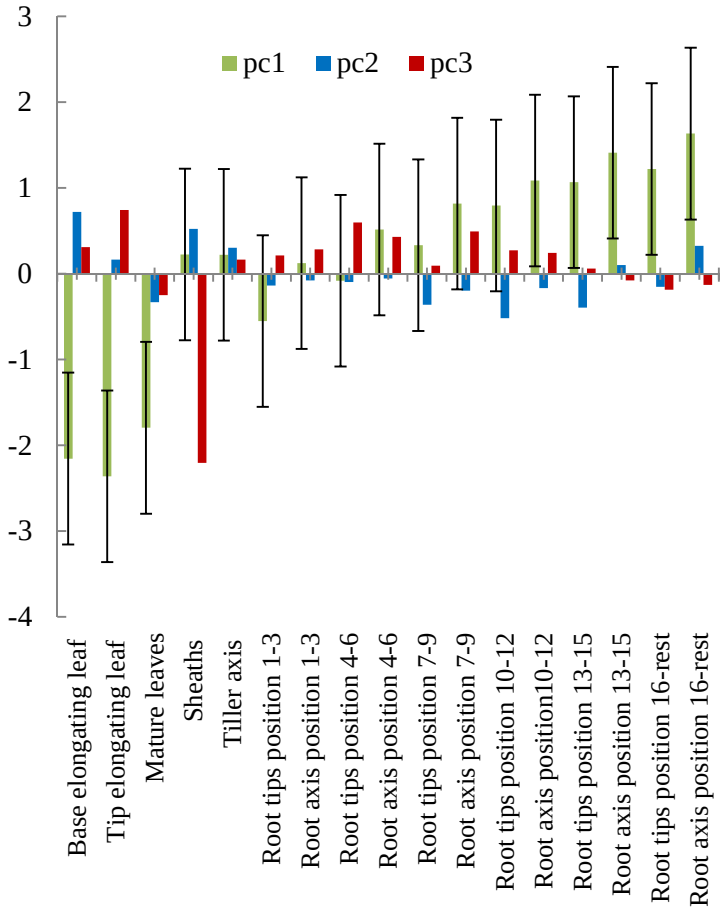


Fig. 7.17 PC scores for the C and N traits of Alto and Aberdart perennial ryegrass cultivars for different root and shoot dissection components.

7.5 Discussion

7.5.1 C exchange between main tiller and daughter tiller

Given a $\delta^{13}C$ value for brewery CO_2 of approximately -28.7‰ (Appendix 7.2), and that the predicted overall fractionation involved in the photosynthetic pathway is 20‰ in favour of ^{12}C (Farquhar et al., 1989), plant parts acting as sinks, such as the base of the elongating leaf, should have had $\delta^{13}C$ much more negative delta values after labelling than brewery CO_2. This clearly did not happen. In hindsight, it is evident that in both Experiments 6.1 and 6.2, the labelling of tillers inside a closed bag with outlet CO_2 concentration often less than 200 ppm would have largely prevented the preferential uptake of ^{12}C that typically occurs during photosynthesis in an open system. Under these

conditions, C isotope ratio inside the bag will be decreased by nearly 10‰ (Appendix 7.11) (H Schnyder and R Schäufele, personal communication).

Another factor reducing the 'signal' in labelled plants would have been the leakage of air into the system at pipe junctions under suction conditions that existed between the 3.5 m^3 gas bladder and the aquarium pumps. In support of this point, the Pearson correlation coefficient showed a moderately strong and positive correlation (r=0.558, p=0.023) between leakage and delta values at the base of the elongating leaves ($\delta^{13}C$,‰). As this was the first stable isotope labelling experiment attempted at the Institute of Natural Resources, Massey University these problems are attributed to inexperience. To overcome these problems in a future experiment options include: (i) increasing the flow rate so that the removal of CO_2 from the gas flow during labelling is no more than 10% (e.g. 400 pm to 360 ppm), (ii) placing the aquarium pumps inside the gas bladder so that the entire system would run at positive pressure, and (iii) devising a labelling system using ^{13}C enrichment instead of ^{13}C depletion so any reduction in photosynthetic isotope discrimination from labelling in a closed bag would reinforce rather than reduce the isotopic signal in labelled plants.

However, even though the labelling signal was weak, the significant variation between two unlabelled tillers for root dissection categories and more negative values for DTL-MT than MTL-DT for cultivar Alto in Experiment 6.1 (Table 7.2) suggested that significantly higher C was translocated from the labelled DT to MT than *vice-versa*. With the cultivar Aberdart in Experiment 6.2 (Fig. 7.10) there was no single dissection component with statistically significant differences in $\delta\ ^{13}C$ among the four tiller groups, but for all 11 plant parts, the $\delta\ ^{13}C$ value was numerically more negative for MTL-DT than for DTL-MT. Under binomial probability theory in non-parametric statistics the probability of this occurring is ½^10 ($p<0.001$). Hence a follow up experiment to clarify if this result was real or an artefact would be desirable. There is a potential biological reason for this difference between cultivars. In a field sward, Aberdart tends to have a higher density of smaller tillers than New Zealand bred cultivars like Alto (Weimar, 2006). Both for a comparison between Grasslands Ruanui (which has a growth habit similar to Aberdart) and Ellett (which has a habit similar to Alto) and for two cultivars of the tropical grass *Panicum maximum* with contrasting tillering patterns (Carvalho et al., 2006), there was evidence that the cultivar with the greater tiller forming habit transmitted more C substrate

to daughter tillers than the cultivar with a lower population density of larger tillers in field swards. These earlier reports indicate cultivar differences in translocation of C between MT and DT that are consistent with the hypothesised difference between Alto and Aberdart when supplied with ^{12}C enriched CO_2 in these two experiments.

7.5.2 N exchange pattern between the main tiller and the daughter tiller

Labelling the roots of the MT with ^{15}N resulted in a significantly lower share of new N reaching the DT than reached the MT of the DTL plants (Fig 7.13). The shoot parts and tiller axis of the DT of the MTL plants also received a significantly lower share of N compared to the MT of the DTL (Fig. 7.12). This result indicates that net gain of total uptake by a grass plant is for MT than adult DTs and thus the process probably keeps DTs comparatively smaller in size by reducing the growth of DTs to a rate comparatively slower than MT (Table 3.3).

Significantly higher new N recovery from the younger leaves and also from the younger roots compared to the older dissection components (Fig. 7.12, 7.13) suggested the requirement of new N varies with age of tissues involved in synthesis of structural and functional protein. Lattanzi et al. (2005b) observed that for mature grass roots, net N accumulation diminishes over time and stops when defoliated, but in growing roots N accumulation continues even after defoliation.

7.5.3 Inter-segmental C-N relations

The N isotope ratio ($\delta^{15}N$) increased from the younger to older leaves (Fig 7.15). Possibly during N remobilization from older leaves at their senescence, ^{15}N was preferentially retained and a greater share of ^{14}N recycled in the N remobilization process. As both new shoot growth receive most recently derived C and N (Lattanzi et al., 2005a), the young shoot and root dissection categories appear to maintain similar N isotope ratios. This suggests that these growing regions receive N from the same substrate pool, whether it is root derived or remobilized N. The difference in C isotopic ratio in the new and old leaf tissues (Fig. 7.16) indicated that the abundance of ^{12}C in the mature leaves compared to growing leaves might be associated with photosynthetic capacity of those tissues which is in turn depended on Rubisco content of the tissues. The increasing pattern of $\delta^{13}C$ (‰) with increasing root age suggests that a decrease in ^{12}C in the old root tissues is associated

with supply of recently assimilated C. It is known that supply of photosynthates diminishes with root aging (Matthew and Kemball, 1997). However, the decrease in $\delta^{15}N$ (Fig. 7.15) and the increase in $\delta^{13}C$ (Fig. 7.16) with increasing root age are observations not previously reported in the literature explored by the author. Further experimentation is therefore needed to explore the possible physiological reasons behind these processes.

7.6 Summary

- Tracing the destination of photosynthesis products was partly successful although the enrichment of ^{12}C in fed-CO_2 was counteracted by a reduced discrimination resulting from closed-system-labelling. Despite this, more negative values of $\delta^{13}C$ (‰) for MT of DTL compared to DT of MTL indicated net C translocation towards MT in cultivar Alto in Experiment 6.1.
- In Experiment 6.2 there was a suggestion of comparatively higher C translocation from MT to DT compared to reverse translocation in cultivar Aberdart, but this was not confirmed as statistically significant for any dissection component except as marginally significant for leaf sheaths. A repeat experiment is needed to clarify if this effect is real or an artefact.
- Similar to C translocation for Alto in Experiment 6.1, N transfer to DT of the MTL plants was much smaller than the reverse transfer from DT to MT of DTL plants in Aberdart. These variations were significantly different for the log-transformed data of the individual tiller, shoot dissection categories and young roots and also marginally significant for the middle aged roots. It would appear from this evidence that N transfer from a DT to its MT may contribute to the DT being smaller than the MT at a given age (see Chapter 3).
- Deposition of ^{15}N label was quite similar in young leaves and roots, consistent with active movement of recently acquired N to leaf and root growth zones;
- Routine analysis of unlabelled plants detected progressive depletion of ^{12}C (increasing $\delta^{13}C$) moving down the tiller axis and progressive depletion of ^{14}N (increasing $\delta^{15}N$) moving up the tiller axis. Further study is needed to explain this observed pattern.

Chapter 8: Evidence of N Concentration Oscillation: Pattern and Possible Causes of N Flux in Barley Shoots

8.1 Introduction and Overview

This chapter further investigated a recently reported phenomenon of cyclic fluctuation in N-concentration in leaves of barley (*Hordeum vulgare* L.). Two separate experiments are described, in which the aim was to confirm the previously reported periodic variation in N concentration in the tiller axis of barley plants, and the relation between N oscillation and leaf appearance interval, in barley plants during their vegetative growth stage.

Chronologically this was the first experiment of the PhD programme and so is referred to here as Experiment 1. Plants were grown in hydroponic culture using the same facility described in Section 4.3 (480 plant capacity: 20 trays of 24 plants) located at the Massey University Plant Growth Unit. Barley was chosen as the test plant because of the comparatively large tiller size, for ease of sampling. It was felt that preliminary familiarisation with barley growth patterns would be desirable but that this could be combined with Experiment 1 with the aim of confirming the previous observations of N-concentration oscillation. It was also decided to introduce leaf excision treatments designed to cause perturbations to the plant internal N cycling, as a means to make logical inference about the N-oscillation phenomenon. A follow up experiment (Experiment 2) was conducted to compare results for plants in Experiment 1 in hydroponic culture with plants of the same barley cultivar grown in soil. A second follow up experiment (Experiment 3) was inconclusive and is not reported.

8.2 Initial concept

Oscillation in N concentration of cell sap was described by LJ Irving and C Matthew in an unpublished manuscript reporting data collected at Massey University in 2006 (Appendix 1). In that report it was presumed that the observed oscillation in N concentration in the tiller axis must arise from asynchrony between N uptake by the elongating leaf and recycling from senescent leaves.

There is little precedent in the literature for an oscillation in plant N-concentration in the Poaceae. Cyclic variations of N uptake are reported in soybean (Tolley and Raper, 1985; Tolley-Henry et al., 1988; Vessey et al., 1990; Raper Jr et al., 1991) and roses (Cabrera et al., 1995). In soybean, cyclic variation of N uptake has been studied both in vegetative and reproductive growth phases. The authors found that periodicity of the oscillation during the vegetative phase corresponded to the leaf emergence interval. In roses, the periodicity of cyclic N uptake could be related to shoot development and harvest (Cabrera et al., 1995). However, the possibility of such an oscillation did seem superficially credible because substantial quantities of N need to flow into a new leaf at leaf development and are known to be released again as the leaf ages (Mae et al., 1983; Makino et al., 1984; Gastal and Nelson, 1994; Skinner and Nelson, 1995; Suzuki et al., 2001; Irving and Robinson, 2006) and these N fluxes are not continuous, but occur for discrete periods of time.

In order to test this hypothesis of N-concentration oscillation, 3 separate measurements on perennial ryegrass and barley plants were made by Irving, with a pulse in N-concentration observed in each case (Appendix 1). Since N analysis by the Kjeldahl method involves destructive sampling, the technique used to detect the oscillation in N-concentration was to synchronise the leaf appearance cycles of a number of plants so that destructive harvesting of individual plants at regular time intervals over the duration of a phyllochron would reveal the relationship between N-concentration in the tiller axis and the development stage of the elongating leaf. Since barley cultivars are developed by inbreeding, different plants of the same cultivar can be regarded as genetically identical for the purposes of providing experimental replication.

Since the N-oscillation data (Appendix 1) on which Experiment 1 was based was interpreted as indicating remobilization of degraded Rubisco from older leaves as a primary reason for periodic pseudostem N increase, the hypothesis was that excision of one or more leaves would interfere with the normal N oscillation, specifically by decreasing the amplitude of influx occurring at the time when the excised leaf would have undergone senescence (Fig. 8.1).

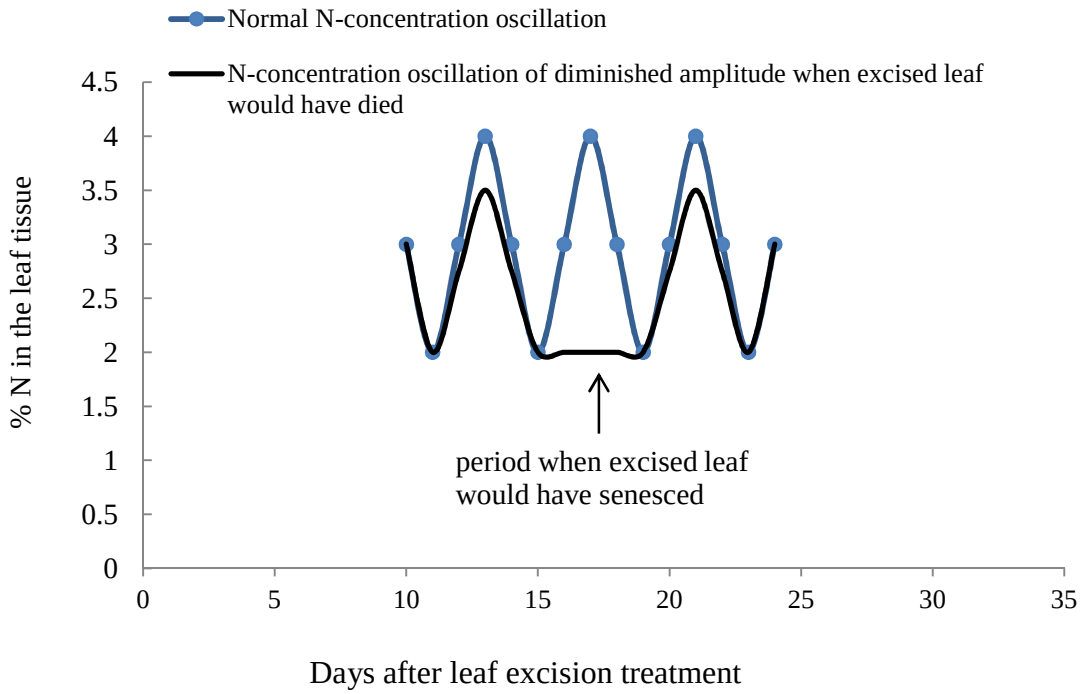


Fig. 8.1 Hypothesised effect of leaf excision on the plant internal N-concentration cycle at the fourth leaf when oldest leaf is excised at Day 0.

In addition to the hypothesised effect on N cycling, leaf excision would reduce the photosynthetic area and thus reduce carbohydrate production. It was not known how a reduction in carbohydrate availability might affect N cycling, so to evaluate this question a leaf shading treatment was also included in Experiment 1 to compare the effects on N oscillation of excising or shading the same leaf of otherwise identical plants.

Accordingly, the following objectives were defined for Experiment 1:

1. Familiarization with barley growth patterns in hydroponic culture;

2. Confirmation of a tiller axis N-concentration oscillation as described in the data reported in Appendix 1;

3. Investigation of involvement of other plant organs beside the tiller axis in the N-concentration oscillation;

4. Comparison of the effects of leaf excision and leaf shading on N-concentration oscillation.

Objectives 2 & 3 were further investigated in a follow up experiment, Experiment 2.

8.3 Experimental

8.3.1 Experiment 1

The hydroponic system used was described in section 4.3. Briefly, the nutrient flow system comprised independent twin tanks (120 L each) and each tank supplied 10 individual trays with nutrient solution as described in Table 8.1. Root aeration was provided to each tray via controlled release of compressed air. The barley (*H. vulgare*) variety used was Cask.

Table 8.1 Nutrient composition used for growing barley plants in Experiment 1

Chemicals	mM strength
NaH_2PO_4	0.6
$MgCl_2$	0.6
KSO_4	0.3
$CaCl_2$	0.3
H_3BO_3	0.05
FeEDTA	0.09
$MnSO_4.5H_2O$	0.009
$ZnSO4.7H_2O$	0.0007
$CuSO_4.5H_2O$	0.0003
$Na_2Mo_4.2H_2O$	0.0001

The N source was NH_4NO_3 which provides both ammonium and nitrate. The nutrient solution was renewed every two weeks. A pH of 5.5±0.1 for the nutrient solution was maintained using 6M HCl and 2M KOH as required. 100g MES (approximately 2mM, see Section 4.3.6) was added to each tank as a buffer to assist in maintaining a stable pH. Seeds were germinated in clean tap water floated in foam net inside the plastic trays. Around 1000 seeds were germinated in 4 trays in order to select 180 healthy seedlings with synchronised leaf appearance at transplanting. A total of 180 seedlings with leaf appearance synchronised in this way were transplanted (Day 0, on 6 July 2008, 7 days after germination) to the hydroponic system following a randomized complete block design (RCBD) and 48 additional seedlings were transplanted and maintained as reserve

plants. Seedlings from the RCB design that became unhealthy were replaced with reserve plants. The leaf appearance intervals were recorded.

Three treatments were included in the experiment design: (i) control, (ii) excision of the first leaf (the oldest leaf) on Day 22 (27 July 2008, 22 days after transplanting), and (iii) shading of first leaf by aluminium foil from Day 22 (Fig. 8.2). Each of the three treatments was replicated three times within the RCBD, and the 180 plants therefore provided for a time series of 20 sampling dates.

(a) (b)

Fig. 8.2 (a) Shading of Leaf 1 (oldest leaf) by aluminium foil; (b) Leaf chlorosis after foil sleeves had been in place for one week.

Excision and shading treatments were imposed at the appearance of leaf four. The leaf lamina of the oldest leaf was removed or placed in a foil wrap for the 60 randomly selected plants of the Excision and Shade, treatments respectively. Plants were sampled every day for the first 11 days after imposing treatments (from Day 22 to Day 32), then from Day 33 onwards the samples were collected only on alternate days. On each sampling day, 9 plants were destructively harvested (3 plants from each treatment). The shoot of each plant was divided into eight different segments: lamina of leaf 2, leaf 3 and leaf 4; sheath of leaf 2, leaf 3 and leaf 4; the main tiller axis and the first daughter tiller (DT1). Samples were stored at -80^{o}C after being snap frozen in liquid N.

After drying at 60^{o}C in a forced air draft oven, samples were cut into small pieces and weighed. The sample weights varied from 1 to 100 mg. Each sample was digested with 4 mL digest acid (concentrated H_2SO_4, with selenium and K_2SO_4) and heated to 360^{o}C in a

microkjeldahl digestion chamber (Ma and Zuazaga, 1942). After the overnight digestion, approximately 46 mL distilled water was added at each digestion tube to make a volume of 50 mL. The N concentration of each segment was estimated in µg g^{-1} (ppm) colorimetrically at 660 nm wavelength (Technicon, 1973) Industrial method no 98/70w, Tarrytown, New York, Technicon). N concentration was then converted from ppm in solution to percentage of DW in the plant tissue (Appendix 8.1).

8.3.2 Experiment 2

A total of 30 synchronized plants were transplanted into separate polybags filled with potting soil. The soil was fertilized with osmocote (a slow release fertilizer, longevity 3-4 months at 21^{o}C temperature, contains 15%N, 4.8% P, 10.8% K, 3% S, 1.2% Mg, 0.02% B, 0.05% Cu, 0.4% Fe, 0.06% Mn, 0.02% Mo, 0.015% Zn) before transplanting. The plants were irrigated by capillary irrigation. The leaf appearance and the tiller appearance intervals at the leaf axes were recorded. Sample collection was commenced from 35 days after transplanting. The samples were collected on every alternate day. Three plants were destructively sampled to obtain the following samples: leaf 4 lamina, leaf 5 lamina, leaf 6 lamina, leaf 7 lamina, tiller axis and the daughter tiller at the axis of leaf 5 (DT 5). Tissue N concentration of the samples was determined as described in section 8.3.1.

8.3.3 Statistical analysis

ANOVA was conducted using the Generalized Linear Model (GLM) command of MINITAB 15 statistical software (Minitab Inc. State College, Pennsylvania). For Experiment 1, a total of 162 data (18 sampling dates x 3 treatments x 3 replications) were used to test the effects of treatments on N concentration, variations between harvest date and date x treatment interaction. Because separate plants were harvested at each date, a repeated measures analysis was not needed. For Experiment 2, a total of 30 data were used for ANOVA, those include three replications for 10 sampling dates.

To test statistically for evidence of oscillation, N concentration of different segments was regressed against the harvest date (expressed as days since start of the experiment) to remove any linear trend of declining N concentration over time, and residuals were stored. For both experiments, residuals for N concentration were then regressed against time

expressed as a sine curve constructed to that each leaf appearance interval represented 360°, to test for any periodicity linked to leaf appearance on the main tiller.

8.4 Results

8.4.1 Evaluation of N concentration oscillation in the tiller axis

In Experiment 1, the tiller axis N concentration showed an oscillation with 4-5 days periodicity between Day 32 and Day 44 after transplanting (Fig. 8.3). Leaf appearance interval during the sampling period was also 4-5 days. The residuals for N concentration at each harvest date of all three treatments showed highly significant dependence on sine values representing leaf appearance interval and hence indicated an oscillation cycle ($p=0.026$) (Fig. 8.3). ANOVA using the GLM procedure also showed statistically significant variation among the different harvest dates for the tiller axis N concentration ($p<0.001$) (Fig. 8.3). The variation for percent N among the three treatments was statistically non-significant ($p=0.16$) (Fig. 8.3). The date x treatment interaction was also non-significant ($p=0.89$) (Fig. 8.3). The rise in N concentration in the tiller axis did not appear to coincide with addition of new N in the nutrient solution. N-concentration fluctuation for excised and shaded plants did not coincide with control plants for the first 10 days after treatment (Fig. 8.3).

