REMOBILIZATION OF LEAF NITROGEN IN STOCKPILED TALL FESCUE

A Dissertation presented to

the Faculty of the Graduate School

at the University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

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MAY 2010

The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

REMOBILIZATION OF LEAF NITROGEN
IN STOCKPILED TALL FESCUE

presented by Melissa Remley,

a candidate for the degree of doctor of philosophy, and hereby certify that, in their opinion, it is worthy of acceptance.

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To
Alma ‘Granny’ Remley,
and my parents,
Richard and Elizabeth Remley

I am forever grateful for your endless support, encouragement, and love.
Thank you with all my heart.

ACKNOWLEDGEMENTS

A tremendous number of people have given time and effort to help me through the many process to complete this body of work. I owe many thanks, more than can possibly be expressed in these few words.

Foremost, I thank my main advisor, Dr. Dale Blevins. His advising has made a tremendous impact on my professional philosophies and goals in both teaching and research. I now know that I “struck gold” when joining his lab and am grateful for the comprehensive training in plant physiology and his balanced advising approach. Dr. Blevins is an incredible mentor and I am forever grateful to have been in his lab. In addition, I also owe many thanks to Dr. Blevins for his help in my dissertation project. He often worked side by side with us, from digging roots in the field on a cold winter morning, to helping hour after hour in lab work. So, thank you, Dr. Blevins. I am honored to have been your student.

I owe a great deal of thanks to my committee members, Dr. David Emerich, Dr. Felix Fritschi, Dr. Robert Kallenbach, and Dr. Randall Miles. I am extremely grateful for their professional guidance and support and willingness to aid me through the entire dissertation process. In addition, I want to thank Dr. Thomas Mawhinney and Dr. James Waters (University of Missouri Agriculture Experiment Station) for aiding with the amino acid profile analyses, Dr. Mark Ellersiek for statistical guidance, Dr. Pat Guinan for helping obtain weather data, and Tim Reinbott for his assistance with the field plots and equipment at Bradford Research and Extension Center.

This research is a result many people's effort in the Blevins Lab Family. Firstly, many thanks are owed to Dr. Krystyna Lukaszewska. I've learned lab techniques, tried to absorb some of your incredible scientific writing skills, and have gained countless life lessons from you. Thank you, Krystyna, for the years of guidance and friendship. Also in the lab, there are several fellow graduate students that I consider my brothers and sisters in science, as well as dear friends. I learned more from each one of them than any book could contain. Dr. Will McClain, thank you for all the years of help and advice. You paved the way many times for me and I acknowledge this with great appreciation. Dr. Maru Kering, "asante sana". Thanks for the many moments of guidance and friendship that only a true rafiki could give. Also, my sister in science, Elizabeth Hamilton, you are the most reliable person I've ever met, and I thank you for all of the help and friendship through these years.

Many undergraduate student workers have given countless hours of work to my project, including many "root parties", consisting of washing and cleaning root samples in the lab to early morning hours. Thank you to all who have helped and worked so hard, including Andrew Byrne, Sean Church, Joe Kamphoefner, Bill Woodard, David Kleinsorge, Matt Brown, Jordan Yoder, Blair Mobley, Meagan Perry, and Emily Reinbott.

Finally, I have to make a blanketed statement to thank all of my family and friends. There are too many of you to list, and I am so proud and grateful to have

that problem. I appreciate every one of you and know I could only get through life with your love and support. So, I love you all and thank you so very much!!

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ABSTRACT

Missouri's beef industry is supported by more than five million hectares of pasture containing the grass tall fescue (*Lolium arundinaceum*). Stockpiled (accumulated autumn growth used for winter grazing) tall fescue leaf macronutrients decrease in concentration from late autumn through winter. The objective of this study was to elucidate the fate of the macronutrient, nitrogen (N), from stockpiled tall fescue leaves during winter and spring. The stable isotope ^{15}N was painted on leaves of established tall fescue during early autumn in central Missouri. Above and belowground plant parts were harvested monthly through spring and analyzed for atom%^{15}N, total N, and N fractions of soluble protein, free amino acids, and nitrate. Recovery of ^{15}N indicated that autumn senescing leaves remobilized 45-60% of leaf N by spring. Through mid-winter, remobilized leaf N supported areas of active growth, including new tillers, crowns, new rhizomes, and a proliferation of new roots. In crowns and new roots, free amino acids were a predominant N storage form in mid-winter, with asparagine (~50%) and glutamine (~20%) predominating. During spring regrowth, N from all tissues was remobilized to support new tiller growth. By late April, remobilized leaf N accounted for 70% of new tiller N. The results of this study offer insight into N dynamics of stockpiled tall fescue and may be used to develop more efficient N fertilization and grazing strategies in tall fescue stockpiling systems.

Chapter 1

Introduction

Tall Fescue

Tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh. = *Schedonorus arundinaceus* (Schreb.) Dumort., formerly *Festuca arundinacea* (Schreb.)] is a cool-season perennial grass that is adapted to the transition zone of the United States. More than 14 million hectares of tall fescue are grown in the United States (Matches 1979), of which five million hectares are pasture in Missouri (Roberts 2004). Tall fescue pastures are integral in supporting Missouri's beef cattle industry, which as a state is ranked third, behind Texas and Oklahoma, in the number of beef cattle (USDA-NASS 2009).

The seasonal growth pattern of tall fescue consists of the greatest shoot growth occurring in spring, followed by culm elongation and flowering in late spring, then a second flush of shoot growth in autumn (Wolf et al. 1979). Stockpiling tall fescue is a management practice in which autumn shoot growth is allowed to accumulate for grazing in late autumn and winter (Matches 1979). This extension of the grazing season reduces the use of other feed inputs, such as stored hay and silage, and reduces winter feeding costs (Poore et al. 2000, Roberts et al. 2009). Tall fescue is favored for stockpiling because it maintains growth in the autumn during the onset of cooler temperatures and is hardy during winter months (Taylor and Templeton 1976, Archer and Decker 1977a, Balasko 1977, Matches 1979, Collins and Balasko 1981a, Sleper and West 1996, Poore et al. 2000, Roberts et al. 2009). Tall fescue leaves grow in minimum air temperatures of 0-3 ^{o}C (Nelson 1996), and increases in aboveground herbage

mass have been reported when the weekly mean air temperatures are between 1 and 4.4 ^{o}C (Burns and Chamblee 1979). However, herbage growth ceases when temperatures fall below 1 ^{o}C (Burns and Chamblee 1979). In addition, Archer and Decker (1977a) found growth of stockpiled tall fescue to be maintained through winter when soil temperatures were about 10 ^{o}C. In Missouri, stockpiled tall fescue may maintain herbage mass in mild winters (Kallenbach et al. 2003), suggesting some new aboveground growth can occur as older leaves senesce.

Although growth can be maintained at relatively low air and soil temperatures, several studies have reported decreased biomass production of tall fescue during the stockpiling period from autumn through winter (Taylor and Templeton 1976, Archer and Decker 1977a, Ocumpaugh and Matches 1977, Rayburn et al. 1979, Burns and Chamblee 2000a). For example, Taylor and Templeton (1976) in Kentucky found dry matter to decline up to 26% from December to February, while Rayburn et al. (1979) reported a 60% reduction between December and February in Virginia. In addition, Ocumpaugh and Matches (1977) reported 14-82% decline in dry matter of stockpiled tall fescue through the winter months in Missouri.