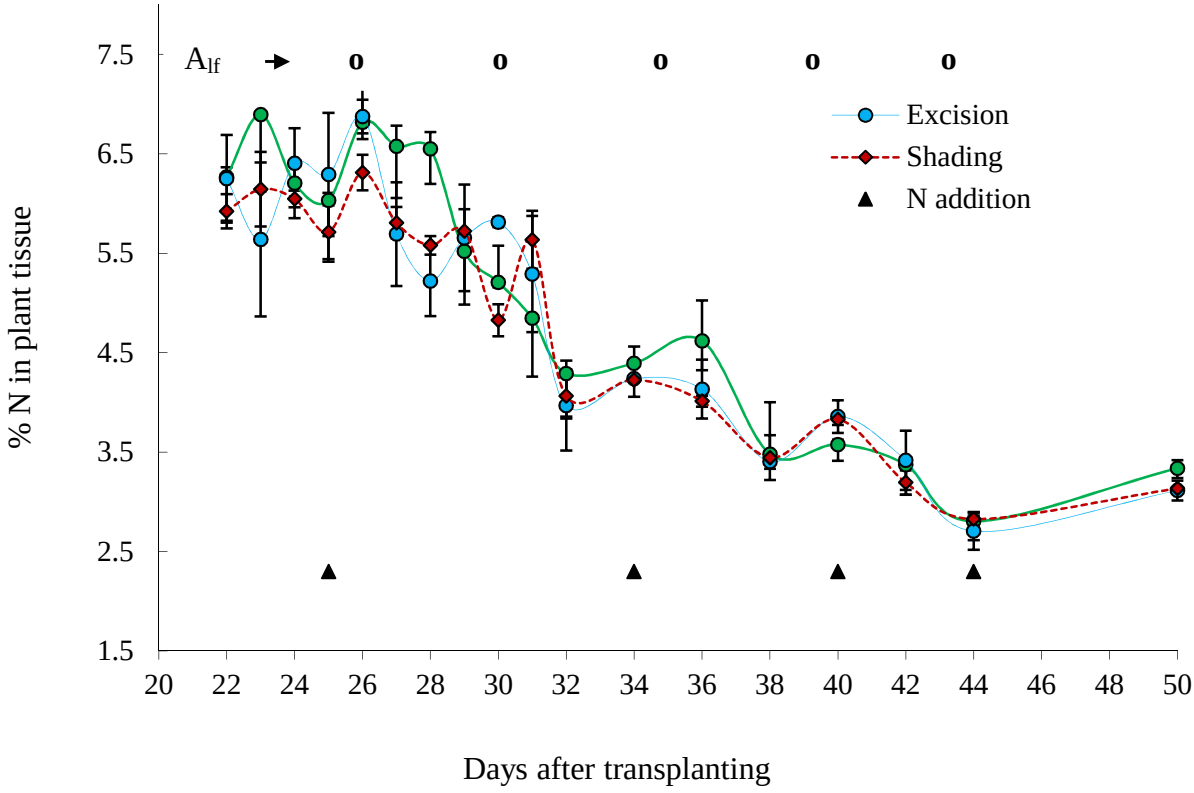


Fig. 8.3 N concentration in the tiller axis of barley plants growing in hydroponic culture during the vegetative growth phase under three treatments in Experiment 1. The treatments were imposed 22 days after transplanting. Vertical bars indicate the standard error of mean at each harvest date. A_{lf}, leaf appearance interval.

In Experiment 2, the same barley genotype growing in soil also showed a decline in tiller axis % N concentration over time ($p<0.001$, Fig 8.4), with an apparent fluctuation of 4-6 days periodicity in the speed of the decline. The periodicity in rate of decline of N concentration in the tiller axis appeared to be matched to the timing of leaf appearance events and a sine test as above showed the evidence of oscillation ($p<0.001$; Fig. 8.4).

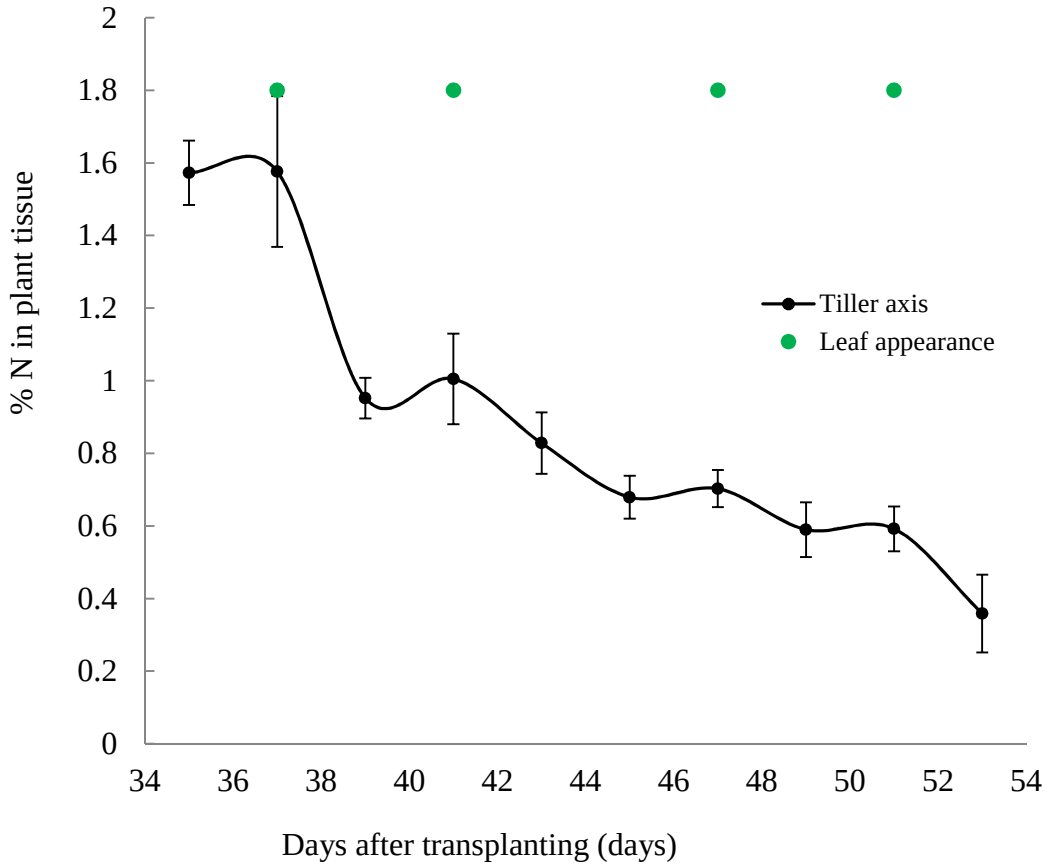


Fig. 8.4 N concentration (%) in the tiller axis at successive destructive harvests in Experiment 2 of barley plants growing in soil.

8.4.2 Evaluation of N concentration oscillation in leaf laminae and sheath segments

In Experiment 1, the trend of rise or fall in N concentration for other shoot parts was dissimilar to that of the tiller axis (Fig. 8.5). N concentration of the other shoot segments decreased at a slower rate with plant age compared to the tiller axis ($p<0.001$; Fig. 8.5). The regression test for residuals of N concentration at the different sampling days against sine values for N concentration fitted with leaf appearance interval was statistically significant for leaf 2 sheath ($p=0.004$), leaf 3 sheath ($p=0.001$) or leaf 4 sheath ($p=<0.001$) and between sampling Day 32 and sampling Day 44 in Experiment 1 (Fig. 8.5).

In Experiment 2, only DT5 among all the dissected leaf segments showed and indication of significant correlation ($p=0.069$) between the residuals from a regression of N concentration on time and sine values representing the passing of time in phyllochrons (Fig. 8.6).

Unlike Experiment 1, all shoot segments followed a similar trend in fluctuation of N concentration and also quite a similar gradient of decreasing N concentration over time as the segments aged (Fig. 8.6). In common with Experiment 1, all shoot segments exhibited a decline in N concentration over time ($p<0.001$, Fig. 8.6).

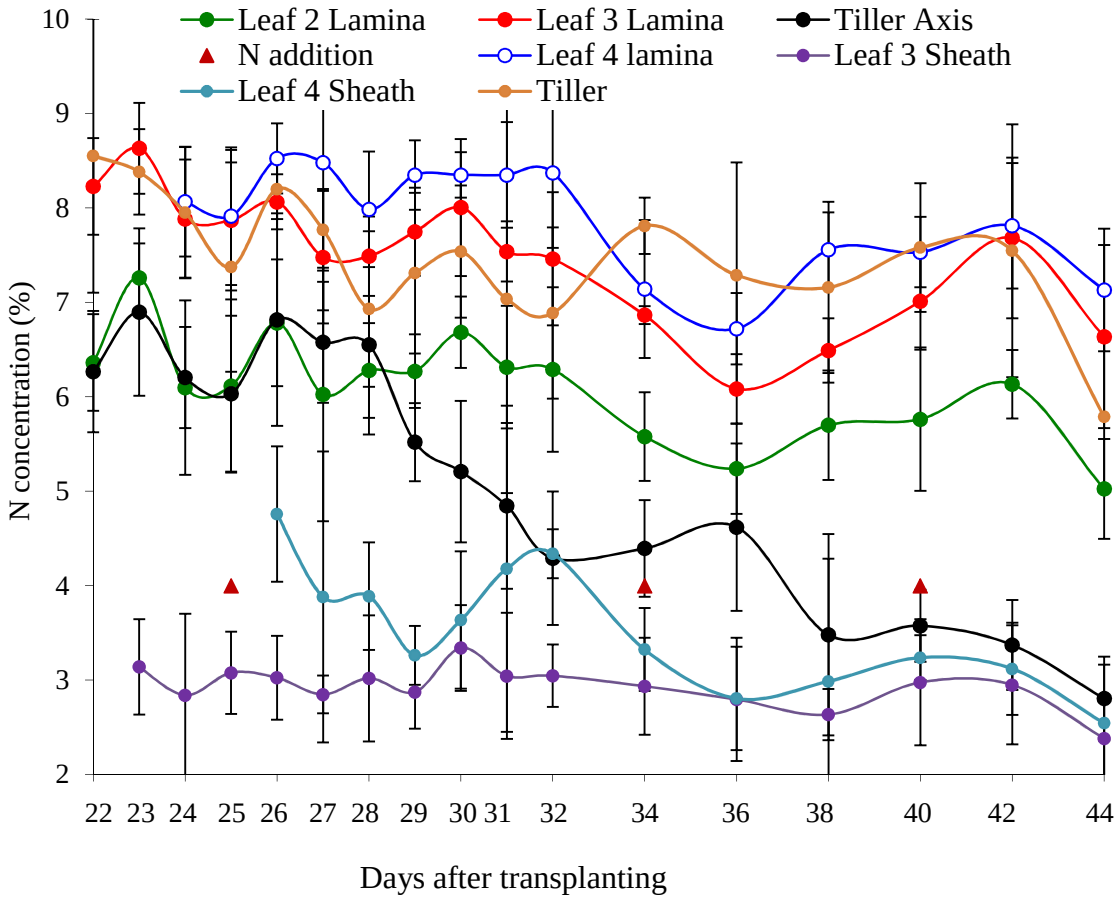


Fig. 8.5 N concentration (%) of different shoot segments of barley plants growing in hydroponic culture for successive harvest dates for the control plants in Experiment 1

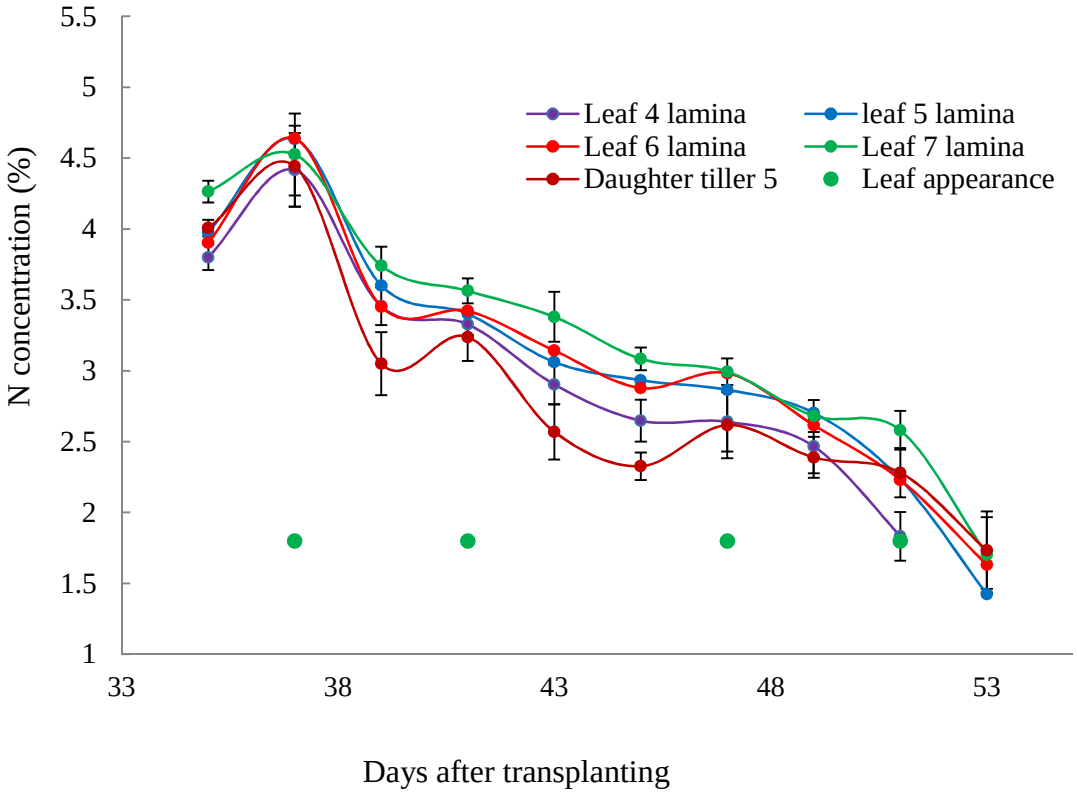


Fig. 8.6 N concentration (%) in the different shoot segments of barley plants at different harvest dates for the plants growing in soil in Experiment 2.

8.4.3 Evaluation of effect of perturbation of photosynthetic area on N flux

Both excision and shading significantly decreased the N concentration for the first 7-8 days after treatment, compared to Control plants (Fig. 8.7 and Table 8.2). Individual shoot segments after 8 days of treatment returned to the N-concentration fluctuation seen in the Control treatment (Day 29 and Day 38 harvests, Fig. 8.7). The N concentration was again significantly lower after 16 days of treatment, for a period of time (Days 38-44, Fig. 8.7, Table 8.2). Like the leaf 3 lamina (see Fig. 8.7) leaf 4 lamina and DT1 also had a similar pattern in N concentration fluctuation (see Table 8.2).

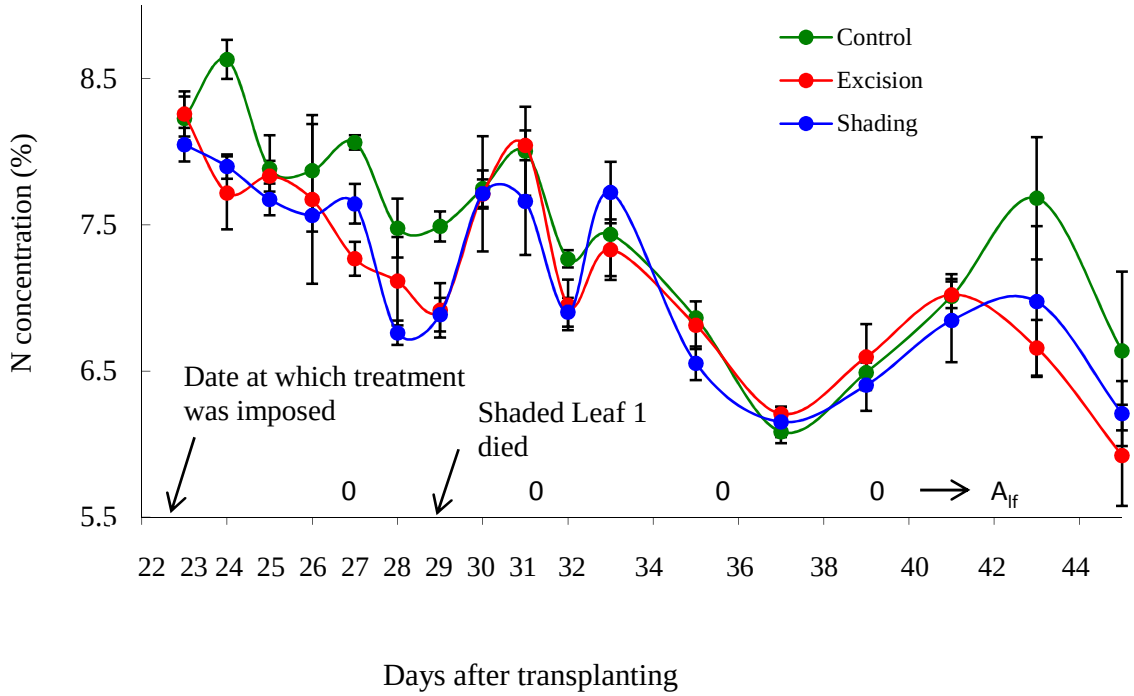


Fig. 8.7 N concentration fluctuation in Leaf 3 Lamina of barley plants during vegetative growth under Control, Excision, and Shading treatments imposed at Day 22. A_{lf}, leaf appearance interval. For Excision and Shading treatments the oldest leaf (Leaf 1) was either excised or shaded, respectively.

Table 8.2 N concentration (%) of leaf 4 lamina (L4) and daughter tiller 1 (DT1) at different sampling dates after transplanting under three different treatments – control, excision of the oldest leaf, shading of the oldest leaf. Treatments were imposed on Day 22.

Treatments	Day 23-29		Day 30-37		Day 38-44	
	L4	DT1	L4	DT1	L4	DT1
Control	8.20	7.88	7.74	7.65	7.25	7.03
Excision	7.79	7.34	7.85	7.08	6.90	6.31
Shading	7.79	7.48	7.68	7.08	6.97	6.16
SE	0.08	0.13	0.087	0.24	0.09	0.16
P(Treatment)	0.001	0.016	0.35	0.17	0.03	0.001
P(Day x Treat)	0.14	0.55	0.54	0.99	0.50	0.63

8.5 Discussion

8.5.1 Evidence of N flux and N oscillation

The evidence of N-concentration oscillation at the tiller axis and leaf sheaths obtained in Experiment 1 was also reproduced in Experiment 2 for tiller axis and youngest DT, but that was not evident in other leaf segments. The rise and fall in N oscillation had 4-6 days periodicity in both of the experiments (Fig. 8.3, 8.4).

Possibly, the fall in N concentration at the tiller axis after leaf appearance might be linked to meet demand for N during cell division and expansion of the newly appeared leaf (MacAdam and Nelson, 1989). Newly formed leaf inside the pseudostem is the site of N deposition for both soluble and insoluble N, with that N later involved in processes like Rubisco formation and chloroplast formation (Fig. 2.3; Skinner and Nelson, 1995).

A trend of decrease in N concentration with time at all shoot segments including the tiller axis in both of the experiments indicated the well-known ontogenetic effect of reduction in total N% as plants grow. One factor contributing to this effect is the increasing percentage of stem tissues and decreasing percentage of leaf tissues (Justes et al., 1994; Marino et al., 2004; Lemaire et al., 2008). In Experiment 2, N concentration of different segments showed a decline with age similar to that of the tiller axis. A slower rate of N concentration decline in the leaf segments compared to the tiller axis (Fig. 8.5) is probably associated with type of N- compounds present in respective segments, e.g. leaf tissues contain Rubisco and chlorophyll are the nitrogenous compounds which have distinct pattern of turnover cycle might be longer than nitrogenous compounds in stem tissues.

8.5.2 Evaluation of leaf shading effects on the pattern of N oscillation

The excision and shading treatments decreased the N concentration of the shoot parts immediately after the treatment imposed (between Day 32-37) (Fig. 8.7, Table 8.2) suggesting that excision/shading of photosynthetic area leads the plant to produce less photosynthate which eventually affects N uptake of the perturbed plants since N uptake by shoots occurs due to exchange of C with roots (Tolley and Raper, 1985). The DW of Leaf 1 at the time excision was around 10 mg which was in between 1/6 to 1/8 parts of total DW of the shoot. The plants at the time of the excision treatment had a maximum of four live leaves and the 4^{th} leaf was just appearing. A DT was also appearing at this time.

Thus the variation between control and excised/shaded plants for N concentration can be photosynthesis driven.

As the leaves aged and plants grew between Day 30 and Day 37, individual leaves (Leaf 3 and Leaf 4) from different treatments retained similar N in their tissues. The variation in N concentration (%) between Day 40 and Day 44 is hard of explain. The following component processes may contribute to that variation: i) the leaves of the stressed plants (shaded and excised plants) started degrading chlorophyll and leaf Rubisco comparatively earlier than the Control plants to bring a significant difference in N concentration between Day 40 and Day 44. The age of Leaf 3 and Leaf 4 was around 36 and 32 days respectively. Therefore it is likely that Rubisco degradation was on-going (Friedrich and Huffaker, 1980; Irving and Robinson, 2006) at that leaf age, ii) the respective leaves (Leaf 3 and Leaf 4) of the control plants received remobilized N at senescence from Leaf 1. However, this explanation is less likely because the leaves in question were too old to receive any significant share of remobilized N from the adjacent older leaves, iii) Leaf 3 and Leaf 4 of the control plants were still comparatively more active than those of treated plants and thus were able to retain more root-derived N at that stage.

8.6 Summary

- N concentration at the tiller axis oscillated with a periodicity that matched leaf appearance interval in both experiments. The oscillation event was found to be statistically significant and that matched with leaf appearance interval but the oscillation peaks were not as high as reported by Irving and Matthew (Appendix 1). Irving and Matthew proposed N oscillation only in the tiller axis but this study also found evidence of oscillation in leaf sheaths and in other shoot segments such as the youngest daughter tillers.

- N-concentration fluctuation in the other shoot parts did not always match that of the tiller axis. This was true in both experiments. It can therefore be concluded that the tiller axis receives a pulse of N concentration for a discrete period. Further study would be needed to determine if or how oscillation in N concentration is related to N-uptake of developing leaves.

- Excision and shading of the oldest (and smallest) leaf decreased N concentration in some shoot segments for the first 7-8 days from the day of treatments imposed and again decreased after two weeks but matched with control plants in between. It can be concluded that excised/shaded young plants immediately after the treatment were in stress for C-assimilation due to reduction in photosynthetic area which eventually resulted in less root-derived N uptake. Lower leaf N concentration at the later stage for the treated plants compared to the Control plants might be due to early degradation of N-compounds from the treated plants.

- Two different studies conducted in two different growing conditions showed weak oscillation of N concentration in the tiller axis. Oscillation of N concentration in the tiller axis although coincided with each leaf appearance interval but further strong evidence is needed for confirmation.

Chapter 9: Overview and Conclusions

9.1 Review of thesis objectives and synthesis of results

9.1.1 Tiller axis morphology and co-ordination between leaf and root appearance

The first objective of the thesis was to describe the morphological and segmental organisation of the tiller axis of *L. perenne* cultivars, Alto and Aberdart. In the Spring experiment, a total of 15 phytomers developed for Alto, 10 of which bore roots; 16 phytomers developed for Aberdart, 11 of which bore roots. In the Autumn experiment, a total of 22 phytomers developed for Alto, and 17 of those bore roots; 23 phytomers developed for Aberdart, 18 of which bore roots. More frequent new leaf appearance in autumn resulted in a significantly higher total number of phytomers per tiller than in spring. The ratio between NPr and NLA differed significantly between the two experiments as NPr was significantly higher than NLA in the Autumn experiment. This result confirmed as hypothesised that in decreasing day length, leaves appeared at a slower rate at successive phytomers compared to roots and in increasing day length that pattern was reversed (Table 5.4, see Section 5.5.1).

9.1.2 Leaf turnover pattern and photosynthetic efficiency associated with leaf turnover

Objective ii of the thesis was to study the pattern of leaf turnover in increasing and decreasing day length and to investigate the photosynthetic efficiency of the individual leaves of known age. Leaves appearing in autumn at a faster rate achieved significantly higher FLL, LDW and LA with significantly shorter LED through significantly faster LER than in spring (Chapter 4). The present study found that in changing growing conditions LER has a vital role in leaf morphogenesis, while Lemaire and Agnusdei (2000) described the central role of LAR influencing tiller morphogenetic and structural traits. The trend of increasing FLL and LDW at successive phytomers in both experiments reflected increasing plant size. A consistent LED over the growing period in spring indicated that the effect of increasing plant size at successive phytomers was partly negated by the increasing day length, whereas decreasing day length in autumn reinforced that effect (Fig. 4.9), resulting in increased LED at successively younger phytomers. Individual leaves

achieved maximum photosynthetic capacity at P2, generally after achieving FLL and between 12.5 and 14.8 d after appearance (Fig. 4.19). NPR decreased at successive phytomers after P3 (Fig. 4.19). NPR of a particular leaf over its life span followed a log normal curve similar to that described for cereals by the Rubisco turnover model of Irving and Robinson (2006) and confirmed for perennial ryegrass by Khaembah (2009).

9.1.3 Dynamics of root production

Objective iii of this thesis was to study the detailed pattern of root turnover of Alto and Aberdart perennial ryegrass cultivars in spring and autumn in a phyllochron time scale. The measures of root size RAL, RDW_i, RSA_i, and RV_i generally increased up to phytomer 6 or 7 in both seasons and also for both of the cultivars (see Chapter 5). Up to four lateral branching orders were recorded (Fig. 5.13-5.15). The fact that lateral root branching of older roots reduces root mean diameter and allows continuing root development with minimal weight gain provides a mechanism for decreased requirement of C for root construction at the lower phytomers, providing adjustment to reduced photo-assimilate supply from the canopy as roots age (see Section 5.5.4, Matthew and Kemball, 1997). Fig. 9.1 provides a tentative tiller C budget including estimated supply of C to successively older Pr of Alto and Aberdart perennial ryegrass cultivars in autumn (see Appendix 9.1 for details about compilation of Fig. 9.1). Assuming 30% of the available photo-assimilate stream is consumed at each Pr, C availability downwards the tiller axis diminishes in an exponential decay curve. As the roots get older, the estimated construction cost based on observed DM deposition decreases and respiration cost increases with increasing DM accumulation (Fig. 9.1). The model output illustrates that at Pr6, canopy can supply only enough C to meet the respiration cost and therefore DM deposition nearly ceases (Fig. 5.6). As roots get older, respiration cost increases but C supply diminishes rapidly (Fig. 9.1). This implied negative energy balance probably triggers the commencement of root decomposition of the older roots in absence of DTs at those Pr. For the autumn roots at the older phytomers below Pr10 a decrease in RV_i was observed along with an increase in RL_i (Fig. 5.17). A decrease of RV_i with corresponding increase of RL_i is mathematically possible when there is loss of length and decomposition of some coarse root branches occurring simultaneously with addition of finer root branches at successively older phytomers (see Section 5.5.5). The genotypes of each cultivar differed significantly for a majority of root dimensions including RDW_i, RL_i, RSA_i, RV_i (Table 5.6, Table 5.11-12;

Crush et al., 2007), whereas none of the root dimensions except RV_i and DMD_p differed significantly between the two cultivars, Alto and Aberdart. The results therefore suggest that selection of genotypes for desired root traits could be possible in breeding *L. perenne* cultivars.

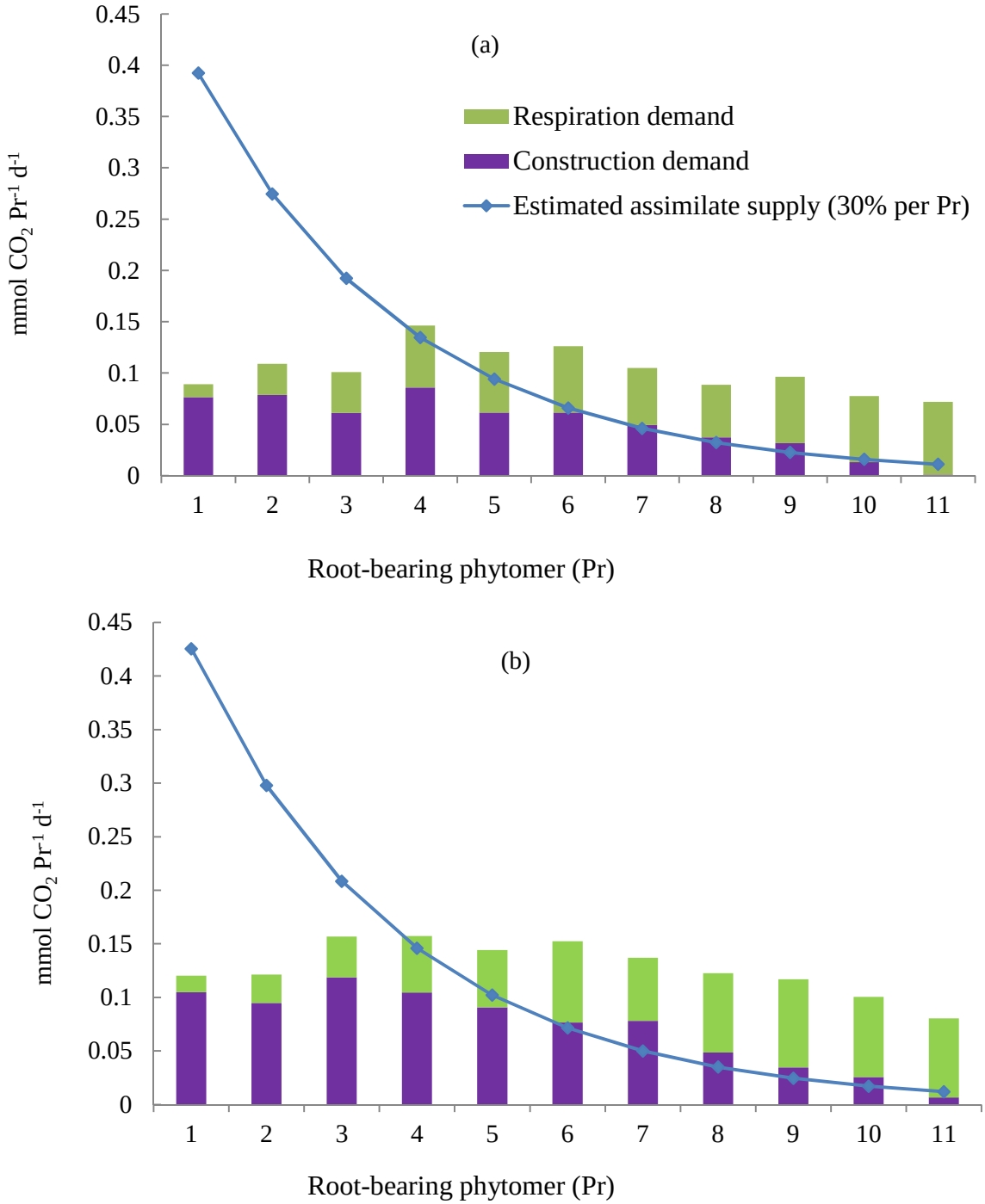


Fig. 9.1 C cost for root construction, root respiration and estimated photo-assimilate supply at different Pr of (a) Alto and (b) Aberdart perennial ryegrass cultivars in the Autumn experiment. The assimilate supply curve assumes 30% of available photo-assimilate consumed at each successive Pr.

9.1.4 Plant architectural signal

Objective iv of this thesis was to explore whether changing phyllochron associated with increasing or decreasing day length in spring and autumn, respectively, might result in seasonal change in root:shoot ratio as hypothesized by Matthew et al. (1998). As stated in Section 9.1.1, the phyllochron in increasing day length in spring was shorter than the rhizochron, and *vice-versa* in decreasing day length in autumn, as indicated by the NPr/NLA ratio (Table 5.4, see Section 5.5.1). The disparity between phyllochron and rhizochron at a particular time was contributed by spatial difference in leaf and root appearance at the tiller axis. As roots at a given phytomer appear 5-6 phyllochron intervals later than leaves at the same phytomer, the ratio between phyllochron (first affected) and rhizochron (not affected until some time later) is subject to change in conditions of increasing or decreasing day length. The ratio NPr:NLA confirmed this effect in both Spring and Autumn experiments (Table 5.4). In field conditions, NLL usually ranges between 3.5 and 4.0 (Fulkerson and Donaghy, 2001), but for both spring and autumn plants NLL was much higher than normal (Table 5.4). On the other hand, plant size in the Autumn experiment was much larger than that of the Spring experiment (Table 6.1). Despite these variations, there was a lower number of live leaves per tiller to feed a comparatively higher number of live roots in Autumn than in Spring. That means for the autumn plants there would be expected to be more C root demand (sink) per unit of C supply from leaves (source) compared to the spring plants. This resulted in a major shift in RDW_p:LDW_t between the two experiments, with greater RDW_p/LDW_t at all phytomers (Fig 6.2; p=0.004). Since leaf area of individual leaves was significantly larger in the Autumn experiment compared to the Spring experiment, to test the hypothesis (Matthew et al. 1998) further a future study might be carried out in controlled environment conditions with half of the plants maintained in simulated spring and half in simulated autumn conditions, and then half of each group of plants changed from spring to autumn or *vice-versa*. In this way, effects of seasonal or plant growth factors other than the manipulated environmental variables (e.g., temperature, PAR) will be same for all groups of plants.

Paradoxically, despite a larger NPr:NLL ratio in autumn than in spring, RDW_t/LDW_t was marginally reduced (Table 6.1) even though TPA_t was significantly higher in autumn (Table 4.7). The increased NPr:NLL ratio was probably associated with significant

reduction of RL_i and RAL (Table 5.6, Fig 5.7, Fig. 5.8), RDW_i ($p=0.037$, Fig. 5.4), RSA_i ($p=0.003$, Fig. 5.11), RV_i (<0.001, Fig. 5.12) and DMD_p ($p<0.01$, Fig. 5.6) at different phytomers in autumn compared to spring (Table 5.6) although tiller size in autumn was much bigger than spring and may influence a part of this picture.

9.1.5 Economics of daughter tiller production

Objective v of this thesis was to explore the exchange pattern of recently assimilated C and N between the MT and the DT. DTs translocated a significantly larger share of N to the MT than MT translocated to the DT (Fig. 7.11, 7.12, 7.13) and this unequal reciprocal transfer process would keep DTs smaller in size by reducing their growth rate. In the presence of two DTs, the MT of DT+ plants in the Autumn experiment remained significantly smaller in size for both LDW_t and RDW_t indicating that DTs withdrew a share of C assimilated by the MT for their development and that caused a size reduction of MT (Table 6.7). The significant reduction of new Pr production (Fig. 6.3) and higher NLL:NPr ratio (Table 6.5) following adult DT excision indicated that plants adjust root:shoot ratio by the cessation of root growth and probably by increasing shoot growth rate. A significant reduction of RDW_p/LDW_t at the older phytomers in the absence of DTs in the Autumn experiment (Fig. 6.8) and significant reduction of RDW for the oldest five Pr of Alto in the Spring experiment (Fig. 6.4) suggested that the growth of older roots of MT is partly supported by C supply from the DT.

9.1.6 N oscillation in the tiller axis

Objective vi of this thesis was to investigate the evidence for recently proposed N concentration oscillation in the tiller axis of *H. vulgare* plants. The tiller axis N concentration increased at each leaf appearance event but the N concentration of the other shoot parts did not always match the N flux of the tiller axis at different harvest dates. A regression test in which the linear-regression-residuals of N concentration at each harvest date were analysed with sine values for the periodicity of leaf appearance confirmed that tiller axis N concentration had an oscillation cycle and the peaks of each cycle coincided with each of the leaf appearance event. The influx in N concentration at the tiller axis for a discrete period at the event of each leaf appearance was possibly meeting immediate high N demand for cell elongation and extension at leaf appearance when LER increases rapidly (see Fig. 2.3 in Section 2.4.2.5).

9.2 Conclusions

This study provided a detailed description of segmental architecture of two perennial ryegrass cultivars of contrasting breeding background growing in two contrasting day length conditions. The study confirmed the hypothesis of Matthew et al. (1998) that for a given phytomer at the tiller axis due to delay between leaf and root appearance, the decrease in phyllochron in increasing day length and increase in phyllochron in decreasing day length, respectively in spring and autumn bring morphogenetic variation in root:shoot ratio. Individual tiller size in autumn was greater than in spring as reflected by LDW_t and RDW_t (Table 6.1). RDW_t was significantly smaller in spring compared to autumn (Table 6.1). However, that variation was not contributed by RDW_i, but rather, variation in R_p between two seasons was one of the key factors along with NPr. It is not easy to understand how autumn tillers achieved more roots per phytomer even though those phytomers appeared at a faster rate than in spring. Possible reasons include: (i) size of the individual tillers (i.e., larger diameter of the tiller axis) provides a larger surface area, allowing for the appearance of more roots at each phytomer; ii) since a greater share of photo-assimilate is transported to the youngest Pr, a much higher net photosynthesis per tiller might trigger release of more roots per phytomer in autumn than spring even though these same roots might face C shortage for their development due to higher NPr:NLL ratio when they are older and moved downwards on the tiller axis by the appearance of new roots above. As number of leaves per tiller in field conditions is generally constant, and ranges between 3 to 4 leaves, it might be worthwhile to explore how root:shoot ratio may vary in field swards. In the present study, variation in total photosynthesis of all leaves per tiller was achieved through larger LA_t (Table 4.7) which was contributed by both a higher number of live leaves per tiller (Fig. 4.18) and significantly larger LA of the individual leaves (Fig. 4.16) in autumn than spring, although unit LA in autumn was less efficient than spring (Table 4.7). Therefore it might be suggested to explore what makes the autumn plants less photo-efficient even though they are from the same cultivars. RAL, RL_i and RSA_i were significantly smaller in autumn compared to spring (Table 5.11) and SRL, SRSA, SRV were significantly higher in autumn than spring (Table 5.12) reflecting a complex situation which deserves further investigation. Negative PC3 coefficients of the autumn roots for the following root dimensions: SRL, SRSA and SRV (Table 5.13) and positive PC3 scores of the spring roots suggested that autumn roots were more branched than spring (Table 5.13). As this study has compared roots of Pr7 in spring with those of

Pr11 in autumn, further investigation in a future experiment would be worthwhile to confirm that this variation is seasonal and not influenced by other factors such as differing phyllochron interval.

This study used data for NPR of leaves of different ages to infer the time course of NPR during the leaf life span (Fig. 4.19), and identified a log-normal trajectory as for leaf Rubisco concentration (Irving and Robinson, 2006). This study also used information on development status of roots at successive phytomers to infer the root development pattern during the first 90 days of their growth. During root development a total of four root branching phases were identified. Root branching reduces the C construction cost of further soil exploration by roots of older phytomers when C supply from the canopy is reduced. Because of lower C supply, roots more than 45 days old appear to start decomposition as evidenced by decrease in RV while RL continued to increase. This observation was evident only in autumn. Fig. 9.1 suggests that older roots suffer severely from negative C balance arising partly from the C cost of root respiration as root size increases, and partly from their distance from the C source. A follow up study is needed to confirm the deduction that this occurs due to the death of root branches under shortage of C. This study also found that DT supply a significant proportion of DT's root derived N to the MT to feed the older roots which are close to the phytomer position of that DT on the tiller axis. Since excision of DTs means a supply-source of C for the older roots is 'switched off', the amputation of the DTs may contribute the early root death as reduction of RDW at the older Pr is evident (Fig. 6.4, 6.8). This study hence identified a number of research questions for further investigation.

9.3 Recommendations and further research

- In this study it was decided that a fixed N concentration would be supplied throughout the growing period in both experiments and no N treatment would be applied when studying the effect of seasonal variation in day length, as temperature and light are the variables of interest. Since in both experiments NLL was much higher than usual, future studies might begin with a lower N concentration and increase N supply proportionally with increasing plant size.
- For the ease of root isolation, the plants were manipulated in both spring and autumn experiments which possibly resulted in higher numbers of live leaves per

plant by accelerating LAR. A further study on root turnover might be suggested keeping the DTs intact, although in that case root isolation would be much more difficult.

- Further research might be conducted to explain why faster growing autumn leaves have a significantly lower photosynthesis rate per unit of leaf area. Is the variation associated with higher dry matter content, or lower chlorophyll content, lower Rubisco content or due to presence of an inefficient biochemical pathway which is again associated with thermal time?
- Root hairs potentially contribute approximately 90% of root surface area and are the functional components of all the branches. Due to time constraints it was not possible to study root hair demography of the different branching phases and at the root axes of roots of various diameters. The addition of root hair data to the root branching (Chapter 5) would complete the detailed description of root system morphology.
- A decrease in RV corresponding with increasing root length and also an increase in tissue density for the older roots are the observations that provide new insight to understand the C economy of older roots, the question being: 'what are the possible sources of C for the respiration and fine root construction of older roots either in presence or absence of DT at each Pr'?
- Since the labelling experiment involving ^{12}C-enriched CO_2 had a planning oversight that resulted in a near undetectable $^{12}CO_2$ signal at the destination segments, and time constraints did not allow the experiment to be repeated, a further study to clarify and confirm the C exchange pattern between main tiller and the adult daughter tiller would be of benefit.

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Appendices

Appendix 1 Irving and Matthew submitted manuscript to Annals of Botany journal

Title: Plants with a pulse: N fluctuations in the tiller axis of graminaceous plants.

Abstract

Mathematical modelling of leaf protein turnover in whole tillers suggests the existence of an oscillation in tiller axis N concentrations through time.

Developmentally synchronised ryegrass and barley plants were destructively sampled through time, and their N levels in the pseudostem / tiller axis quantified. In the barley plants, soluble N, soluble protein N and nitrate N levels were also measured. In a third experiment, we quantified amino N levels.

A regular oscillation was noted in both species which corresponded to the leaf appearance interval (phyllochron). In barley, this oscillation was largely attributable to changes in amino N levels in the tiller axis. These amino acids are presumably the result of leaf protein turnover.

Perturbations in this oscillation precede changes in leaf growth rates, suggesting that this represents a cause, rather than an effect of, leaf growth, and may represent a timing mechanism for leaf developmental processes, and may have implications in controlling grain number in cereals.

Note: Please see the full manuscript in the enclosed CD (folder: *Appendix 1.1*).

Appendix 4.1 ANOVA structure to estimate statistical variation between experiments, cultivars, genotypes within experiment and cultivar, phytomers within experiment and cultivar following general linear model (GLM) in MINITAB 15 statistical software package. Exp, Experiment; Cul, Cultivar; Geno, Genotypes; ClonalRep, Clonal replicates. A_{lf}, phyllochron, as an example.

MTB> GLM A_{lf}= Exp | Cul Geno (Exp Cul) ClonalRep (Exp Cul Geno) Phytomer (Exp Cul) ;
SUBC> means Exp | Cul Geno (Exp Cul) ClonalRep (Exp Cul Geno) Phytomer (Exp Cul);
SUBC> test Exp/ Geno (Exp Cul);
SUBC> test Cul/ Geno (Exp Cul);
SUBC> test Exp*Cul/ Geno (Exp Cul);
SUBC> test Geno(Exp Cul)/ ClonalRep (Exp Cul Geno);
SUBC> test Phytomer (Exp Cul)/ ClonalRep (Exp Cul Geno).

Folder name in the CD for data: *"Chapter 4 / Leaf data – 1082"* and *"Chapter 5 / Root data – 1082"*

Appendix 4.2 Leaf morphological data for the individual tillers of Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments, mean phyllochron (A_{lf}) in days, leaf elongation duration (LED) in days, leaf elongation rate (LER) mm d^{-1}, number of visible elongating leaves (NEL), final leaf lamina length (FLL) in cm $leaf^{-1}$, mean leaf dry weight (LDW_i) in mg $leaf^{-1}$, mean leaf width (LW) in cm, leaf area per (LA_i) in cm^2 $leaf^{-1}$, specific leaf area (SLA) in cm^2 g^{-1}, leaf life span (LLS) in days, number of live leaves per tiller (NLL) and net photosynthetic rate (NPR) in µmol CO_2 cm^{-2} s^{-1}.