Several studies have suggested that the decline in dry weight through winter is due to a decrease in percent green (live) tissue compared to brown (dead) tissue. The proportion of dry weight as green tissue in stockpiled tall fescue has been reported to decrease from 80% in October to about 20% by February in Kentucky (Taylor and Templeton 1976), from 80% to 54% from

autumn to early winter in Maryland (Archer and Decker 1977b), and 76% to 36% from November to January in North Carolina (Burns and Chamblee 2000b). Although dependent on harvest dates and weather conditions, the decline in dry matter and green tissue of tall fescue has been suggested to be from losses in leaf senescence, respiration, translocation, leaf degradation, and leaching of water-soluble constituents as a result of cell membrane rupture from freezing and thawing (Taylor and Templeton 1976, Archer and Decker 1977a, b, Ocumpaugh and Matches 1977, Rayburn et al. 1979, Taylor et al. 1989, Willms et al.1998).

In addition to losses of dry matter and green tissues during the stockpiling period, tall fescue leaf mobile macronutrients, including nitrogen (N), phosphorus (P), potassium (K), and magnesium (Mg) decrease in concentration from late autumn through winter (Fleming and Murphy 1968, Taylor and Templeton 1976, Balasko 1977, Ocumpaugh and Matches 1977, Ross and Reynolds 1979, Collins and Balasko 1981b, Fribourg and Bell 1984, Opitz von Boberfeld and Banzhaf 2006, McClain and Blevins 2007, 2009). The decline in macronutrient concentrations in stockpiled tall fescue forage can result in lowered dietary value for grazing animals, and has been associated with an increased susceptibility of grazing animals to nutritional disorders, such as grass tetany.

The concurrent decline in macronutrients, biomass, and green tissue in stockpiled tall fescue leaves suggests a loss of leaf contents over time. However, the processes of leaf content loss, such as through leaching or remobilization to other plant tissues, and the extent of loss have not been

determined. In purple moor grass (*Molina caerulea* L.), the fate of leaf nutrients from senescing leaves was investigated by Morton (1977). Changes in leaf macronutrient concentrations were examined in leaves exposed to rain compared to covered leaves. Concentrations of K, Mg, and Ca declined in leaves exposed to rain, while covered leaves did not change in concentration, suggesting these nutrients are leached from the leaf tissue by rain as the leaves senesce. However, N and P declined steadily in both covered and exposed leaves, indicating the decline of N and P was due to internal remobilization.

Several studies in grasses have provided strong evidence that leaf N is remobilized to other plant parts during winter (Clark 1977, Robson and Deacon 1978, Li et al. 1992, Bausenwein et al. 2001a, Partala et al. 2001, Gloser 2005). In stockpiled tall fescue, the loss of leaf N through winter can be substantial. McClain and Blevins (2007, 2009) reported a decrease of N by more than 40% in the most recently-collard leaves from October through January in Missouri. In total forage, N concentrations have been reported to decrease as much as 67% from October through January in Tennessee (Ross and Reynolds 1979), 50% from September to January in Tennessee (Fribourg and Bell 1984), and 40% from October through January in Missouri (Ocumpaugh and Matches 1977). The decline in N content of stockpiled tall fescue over winter is likely to be a result of an increase in brown tissue content, (Taylor and Templeton 1976, Ocumpaugh and Matches 1977) as well as losses from green tissues (Taylor and Templeton 1976). Therefore, I hypothesize that the loss of leaf N in stockpiled tall fescue

leaves from autumn through winter is a result of the internal remobilization to other plant parts.

The growth and N content of the entire plant must be considered when investigating the fate of leaf N in stockpiled tall fescue. Preliminary investigations on the growth pattern of entire field-grown tall fescue plants during the winter months by Blevins et al. (unpublished) discovered that the tall fescue root mass increased through the winter months during the same time period that N concentrations declined in the leaves (data not shown). My hypothesis is that N is remobilized from leaf tissue to support the proliferation of new root growth during winter.

In order to investigate N remobilization in stockpiled tall fescue, it is important to review the use of N by plants, the methods of acquisition, accumulation, and storage of N in plant tissues, plus the remobilization of N within plants. In addition, it is important to review methods used to study N remobilization in plants, especially seasonal N dynamics of grasses.

Nitrogen Uptake and Assimilation

Nitrogen (N) is an essential chemical element for plant growth and development. To be essential, an element must be required by the plant to complete its life cycle, be involved directly in plant metabolism, or its functionality cannot be replaced by another element in the plant (Arnon and Stout 1939). Nitrogen is also defined as a macronutrient in higher plants, meaning it is needed

in relatively greater concentrations for growth than other nutrients, averaging 1.5% in plant shoot dry matter (Marschner 1995).

Plant roots acquire inorganic forms of N, mainly ammonium (NH_4^+) or nitrate (NO_3^-). Ammonium is the preferred form for root uptake. Once in the plant, all forms of N are reduced to ammonium and then incorporated into organic molecules such as amino acids or amides (Raven 1986, Blevins 1989). Nitrate is reduced to nitrite (NO_2^-) by nitrate reductase (Evans and Nason 1953) in the cytoplasm of both leaves and roots. Nitrite is then reduced to ammonia (NH_3) by nitrite reductase in chloroplasts of leaves or proplastids in roots (Dalling et al. 1972, Oaks and Hirel 1985). The resulting ammonia is toxic at low concentrations; therefore it must be converted to amino acids, amides, and other organic molecules rapidly. The compounds transported out of the roots in the xylem usually have a high N:C ratio, such as glutamine, asparagine, arginine, and ureides (Streeter 1979, Blevins 1989). The low molecular weight N compounds (such as amino acids, amides, and amines) derived from ammonium, nitrate, and urea serve as transient storage for the plant and are often utilized to transport reduced N over long distances (Marschner 1995). They can be further assimilated into larger compounds including nucleic acids and proteins.

Nitrogen Remobilization

Once N is taken up and assimilated into organic compounds, it may be imported and exported from different organs and tissues throughout the lifecycle of a plant. The remobilized molecules often arise from the breakdown of cellular constituents or from vacuolar pools. In general, macronutrient remobilization is more prevalent at times of seed germination, vegetative growth when nutrient supply to the roots is limited, reproductive growth, and before leaf abscission in perennials (Marschner 1995). The ability to reuse N within the plant allows it to maintain growth or buffer against reduced growth during periods when the N nutrient supply is limited or when the metabolic mechanisms for N acquisition is limited in roots (Ourry et al. 2001). Remobilized N molecules can originate from accumulated or stored pools within plant tissues.

Nitrogen Accumulation and Storage

Nitrogen storage has been defined as N accumulation resulting from plants taking up greater amounts of N than needed for current growth (Pate 1983). However, Millard (1988) states that this definition does not include the capacity of N-deficient plants to store N. If the plant is taking up more N than needed for optimal growth, accumulation occurs. Accumulation must be in relation to whole plant growth. Stored N is an N pool that has the ability to be mobilized from one tissue to support the growth and maintenance of another tissue and is independent of the growth rate (Millard 1988).