Cultivar-Experiment	A_{lf}	LED	LER	NEL	FLL	LDW_i	LW	LA_i	SLA	LLS	NLL	NPR
Alto-Spring	7.56	11.3	16.2	1.45	18.2	40.9	0.61	7.81	199	55	7	16.1
Alto-Spring	7.44	11.0	18.7	1.45	20.6	47.4	0.59	8.46	215	58	8	15.6
Alto-Spring	7.44	10.2	16.4	1.38	16.6	35.5	0.62	7.14	180	65	8	12.5
Alto-Spring	8.33	12.3	17.1	1.47	19.6	46.9	0.68	9.33	199	58	7	13.8
Alto-Spring	5.78	8.9	20.4	1.58	17.7	42.5	0.66	8.21	211	52	8	22.2
Alto-Spring	7.89	12.2	17.7	1.64	20.9	49.1	0.72	10.51	280	66	8	13.2
Alto-Spring	8.00	11.0	21.0	1.38	21.5	38.7	0.65	9.82	254	55	6	18.7
Alto-Spring	8.33	10.7	20.3	1.29	21.7	41.7	0.65	9.90	236	56	7	17.4
Alto-Spring	8.33	10.6	19.0	1.24	20.9	41.7	0.67	9.84	236	62	7	19.4
Alto-Spring	8.89	11.2	14.6	1.39	14.8	23.7	0.67	6.93	234	54	6	18.4
Alto-Spring	7.63	11.1	15.1	1.49	17.0	32.2	0.68	8.12	257	58	7	12.6
Alto-Spring	7.44	10.8	16.3	1.48	18.0	34.1	0.42	5.30	185	73	8	16.6
Alto-Spring	7.33	10.1	18.4	1.40	18.6	33.3	0.49	6.31	213	52	6	14.0
Alto-Spring	8.00	12.0	16.5	1.59	19.1	51.7	0.44	5.90	249	46	6	15.0
Alto-Spring	7.89	11.7	15.2	1.55	17.7	47.8	0.62	7.66	180	61	7	14.5
Alto-Spring	8.22	11.9	17.6	1.54	19.0	50.4	0.63	8.45	211	51	6	16.0
Aberdart-Spring	8.22	11.2	17.4	1.40	18.7	31.7	0.47	6.14	194	77	9	17.1
Aberdart-Spring	7.00	10.5	21.4	1.53	21.4	36.4	0.62	9.24	254	56	8	14.6
Aberdart-Spring	7.33	10.6	17.6	1.59	16.9	28.7	0.62	7.32	281	77	10	14.3
Aberdart-Spring	7.67	11.1	17.4	1.40	19.8	51.4	0.71	9.76	190	63	8	14.4
Aberdart-Spring	8.33	11.8	16.7	1.49	21.4	55.6	0.75	11.26	262	70	8	15.4
Aberdart-Spring	7.56	11.1	19.5	1.50	22.6	54.6	0.70	11.12	249	72	9	13.9
Aberdart-Spring	6.89	10.9	19.2	1.61	20.6	45.4	0.65	9.39	182	48	7	17.4
Aberdart-Spring	7.00	10.9	18.3	1.60	20.1	43.2	0.72	10.06	242	59	8	10.7
Aberdart-Spring	8.78	12.6	18.8	1.51	22.0	55.1	0.76	11.72	279	70	7	20.6
Aberdart-Spring	7.67	12.1	17.9	1.60	21.0	44.0	0.75	10.95	237	68	8	18.2
Aberdart-Spring	8.56	11.8	16.9	1.38	18.8	41.4	0.83	10.95	264	73	10	13.1
Aberdart-Spring	8.33	11.8	19.2	1.47	22.1	48.6	0.58	8.99	136	70	9	13.8
Aberdart-Spring	7.00	10.9	20.6	1.54	21.6	64.9	0.60	9.02	139	68	8	15.0
Aberdart-Spring	6.56	10.9	21.7	1.65	22.5	63.2	0.62	9.68	196	76	9	15.0
Aberdart-Spring	6.89	10.4	19.1	1.45	21.4	34.3	0.64	9.63	281	70	8	16.0
Aberdart-Spring	7.78	11.8	12.1	1.50	13.7	20.7	0.66	6.30	287	75	10	14.0
Alto-Autumn	5.85	7.5	39.8	1.35	27.6	82.9	0.63	12.08	149	64	8	18.6

Appendix 4.2 (continued)

Alto-Autumn	5.62	7.1	43.1	1.38	30.5	82.9	0.69	14.78	164	58	8	15.6
Alto-Autumn	4.86	7.2	31.9	1.60	22.5	58.1	0.53	8.31	142	53	8	14.6
Alto-Autumn	5.92	9.0	35.2	1.64	30.6	113	0.70	14.93	131	70	9	10.9
Alto-Autumn	5.21	7.8	35.9	1.53	27.8	93.4	0.66	12.91	145	46	7	16.0
Alto-Autumn	5.46	7.7	37.2	1.57	28.6	95.9	0.70	13.92	128	57	7	13.5
Alto-Autumn	5.85	7.9	34.5	1.38	26.1	84.2	0.57	10.36	125	62	8	20.5
Alto-Autumn	5.69	8.0	39.6	1.53	31.2	62.7	0.48	10.54	174	74	9	15.1
Alto-Autumn	5.07	7.0	35.0	1.56	23.9	57.4	0.53	8.92	167	65	9	17.6
Alto-Autumn	5.13	8.0	34.2	1.63	26.0	71.9	0.57	10.29	156	59	9	20.9
Alto-Autumn	5.15	7.5	38.1	1.48	28.0	67.4	0.57	11.17	169	68	9	11.7
Alto-Autumn	5.33	7.7	35.5	1.54	26.4	57.6	0.48	8.84	149	56	8	14.5
Alto-Autumn	5.57	8.8	36.8	1.66	30.5	68.0	0.56	12.03	186	62	9	17.4
Alto-Autumn	5.20	8.8	36.6	1.76	30.1	78.6	0.59	12.39	163	56	9	20.1
Alto-Autumn	5.21	7.7	40.7	1.56	31.0	91.7	0.68	14.74	154	65	10	17.3
Alto-Autumn	6.25	7.9	43.2	1.37	33.8	86.7	0.59	13.91	155	60	7	17.0
Aberdart-Autumn	6.67	9.9	41.0	1.56	36.8	122	0.71	18.36	155	67	9	17.9
Aberdart-Autumn	5.92	9.8	34.9	1.72	32.3	127	0.69	15.67	157	69	9	13.2
Aberdart-Autumn	4.56	8.9	35.8	2.01	30.5	96.1	0.63	13.44	141	74	14	15.8
Aberdart-Autumn	4.69	9.0	32.8	2.43	25.4	101	0.60	10.69	128	70	12	19.9
Aberdart-Autumn	4.56	9.4	35.4	2.13	30.2	104	0.66	13.88	139	68	14	14.9
Aberdart-Autumn	4.31	8.8	32.6	2.21	25.7	105	0.60	10.74	116	63	14	14.2
Aberdart-Autumn	4.06	8.6	36.0	2.50	29.0	113	0.62	12.56	119	66	14	14.8
Aberdart-Autumn	4.63	8.4	32.7	1.90	24.6	113	0.63	10.93	112	72	14	14.9
Aberdart-Autumn	5.08	8.0	34.8	1.69	28.8	77.1	0.65	13.07	158	80	11	14.2
Aberdart-Autumn	4.93	8.7	36.3	1.89	31.2	100	0.66	14.32	147	65	11	14.7
Aberdart-Autumn	5.00	8.4	36.5	2.30	30.3	82.9	0.63	13.36	199	72	12	11.5
Aberdart-Autumn	4.88	9.4	35.6	1.94	31.4	113	0.62	13.60	141	80	15	12.9
Aberdart-Autumn	5.07	8.9	35.8	1.82	30.4	104	0.70	14.83	144	65	11	14.7
Aberdart-Autumn	5.19	8.4	38.2	1.75	28.8	104	0.67	13.45	136	72	12	13.9
Aberdart-Autumn	5.40	9.3	35.6	1.79	31.1	92.5	0.66	14.38	151	84	12	12.8
Aberdart-Autumn	5.47	9.2	36.3	1.76	30.2	86.2	0.56	11.80	139	67	11	12.0

Appendix 4.3 Leaf area and net photosynthetic rate at different phytomer positions (P) of Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments. P1 is the youngest phytomer.

	Leaf area (cm^2)				Photosynthetic rate $leaf^{-1}$ µmol CO_2 $m^{-2}s^{-1}$			
P	Alto-Spring	Aberdart-Spring	Alto-Autumn	Aberdart-Autumn	Alto-Spring	Aberdart-Spring	Alto-Autumn	Aberdart-Autumn
1	5.30	4.12	6.54	3.85	15.4	13.7	13.2	12.1
2	13.2	16.5	15.4	12.6	19.2	17.6	18.0	15.0
3	12.6	18.2	16.9	16.6	16.9	16.9	18.2	16.7
4	10.8	15.9	16.3	16.9	16.0	14.0	17.6	15.2
5	9.24	13.1	15.7	17.4	14.3	12.9	16.5	14.3
6	8.10	14.5	14.5	17.2	12.6	11.1	14.6	13.2
7	5.91	9.30	12.7	17.3	11.7	9.63	12.4	11.4
8		7.05	10.8	15.3		8.45	10.6	9.82
9			9.02	13.9			9.18	8.55
10			9.90	14.9			7.98	8.00
11				13.1				7.59
12				12.5				7.14

Appendix 4.4 PC scores for the leaf morphological traits of the individual tillers of Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments (Experiment 4 & 5, respectively).

Coding: Experiment 1 = Spring experiment, Experiment 2 = Autumn experiment; Cultivar 1 = Alto, Cultivar 2 = Aberdart.

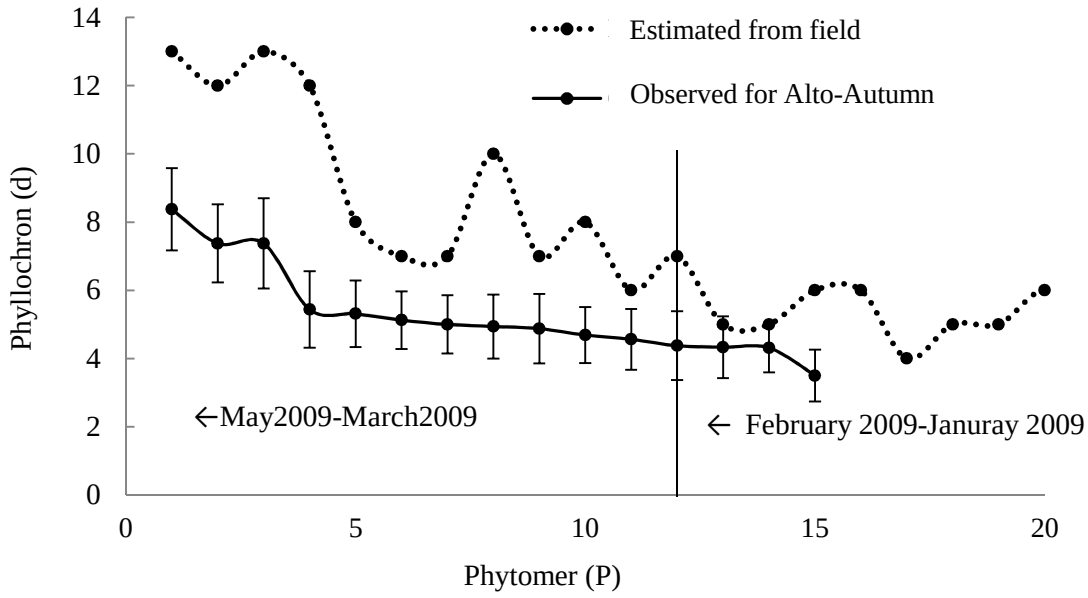
Experiment	Cultivar	PC1	PC2	PC3
1	1	-2.72	0.65	-0.17
1	1	-2.14	0.43	-0.34
1	1	-2.34	-0.18	-1.06
1	1	-2.64	-0.64	0.49
1	1	-1.56	1.86	0.41
1	1	-2.35	-2.04	0.59
1	1	-2.77	0.79	1.29
1	1	-2.58	0.56	1.14
1	1	-2.69	0.41	1.32
1	1	-4.13	0.69	0.53
1	1	-3.14	-0.72	0.02
1	1	-2.46	1.00	-2.72
1	1	-2.85	1.82	-1.28
1	1	-3.37	1.73	-1.41
1	1	-2.55	-0.09	-0.59
1	1	-2.88	0.54	0.43
1	2	-2.56	0.24	-2.18

Appendix 4.4 (continued)

1	2	-2.00	0.18	-0.07
1	2	-2.41	-1.54	-1.47
1	2	-1.95	-0.73	0.54
1	2	-2.23	-1.95	1.27
1	2	-1.46	-1.81	0.48
1	2	-1.81	1.08	0.50
1	2	-1.85	-1.37	0.22
1	2	-2.65	-1.39	2.14
1	2	-2.10	-1.33	1.14
1	2	-2.46	-3.10	1.24
1	2	-1.47	-0.58	-0.93
1	2	-0.84	0.01	-0.69
1	2	-0.59	-1.02	-0.79
1	2	-2.11	-0.64	0.07
1	2	-3.57	-1.99	-1.39
2	1	1.44	1.73	1.16
2	1	2.11	1.19	2.13
2	1	0.61	2.43	-1.18
2	1	2.63	-1.12	1.01
2	1	1.52	2.02	1.66
2	1	2.07	0.84	1.56
2	1	1.01	2.45	0.43
2	1	1.55	1.14	-1.00
2	1	0.90	1.97	-0.97
2	1	1.13	2.27	-0.01
2	1	1.60	0.58	-0.55
2	1	0.78	2.45	-1.18
2	1	1.34	1.19	0.19
2	1	1.69	1.79	0.63
2	1	2.72	0.56	1.46
2	1	1.89	1.91	1.65
2	2	2.99	-0.39	3.04
2	2	2.70	-1.11	1.45
2	2	3.54	-1.04	-0.79
2	2	2.91	-0.01	-1.48
2	2	3.63	-1.24	-0.58
2	2	3.25	-0.42	-1.91
2	2	4.29	-0.87	-1.61
2	2	3.14	-0.73	-1.36
2	2	2.46	-0.90	-0.28
2	2	3.05	-0.37	0.37
2	2	3.10	-1.63	-1.08
2	2	3.85	-2.02	-1.16
2	2	2.96	-0.63	0.84
2	2	3.07	-0.82	0.03
2	2	2.99	-2.00	-0.23
2	2	2.31	-0.12	-0.98

Appendix 5.1 Estimation of age of the the root-bearing phytomers of unknown phyllochron for perennial ryegrass cultivar Alto in the Autumn experiment. For the cultivar Alto in the Spring experiment and for the cultivar Aberdart in Spring and Autumn experiments root age was decided similarly.

To estimate the age of Pr of the phytomers for which A_{lf} was unknown phyllochron of those Pr was extrapolated from the weather data assuming 80 ^{o}C.d thermal time per phyllochron (see Fig. below). Once phyllochron was extrapolated rhizochron of the respective phytomers was assumed equal to phyllochron at those phytomers when leaf was formed (see Table below).



Appendix 5.1a Phyllochron expressed in days for Alto perennial ryegrass cultivar in the Autumn experiment (Experiment 5)

Appendix 5.1b Phyllochron (A_{lf}), leaf age, rhizochron and estimated root age at different phytomers. *de*, delay between leaf and root appearance at a particular phytomer for cultivar Alto in the Autumn experiment (Experiment 5)

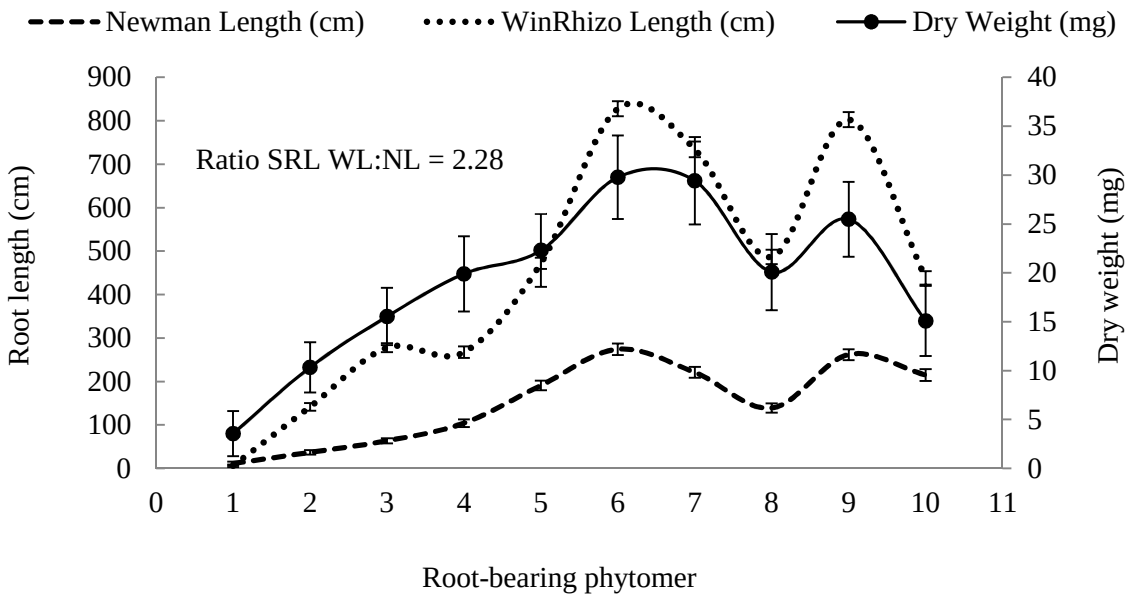
Phytomer	Phyllochron	Leaf age	Rhizochron	Root age
1	-	6		
2	8	14		
3	7	21	*de*	
4	7	28		
5	5	33		
6	5	38	5	6
7	5	43	5	11
8	5	48	5	16
9	5	53	5	21
10	5	58	5	26
11	5	63	5	31
12	5	68	5	36
13	4	72	4	40
14	4	76	4	44
15	4	80	4	48
16			4	52
17		Estimated A_{lf} in field →	5	57
18		assuming phyllochron =	6	63
19		80 ^{o}C d) thermal time and	6	69
20		is equal to	4	73
21		rhizochron in thermal	5	78
22		time	-	83

Appendix 5.2 Number of leaves appeared since transplanting (NLA), root-bearing phytomers (NPr) and length of individual roots at particular phytomers at Day 16, Day 22 and Day 27 after transplanting for 6 tillers of Alto (A) and Aberdart (B) perennial ryegrass. Phytomer positions are as at Day 27 and observations in the same column show the development over time of roots at that phytomer position.

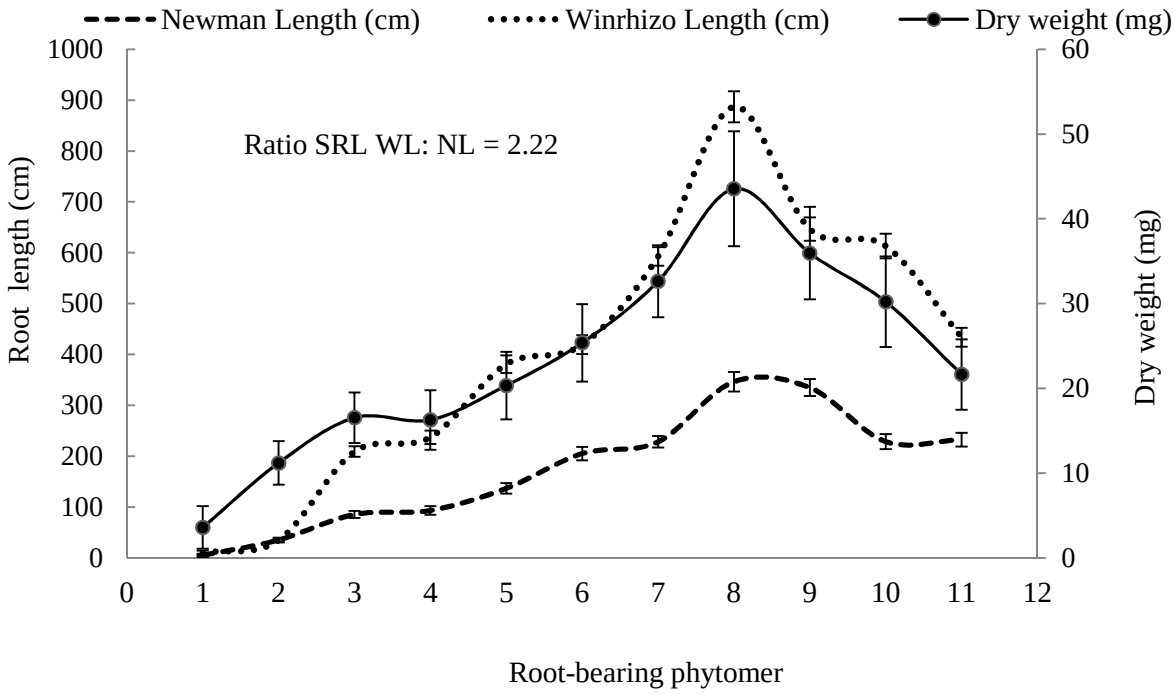
				Root length in cm at different phytomer position (Pr)							
Plant	Day	NLA	NPr	**Oldest Pr**						**Youngest Pr**	
			Pr	7	6	5	4	3	2	1	
A35	16	4	4	35,36	26	14	8,8,9,9				
	22	6	6	37,46	36	26	16,18,19,23	12	6,6		
	27	6	7	43,53	40	38	27,27,32,32	18	6,7	4,6,6,6	
			Pr	7	6	5	4	3	2	1	
A39	16	4	5	37,37	27	22	9	5,5,5,5			
	22	6	6	41,41	36	27	22	17,18,19,21	5,7,8		
	27	6	7	49,53	40	36	34	18,21,30,32	9,9,14	4,4,6	
			Pr	5	4	3	2	1			
A44	16	4	4	51	40,41	15	10,11,11				
	22	6	5	69	46,49	33	26,28,29	7,10,10			
	27	6	5	79	50,63	49	40,46,46	11,26,28			
			Pr	6	5	4	3	2	1		
A50	16	5	4	41	34	9,7	1				
	22	6	5	49	41	22,23	10,11,11	1			
	27	8	6	53	36	32,35	22,24,25	5,5,5	1		
			Pr	7	6	5	4	3	2	1	
A52	16	5	5	31	30,30	8,8,8	4,6	1			
	22	6	6	43	30,33	24,25,26	17,17	7,8,8	2		
	27	5	7	48	35,41	29,32,34	25,28	8,9,10	7	2,2,3	
			Pr	7	6	5	4	3	2	1	
A60	16	5	5	36	34	10	8	3,3			
	22	5	6	45	39	16	13	7,8,10	3,4,4,4		
	27	5	7	46	39	32	27	20,20,22	8,11,15,18	6,7	
			Pr	7	6	5	4	3	2	1	
B35	16	5	6	35	29	17,17	11	8,8	2		
	22	7	7	37	32	27,27	20	13,13	11	5,1,1	
	27	8	7	45	40	29,33	22	14,16	11	6,6,6,7	
			Pr	6	5	4	3	2	1		
B39	16	5	4	39,40	27	13,13	1				
	22	6	5	47,51	42	23,26	13	1,2,2			
	27	6	6	52,64	45	42,44	32	8,11,14	5,5,6		
			Pr	6	5	4	3	2	1		
B44	16	5	5	32,33,35	22,24	13	9	1			
	22	6	6	42,42,45	30,35	15	23	12	3,3		
	27	6	6	45,52,55	38,39	37	27	16	11,15		
			Pr	6	5	4	3	2	1		
B50	16	5	5	38,39	19,20	17	11	1,1,1			
	22	5	6	43,48	34,35	29	12	4,1,1			
	27	6	6	54,62	49,52	43	13	14,16,22	4,5,7		
			Pr	8	7	6	5	4	3	2	1
B52	16	5	5	27,34,38	20	10,10,11,12	9,9	1,3,3			
	22	6	6	38,40,42	36	20,22,23,24	18,18	9,11,12	2,3		
	27	7	8	43,52,53	42	32,34,38,38	26,27	24,26,26	17,19	11	6,3,3,3
			Pr	7	6	5	4	3	2	1	
B60	16	5	5	29,30	24	8,8,8	4	1			
	22	6	6	38,40	35	19,19,20	10	8	5		
	27	7	7	48,51	46	33,33,33	25	14	9	7,7	

Appendix 5.3 Comparison between root length at the different phytomer positions of Alto and Aberdart perennial ryegrass cultivars in the Spring experiment (Experiment 4) obtained from modified Newman Method and WinRhizo Method. SRL, specific root length; WL, WinRhizo length; NL, Newman Length.