Nitrogen is often accumulated as nitrate (Smirnoff and Steward 1985) and free amino acids (Boudet et al. 1981) in vacuoles of plant cells. Nitrogen can also accumulate in the form of proteins without having as great an impact on osmotic potential. The main forms of N used for storage have been identified as nitrate, free amino acids, and proteins (Millard 1988, Ourry et al. 1988, Chapin et al. 1990, Hendershot and Volenec 1993, Thornton and Millard 1993). Nitrate-N may be used as a short-term N store in vacuoles, but is most likely assimilated into an organic form before it is transported (Smirnoff and Stewart 1985). Although more costly in terms of energy to produce, amino acids and proteins are more often used for N stores for longer periods of time. Storing N as proteins offers potential advantages for the plant in terms of osmotic regulation and possible additional catalytic capability (Millard 1988). Storage proteins have been defined as proteins not being directly involved in the structural and metabolic roles and serve as a form to store N (Staswick 1994, Bewley 2002). They are formed and are biologically inactive until degraded for the purpose of freeing their N reserves (Staswick 1994). Tissues accumulate and store N compounds when the tissue is a net importer, or a "sink" for N. In turn, a tissue can be a net exporter of N and behave as a "source" of N (Ourry et al. 2001). Nitrogen is accumulated, stored, and mobilized from different tissues as sources and sinks change to support growth throughout the lifecyle of a plant.

Leaves may also become a major source of remobilizable N during senescence. Leaf senescence is the final stage in the lifecycle of a leaf where

photosynthetic capacity declines and the tissue undergoes a controlled and sequential process of catabolism (Thomas and Stoddart 1980, reviewed by Feller and Fischer 1994). The process of senescence involves the breakdown of cellular constituents and the subsequent remobilization of nutrients out of the leaf tissue to other tissue sinks. Protein and nucleic acids are abundant forms of N in leaf tissue (Hortensteiner and Feller 2002). The products of protein hydrolysis, free amino acids and amides, are converted to transportable forms of N (Thomas 1978) and remobilized to sink tissues.

Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase) is the most abundant plant protein, comprising up to 80% of the total soluble leaf protein in C3 plants (Huffaker 1982) and up to 25% in C4 plants (Ku et al. 1979, Schmitt and Edwards 1981). Plants produce more Rubisco with increasing N supply (Lawlor et al. 1987). Rubisco is preferentially degraded during leaf senescence (Wittenbach 1979), accounts for a large proportion of N exported from the senescing leaves (Callow 1974), and has been suggested to be an N storage compound (Millard 1998). However, the role of Rubisco as a storage protein has been debated since it is not sequestered and biologically inactive until degradation (Staswick 1994). Nevertheless, Rubisco is a large N source remobilized from senescing leaves.

Annual plants remobilize N from vegetative growth, such as senescing leaves, to support the sink strength of reproductive growth and grain fill (Williams 1955, Thomas and Stoddart 1980). Perennial plants mainly use N stores during

periods when N acquisition is limited and rapid growth is needed. Nitrogen uptake is limited in early spring when soil temperatures are low (Clarkson et al. 1992). Nitrate uptake is also reduced after a defoliation event (Clement et al. 1978, Ourry et al. 1988, 1989, Jarvis and Macduff 1989, Louahlia et al. 1999, Kavanová and Gloser 2005) and possible nitrate efflux from roots can occur (Macduff and Jackson 1992, Kavanová and Gloser 2005). During these periods of reduced N uptake, plants must restore their photosynthetic tissues in order to maintain a positive carbon balance. Therefore, many deciduous woody species and other perennial plants have been found to accumulate and subsequently use N stores to meet the N demands during times of spring regrowth, or regrowth after a defoliation event.

In many perennial species, seasonal fluctuations and patterning of amino acid and protein content of tissues have been identified to suggest the accumulation of N stores and subsequent degradation and remobilization for regrowth. This includes an increase of amino acids and proteins in overwintering plant tissues in the autumn (end of vegetative season), remaining stable through winter, and a decline in spring when new vegetative growth occurs. Gloser (2005) stated that a combination of several processes can affect the accumulation of N storage compounds in overwintering tissues, including increased soil N availability, greater N uptake than N assimilation and use in growth, translocation of N from senescing shoots, and accumulation of cryoprotective compounds. However, the relative importance of these processes

to the final amount of stored N is not well known. The status of N storage compounds at the end of the growing season in overwintering tissues have been shown to be of great importance, being related to winter survival and spring regrowth potential (Chapin et al. 1990, Volenec et al. 1996, Gloser 2005).

The accumulation of N stores throughout the lifecyle of a plant is also utilized after a defoliation event. After defoliation, N stores are utilized for new leaf growth (Phillips et al. 1983, Ourry et al. 1988, Millard et al. 1990, Barber et al. 1996, Kim et al. 1993b, Thornton et al. 1993a, 1994, 2002, Thornton and Millard 1997, Louahlia et al. 1999, Thornton and Bausenwein 2000, Kavanová and Gloser 2005, Gloser et al. 2007). Once the photosynthetic tissues are restored to provide energy needed to reaccumulate reserves, N reserves are once again accumulated. The amount of N reserves at the time of defoliation has been found to affect the regrowth potential of the plant, with greater N reserves increasing regrowth potential (Ourry et al. 1994, Thornton and Millard 1996, Volenec et al. 1996, Avice et al. 1997, Louahlia et al. 1999, Gloser et al. 2007).

Nitrogen Storage Compounds

Seasonal fluctuations of free amino acids, including a preferential accumulation in overwintering tissues, followed by a decline during spring regrowth, have been documented in several species (Sagisaka 1974, 1983, 1987, Rosnitschek-Schimmel 1985b, Cyr et al. 1990, Hendershot and Volenec

1993, Volenec et al. 1996, Nordin and Näsholm 1997, Gloser 2002). The depletion of amino acid pools in tissues often corresponds with the occurrence of new tissue growth, suggesting a main role of free amino acids in N storage (Sagisaka 1983,1987, Rosnitschek-Schimmel 1985b, Läkdesmäki et al. 1990, Gloser 2002). The constituents of the amino acid pool are species dependent, however most of the dominant amino acids are efficient compounds for N storage having a high N:C ratio. In woody perennials, the amino acid profile in buds, living bark, and xylem is dominated by arginine and/or proline (Sagisaka 1974, 1983). Herbaceous perennials have been found to accumulate arginine, proline, glutamate, glutamine, and asparagine in aboveground tissues during winter (Sagisaka 1987). Arginine, glutamine, asparagine, and alanine often are the accumulated free amino acids in belowground tissues of herbaceous perennials (Rosnitschek-Schimmel 1985b, Nordin and Näsholm 1997, Gloser 2002), and asparagine and glutamine commonly predominate in grasses (Sagisaka 1987, Nordin and Näsholm 1997, Gloser 2002).

In several species, the N concentrations in rhizomes and roots have been found to increase from summer to autumn (Nordin and Näsholm 1997). This increase of total N was mainly due to an increase in free amino acid-N. In the shrubs lingonberry (*Vaccinium vitus-idaea* L.) and bilberry (*Vaccinium myrtillus* L.), and herbaceous perennials fireweed (*Epilobium angustifolium* L.), May lily (*Maianthemum bifolium* L.), woodland geranium (*Geranium sylvaticum* L.), and Arctic starflower (*Trientalis europaea* L.), arginine dominated the free amino acid

pool in rhizomes and roots. The grass, wavy hairgrass [*Deschampsia flexuosa* (L) Trin.], and herb, goldenrod (*Solidago virgaurea* L.), had both asparagine and arginine dominate the amino acid pool, while the fern, western oakfern [*Gymnocarpium dryopteris* (L) Newm.], accumulated glutamine in roots. Läkdesmäki et al. (1990) also found bilberry to exhibit seasonal storage in roots of free amino acids, dominated by arginine, while seasonal fluctuation of protein was not found. Free amino acids were also found to dominate (>70%) overwinter stored N in taproots of the biennial, bull thistle [*Cirsium vulgare* (Savi) Ten.] (Heilmeier et al.1994).

Chapin et al. (1986) found stem tissues of the sedge, tussock cottongrass (*Eriophorum vaginatum* L.), were the site of overwinter storage and seasonal fluctuations of N compounds. Growing on the Alaskan tundra, the N stores in stems were important for growth during periods when the roots were still frozen in the ground. The amino acid fraction contained more N and exhibited greater seasonal fluctuations than the protein fraction. In addition, arginine constituted more than 78% of the amino acids.

The herbaceous perennial, common sorrel (*Rumex acetosa* L.), utilizes taproot N stores to support up to 58% of early spring growth. A majority of the remobilized N was from protein (60%), however no specific storage proteins were identified. Arginine dominated the amino acids used for spring growth, supplying 82% of the amino acid-N (Bausenwein et al. 2001b).

Plants contain many proteins that have both structural and metabolic roles, and these proteins can comprise a majority of the protein fraction. Proteins with a structural or metabolic role are generally synthesized and degraded according to the needs of the plant. Some proteins are not directly involved in structural and metabolic roles (Bewley 2002) and serve solely as N storage forms. These proteins, termed storage proteins, are biologically inactive until degraded for their N reserves (Staswick 1994). Some storage proteins are found in vegetative tissues. By definition, vegetative storage proteins (VSPs) (i) constitute a significant fraction of the total nitrogen, (ii) are preferentially synthesized in developing storage organs, and (iii) are preferentially degraded during reactivation of meristems (Cyr and Bewley 1990a, Staswick 1994, Volenec et al. 1996). Some VSPs have been found to have enzymatic activity or have similar amino acid sequences as active proteins (Racusen 1986, Andrews et al. 1988, Boyce and Volenec 1992, Van Damme et al. 2000, Goulas et al. 2001, 2002). Therefore, Staswick (1994) suggested that some VSPs may have been derived from biologically active proteins.

Vegetative storage proteins have been identified and extensively characterized in the leaves and seed pods of soybean (*Glycine max* L.) (Wittenbach 1983). Two proteins, termed VSPα and VSPβ, accumulate in developing leaves, seed pods, and roots acting as sinks, then are later degraded and used as sources of N for the developing seed (Wittenbach 1983, Mason and Mullet 1990). Within a single growing season, the VSPs accumulate during

flowering in vacuoles of leaf paraveinal mesophyll and bundle sheath cells (Franceschi and Giaquinta 1983, Franceschi et al. 1983), and vascular tissues in stems and seed pods (Huang et al. 1991) then are remobilized during seed fill (Wittenbach 1983, Staswick 1989).

Another annual crop, canola (*Brassica napus* L.), remobilizes N from senescing leaves to accumulate a 23 kDa VSP in taproots during flowering. This VSP is fully hydrolyzed later during seed fill, acting as a “time” buffer for N utilization from leaf senescence to grain fill (Rossato et al. 2001, 2002, Noquet et al. 2004).

Storage proteins can serve as N reserves to be utilized for the next growing season in woody perennials. In autumn, deciduous trees translocate N from senescing leaves to the bark of the trunk and older roots for overwinter storage. The N is stored in the form of bark storage proteins (BSP) and degraded and utilized for spring regrowth (Tromp and Ovaa 1973, Kang et al. 1982). Bark storage proteins of 38 and 56 kDa were identified in apple (*Malus domestica* Borkh. cv. Golden Delicious) (Kang et al. 1982). Willow (*Salix* x *smithiana* Willd.) and eastern cottonwood (*Populus deltoids* Bartram ex. Marsh.) accumulate a 32 kDa protein and sugar maple (*Acer saccharum* Marsh.) accumulates 16 and 24 kDa proteins. These BSPs constitute about 30% of the total extractable bark proteins and are located in protein bodies in parenchyma of the inner bark only during winter months (Wetzel et al. 1989).

Vegetative storage proteins have also been identified in several tuberous root crops. Although generally low in protein, tubers can contain a single VSP that constitutes a large portion of the protein fraction. Sporamin, a 25 kDa VSP in sweet potato (*Ipomoea batatas* Lam.), comprises up to 80% of the tuber soluble protein (Li and Ôba 1985, Maeshima et al. 1985) and has activity as a trypsin inhibitor (Yeh et al. 1997). Common potato (*Solanum tuberosum* L.) also contains patatin, a 40 kDa VSP that constitutes 40-45% of the soluble protein (Racusen and Foote 1980, Paiva et al. 1983, Park et al. 1983). Patatin is a glycoprotein found in the vacuoles of tuber parenchyma cells (Sonnewald et al. 1989) and has been found to function as an esterase (Racusen 1986), and has activity as an lipid acyl hydrolase (Andrews et al. 1988). Both sporamin and patatin have been shown to preferentially degrade during sprouting (Paiva et al. 1983, Maeshima et al. 1985, Staswick 1994). Three tuber-specific VSPs have been identified in Jerusalem artichoke (*Helianthus tuberosus* L.). Two proteins, 16 and 16.5 kDa, are glycosylated, while an 18 kDa VSP is not glycosylated. The 18 kDa VSP degraded during sprouting and did not associate with antibodies from patatin (Mussigmann and Ledoigt 1989).

Weedy species characteristically have a persistent regrowth potential and ability to tolerate various environmental conditions. Nitrogen stores in roots are thought to be utilized for these characteristics and several VSPs have been identified to accumulate in the taproots of some perennial weeds. Chicory (*Cichorium intybus* L.) and dandelion (*Taraxacum officinale* Weber) taproots

have been found to have an increase in free amides, with asparagine and glutamine predominating, and soluble proteins during the autumn. These N pools then decline in spring during regrowth (Cyr et al. 1990). An 18 kDa VSP has been identified in the roots of both chicory and dandelion (Cyr and Bewley 1990a). Both 18 kDa proteins were recognized by an antibody from the 18 kDa VSP identified in Jerusalem artichoke. The chicory VSP was also reported by Limami et al. (1996) as a 17 kDa VSP used during flowering. Xu et al. (2000) determined that the dandelion 18 kDa VSP transcript was regulated by temperature, being up regulated with cold-shock treatments and down regulated with warm-shock treatments. However, the amount of protein did not coincide with the transcript levels nor did the accumulation and degradation of this protein follow that of a VSP after defoliation. Therefore, they propose that the 18 kDa protein in dandelion is not a VSP, but rather related to cold protection.

Leafy spurge (*Euphorbia esula* L.) is another noxious perennial weed that shows seasonal and post-defoliation fluctuations of root N pools similar to chicory and dandelion. However, the free amino acids\amides most abundant in roots were asparagine, glutamine, proline, and arginine (Cyr and Bewley 1989) and the identified VSP in roots was a 26 kDa protein (Cyr and Bewley 1990b). Van Damme et al. (2000) identified a 28 kDa VSP in rhizomes of hedge bindweed (*Calystegia sepium* L.) as an inactive RNase-like protein.