Appendix 5.3a Alto perennial ryegrass cultivar in spring

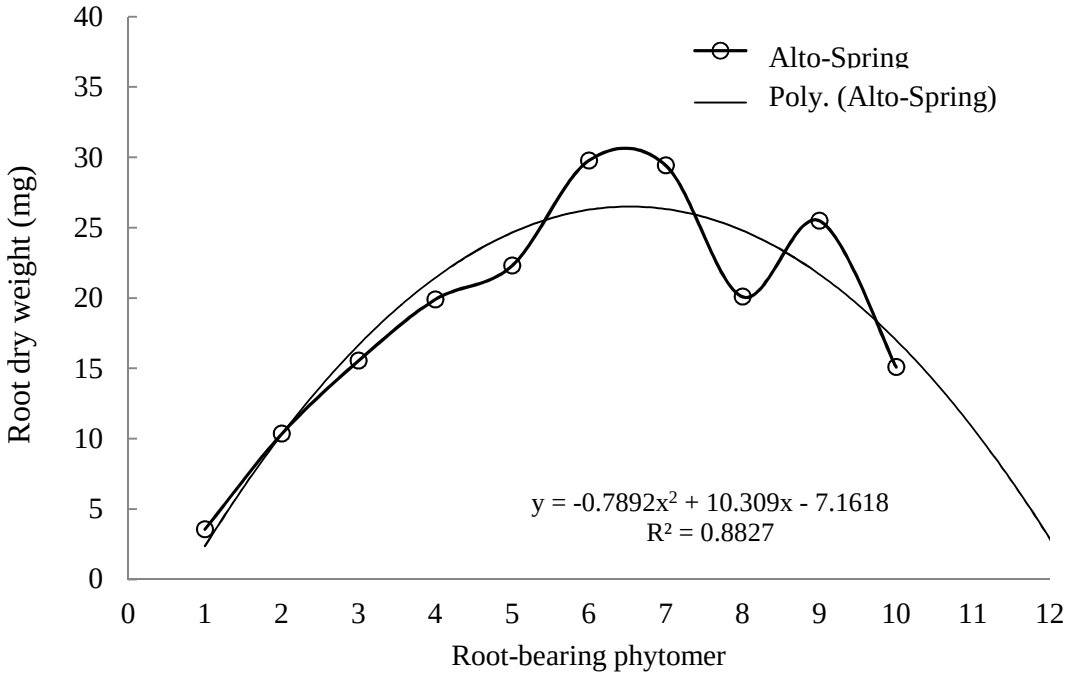


Appendix 5.3b Aberdart perennial ryegrass cultivar in spring

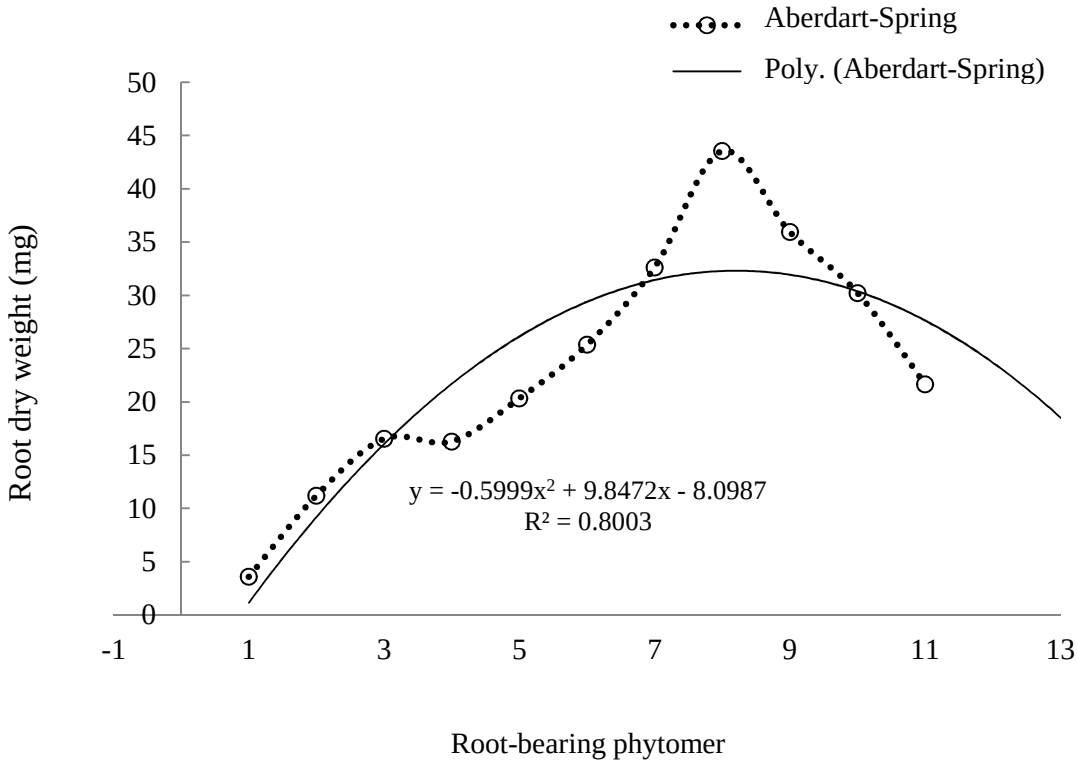


Appendix 5.4 Quadratic curves for estimating the root dry matter deposition rate at different phytomer (DMD_p) positions of Alto and Aberdart perennial ryegrass cultivars in the Spring and Autumn experiments.

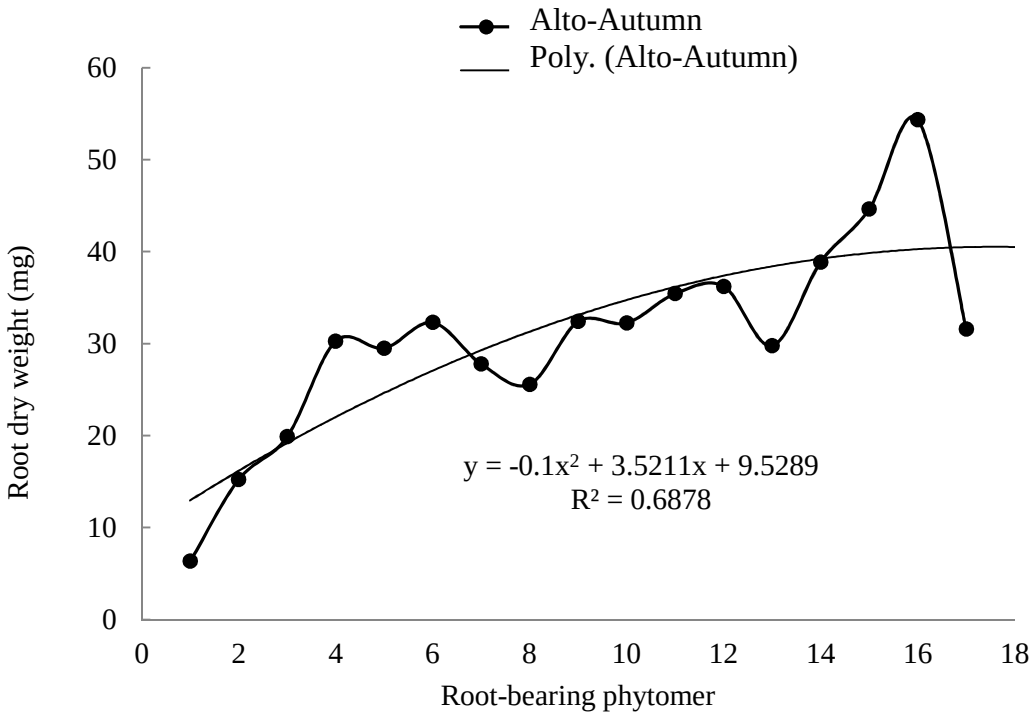
Appendix 5.4a Alto in the Spring experiment



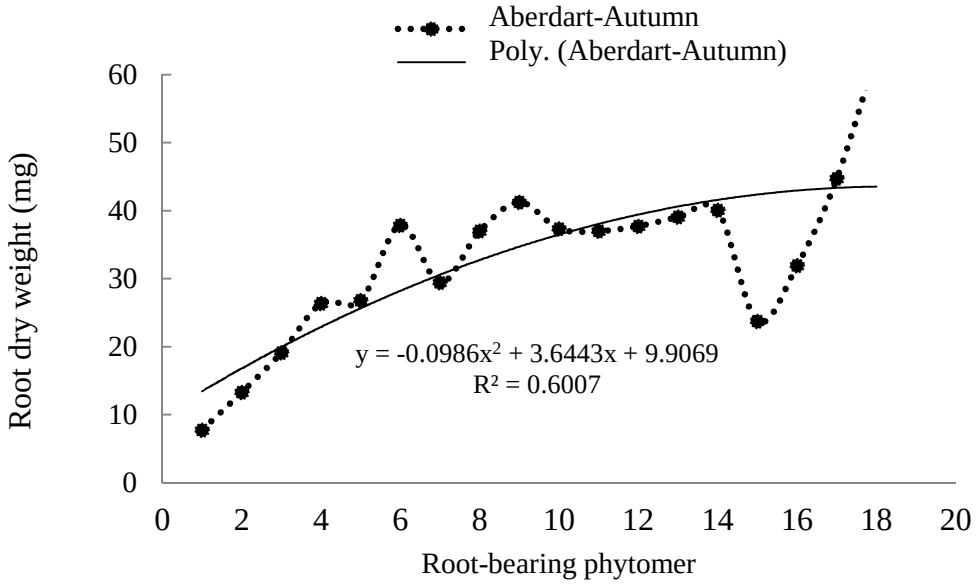
Appendix 5.4b Aberdart in the Spring experiment



Appendix 5.4c Alto in the Autumn experiment

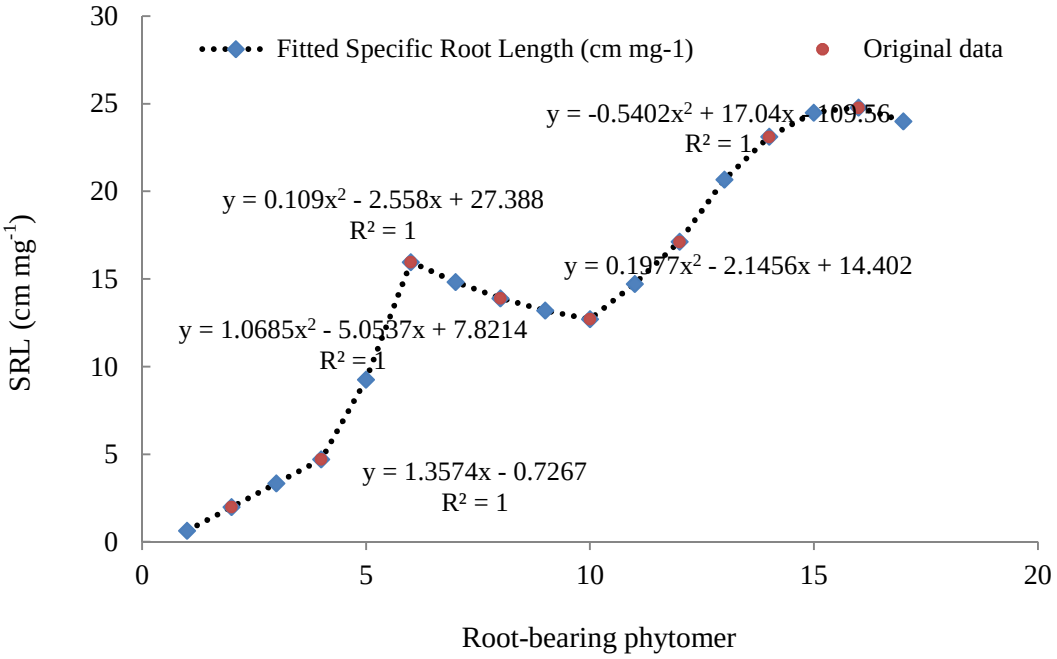


Appendix 5.4d Aberdart in the Autumn experiment

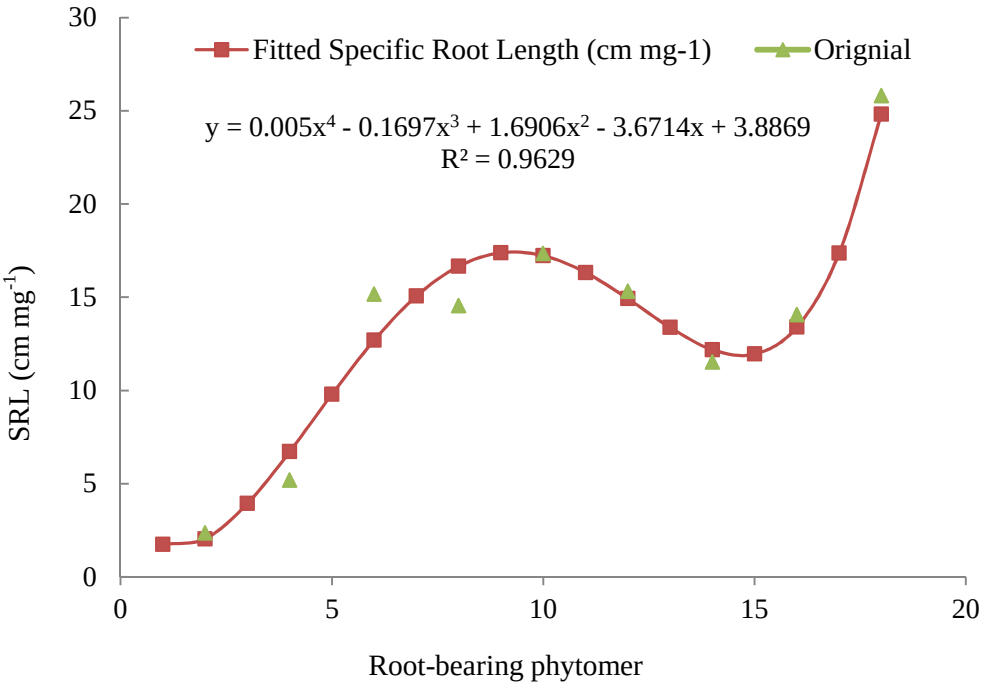


Appendix 5.5 Estimation of specific root length, specific root surface area and specific root volume for Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments (Experiment 4 & 5, respectively).

Appendix 5.5a Estimation of specific root length at the phytomer positions of unknown root length

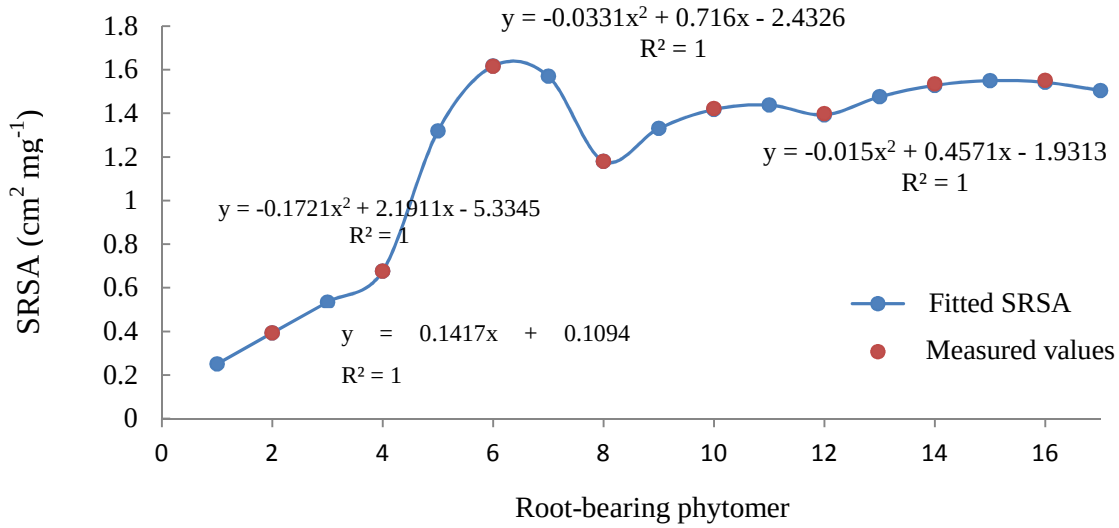


Specifc root length (SRL, cm mg^{-1}) at different phytomer positions of Alto perennial

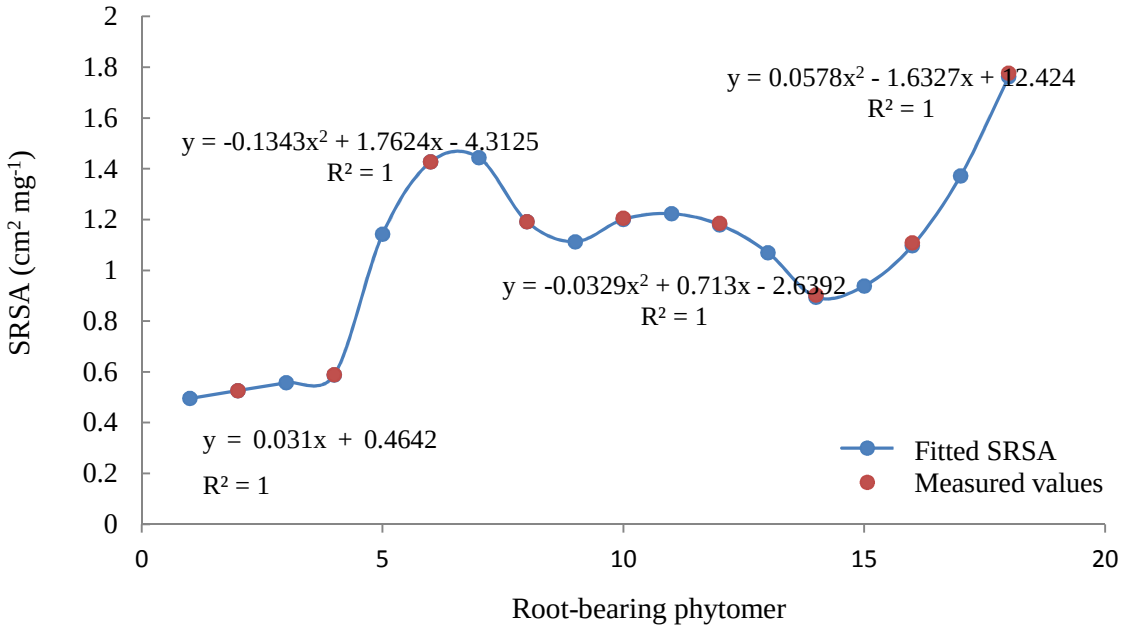


Specific root length (SRL, cm mg^{-1}) at different phytomer positions of Aberdart perennial ryegrass cultivar in Autumn experiment (Experiment 5)

Appendix 5.5b Estimation of specific root surface area at the phytomer positions of unknown surface area

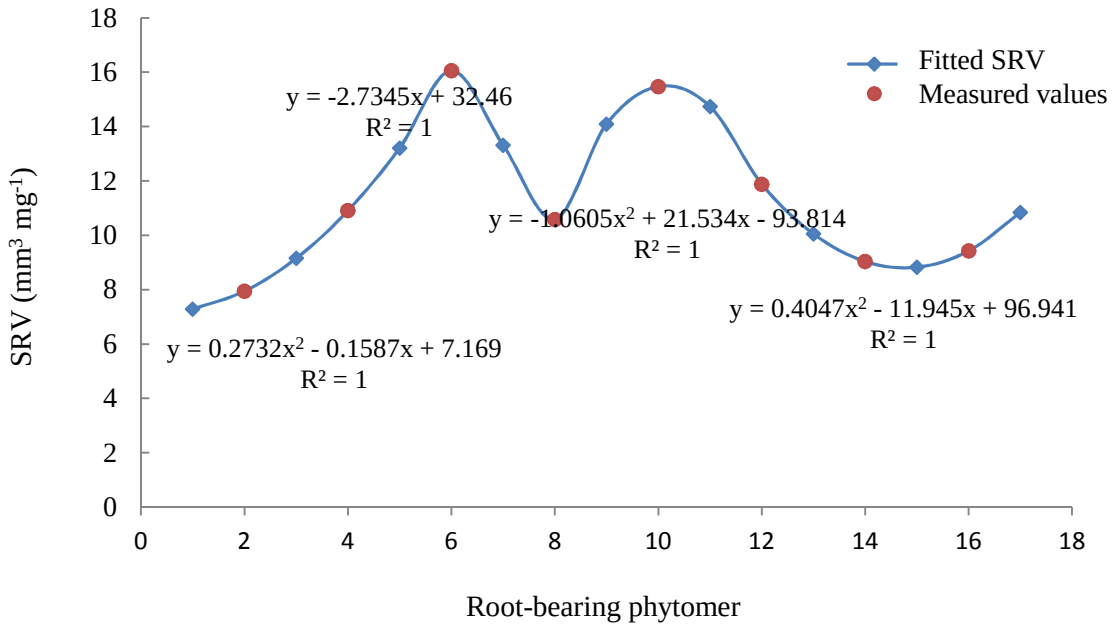


Specifc root surface area (SRSA, cm^2 mg^{-1}) at different phytomer positions of Alto perennial ryegrass cultivar in Autumn experiment (Experiment 5)