Stinging nettle (*Urtica dioica* L.) and purple nutsedge (*Cyperus rotundus* L.) are rhizomatous perennial weeds that both utilize stored protein and amino

acid-N in roots and rhizomes (Rosnitschek-Schimmel 1985a, Fischer et al. 1995). Free amino acids were found to be the most important N storage pool in early spring growth with arginine and asparagine dominating in both species, reported as 70% of the free amino acid fraction in purple nutsedge (Fischer et al. 1995). In stinging nettle, arginine was preferentially accumulated and remobilized in older rhizomes and roots while asparagine accumulated more in young rhizomes (Rosnitschek-Schimmel 1985b).

Alfalfa

The perennial forage legume, alfalfa (*Medicago sativa* L.), is often subjected to many defoliation events throughout a growing season as a result of mechanical harvesting or grazing. In order to persist, it must have the potential to regrow shoots after these events and during spring regrowth. During early spring growth or post-cutting regrowth, the N demand is greater to support new growth, however alfalfa plants have been shown to have reduced nitrate and ammonium acquisition (Vance and Heichel 1981, Kim et al. 1991, 1993a, Ourry et al. 1994), and reduced symbiotic N_2 fixation (Vance et al. 1979, Ta et al. 1990, Kim et al. 1993b) during these periods of time. Since acquisition of external N, from both root uptake and N_2 fixation, is suppressed after defoliation, the N used in regrowth during these periods must be previously acquired and present in the remaining tissues. In fact, Vance et al. (1979) found that nodulated alfalfa had reduced N_2 fixation during the first 10 d after cutting and 80% of the N used in the

regrowing shoots was from root reserves. Use of root N reserves for shoot regrowth was also similarly shown in non-nodulated alfalfa (Kim et al. 1991, 1993a). Alfalfa has a large taproot that is a storage organ for N reserves. Through ^{15}N labeling, 90% (Barber et al.1996) up to 100% (Kim et al. 1993b) of the N in regrowth 10 d after cutting was found to have originated from the taproot N pools.

Taproot N compounds have been extensively studied for seasonal fluctuations and their roles in shoot regrowth. Amino acids and soluble proteins have been found to accumulate in alfalfa taproots in autumn. They are maintained at these levels through winter months when the plant is dormant, then decline rapidly once spring regrowth begins (Volenec et al. 1991, Hendershot and Volenec 1992). These taproot N pools were also found to deplete after defoliation, then reaccumulate in the taproot after 21 d of regrowth (Hendershot and Volenec 1993). Within the taproot, bark tissues (phloem and phloem parenchyma) had greater amounts of N than wood tissues (xylem and xylem parenchyma). Protease activity within the bark tissues increased over two-fold during spring regrowth when the protein pool was declining compared to activity in autumn and winter. This suggested that the protein pools were degraded and remobilized for the new shoot growth. Within the soluble protein pool of taproots, proteins of 15, 19, 32, and 57 kDa were degraded as the total soluble protein declined during spring regrowth (Volenec et al. 1991, Hendershot and Volenec 1993) and three proteins of 15, 19, and 32 kDa were identified as being most

abundant (Hendershot and Volenec 1992). The free amino acid pool consisted of nearly 50% asparagine and aspartate, and these two amino acids were preferentially utilized compared to other amino acids during shoot regrowth and after defoliation (Hendershot and Volenec 1993). Similarly, Dhont et al. (2003) also found an increase in taproot total amino acid concentrations going into autumn that remained throughout the winter, which was then utilized during spring regrowth. Asparagine accounted for 40-60% of this amino acid pool. In support, Kim et al. (1993a) found asparagine to be the main amino acid transported in xylem sap of alfalfa in both nodulated and non-nodulated plants. In non-nodulated alfalfa, 75% of xylem sap amino acid content was asparagine, and during 10 d of regrowth asparagine was preferentially reduced to 67%.

Cunningham and Volenec (1996) defined the 15, 19, and 32 kDa proteins in alfalfa taproots as VSPs. Comprising about 20% of the taproot soluble proteins, these proteins are dominant in the soluble protein profile, are preferentially accumulated and degraded during times of shoot regrowth in the spring (Hendershot and Volenec 1992) and after defoliation (Hendershot and Volenec 1993). Avice et al. (1996) reported that these three VSPs represented 28% of the soluble proteins in alfalfa (cv. Europe), and after defoliation, decreased 38% after 10 d of regrowth.

The three defined VSPs were only located in taproot tissues in alfalfa and not in nodules or other plant segments (Cunningham and Volenec 1996). Avice et al. (1996) used immunolocalization techniques to locate the VSPs in the

taproot tissues and found them to be predominately in the tissue wood ray parenchyma cells. Within the cell, VSPs were in the fibrillar material in vacuoles and along the periphery of amyloplasts. Cunningham and Volenec (1996) also determined these three VSPs to be glycosylated and the amino acid composition of the proteins were all >10 mole% asparagine, aspartate, and leucine.

In addition to the identified 15, 19, and 32 kDa VSPs, a 57 kDa protein was found to behave like a VSP in that it accumulated in autumn and decreased at the time of spring regrowth in alfalfa taproots (Volenec et al. 1991, Hendershot and Volenec 1993, Li et al. 1996). This 57 kDa protein represented up to 8% of the soluble protein pool and was identified as β-amylase (Boyce and Volenec 1992). Beta-amylase accounts for >99% of total amylase specific activity in alfalfa taproots (Doehlert et al. 1982) and was hypothesized to be utilized in the breakdown of starches within the taproot at the time of regrowth. However, β -amylase specific activity was found to decrease at the time of spring regrowth, suggesting it may be utilized as a VSP for its N reserves rather than to hydrolyze starches (Volenec et al. 1991, Gana et al 1998). Beta-amylase specific activity is also prevalent in red clover (*Trifolium pretense* L.) roots and this activity declines during the time of rapid spring regrowth (Li et al. 1996). This also supports the role of β -amylase as a VSP, rather than a role in starch hydrolysis during the time of spring regrowth.

Several studies have explored the role of N root reserves in the regrowth potential of alfalfa. Ourry et al. (1994) found that the amount of N present in

taproots before defoliation greatly affected shoot regrowth. The amount of N present in the roots correlated with the regrowth shoot dry matter and N remobilization to new tissues. In contrast, the initial starch content of the roots did not correlate with these factors. They concluded that N reserves in taproots have a greater effect on regrowth potential of the plant than starch reserves. Avice et al. (1997) found that the regrowth potential of alfalfa after a summer cutting was dependent on the total soluble protein and the concentrations of the identified VSPs present in the taproot on the day of defoliation. Similar relationships to spring regrowth potential were found with root N and VSPs contents in autumn. Alfalfa plants with greater root soluble protein and VSPs in autumn had more rapid regrowth, greater rates of leaf area index expansion, and overall greater forage production during spring regrowth (Justes et al. 2002). Many researchers suggest that VSPs could also be involved in cold tolerance, winter hardiness, and spring growth vigor (Cunningham and Volenec 1998, Dhont et al. 2003).