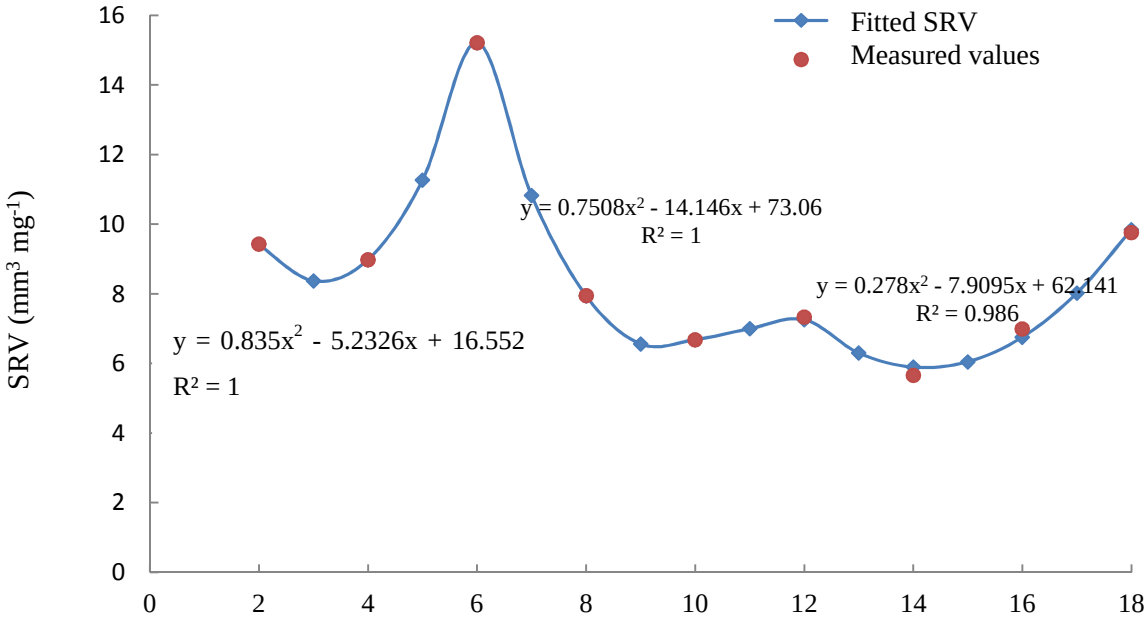


Specific root surface area (SRSA, cm^2 mg^{-1}) at different phytomer positions of Aberdart perennial ryegrass cultivar in Autumn experiment (Experiment 5)

Appendix 5.5c Estimation of specific root volume at the different phytomer positions of unknown root volume



Specifc root volume (SRV, mm^3 mg^{-1}) at different phytomer positions of Alto perennial ryegrass cultivar in Autumn experiement (Experiment 5)



Specifc root volume (SRV, mm^3 mg^{-1}) at different phytomer positions of Aberdart perennial ryegrass cultivar in Autumn experiment (Experiment 5)

Appendix 5.6 ANOVA for estimating experiment × phytomer interaction (RL, root length as an example). Exp, experiment; Cul, cultivar; Geno, Genotypes; ClonalRep, clonal replicates; Pr, phytomers.

```
GLM RL= Exp | Pr Cul(Exp) Geno(Exp Cul) ClonalRep(Exp Cul Geno);
SUBC> means Exp | Pr Cul(Exp) Geno(Exp Cul) ClonalRep(Exp Cul Geno);
SUBC> test Exp*Pr/ ClonalRep(Exp Cul Geno).
```

Folder name in the CD for data: *"Chapter 5 / Root data – 1082"*

Appendix 5.7 ANOVA for estimating variation between two experiments, Spring and Autumn experiments; two cultivars, Alto and Aberdart; genotypes of each cultivar for different root dimensions at different root-bearing phytomers (SRL, specific root length as an example).

```
MTB > GLM SRL = c1|c2 c3(c1 c2) c4(c1 c2);
SUBC> means  c1|c2 c3(c1 c2) c4(c1 c2);
SUBC> test c1/c3(c1 c2);
SUBC> test c2/c3(c1 c2);
SUBC> test c1*c2/c3(c1 c2);
SUBC> test c4(c1 c2)/c3(c1 c2).
```

Where,
C1= Experiment
C2= Cultivar
C3= Plant/Genotype/Replication
C4= Phytomer

Floder name in CD for data: *"Chapter 5 / detailed root morphology-81 data"*

Appendix 5.8 Non-orthogonal linear contrast: SAS code used for non-orthogonal linear contrast

"A contrast matrix consists of so-called contrast coefficients that (in the end) all have to sum to zero. This means, those things we want to compare have to get the opposite sign (e.g., +1 and –1), while those things we don't want to compare will receive a value of zero."

A block design that does not contain all treatments are incomplete and hence incomplete block designs are non-orthogonal designs (Hinkelmann and Kempthorne, 2007).

The following SAS command was used in this test:

```
proc glm order = data;
class position;
model PC1  PC2 PC3 PC4 = position;
contrast 'linear'  position  1  -1   0;
contrast 'linear' position   0   1  -1;
run;
```

Folder name in CD for SAS analysis: *"Chapter 5 / contrast 5 7 11 – pc scores"*

Appendix 5.9 Derived measures for new root dry weight (RDW), root length (RL), root surface area (RSA), root volume (RV), root diameter at each root-bearing phytomer position (Pr) considering root growth at each phytomer position in steady-state and derived root diameter of the all roots at each phytomer position for two perennial ryegrass cultivars, Alto and Aberdart in two different experiements, the Spring and Autumn experiements. The new root growth at each position was calculated from a quadratic equation. Pr1 refers the youngest root-bearing phytomer.

Appendix 5.9a Alto in the Spring experiment

Branching level	Pr	New RDW (mg)	New RL (cm)	New RSA (cm2)	New RV (mm3)	Diameter all roots (mm)	Diameter new roots (mm)	Deductions
Main axis elongation	1	1.55	2.50	0.55	10.8	0.69	0.69	Phase 0
1^{o} branching	2	4.27	68.1	6.95	58.6	0.34	0.32	Phase 1
	3	3.85	38.3	3.87	21.8	0.33	0.32	
2^{o} branching	4	3.29	51.6	4.78	34.8	0.32	0.29	Phase 2
	5	2.74	52.9	4.18	28.6	0.30	0.25	
3^{o} branching	6	2.19	54.1	3.59	22.3	0.28	0.21	Phase 3
	7	1.63	55.3	3.00	16.0	0.27	0.17	
	8	1.08	56.6	2.41	9.77	0.25	0.14	Fine roots (+)
	9	0.53	57.8	1.82	3.50	0.23	0.10	

Appendix 5.9b Aberdart in the Spring experiment

Branching level	Pr	New RDW (mg)	New RL (cm)	New RSA (cm^2)	New RV (mm^3)	Diameter all roots (mm)	Diameter new roots (mm)	Deductions
Main axis elongation	1	1.18	2.90	0.57	8.17	0.63	0.63	Phase 0
	2	3.93	13.1	2.47	38.6	0.61	0.61	
1° branching	3	2.89	92.5	9.06	63.6	0.36	0.31	Phase 1
	4	2.89	60.0	5.47	39.3	0.33	0.29	
2° branching	5	2.64	56.3	4.65	29.9	0.31	0.26	Phase 2
	6	2.38	52.7	3.83	20.5	0.30	0.23	
3° branching	7	2.13	49.1	3.01	11.1	0.28	0.20	Phase 3
	8	1.87	45.5	2.19	1.64	0.27	0.15	
	9	1.61	41.9	1.37	-7.77	0.25	0.10	Fine roots (±)

Appendix 5.9c Alto in the Autumn experiment

Branching level	Pr	New RDW (mg)	New RL (cm)	New RSA (cm^2)	New RV (mm^3)	Diameter all roots (mm)	Diameter new roots (mm)	Deductions
Main axis elongation	1	2.67	1.8	0.50	19.5	0.89	0.89	Phase 0
	2	2.99	8.9	1.56	25.6	0.63	0.63	
	3	1.74	13.5	1.73	22.8	0.51	0.41	
1° branching	4	2.69	28.6	3.72	56.0	0.46	0.41	Phase 1
	5	1.87	59.2	6.87	44.5	0.41	0.37	
2° branching	6	1.60	29.7	3.03	27.9	0.39	0.32	Phase 2
	7	1.34	28.7	2.56	21.1	0.37	0.28	
	8	1.08	27.8	2.10	14.3	0.36	0.24	
3° branching	9	0.82	26.9	1.64	7.49	0.34	0.19	Phase 3
	10	0.56	25.9	1.17	0.67	0.32	0.14	
4° branching	11	0.30	25.0	0.71	-6.14	0.30	0.09	Phase 4
	12	0.04	24.0	0.24	-13.0	0.28	0.03	Fine roots (±)
	13	-0.22	23.1	-0.22	-19.8	0.25	-0.03	
	14	-0.49	22.1	-0.68	-26.6	0.23	-0.10	

Appendix 5.9d Aberdart in the Autumn experiment

Branching level	Pr	New RDW (mg)	New RL (cm)	New RSA (cm^2)	New RV (mm^3)	Diameter all roots (mm)	Diameter new roots (mm)	Deductions
Main axis elongation	1	2.93	5.16	1.47	8.88	0.90	0.90	Phase 0
	2	2.61	6.48	1.54	45.1	0.82	0.76	
1° branching	3	2.52	21.1	1.62	15.7	0.45	0.25	Phase 1
	4	0.73	64.3	5.07	23.2	0.32	0.25	
	5	1.28	34.0	2.56	15.0	0.30	0.24	
2° branching	6	1.14	29.2	2.12	10.7	0.29	0.23	Phase 2
	7	1.01	24.3	1.68	7.22	0.28	0.22	
	8	0.87	19.5	1.25	3.71	0.27	0.20	
3° branching	9	0.74	14.7	0.81	0.20	0.26	0.18	Phase 3
	10	0.61	9.82	0.37	-3.31	0.26	0.12	Root branches (±)
4° branching	11	0.47	4.98	-0.06	-6.82	0.25	-0.04	Phase 4
	12	0.34	0.14	-0.50	-10.3	0.24		
	13	0.20	-4.70	-0.94	-13.8	0.24		
	14	0.07	-9.54	-1.38	-17.4	0.23		Branches dying

Appendix 5.10 PC scores for different measured and derived root dimensions of phytomer (Pr) 5 and Pr 7 of Spring experiment, Pr 11 of Autumn experiment for Alto and Aberdart perennial ryegrass cultivars.

Coding: Experiment 1 = Spring experiment, Experiment 2 = Autumn experiment; Cultivar 1 = Alto, Cultivar 2 = Aberdart.

Exp	Pr	Cultivar	Genotypes	Replications	PC1	PC2	PC3	PC4
1	5	1	1	1	5.08	-3.7	-0.78	0.98
1	5	1	1	2	1.66	-4.6	-1.5	0.23
1	5	1	1	3	-0.93	-1.76	-0.52	0.06
1	5	1	2	1	-0.78	2.03	-0.96	0.56
1	5	1	2	2	-1.2	4.19	-0.2	0.37
1	5	1	3	1	-5.94	0.91	-0.06	0.17
1	5	1	3	2	-6.01	2.29	0.45	-0.07
1	5	1	4	1	1.27	1.76	-1.03	-0.82
1	5	1	4	2	0.8	2.77	-0.67	-0.89
1	5	1	5	1	-3.83	2.85	-0.12	0.57
1	5	1	5	2	0.97	0.62	-0.83	0.54
1	5	1	6	1	-5.17	1.55	-0.09	0.07
1	5	1	6	2	-3.08	1.52	0	-0.1
1	5	1	6	3	2.05	0.39	-1.11	-0.42
1	5	1	6	4	-0.06	1.93	-0.49	-0.34
1	5	2	1	1	-1.83	0.03	-0.33	1.03
1	5	2	1	2	-2.86	-1.32	0.06	0.43
1	5	2	1	3	-0.93	-1.99	0.18	0.4
1	5	2	1	4	-3.66	-0.02	0.54	0.27
1	5	2	1	5	-1.91	-2.1	-0.23	0.36
1	5	2	2	1	0.5	3.67	0	0.53
1	5	2	2	2	1.47	1.02	-0.86	0.72
1	5	2	2	3	2.86	0.39	-1.49	0.48
1	5	2	3	1	-4.45	-1.44	-0.96	0.28
1	5	2	3	2	-1.16	-2.54	-0.64	0.33
1	5	2	4	1	-3.09	-1.49	-1.3	0.3
1	5	2	4	2	-1.62	0.66	-0.25	-0.02
1	5	2	5	1	-4.02	0.69	-0.09	-0.04
1	5	2	5	2	-3.39	0.53	-0.19	-0.13
1	5	2	5	3	-2.71	-0.5	-0.56	1.05
1	5	2	6	1	-4.79	0.33	-0.12	0.21
1	5	2	6	2	-1.66	-0.79	0.08	0.17
1	7	1	1	1	-1.19	-1.04	-1.35	0.8
1	7	1	1	2	-2.57	-1.46	-0.64	1.02
1	7	1	2	1	3.62	0.16	-1.38	0.35
1	7	1	2	2	4.27	4.99	-0.84	-0.09
1	7	1	3	1	1.64	0.92	-0.65	0.58
1	7	1	3	2	3.82	2.57	-0.64	0.27

1	7	1	4	1	3.6	2.11	-0.17	0.31
1	7	1	4	2	3.34	-0.05	-1.32	0.59
1	7	2	1	1	2.48	1.52	-0.36	-0.47
1	7	2	1	2	2.32	1.38	-0.46	-0.43
1	7	2	1	3	3.91	-0.99	-0.62	-0.33
1	7	2	1	4	2.74	-2.66	-1.7	-0.13
1	7	2	2	1	-0.18	-1.36	-0.24	0.31
1	7	2	2	2	-2.23	-0.54	-0.57	0.11
1	7	2	2	3	3.31	1.13	-0.89	-0.46
1	7	2	2	4	3.49	0.1	-1.27	-0.34
1	7	2	3	1	-0.2	-0.13	-0.12	0.74
1	7	2	3	2	-1.52	1.74	0.56	0.63
1	7	2	4	1	3.17	-0.56	1.02	-0.47
1	7	2	4	2	-1.7	-2.29	-0.97	0.19
1	7	2	4	3	2.34	-1.95	-1.03	1.16
2	11	1	1	1	5.34	0.79	-0.85	-0.18
2	11	1	1	2	6.0	4.18	-0.04	-1.22
2	11	1	2	1	3.27	-2.38	3.06	1.06
2	11	1	2	2	2.85	-2.08	2.53	0.77
2	11	1	3	1	2.78	0.21	1.48	1.11
2	11	1	3	2	3.11	-1.18	1.39	-1.01
2	11	1	3	3	-0.04	-0.4	0.75	-1.05
2	11	1	3	4	1.25	-1.88	0.41	-0.79
2	11	1	4	1	-2.83	0.47	-0.56	-0.24
2	11	1	4	2	-3.24	0.36	-0.59	-0.17
2	11	1	5	1	1.23	-0.2	-0.48	-0.33
2	11	1	5	2	-0.56	-0.4	-0.97	-0.28
2	11	1	6	1	-2.58	0.47	1.75	0.46
2	11	1	6	2	0.69	-0.09	3.46	0.67
2	11	2	1	1	-2.8	0.83	2.74	-4.68
2	11	2	1	2	0.31	-3.12	0.77	-4.79
2	11	2	2	1	0.83	-1.29	1.06	-0.73
2	11	2	2	2	-0.88	-1.71	0.13	-0.7
2	11	2	3	1	-0.6	1.44	0.84	0.01
2	11	2	3	2	-0.46	1.39	0.93	0
2	11	2	3	3	0.44	0.99	4.84	1.07
2	11	2	3	4	3.09	-0.73	5.76	1.48
2	11	2	3	5	-1.41	0.27	1.6	0.13
2	11	2	3	6	-0.9	-1.5	0.65	0.23
2	11	2	4	1	0.16	-0.86	-0.41	-1.2
2	11	2	4	2	-0.99	-1.97	-1.09	-1.06
2	11	2	4	3	0.34	-1.66	-0.87	-0.21
2	11	2	4	4	-0.43	-1.43	-0.55	0.03

Appendix 6.1 ANOVA commands for testing statistical significance of effects of experiment, cultivar, the experiment x cultivar interaction, and genotypes within experiment and cultivar for tillers of Alto and Aberdart perennial ryegrass in the Spring and Autumn experiments. LDW_t, leaf dry weight per tiller.

GLM LDW_t= c1|c2 c3(c1 c2) c4(c1 c2 c3);
SUBC> means c1|c2 c3(c1 c2) c4(c1 c2 c3);
SUBC> test c1/c3(c1 c2);
SUBC> test c2/c3(c1 c2);
SUBC> test c1*c2/c3(c1 c2);
SUBC> test c3(c1 c2)/c4(c1 c2 c3).

Where,
C1= Experiment (Spring or Autumn)
C2= Cultivar (Alto or Aberdart)
C3= Genotype (within each cultivar within experiments)
C4= Clonal replicate of each genotype

Folder name in the CD for data: *"Chapter 6 / Root-shoot ratio PCA and correlation-89 tillers"*

Appendix 6.2 PC scores for root-shoot morphological traits of Alto and Aberdart perennial ryegrass cultivars in Spring and Autumn experiments (Experiments 4 & 5, respectively).

Coding: Experiment 1 = Spring, 2 = Autumn; Cultivar 1 = Alto, 2 = Aberdart

Experiment	Cultivar	Genotype	PC1	PC2
1	1	1	-3.14	-1.56
1	1	1	-2.51	-0.48
1	1	1	-2.15	0.28
1	1	2	-2.78	-1.18
1	1	2	-2.36	-0.83
1	1	2	-1.98	0.06
1	1	3	-1.84	0.88
1	1	3	-0.62	2.39
1	1	3	-0.81	1.94
1	1	4	-2.38	-0.29
1	1	4	-1.88	0.53
1	1	4	-1.84	-0.13
1	1	5	-3.61	0.08
1	1	5	-2.09	5.08
1	1	5	-2.79	2.89
1	1	6	-3.11	-0.48
1	1	6	-2.54	-0.12
1	1	6	-2.41	0.13
1	1	7	-2.18	1.84
1	1	7	-2.24	0.06
1	1	7	-1.78	1.06
1	1	8	-2.18	-0.52
1	1	8	-1.38	1.89
1	1	8	-0.43	1.63
1	1	9	-3.38	-0.86
1	1	9	-1.96	0.52
1	1	9	-3.16	-0.03
1	2	1	-2.85	-0.98
1	2	1	-1.73	0.25
1	2	1	-2.22	0.35
1	2	2	-1.83	-0.49

Appendix 6.2 (continued)

1	2	2	-1.14	0.08
1	2	2	-0.13	-0.12
1	2	3	-0.96	0.65
1	2	3	-0.87	0.43
1	2	3	-1.41	-0.06
1	2	4	-1.20	-0.06
1	2	4	-1.01	-0.46
1	2	4	-0.36	0.19
1	2	5	0.89	3.77
1	2	5	-2.23	-1.34
1	2	5	-1.92	-1.93
1	2	6	-2.55	-2.46
1	2	6	-1.56	-2.51
1	2	6	0.39	0.59
1	2	7	-2.50	-0.95
1	2	7	-1.46	0.33
1	2	7	-1.49	3.73
1	2	8	-0.74	-1.58
1	2	8	0.03	-1.97
1	2	8	0.13	-1.08
1	2	9	-1.72	-0.86
1	2	9	-1.10	-1.11
1	2	9	0.44	1.20
1	2	10	-1.23	0.05
1	2	10	-0.41	0.51
1	2	10	-1.84	-0.44
2	1	1	2.78	0.72
2	1	1	1.63	-0.05
2	1	2	1.11	1.05
2	1	2	5.51	1.23
2	1	3	3.92	3.53
2	1	3	3.39	2.26
2	1	4	2.52	2.42
2	1	4	1.45	1.23
2	1	5	1.14	1.09
2	1	5	1.81	0.00
2	1	6	1.48	0.31
2	1	6	2.53	2.84
2	1	7	2.65	1.52
2	1	7	4.12	1.95
2	1	8	2.92	-0.65
2	1	8	2.42	1.96
2	2	1	3.10	0.08
2	2	1	5.07	1.00
2	2	2	3.26	-3.11
2	2	2	0.75	-3.64
2	2	3	2.86	-3.59
2	2	3	1.43	-4.56
2	2	4	3.48	-3.16
2	2	4	2.61	-3.87
2	2	5	1.81	-1.60
2	2	5	3.20	-1.47
2	2	6	2.80	-1.40
2	2	6	7.96	-0.56
2	2	7	5.35	0.66
2	2	7	5.40	-0.72
2	2	8	1.59	-2.64
2	2	8	2.02	-1.34

Appendix 6.3 Description of the tiller axis for the main tiller, MT (age 90 d) and two daughter tillers, DT1 and DT2 at the phytomer level for Alto and Aberdart perennial ryegrass cutlivars in the Spring and Autumn experiments (Experiments 4 and 5, respectively). Data obtained from the destructive havest. MT data is presented in Chapter 4 and 5 for shoot and roots, respectively. FLL, final leaf length (cm); LW, leaf width (mm); LDW, leaf dry weight (mg); LA, leaf area (cm^2); SLA, specific leaf area ($cm^2\ g^{-1}$); R_p, number of roots per phytomer; RAL, main root axis length (cm); RDW_i, dry weight of per root (mg) at each phytomer position. SE, standard error of mean when presented as % is back-transformed from the log-transformed data.

Appendix 6.3a Alto perennial ryegrass cultivars in Spring experiment (Experiment 4) before the DT excision treatment. Age of MT, DT1 and DT2 were around 90, 80 and 70 d, respectively.