The VSPs of 15, 19, and 32 kDa were also found in other perennial *Medicago* species (*M. cancellata*, *M. ruthenica*, *M. suffruticosa*, *M. sativa* subsp. *sativa*, *M. sativa* subsp. *falcata*, *M. sativa* subsp. *glomerata*, *M. sativa* subsp. *caerulea*, and *M. sativa* var. *viscose*). However, these VSPs were scarce or absent in annual *Medicago* species. Annual *Medicago* species are not known for winter hardiness or regrowth potential after harsh defoliation. It was concluded

that these VSPs may be important for winter hardiness and regrowth potential after defoliation in *Medicago* species (Cunningham and Volenec 1996).

Other Forage Legumes

Along with alfalfa, white clover (*Trifolium repens* L.) is a perennial forage legume that exhibits seasonal patterning of N compounds. Nitrogen from roots and stolons has been shown to mobilize and be utilized for shoot regrowth in spring (Bouchart et al. 1998, Corbel et al. 1999) and after a defoliation event (Corre et al. 1996, Goulas et al. 2001, 2002). A 17.3 kDa VSP was identified in stolons and roots and two 15 kDa VSPs were identified in roots (Corre et al. 1996). However, Goulas et al. (2001, 2002) found that the two 15 kDa VSPs were present only in nodules and were identified as leghemoglobin. The accumulation of 17.3 kDa VSP in roots was found to be induced by chilling and is thought to be involved in cold stress tolerance (Goulas et al. 2007). In contrast, Corbel et al. (1999) found cultivars of different cold hardiness capacities to accumulate the same amount of VSP in stolons, suggesting this VSP is not a factor for cold hardiness of white clover. Instead, cold hardiness has been attributed to starch reserves (Turner and Pollock 1998).

Root soluble protein concentrations of red clover (*Trifolium pretense* L.), sweetclover (*Melilotus officinalis* L.), birdsfoot trefoil (*Lotus corniculatus* L.) (Li et al. 1996), and subterranean clover (*Trifolium subterraneum* L.) (Culvenor and Simpson 1991) followed the pattern of accumulation and degradation as alfalfa

during spring regrowth and after defoliation, however birdsfoot trefoil did not show a change in protein concentrations after defoliation and did not reaccumulate a protein pool after spring regrowth (Li et al. 1996). Although no VSPs were identified in these species (Cunningham and Volenec 1996, Li et al. 1996), a 17 kDa protein was found to accumulate in roots from December through March, but did not reaccumulate after spring regrowth occurred. It was speculated that this protein may have a role in cold acclimation (Li et al. 1996).

Grasses

Many studies have shown that internal N pools are remobilized to support regrowth after a defoliation event in grasses, including perennial ryegrass (*Lolium perenne* L.) (Ourry et al. 1988, 1989, 1990a ,b, 1996, Macduff et al. 1989, Millard et al. 1990, Lefevre et al. 1991, Thornton et al. 1993a, b, 1994, Thornton and Millard 1996, 1997, Louahlia et al. 1999, 2000, Morvan-Bertrand et al. 1999, Lestienne et al. 2006), red fescue (*Festuca rubra* L.) (Thornton et al. 1993a, 1994, 2002), highland bent grass (*Agrostis castellana* Boiss. and Reuter), rough bluegrass (*Poa trivialis* L.) (Thornton et al. 1993a, 1994), soft brome grass (*Bromus mollis* L.) (Phillips et al. 1983), and purple moor grass (Thornton and Millard 1993, Thornton and Bausenwein 2000). Although relatively different among species, grasses utilized both remobilized N and N from root uptake when regrowing leaves after defoliation. Generally, when a grass shoot is left intact, new leaf growth is supported by N uptake from roots, not supported by N stores

remobilized from root tissue (Bausenwein 2001a, Thornton et al. 2002). However, after a defoliation event, internal N from remaining tissues is used to support new leaf growth. Such remobilization occurs to a greater extent during the first seven days following defoliation, and has been shown to constitute up to 60% of the N used in regrowth in perennial ryegrass, red fescue, highland bent grass, and rough bluegrass shoots (Thornton et al. 1993a). Remobilization of N from stubble and root tissue in perennial ryegrass (Ourry et al. 1988, Millard et al. 1990), root tissue in red fescue (Thornton et al. 2002), crown and roots in soft brome grass (Phillips et al. 1983) and basal internodes and roots in purple moor grass (Thornton and Millard 1993) has been documented to support new shoot growth after defoliation.

Seasonal N dynamics in grasses has also been documented based on changes in N content of different plant parts. Several studies have found belowground structures, like roots and rhizomes, to increase in mass, total N, and N concentration during autumn and winter. A subsequent decrease of N in these structures during spring growth suggests that the N in the underground tissues is utilized for spring regrowth (McKendrick et al. 1975, Clark 1977, Power 1986, Nordin and Näsholm 1997). For example, McKendrick et al. (1975) found rhizomes of big bluestem (*Andropogon gerardi* Vitman) and indiangrass [*Sorghastrum nutans* (L.) Nash] increased in mass and N concentration in winter and declined in spring. Power (1986) reported total N sequestered in the root system was the greatest in autumn and declined during spring regrowth in

several cool-season grasses including western wheatgrass [*Pascopyrum smithii* Rybd. (Love)], crested wheatgrass [*Agropyron desertorum* (Fisch. Ex Link) Schult.], intermediate wheatgrass [*Elytrigia intermedia* (Host) Nevski], smooth bromegrass (*Bromus inermis* Leyss.), Russian wildrye [*Psathyrostachys juncea* (Fisch.) Nevski], green needlegrass (*Stipa viridula* Trin.), and Garrison creeping foxtail (*Alopecurus arundinaceus* Poir). Nordin and Näsholm (1997) also reported similar N cycling in roots of wavy hairgrass [*Deschampsia flexuosa* (L.) Trin]. However, observations of seasonal fluctuations of total N or N concentration in plant parts does not differentiate N from root uptake or remobilized stored N being used for new growth (Jonasson and Chapin 1985, Bausenwein et al. 2001a).

Several studies have utilized ^{15}N tracer techniques to discriminate internal N remobilization or N from current root uptake as the source of N used for new growth in grasses. Clark (1977) found 33% of leaf N of blue grama [*Bouteloua gracilis* (H.B.K.) Lag.] was remobilized to underground tissues, including crowns and roots, by late autumn, and this N was recycled for new shoot growth in spring. Partala et al. (2001) reported a 49% decline in leaf labeled N of reed canary grass (*Phalaris arundinacea* L.) during winter, while it increased in rhizomes and roots in winter, and was then subsequently used for spring regrowth. Purple moor grass also utilized stored N from roots and basal internodes for new shoot growth from January though June when root uptake was inhibited. Remobilized N constituted up to 55% of new shoot N (Thornton

and Millard 1993). Although basal internodes remobilized a great proportion of stored N from its tissue, over 90% compared to about 64% of root N, roots contributed over twice the amount of N to shoots than basal internodes (Thornton and Bausenwein 2000). Protease activity was also correlated with N remobilization from roots and basal internodes, suggesting degradation of protein pools for N remobilization (Thornton and Bausenwein 2000).