	FLL			LW			LDW			LA			SLA			R_p			RAL			RDW_i		
Phytomer	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2
1	23.4	11.1	22	6.3	4.85	5.95	34	9	25.5	10.9	4.2	10.3	311	570	436									
3	37.3	31	32.7	8.4	7.5	7.25	87	47.5	53	21.9	16.4	16.6	252	349	316									
3	34.2	33.5	32.7	7.8	7.3	7.1	80	63	58.5	18.7	17.2	16.2	234	272	277									
4	31.5	28.9	26.2	7.8	6.5	6	75.5	55	49.5	17.2	13.3	11	228	239	224									
5	30.4	23.7	24.1	7.2	5.85	5.25	73	47	42	15.4	9.86	9.04	210	206	212									
6	26.7	20.8	22	6.55	5.05	6.1	56.5	36	33	12.3	7.45	9.39	217	205	285	1.5	1	2	1	4.75	10.7	0.5	1.2	2.4
7	23.7			5.8			50			9.62			192			2	1	1	24.1	19	22	17.4	6.2	5.05
8																1	1.5	1	19.5	17.8	25.5	4.55	6.63	6.2
9																1	1.5	1.5	28.3	20.1	35	10.4	4.73	10
10																1	1.5	1.5	53.5	27.3	26.8	19.2	9	6.9
11																1.5	1	1	27	19.3	36.5	19.3	4.5	13.7
12																1	1.5	1.5	40.5	44	28.8	23.6	10.7	6.07
13																1.5	1	2.5	45.8	37	27.5	36.1	11.7	4.16
14																1	1	1.5	22.8	48.5	29	10.1	18.3	10.4
15																1.5	1.5	1.5	49.5	44.6	15.5	60.5	10.6	2.87
16																2			15.2			12.6		
Total	-	-	-	-	-	-	456	258	262	106	68.4	72.5	-	-	-	15	12.5	15	-	-	-	214	119	115
SE	1.25	1.46	1.48	0.24	0.28	0.29	3.28	3.31	3.58	0.97	0.98	1.06	13.6	13.6	14.9	0.15	0.15	0.15		10.1%			11.1%	

Appendix 6.3b Aberdart perennial ryegrass cultivars in the Spring experiment (Experiment 4) before the DT excision treatment. Age of MT, DT1 and DT2 were around 90, 80 and 70 d, respectively.

	FLL			LW			LDW			LA			SLA			R_p			RAL			RDW_i		
P	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2
1	26.2	21.6	20.8	7.55	5.35	5.3	39	18.5	18	13.8	8.08	8.06	354	438	455									
3	43.1	44	40.8	10	7.95	7.8	110	75	63.5	30.1	24.6	22.3	277	329	351									
3	40.8	39.8	36.9	9.2	7.75	7.1	106	78	64	26.2	21.6	18.4	250	283	287									
4	36.8	33.6	31.6	8.7	6.6	6	96.5	66.5	55	22.4	15.8	13.3	234	238	241									
5	31.8	26.6	19.9	7.05	6.15	5.85	73.5	46.5	34.5	15.7	11.5	8.09	214	253	235									
6	27.8	23.2		6.2	5.3		56.5	43		12.1	8.61		213	200	455	1.5	1.5	1.5	2.92	1.1	6.3	1.95	0.5	1.43
7	21			5.6			38			8.26			217			2	2	1.5	26.8	13.6	19.8	14.6	3.83	5.07
8	19.8			4.85			30			6.7			224			2.5	1.5	2	28.2	26.8	17.3	19.5	8.27	6.15
9																4	1	1	25.4	26.8	16	31.3	7.25	4.6
10																1.5	1.5	1	39.2	16.1	34.5	20.7	3.37	11.8
11																1.5	1	1	33.2	37.5	35	14.8	16.1	11.6
12																1	2	1	39	21.6	44	15.9	7.18	18.9
13																1.5	1.5	1.5	13.1	17.1	44.6	5.9	4.6	11.4
14																1	1	1	56	37	21	22.5	12.7	6
15																2	1	1	36.8	45.5	35.5	21.1	15.9	6.5
16																1	2		63	30.5		23.4	9.5	
17																2			59.2			40.7		
Total	-	-	-	-	-	-	550	328	235	135	90.2	70.2	-	-	-	21.5	16	12.5	-	-	-	192	118	98.5
SE	1.25	1.46	1.48	0.24	0.25	0.28	3.07	3.51	3.62	0.91	1.06	1.01	12.6	14.9	14.9	0.13	0.15	0.17		10.4%			11.5%	

Appendix 6.3c Alto perennial ryegrass cultivars in the Autumn experiment (Experiment 5). Age of MT, DT1 and DT2 were around 90, 85 and 80 d, respectively.

	FLL			LW			LDW			LA			R_p			RDW_i		
P	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2
1	14.8	9.5	17	3.6	2.8	4.25	24.5	13.5	27.5	3.81	2.2	5.06						
3	30.1	25	33.5	5.9	5.45	5.75	78	56	84	12.4	9.9	13.5						
3	34.5	29.8	35.5	6.55	6.3	6.3	96.5	83	88	15.8	13.1	15.6						
4	35.6	29.8	34.1	6.8	7	6.55	99	108	89	16.9	14.6	15.6						
5	34.9	34	31.7	6.55	6.85	6.15	102	99.5	84.5	16	16.4	13.5						
6	33.5	33.9	30.8	6.55	6.55	6.4	98	104	88	15.4	15.5	13.6	3	3	2	1.68	2.38	2
7	29.5	32.6	33	5.85	6.55	6.05	93	104	81	12.1	14.9	14.1	3.5	3.5	2.5	2.88	7.45	7.17
8	26.6	29.8	13.8	5.85	6.65	2.85	93	97.5	98	10.9	13.8	10.9	3.5	3	2	5.74	13	5.17
9	28.9	29		5.31	5		92	90		10.7	10.1		3.5	2.5	2	8.42	5.67	2.83
10	26.5			5.91			81.1			10.9			3	2	2	11	14.3	10
11													3	3	1.5	15.4	11.5	13.7
12													3	2	2	6.91	10.8	10.8
13													2	2	2.5	12.6	18	26.3
14													3	1.5	1.5	12.9	18	5.75
15													2.5	2	2	14.6	18.3	17.3
16													2	3.5	2.5	27.2	20.1	21.8
17													2	2	2	23.1	19.5	15.8
18													4	3	2.5	8.33	13.1	7.75
19													3	2	4	14.8	12.5	13.8
20													4	2		6.79	17.5	
21													3.5	5		10.3	8.2	
22													2.2			8.94		
Total	295	253	229	-	-	-	857	756	640	125	111	102	50.7	42	31	526	520	370
SE	1.52	1.52	1.62	0.21	0.21	0.22	7.07	7.07	7.56	0.97	0.97	1.04	0.22	0.25	0.24		6.82%	

Appendix 6.3d Aberdart perennial ryegrass cultivars in the Autumn experiment (Experiment 5). Age of MT, DT1 and DT2 were around 90, 85 and 80 d, respectively.

	FLL			LW			LDW			LA			Rp			RDW_i		
P	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2	MT	DT1	DT2
1	23.8	30.1	32.3	3.69	4.8	4.85	50	49.5	57.5	5.86	10.3	11.1						
3	34.1	39.8	39.8	6.5	6.95	6.75	106	100	98.5	15.8	19.3	18.8						
3	37.8	42.5	41	6.7	7.05	7.15	120	107	104	18.1	21	20.5						
4	35.1	43.6	40.9	6.7	7.5	7.15	116	113	102	16.8	22.9	20.5						
5	34.3	41.6	41.6	6.4	6.85	6.7	114	114	115	15.7	20	19.6						
6	33.3	31	39.5	6.4	6.55	6.7	115	97	104	15.2	14.1	18.5	3.7	2.5	2.5	5.84	1.83	2.67
7	24	37.8	35	6.9	6.3	6.25	117	113	94	11.8	16.7	15.3	3.7	4.5	4.5	11.5	4	5.55
8	31.5	32.5	32.1	6.8	6.15	6.1	126	100	86.5	15.3	14	13.7	3.7	4.5	3	12.5	5.13	7.1
9	28.3	28.3	33.8	6.9	6.25	6.25	133	93	105	13.9	12.4	14.8	4.7	4	4.5	16.5	6.5	11.6
10	30.3	27	24.8	6.4	5.1	5.15	125	72.5	63.5	13.8	9.64	8.94	2.7	3	3	16	7.83	14.6
11	27.9	11.3	15	5	2.05	2.05	111	49	53	9.78	6.46	7.75	5.7	4	4	20.5	9.38	11.7
12													3.7	2.5	2	16.3	11.3	15
13													4.7	2.5	2.5	15.2	14.7	18.1
14													2.7	4	3.5	16	6.5	12.5
15													2.7	3	3	7.51	11.6	13.6
16													1.7	3	3.5	15.5	11.3	12.1
17													1.7	3	3	18.5	12	11.3
18													2.7	2	2	22.5	8.21	11
19													3.7	1.5	2	19	15.5	17.3
20													3.7	2	2.5	14.8	8.25	10.1
21													2.7	3	3	12.8	11	8.38
22													6.7	1.5	4	15	11.8	4.5
23													6.7			15.5		
Total	340	366	376	-	-	-	1230	1010	983	152	167	169	67.6	50.5	52.5	1030	435	557
SE	3.13	1.41	1.35	0.43	0.19	0.19	14.6	6.55	6.32	2.01	0.9	0.87	0.65	0.22	0.22		11.6%	

Appendix 6.4 A comparison for the morphological traits at the individual tiller level of the main tiller and the two daughter tillers of two perennial ryegrass cultivars Alto and Aberdart in the Spring and Autumn experiments (Experiment 4 & 5, respectively). LDW, leaf lamina dry weight per tiller (mg); LA, leaf area of the live leaves per tiller (cm^2); SLA, specific leaf area ($cm^2\ g^{-1}$); NLL, number of live leaves; NPr, number of live root-bearing phytomers; NR_t, number of roots per tiller; RDW, root dry weight per tiller; R:S, root-shoot ratio; DT:MT, ratio between leaf dry weight of daughter to main tiller; MT, main tiller; DT, daughter tiller; p, statistical probability.

	Tiller	LDW	LA	SLA	NLL	NP_r	NR_t	RDW_t	R:S	DT:MT
Alto-Spring	MT	456	106	235	7.0	11.0	15.0	214	0.47	
	DT1	258	68.4	307	6.0	10.0	12.5	119	0.46	0.57
	DT2	262	72.5	292	6.0	10.0	15.0	115	0.44	0.57
Aberdart- Spring	MT	550	135	248	8.0	12.0	21.5	192	0.35	
	DT1	328	90.2	290	6.0	11.0	16.0	118	0.36	0.60
	DT2	235	70.2	314	5.0	10.0	12.5	98.5	0.42	0.43
Alto- Autumn	MT	857	125	146	10.0	17.0	50.7	526	0.61	
	DT1	756	111	150	9.0	16.0	42.0	520	0.69	0.88
	DT2	640	102	165	8.0	14.0	31.0	370	0.58	0.75
Aberdart -Autumn	MT	1230	152	131	11.0	18.0	67.6	1030	0.84	
	DT1	1010	167	165	11.0	17.0	50.5	435	0.43	0.82
	DT2	983	169	168	11.0	17.0	52.5	537	0.55	0.80
Experiment 4		0.12	0.09	0.45						
P (Cultivar)		0.01	0.15	0.002			0.99	0.75		
P(Tiller)		0.76	0.93	0.18			0.72	0.91		
P(TillerxCultivar)		0.14	0.30	0.002			0.82	0.97		
P(Phytomer)							0.99	0.87		
Experiment 5										
P (Cultivar)		0.038	0.022	0.942			0.265	0.804		
P(Tiller)		0.125	0.728	0.193			0.727	0.543		
P(Tiller x Cultivar)		0.393	0.509	0.548			0.725	0.202		
P(Phytomer)		0.178	0.038	0.904			1.00	0.719		

Appendix 6.5 A **c**omparison between DT+ and DT- plants for main tiller morphological development at the successive phytomers after the daughter tiller excision treatment in perennial ryegrass cultivar cv. (a) Alto and (b) Aberdart in the Spring experiment (Experiment 4). Tillers were excised 98 days after transplantation and harvested between 12 and 20 d later. FLL, final leaf length (cm); LDW, Leaf dry weight (mg), LA, leaf area (cm^2); SLA, specific leaf area ($cm^2\ g^{-1}$); $R_{p,}$ number of roots per phytomer, $RDW_{i,}$ root dry weight of the individual roots at each phytomer (mg). Net RDW, change in RDW per phytomer at the treatment period. SE, standard error of mean; where given as % is for log transformed data.

Appendix 6.5a Alto perennial ryegrass cultivar.

Phytomer	FLL		LDW		LA		SLA		R_p		RDW_i		Net RDW	
	DT+	DT-	DT+	DT-	DT+	DT-	DT+	DT-	DT+	DT-	DT+	DT-	DT+	DT-
1	13.1	20.7	24.3	39.9	5.61	10.7	203	291						
2	32.3	35.9	81.9	97.9	20.1	22.3	256	238						
3	36.2	38.6	102	105	22.6	23.3	221	233						
4	37.9	36.8	105	106	22.2	21.3	215	213						
5	34.4	32.6	92.8	82.5	18.6	17.2	206	216						
6	29.5	28.8	76.1	81.6	15.5	15.8	207	188	1.44	1.50	1.91	1.54	2.83	1.88
7	27.8	28.3	72.4	69.9	14.1	14.3	198	204	1.67	1.12	7.30	10.3	9.90	9.41
8	24.4	25.6	53.6	50.1	10.8	9.65	215	200	1.22	1.38	15.0	16.0	14.2	11.9
9	26.8	21.2	69.7	24.5	12.4	5.66	186	210	1.00	1.50	13.3	12.5	9.31	11.8
10	22.9		63.8		11.8		172		1.33	1.13	17.2	17.5	13.0	5.82
11									1.11	1.38	22.9	17.9	12.5	-0.96
12									1.11	1.13	15.6	14.8	-1.72	0.21
13									1.33	1.13	20.8	15.2	-2.53	-9.94
14									1.22	1.25	27.3	16.5	1.83	-13.5
15									1.11	1.25	29.0	23.5	5.30	-13.5
16									1.33	1.13	27.9	32.3	5.30	4.56
17									1.13	1.19	27.4	25.8	-4.05	13.3
18									1.13	1.19	24.9	25.2	7.90	9.38
19									1.33		40.5		24.4	5.94
Total	285	269	742	657	154	140			17	16	291	229		
SE	0.89	0.85	3.55	3.38	0.73	0.69	18.0	17.2	0.06	0.06	8.2%	11.6%	15.1%	17.4%

Appendix 6.5b Aberdart perennial ryegrass cultivar.

Phytomer	FLL		LDW		LA		SLA		R_p		RDW_i		Net RDW	
	DT+	DT-	DT+	DT-	DT+	DT-	DT+	DT-	DT+	DT-	DT+	DT-	DT+	DT-
1	19.9	15.2	30.1	16.9	8.33	5.50	300	310						
2	35.9	34.4	92.0	75.6	21.6	20.5	262	279						
3	40.2	41.6	112	127	25.2	27.3	285	223						
4	41.3	44.2	122	141	25.6	29.1	221	214						
5	41.4	42.1	111	129	24.9	26.5	232	209						
6	41.3	37.2	102	118	22.7	23.0	224	196	1.13	1.56	1.60	1.03	2.09	1.53
7	38.6	37.8	97.3	90.0	20.4	22.7	216	412	1.38	1.67	13.4	12.6	16.4	16.6
8	36.3	34.8	79.9	92.1	16.7	19.6	209	213	1.38	1.33	14.3	13.4	18.8	15.2
9	30.7	31.0	74.9	77.9	15.3	16.9	198	214	1.88	1.44	11.9	19.7	18.2	21.6
10	29.9	25.9	75.3	61.6	15.6	12.2	224	186	1.25	1.22	12.5	17.2	7.27	11.7
11	29.7	27.3	55.9	64.0	11.8	11.8	212	208	1.25	1.67	10.5	15.6	0.68	10.2
12									1.25	1.44	12.8	16.7	0.33	6.73
13									1.13	1.44	17.2	14.8	2.34	-2.02
14									1.25	1.56	20.5	16.8	5.23	-3.83
15									1.50	1.44	26.2	15.5	7.97	-7.95
16									1.38	1.89	20.7	24.7	2.49	11.7
17									1.50	1.28	30.1	33.9	1.63	10.6
18									1.88	1.81	43.7	18.7	34.0	-24.3
19									1.50		30.4		-6.05	
Total	385.2	371.5	952.4	993.1	208.13	215.1			19.66	19.8	393	334		
SE	0.69	0.64	2.74	2.52	0.56	0.52	13.9	12.8	0.06	0.06	8.0%	11.5%	13.9%	18.5%

Appendix 6.6 Tiller axis shoot and root traits for daughter tiller excised (DT-) plants of Alto and Aberdart (Aber) perennial ryegrass cultivars at the destructive harvest after 93 d growth in the Autumn experiment (Experiment 5). FLL, final leaf length (cm); LW, leaf width (mm); LDW, leaf dry weight (mg); LA, leaf area (cm^2); SLA, specific leaf area ($cm^2\ g^{-1}$); R_p, number of roots per phytomer, RDW_p, root dry weight per phytomer; RDW_i, root dry weight per root of the individual roots at each phytomer; RDW_i/LDW_t, RDW at each phytomer position standardized against leaf dry weight per tiller; SE, standard error of mean; where given as % is for log transformed data; p, probability of statistical significance.

Phytomer	FLL		LW		LDW		LA		SLA		R_p		RDW_p		RDW_i		RDW_i/LDW_t	
	Alto	Aber	Alto	Aber	Alto	Aber	Alto	Aber	Alto	Aber	Alto	Aber	Alto	Aber	Alto	Aber	Alto	Aber
1	22.0	14.1	4.20	3.13	45	24	6.5	3.5	146	147								
2	33.8	31.1	6.13	5.68	117	95	14.4	12.6	132	130								
3	36.6	34.0	6.33	6.90	131	143	16.2	16.5	128	117								
4	31.6	34.0	6.35	6.83	116	149	14.5	16.3	124	113								
5	34.3	33.3	6.20	7.03	161	148	14.9	16.5	96	115								
6	31.0	33.7	6.43	7.03	117	156	14.1	16.7	122	114	2.50	3.00	6.0	4.0	2.8	1.4	4.2	2.7
7	32.9	30.4	6.13	6.70	132	129	14.3	14.3	112	112	2.75	4.50	17.0	25.0	6.7	5.1	14.6	14.3
8	32.0	28.6	5.55	6.48	119	121	12.5	13.0	115	107	4.50	5.00	59.0	41.5	14.2	9.6	45.5	23.3
9	27.7	22.1	5.67	6.65	118	113	11.3	10.3	103	89	3.00	3.75	22.3	20.0	8.3	8.3	23.6	11.5
10	27.0	29.8	4.43	6.73	105	130	8.5	14.0	82	109	2.75	4.50	66.0	63.5	13.6	12.1	39.3	33.8
11	22.5	27.9	4.70	6.13	105	116	7.4	11.8	70	103	3.25	4.50	72.3	57.3	24.3	12.3	61.0	35.3
12	19.7	28.4	5.00	6.05	89	117	6.7	12.3	75	105	2.50	3.25	73.5	69.3	29.9	19.7	72.4	44.2
13		24.7		6.13		105		10.6		101	3.00	3.75	69.5	65.5	25.1	15.8	61.3	38.5
14											2.25	3.25	61.3	58.0	29.5	15.6	55.7	36.1
15											2.25	4.25	66.3	70.8	29.4	16.5	55.5	42.6
16											2.00	4.00	40.3	78.5	18.6	18.9	31.2	49.1
17											1.75	2.25	35.8	48.5	24.9	25.9	30.6	32.0
18											2.00	2.50	47.0	42.0	23.0	16.9	35.0	28.1
19											2.00	2.50	46.3	58.3	22.2	22.1	36.1	37.6
20											2.50	2.25	48.5	61.0	18.6	26.0	42.0	39.5
21											2.00	2.25	42.7	56.3	36.3	24.4	39.7	36.3
22											2.67	2.75	114	79.5	43.7	27.9	117	50.2
23												2.67		60.3		22.5		35.2
Total	351	372	-	-	1350	1550	141	168	-	-	43.7	60.9	888	959	-	-	-	-
SE	10.5	8.88	0.11	0.09	5.54	4.71	0.56	0.47	3.55	3.01	0.20	0.17	5.59	4.96	1.54	1.36	4.05	3.59

Appendix 7.1 Protocol for recharging the air tank

Several measures were developed to minimise the CO_2 concentration of scrubbed air inside the air tank. The first time the air tank was filled, internal CO_2 concentration was initially at 25 ppm but gradually fell to between 5 to 6 ppm over a period of approximately 36 hours. It was then discovered that the air flow through the scrubber was carrying soda-lime dust inside the air tank resulting in CO_2 absorption inside the tank. To combat this problem a dust trap was constructed and inserted into the air line. Air leaving the scrubber was bubbled through a 20 L container of water before passing to the air tank.

After placing the dust trap, with a comparatively fast flow rate to fill the air tank quickly, the CO_2 in the air tank was recorded as >80 ppm. This was a higher level of non-labelled CO_2 than desired, so the air tank was emptied and refilled at a much slower flow rate than before. The routine adopted for the experiment was to fill the air tank overnight, taking more than 12 hours and this resulted in a CO_2 concentration inside the tank of <20 ppm. CO_2 concentration in the tank was noted to increase slowly after filling, at a rate a little under 2 ppm per hour, but since the tank contents were used for labelling each day after filling overnight, this low level of leakage (which would indicate potential for exchange with the atmosphere after the tank was charged with brewery CO_2) was considered unimportant.

Appendix 7.2 C isotope ratio in the labelling samples as read by isotope ratio mass spectrometer. Samples were collected in 10 mL vacutainers from the air tank using a double-ended needle. The gas samples were carried from Palmerston North to Freising, Germany in a luggage for measuring C isotope mass ratio.

Sample collected from	Date of sample collection	Replication	$\delta^{13}C$ (‰)
Air tank	11-05-2009	1	-29.05
		2	-28.83
		3	-28.74
		4	-29.41
		5	-28.76
	12-05-2009	1	-27.96
		2	-28.27
		3	-27.80
		4	-28.27
		5	-28.82
	13-05-2009	1	-28.39
		2	-28.04
		3	-27.80
		4	-27.89
		5	-27.92
	14-05-2009	1	-28.45
		2	-28.02
		3	-29.16
		4	-28.60
		5	-29.41
	15-05-2009	1	-29.14
		2	-29.42
		3	-29.74
		4	-29.35
		5	-28.72
	16-05-2009	1	-28.71
		2	-29.41
		3	-29.30
		4	-29.01
		5	-29.74
	17-05-2009	1	-29.13
		2	-28.64
		3	-28.05
		4	-27.51
		5	-27.94
			-28.7

Appendix 7.3 Calculation for labelling CO_2 gas required for the air tank

400 ppm CO_2

= 400 µL/L air

= 400 mL/1000L air

= 1200 mL in $3m^3$

Actual volume needed was 1400 mL means the volume of the air tank when it was completely full was 1400÷400= 3.5 m^3

Appendix 7.4 CO_2 concentration (ppm) recorded at the outlet of the bags feeding the tillers at different times of the day (CO_2 concentration inside the air tank was 420 ppm in Experiment 6.1 and 430 ppm in Experiment 6.2).

Pump	Experiment 6.1 (Day 1)					Exp 6.1 (Day 2)			Exp 6.2 (Day 1)		Experiment 6.2 (Day 2)				
	Initial Flow rate mL air min^{-1}	CO_2 at 11.00 am	Increased Flow rate (mL air min^{-1})	CO_2 at 02.30 pm	CO_2 at 05.00 pm	CO_2 at 11.00 am	CO_2 at 02.00 pm	CO_2 at 04.00 pm	Flow rate mL air min^{-1}	CO_2 at 03.40 pm	CO_2 at 09.00 am	CO_2 at 11.00 am	CO_2 at 01.30 pm	CO_2 at 03.30 pm	CO_2 at 05.00 pm
1	30	112	270	242	410	304	320	286	550	289	311	203	200	308	413
2	150	254	225	333	413	368	333	361	240	230	222	168	175	274	397
3	90	122	260	203	397	254	234	261	700	258	253	218	202	324	413
4	90	98	210	132	379	220	130	110	560	222	240	174	184	285	409
5	100	85	400	210	400	263	272	285	520	312	301	207	227	352	422
6	60	112	380	231	410	248	245	285	720	245	220	198	206	309	407
7	130	200	150	259	408	280	296	326	480	266	240	223	252	328	418
8	30	119	380	260	410	322	308	345	600	228	208	179	180	297	391

Appendix 7.5 Estiamtion of change in C isotope mass ratio due to leakage within labelling system

Appendix 7.5a Calibration of the labelling system: change in C isotope mass ratio at the outlets of the pumps due to leakage within the system

Treatment	CO_2 concentration at supply (ppm)	CO_2 inside the air tank (ppm)	Atomospheric CO_2 concentration	Fraction of bladder air	Delta of mixture (assuming, in air = -12 ‰, in air tank= -25 ‰)	Source of leakage	Delta reduced by % of air tank
Main	92	26	392	0.82	22.66	pump	9.38
Main	124	26	392	0.73	21.52	pump	13.92
Main	30	26	392	0.99	24.86		0.57
Main	58	26	392	0.91	23.86		4.55
Daughter	32	26	392	0.98	24.79		0.85
Daughter	33	26	392	0.98	24.75		0.99
Daughter	75	26	392	0.87	23.26	pump	6.96
Daughter	107	26	392	0.78	22.12	pump	11.51

Appendix 7.5b Calculation of the estimated change in C isotope ratio of the supplied CO_2 mixture due to measured leakage into the system

Assuming, CO_2 in labelling gas	420 ppm
CO_2 in air	370 ppm
CO_2 in mixture	400 ppm
Equation 1:	
X = fraction of labelling gas	
Y = fraction of air	$(X * 420) + (Y*370) = 400$
	$X + Y = 1$
	Therefore substitute (1-X) for Y and solve
Fraction of labelling gas	0.6
Delta of labelling gas (Appendix 7.2)	-28.7 ‰
Delta of air (assuming)	-12 ‰
Delta of mixture	-22 ‰

Appendix 7.6 Calculation for $(^{15}NH4)_2\,SO_4$ required to prepare 1 atom% solution

Molecular weight of $(^{15}NH4)_2\,SO_4$ = 134 g

Molecular weight of $(^{15}NH4)_2\,SO_4$ = 134 g

1 Molar solution = 134 g /L

1 mM $(^{15}NH_4)_2\,SO_4$ = 134 mg /L

1 mM ^{14}N= 132/2=66 mg $(NH_4)_2\,SO_4$ /L

or, 1 mM ^{15}N= 67 mg $(^{15}NH_4)_2\,SO_4$ /L

Now, 66 mg $(NH_4)_2\,SO_4$ contains 0.37 atom% ^{15}N as the natural abundance.

The remaining 0.63 atom% ^{15}N needs to be supplied from the $(^{15}NH_4)_2\,SO_4$ to prepare 1 atom% ^{15}N solution.

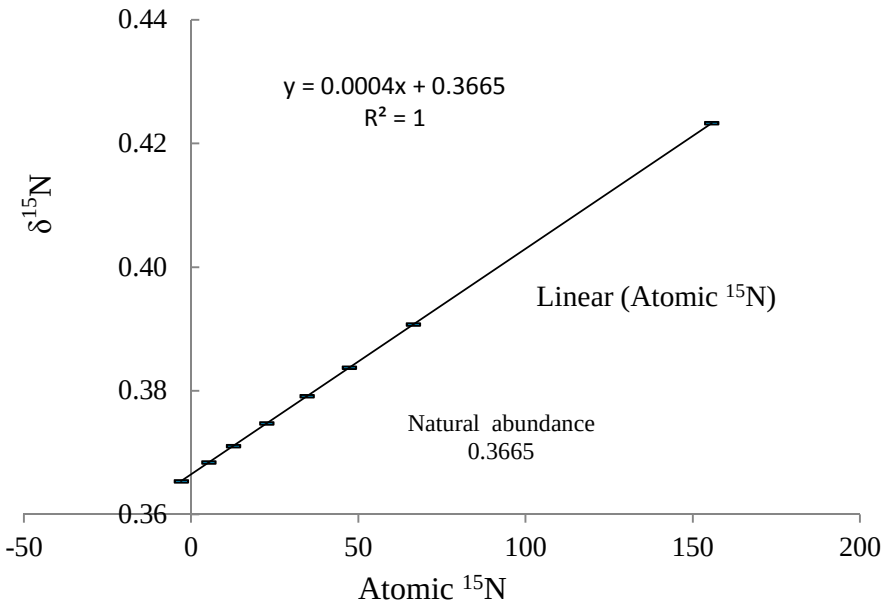
Thus, the required amount of $(^{15}NH_4)_2\,SO_4$ is 0.63% of (67 mg – 0.37% of 66 mg)

= 0.63% of 66.75 mg

= 0.42 mg

$(^{15}NH_4)_2\,SO_4$ was 98% pure therefore the actual amount was needed for per L solution is 0.43 mg.

Appendix 7.7 Standard curve for estimating the natural abundance of ^{15}N



Appendix 7.8 ANOVA structure for the C labelled plants to estimate the statistical variation between labelled and unlabelled tillers after GLM procedure: root and shoot, and different shoot and root dissection categories. (1. MTL-MT, main tiller of the main tiller labelled plant; 2. MTL-DT, daughter tiller of the main tiller labelled plant; 3. DTL-MT, main tiller of the daughter tiller labelled plant; 4. DTL-DT; daughter tiller of the daughter tiller labelled plant.)

Effect tested	Command syntax

a) Orthogonal linear contrast (in SAS):
 	
Labelled Vs unlabelled tiller
Unlablled MT vs DT
Labelled MT vs DT
	`proc glm;`
	`class LabTiller;`
	`model BaseEL TipEL otherleaves sheaths Tilleraxis uppertips uppperaxis middletip midddleAxis lowertip LowerAxis = LabTiller ;`
	`contrast 'linear' LabTiller 1 -1 -1 1;`
	`contrast 'linear' LabTiller 0 1 -1 0;`
	`contrast 'linear' LabTiller 1 0 0 -1;`
	`run;`

b) Compartment (Shoot , tiller axis and roots) for C and N traits (in MINITAB)	MTB> GLM Treatment Compartment Plant (Treatment); SUBC> means Treatment Compartment Plant (Treatment); SUBC> test Compartment / Plant (Treatment).
c) Tiller dissection categories for C and N traits (in MINITAB)	MTB> GLM Base EL = treatment | tiller segment (treatment tiller) plant (treatment); SUBC> means treatment | tiller segment (Treatment tiller) plant (treatment); SUBC> test segment (treatment tiller) / plant (treatment).
d) Compartment (shoot, root, tiller axis) for PC scores (in MINITAB)	MTB> GLM PC1-PC3= tiller Compartment plant (tiller); SUBC> means tiller unit plant (tiller); SUBC> test tiller/ plant (tiller); SUBC> test Compartment /plant (tiller).
e) Dissection categories for PC scores (in MINITAB)	MTB > GLM PC1-PC3 = Tiller DissectionCategories (Tiller) Plant(Tiller); SUBC> means Tiller DissectionCategories (Tiller) Plant(Tiller); SUBC> test DissectionCategories t (Tiller)/Plant(Tiller).

Note: For the data related to this appendix please see folders "*Chapter 7/ Exp 6.1-C labelling'* and "*Chapter 7/ Exp 6.2 -C and N labelling*" in the data CD.

Appendix 7.9 ^{15}N uptake (mg g^{-1} Dissection category) by different dissection categories of main tiller and daughter tiller of Aberdart perennial ryegrass cultivar plants for the labelled plants with $^{15}(NH_4)_2SO_4$ for 5 days. (MTL, main tiller labelled; DTL, daughter tiller labelled, (asterisks indicates significant relations). Data for some of the selected dissection categories are also presented in Fig. 7.12 and 7.13.

Tiller	Dissection Category	MTL	±SE	DTL	±SE
Main	Base elongating leaves (1-2)	5.37	1.84	1.64	0.68
	Tip elongating leaves (1-2)	3.21	1.54	1.26	0.68
	Leaves lamina (3-4)	1.22	0.61	0.56	0.22
	Leaf sheathes (3-4)	1.55	0.85	0.55	0.21
	Leaves lamina (5-rest)	0.74	0.35	0.44	0.19
	Sheaths and pseudostem (5-rest)	0.39	0.15	0.19	0.06
	Root tips (position 1-3)	1.52	0.70	0.61	0.05
	Root axis (position 1-3)	0.72	0.46	0.32	0.03
	Root tips (position 4-6)	1.40	0.71	0.33	0.05
	Root axis (position 4-6)	0.71	0.29	0.25	0.02
	Root tips (position 7-9)	0.25	0.11	0.21	0.04
	Root axis (position 7-9)	0.26	0.11	0.20	0.03
	Root tips (position 10-12)	0.18	0.06	0.13	0.02
	Root axis (position10-12)	0.26	0.12	0.15	0.01
	Root tips (position 13-15)	0.05	0.02	0.08	0.03
	Root axis (position 13-15)	0.09	0.05	0.09	0.02
	Root tips (position 16-rest)	0.05	0.02	0.07	0.04
	Root axis (position 16-rest)	0.09	0.07	0.09	0.04
	Tiller axis	1.07	0.30	0.34	0.02
Daughter	Base elongating leaves (1-2)	0.37	0.23	3.83	1.27
	Tip elongating leaves (1-2)	0.20	0.09	2.16	0.80
	Other leaves lamina	0.11	0.04	1.15	0.36
	Sheaths and Pseudostem	0.09	0.02	1.12	0.55
	Root tips (position 1-5)	0.10	0.02	0.31	0.12
	Root axis (position 1-5)	0.10	0.02	0.38	0.17
	Root tips (position 6-10)	0.07	0.01	0.21	0.09
	Root axis (position 6-10)	0.08	0.01	0.20	0.11
	Root tips (position 11-rest)	0.08	0.04	0.10	0.04
	Root axis (position 11-rest)	0.04	0.02	0.13	0.05
	Tiller axis	0.19	0.14	1.14	0.24
	^{15}N labelled root tips	7.19	2.22	4.45	1.34
	^{15}N labelled root axis	2.06	0.77	1.17	0.17
p value		<0.01		<0.01	

Appendix 7.10 %C, % N, C:N ratio, $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) at different root and shoot dissection categorires of Alto and Aberdart perennial ryegrass cultivar at destructive harvest in Experiment 6.1 & 6.2 (Aberdart root positions are in parenthesis)

Appendix 7.10a %C, % N and C:N ratio

	%N				%C				C:N			
	Alto		Aber		Alto		Aber		Alto		Aber	
Main tiller	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
Base elongating leaf 1-2	5.31	0.35	4.08	0.13	40.9	0.50	42.1	0.19	7.96	0.47	10.4	0.39
Tip elongating leaf 1-2	5.29	0.21	5.28	0.19	41.7	0.28	43.6	0.29	8.01	0.33	8.36	0.32
Other leaves	4.46	0.13	4.36	0.14	40.1	0.34	40.4	0.43	9.05	0.27	9.34	0.92
Sheaths and pseudostem	2.31	0.13	1.70	0.10	36.5	1.51	38.1	0.27	16.1	0.78	22.9	1.23
Tiller axis	2.76	0.13	3.05	0.09	44.8	0.25	44.4	0.24	16.5	0.86	14.7	1.24
Root tips position 1-2 (1-3)	3.33	0.22	3.57	0.47	44.4	1.77	42.1	0.22	14.01	1.36	10.6	0.43
Root axis position 1-2 (1-3)	3.02	0.28	2.84	0.48	46.7	1.59	42.7	0.31	16.2	1.83	12.9	0.41
Root tips position 3-4 (4-6)	3.29	0.21	3.71	0.17	47.5	1.70	42.5	0.14	15.2	1.51	11.7	0.38
Root axis position 3-4 (4-6)	2.69	0.21	3.42	0.07	47.9	1.29	42.7	0.17	18.9	1.79	12.5	0.56
Root tips position 5-6 (7-9)	3.18	0.18	3.03	0.24	45.1	2.36	42.9	0.14	14.9	1.66	15.5	0.29
Root axis position 5-6 (7-9)	3.05	0.27	2.87	0.22	48.4	2.03	43.5	0.18	17.2	2.00	16.6	2.04
Root tips position 7-8 (9-12)	3.13	0.21	2.69	0.19	46.7	1.99	43.2	0.19	15.6	1.45	16.9	2.21
Root axis position 7-8 (9-12)	2.67	0.20	2.69	0.19	47.4	1.04	43.6	0.17	18.8	1.67	17.6	1.50
Root tips position 9-12 (13-15)	2.94	0.20	2.38	0.18	45.9	0.84	43.7	0.27	16.4	1.25	19.3	2.27
Root axis position9-12 (13-15)	2.41	0.18	2.22	0.12	46.5	0.96	43.9	0.16	20.7	2.53	20.5	1.59
Root tips 13-rest (16-rest)	2.78	0.22	2.13	0.13	45.0	0.67	43.8	0.18	17.4	1.81	21.5	1.54
Root axis 13-rest (16-rest)	2.27	0.21	2.06	0.12	46.8	0.56	44.1	0.13	22.6	2.46	21.9	1.82
Daughter tiller												
Base EL	5.16	0.30	4.02	0.18	40.3	0.45	42.4	0.22	8.06	0.47	10.7	0.51
Tip EL	5.55	0.17	4.80	0.18	41.9	0.33	43.6	0.31	7.62	0.25	9.19	0.36
Other leaf	4.50	0.19	4.38	0.11	40.1	0.32	41.5	0.27	9.06	0.41	9.5	0.27
Pseudostem	2.44	0.12	2.34	0.14	38.2	0.45	39.3	0.30	16.1	0.94	17.5	1.39
Tiller axis	2.85	0.15	3.03	0.06	44.1	0.34	44.2	0.23	15.7	0.86	14.6	2.93
Root tips 1-5	3.41	0.18	3.41	0.25	44.1	0.85	42.6	0.19	13.4	1.04	13.3	0.39
Root axis 1-5	2.97	0.18	2.86	0.21	44.9	0.76	43.14	0.24	15.7	1.23	16.1	1.81
Root tips 5-10	3.41	0.25	2.86	0.23	44.2	0.82	43.2	0.19	13.8	1.30	16.2	2.59
Root axis 5-10	2.64	0.28	2.75	0.21	47.1	0.91	43.4	0.13	19.9	2.11	16.6	2.47
Root tips 11-rest	2.63	0.15	2.51	0.14	46.0	0.77	43.4	0.17	18.1	1.20	17.9	2.29
Root axis 11-rest	2.13	0.22	2.37	0.10	46.6	0.68	43.8	0.17	24.0	2.50	18.8	2.83
P (dissection component)	<0.01		<0.01		<0.01		<0.01		<0.01		<0.01	
Dissection component (N label)			0.313						0.965			
P (Tiller)	0.341		0.807		0.274		0.730		0.499		0.667	

Appendix 7.10b $\delta^{15}N$ and $\delta^{13}C$ isotope mass ratio (‰)

Main tiller	$\delta^{15}N$ (‰)				$\delta^{13}C$ (‰)			
	Alto	±SE	Aber	±SE	Alto	±SE	Aber	±SE
Base elongating leaf 1-2	0.55	0.18	1.21	0.45	-27.31	0.27	-28.18	0.36
Tip elongating leaf 1-2	1.03	0.17	1.31	0.02	-27.59	0.17	-28.84	0.28
Other leaves	1.51	0.15	1.51	0.47	-27.59	0.15	-28.72	0.22
Sheaths and pseudostem	2.22	0.21	2.03	0.12	-27.34	0.16	-28.64	0.28
Tiller axis	-0.89	0.46	1.18	0.01	-27.13	0.21	-27.42	0.21
Root tips position 1-2 (1-3)	0.78	0.38	1.75	0.48	-26.84	0.18	-28.18	0.28
Root axis position 1-2 (1-3)	0.40	0.75	1.42		-26.75	0.23	-28.84	0.25
Root tips position 3-4 (4-6)	-0.03	0.47	2.19	0.34	-26.81	0.22	-28.72	0.26
Root axis position 3-4 (4-6)	0.84	0.40	1.25		-26.52	0.22	-28.64	0.28
Root tips position 5-6 (7-9)	-0.44	0.45	1.69	0.51	-26.67	0.25	-27.97	0.20
Root axis position 5-6 (7-9)	0.23	0.46	1.48	0.28	-26.49	0.27	-27.74	0.15
Root tips position 7-8 (9-12)	-0.77	0.37	0.65	0.27	-26.39	0.19	-27.42	0.15
Root axis position 7-8 (9-12)	0.04	0.46	-0.62	2.09	-26.43	0.20	-27.07	0.14
Root tips position 9-12 (13-15)	-0.89	0.54	-1.32	0.78	-26.31	0.17	-27.16	0.15
Root axis position9-12 (13-15)	-0.15	0.55	-1.44	0.52	-26.59	0.17	-27.12	0.15
Root tips 13-rest (16-rest)	-0.55	0.56	-1.94	0.88	-26.31	0.18	-26.70	0.15
Root axis 13-rest (16-rest)	-1.64	0.51	-0.96	1.03	-26.90	0.18	-26.78	0.20
Daughter tiller								
Base EL	0.45	0.20	1.03	0.32	-27.50	0.21	-28.34	0.37
Tip EL	0.86	0.14	1.13	0.02	-27.67	0.22	-28.95	0.28
Other leaf	1.59	0.13	1.45	0.72	-27.67	0.23	-28.51	0.26
Pseudostem	2.14	0.23	2.93	0.67	-27.48	0.22	-28.65	0.29
Tiller axis	-0.25	0.43	0.50	1.50	-26.97	0.28	-27.64	0.14
Root tips upper 5	1.16	0.43	2.12	0.47	-26.78	0.23	-27.23	0.23
Root axis upper 5	1.22	0.41	4.73	0.18	-26.77	0.25	-27.38	0.18
Root tips middle upto 10	0.15	0.40	1.16		-26.48	0.20	-26.99	0.27
Root axis middle upto 10	0.75	0.29	2.85	0.90	-26.70	0.27	-27.04	0.22
Root tips lower 11-rest	-0.33	0.37	0.69	2.04	-26.48	0.19	-26.72	0.18
Root axis 11-rest	-0.57	0.41	1.51	1.10	-26.90	0.16	-27.05	0.15
P (segment)	<0.01		<0.01		<0.01		<0.01	
Segment (N label)			0.698				0.999	
P (Tiller)	0.450		0.481		0.575		0.268	

Appendix 7.11 C mass isotope ratio inside the bag (Farquhar et al., 1989).

$$\Delta = \frac{\xi(\delta o - \delta e)}{1000 + \delta o - \xi(\delta o - \delta e)}$$

Where,

$\xi = C_e/(C_e - C_o)$

C_e= CO_2 concentration at inlet

C_o= CO_2 concentration at outlet

δe= isotopic ratio at supply
δo = isotopic ratio inside the bag
Δ= Carbon isotope discrimination by plants

C_e	C_o	ξ	δ_e	δ_o inside the bag	$\delta_o - \delta_e$	Δ
400	300	4.00	-24	-18.72	5.28	22
400	270	3.08	-24	-17.12	6.88	22
400	250	2.67	-24	-16.06	7.94	22
400	230	2.35	-24	-14.99	9.01	22
400	210	2.11	-24	-13.92	10.08	22
400	190	1.90	-24	-12.84	11.16	22
400	170	1.74	-24	-11.77	12.23	22
400	150	1.60	-24	-10.69	13.31	22
400	130	1.48	-24	-9.61	14.39	22

Appendix 8.1 Calculation for % N concentration

For example,

for a particular sample machine reads 20 µg N ml^{-1}

= 20 x 50 µg N 50 ml^{-1}

= 1000 µg N 30 mg sample (sample DW = 30 mg)

= 1000/30 µg N mg^{-1} sample

= 1/30 mg N mg^{-1} sample

= 3.33 % N in sample

Appendix 9.1 Calculation for C demand and supply at the different root-bearing phytomers (Pr) of the tiller axis of Alto and Aberdart perennial ryegrass cultivars: a) estimated photo-assimilate C supply at different Pr, b) C required for root construction, and c) C required for root respiration.

Note: For step by step calculation please see the enclosed CD.

a) Estimation of C supply at different Pr

As stated in Section 5.3.10, that total amount of net CO_2 exchange per tiller per day in Spring and Autumn experiments was calculated form the data presented in Appendix 4.3 taking leaf area of all leaves per tiller and their net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) into account. It was then assumed that 40% of total assimilated CO_2 is respired immediately (Danckwerts and Gordon, 1987) and 15% of remaining CO_2 is allocated to the root system (Parsons and Robson, 1981). It was further assumed that 30% of available C is taken up by a particular Pr when C moves downwards to the tiller axis.

b) C cost for root construction

The root construction cost for the individual Pr in mmol CO_2 Pr^{-1} d^{-1} was calculated from DMD_p (mg Pr^{-1} d^{-1}) assuming 45% C present in the root tissues (see Chapter 7). Respiration cost during root growth at the individual Pr was assumed 17% of DMD_p (Eissenstat and Yanai, 1997). Thus, the C cost for root construction was calculated as C required for DM deposition and that required for respiration during root growth.

c) C cost for root respiration

Root respiration cost at different phytomer positions was calculated assuming 2.0 mmol CO_2 required per g RDW per d (Eissenstat and Yanai, 1997).