Nitrogen from senescing leaf tissue was found to support early spring growth of new leaves and new tillers in red fescue and colonial bentgrass (*Agrostis capillaries* L.) (Bausenwein et al. 2001a). Using ^{15}N, they found up to 70% (red fescue) and 82% (colonial bentgrass) of the total N in new leaf tissue was from remobilized old leaf N. Root mass was found to increase from late January through spring, however this growth was supported by N uptake by roots, not from remobilized N. Furthermore, root N was not remobilized for spring shoot growth. By late spring, the N from vegetative tillers was again remobilized to support reproductive tillers.

Li et al. (1992) found solution cultured perennial wheatgrass [*Agropyron dasystachyum* (Hook.) Scribn.] remobilized ^{15}N from old leaves, crowns, and roots to support new growth. When grown in different concentrations of available N, unstressed plants had greater root N uptake to support new growth. This resulted in unstressed plants having a smaller proportion of remobilized N in new leaves and tillers, about 12% compared to about 53% in N-stressed plants.

However, the proportion of N remobilized from old leaves was independent of N supply.

Perennial Ryegrass

Remoblization dynamics of N have been extensively studied in perennial ryegrass. Many studies have focused on the contributions of N uptake by roots and remobilized N from remaining tissues to support shoot regrowth after a single or repeated defoliations. As with alfalfa, N uptake and assimilation is reduced in perennial ryegrass after a defoliation event. Near cessation of uptake was observed during the first two days after defoliation (Clement et al. 1978, Ourry et al. 1996), and remained reduced through day six (Ourry et al. 1988, 1989, 1996, Jarvis and Macduff 1989, Macduff et al. 1989, Louahlia et al. 1999, 2000). Root growth and mass is also reduced during the first four days (Ourry et al. 1988, Jarvis and Macduff 1989), and some root death occurred (Jarvis and Macduff 1989). Reduced N uptake and root mass were also observed in field grown plants for the first seven days following defoliation (Louahlia et al. 2000). Further reduction in N uptake and root mass can occur with repeated defoliations (Thornton and Millard 1997) and under more severe defoliation intensity (Lestienne et al. 2006).

Another factor affecting N uptake after defoliation is the initial N status of the plant at the time of defoliation. Unlike alfalfa where N uptake rates were reduced independent of the N status of the plant (Ourry et al. 1994), perennial

ryegrass with high N status exhibited a more severe reduction of N uptake than low N plants during the first seven (Lestienne et al. 2006) and ten days of regrowth (Louahlia et al. 1999, Macduff et al. 1989). Ourry et al. (1996) found low N plants to maintain N uptake. This ability for low N plants to be less inhibited in N uptake after defoliation may be a compensation mechanism for lower N reserves (Louahlia et al. 1999, Morvan-Bertrand et al. 1999, Lestienne et al. 2006). Since N uptake is severely inhibited after defoliation, initial regrowth of perennial ryegrass shoots after a defoliation event is reliant on N reserves from remaining tissues.

It has been well established that perennial ryegrass remobilizes N from roots and remaining stubble (includes leaf sheaths of fully expanded leaves, basal parts of expanding leaves, and meristems) to support new leaf growth after a single defoliation (Ourry et al. 1988, 1989, 1990a, b, 1996, Lefevre et al. 1991, Louahlia et al. 1999, 2000) and repeated defoliations (Millard et al. 1990, Thornton and Millard 1996, 1997). Whether the majority of remobilized N in regrowing shoots originated from the roots or stubble is dependent on several factors, including defoliation height and frequency, and the N status of the plant at the time of defoliation. Stubble can be the largest source of remobilized N used for leaf regrowth (Ourry et al. 1988, 1989, 1990b). However, at lower defoliation heights where less stubble remains, roots have been found to supply the majority of N to new leaf growth after a single defoliation (Thornton and Millard 1996, Louahlia et al. 1999, 2000, Lestienne et al. 2006). After the first

defoliation event, the overall root mass is reduced, and this reduces the available N for mobilization from roots to support new shoot growth for subsequent defoliations. Therefore, in subsequent defoliations, most remobilized N originated from stubble (Thornton and Millard 1996).

The first six days of regrowth following defoliation are supported mainly by remobilized N reserves from roots and stubble (Ourry et al. 1989, Lefevre et al. 1991, Louahlia et al. 1999, 2000). Stored N in roots and stubble were reduced by 47 and 43%, respectively, during seven days of regrowth in field grown plants, while the total amount of N increased in new leaves (Louahlia et al. 2000). Through the use of ^{15}N, Ourry et al. (1989) found 70% of the new leaf growth N originated from roots and stubble after six days of regrowth. Soluble proteins, the main source of N remobilized from these sources, were reduced in roots by 40% and in stubble by 45% during this period. The activity of peptidases increased in roots and stubble during this period of time, supporting the hypothesis that soluble proteins are broken down to amides and amino acids, and these N forms are the main N transport compounds exported from source organs and transported to regrowing shoots (Ourry et al. 1989). Lefevre et al. (1991) found free amino acid and amides from roots and stubble to be the main source of remobilized N to support new leaf growth, followed by protein N. They found amino acid content to increase in leaves, decrease in roots and remain unchanged in stubble during the first six days of regrowth. Before defoliation, asparagine and glutamine dominated the amino acid pool in stubble, 48 and

21%, respectively, and in roots 57 and 16%, respectively. After six days of regrowth, root asparagine and glutamine declined to about half their initial concentration. However, in stubble, asparagine increased during the first three days. This was accompanied by a 3-fold increase in asparagine in new leaves during the first day of regrowth. Lefevre et al. (1991) stated that these changes in the content of free amino acids in N sources (roots and stubble) and sinks (new leaves) were the result of changes in proteolysis and the conversion of the resulting amino-N into preferred forms of N transport.

Stubble tissue includes older leaves, which can be a major source of remobilized N during the senescence processes. Robson and Deacon (1978) found up to 70% of leaf N was remobilized from intact tillers before leaf death occurred in perennial ryegrass. In grasses, glutamine and asparagine accumulate in senescing leaf tissue and are thought to be major forms of transportable N resulting from protein breakdown (Thomas 1978). Regulation of enzymes involved in the synthesis of glutamine and asparagine during leaf senescence of Darnel ryegrass (*Lolium temulentum* L.) suggests that deamination of amino acids results in free ammonia, which is incorporated into aspartate and glutamate to produce glutamine and asparagine (Thomas 1978). After six days of senescence, asparagine:glutamine ratio increased in senescing leaves as a result of increased asparagine. Therefore, asparagine could be a preferred transported N form from senescing stubble tissue to support the new

leaf growth. This is supported by an increase in asparagine in stubble and new leaves found by Lefevre et al. (1991).

In order to further elucidate the amino acid dynamics in N remobilization between the source and sink tissues, xylem exudates were examined. Bigot et al. (1991) found in perennial ryegrass, asparagine and glutamine dominated the amino acid content in xylem exudates just after defoliation, representing 20 and 52% of the total amino acids, respectively. Glutamine, therefore, was the main source of translocated N before defoliation, when N uptake was not inhibited. After defoliation, nitrate uptake and xylem amino acid concentrations decline dramatically within one day, the reduction of glutamine was greater than the reduction of asparagine (Thornton and Macduff 2002), resulting in an increased xylem asparagine:glutamine within three days (Bigot et al. 1991). Thornton and Macduff (2002) suggest that the reduction in glutamine is the result of decreased nitrate uptake and reduction during the first day after defoliation. This change in xylem exudate amino acid composition occurs concurrently as the relative abundance of asparagine increases in new leaves. Bigot et al. (1991) suggest that these changes in xylem sap amino acids over several days indicate that roots and stubble become sources of amino acids for remobilization to new leaf tissue during this period.

As with N uptake, the status of N reserves also affects the regrowth potential in perennial ryegrass. Perennial ryegrass with low N status at the time of cutting has been found to have decreased regrowth, and decreased N

remobilization from N stores compared with plants having high N status at the time of defoliation (Louahlia et al. 1999, Morvan-Bertrand et al. 1999). Sheath soluble protein pools were also greater in plants having high N status, however N status did not affect protein concentrations in roots (Louahlia et al. 1999). In addition, Ourry et al. (1990b) found using ^{15}N that plants with equal initial N reserves at the time of defoliation, but subsequently given high N, had greater leaf regrowth, with remobilized N contributing 40% of new leaf N after 14 d of regrowth. However, plants given low N had reduced leaf regrowth and relied more on remobilized N, contributing about 69% of new leaf N. This suggests that the use of remobilized N in grasses is reliant on N availability and the amount of N reserves present at the time of defoliation (Ourry et al. 1990b, Li et al. 1992, Thornton and Millard 1997, Gloser 2005).

Louahlia et al. (1999) identified specific leaf sheath proteins of 24, 36.6, and 55 kDa that depleted during initial days of regrowth and then reaccumulated after seven days, following a pattern of VSP accumulation and degradation. These putative VSPs also were more abundant in high N plants, suggesting their role in N storage. However, collectively these proteins were <8% of the soluble protein pool and therefore did not meet the VSP requirement of representing a significant fraction of total protein.

Although the remobilization from roots and stubble to support regrowth is rapid after defoliation, similar remobilization could occur over a longer period of time to support seasonal regrowth during spring. However, few studies have

examined seasonal N remobilization and growth dynamics in perennial ryegrass. Louahlia et al. (1999) studied the seasonal fluctuations of N compounds in field grown perennial ryegrass and found soluble protein concentrations in leaf sheaths to accumulate in autumn and winter and decline in spring. The three putative VSPs also accumulated in field grown leaf sheaths, however they were not confirmed as VSPs based on their degradation pattern (Louahlia et al. 1999). It is suggested that the 82% decline in sheath proteins during the spring is from leaf senescence of some of the sheath tissues and not from VSP accumulation/degradation (Louahlia et al. 2000).

Bushgrass

Another extensively studied grass is bushgrass (*Calamagrostis epigejos* L. Roth.), a rhizomatous perennial grass known for its competitiveness and ability to adapt and survive in many environmental conditions. Several studies in the Czech Republic examined N storage dynamics through changes in biomass and N content of different plant parts, without the use of ^{15}N (Gloser 2002, 2005, Gloser et al. 2004, 2007, Kavanová and Gloser 2005). Defoliation studies of bushgrass grown in inorganic medium with nutrient solution in a chamber or greenhouse environment, found roots and stubble to be major sources of remobilized N for new leaf growth (Kavanová and Gloser 2005, Gloser et al. 2007). Similarly to perennial ryegrass, nitrate uptake is severely inhibited during the first six days following defoliation (Kavanová and Gloser 2005). Free amino

acids and protein decreased in bushgrass roots about 58 and 54%, respectively during regrowth. Protein in stubble also declined about 38% during the first six days following defoliation (Kavanová and Gloser 2005). Specific proteins of 63.5, 55, 41, and 28 kDa decreased in abundance in stubble and roots. However, these proteins were not abundant in the total protein profile, and were not deemed VSPs (Kavanová and Gloser 2005). Instead, these proteins are suspected of being involved in inhibiting N uptake and assimilation. Unlike roots and stubble, rhizomes were not a source of remobilized N. Protein did not change and free amino acids actually increased in rhizomes following defoliation. These findings, as well as the fact that total N in rhizomes was lower than in roots and stubble (Gloser 2002), suggest that rhizomes transport N from sources (mature tillers) to sinks (young tillers) and are minor in N storage (Kavanová and Gloser 2005).

It is estimated that internal N reserves are able to support regrowth of bushgrass for about 11 d (Kavanová and Gloser 2005). Repeated defoliations reduced regrowth and resulted in reduced amino acids in roots and rhizomes (Gloser et al. 2004). Nitrogen status also affected N reserves, increasing N storage pools with increasing N supply, and this resulted in faster regrowth rates, mainly as greater leaf area development (Gloser et al. 2007).

Seasonal patterns of storage N forms were studied in field-grown plants of bushgrass (Gloser 2002). In rhizomes, roots, and stubble, free amino acids and soluble protein concentrations increased through the winter and early spring and

declined in concentration once spring growth began. Nitrate concentrations in all plant parts increased toward the end of the vegetative growing season, were the greatest through winter, and declined by early spring. Through winter, the free amino acid concentration was greater than protein and nitrate fractions in all plant parts. In early spring (March), rhizomes contained the greatest concentration of free amino acids and this N pool declined more than 40% by April and continued to decline through the rest of the growing period. Although not as dramatic as in rhizomes, free amino acids also declined in root and stubble (Gloser 2002).

The contribution of N from each N pool in seasonal dynamics was further studied in bushgrass grown in outdoor pots of inorganic media and nutrient solution (Gloser 2002). These plants also showed a decline in N pool concentrations from early spring (March) through summer (June) (Gloser 2002). In all plant parts, amino acid-N was the predominate N pool, followed by nitrate-N and then soluble protein-N. Comparing plant parts, roots contained lower amounts of amino acid-N in March than rhizomes or stubble, however by June all plant parts were reduced to similar amounts. In all plant segments, asparagine was the dominant amino acid-N source, followed by arginine and glutamine, with all three representing about 90% of the amino acid-N fraction. It was concluded that amino acids in roots and stubble are probably the most important N-storage compound used for spring regrowth in bushgrass (Gloser 2002, 2005).

In similar growth conditions as Gloser (2002) grown in outdoor pots of inorganic media and nutrient solution, bushgrass given high rates of N in autumn increased N storage pools compared to plants given lower amounts of N (Gloser 2005). Higher rates of N also altered the amino acid composition, increasing asparagine content in all tissues. When examining changes of leaf N overwinter, Gloser (2005) suggests that leaves, rather than roots, are the main source of remobilized N utilized for spring regrowth. High N plants are less efficient at remobilizing leaf N to support storage pools in other tissues than low N plants. It was concluded that N availability in late autumn affects the amount and composition of N reserves available for spring growth (Gloser 2005).

Even though N remobilization has been studied in several grasses, to my knowledge, nothing has been reported in tall fescue. In addition, whole plant seasonal N dynamics have not been reported for tall fescue. Therefore, in order to properly determine seasonal N remobilization dynamics in tall fescue, methods used to study seasonal N remobilization in grasses must be considered.

Use of ^{15}N Labeling in Nitrogen Remobilization Studies in Grasses

The source of N utilized for growth in grasses is either from remobilized endogenous N or from root uptake of external N. Several studies have examined N mobilization by measuring changes in biomass and N content of different plant parts in grasses (Power 1986, Nordin and Näsholm 1997, Gloser 2002, 2005, Gloser et al. 2004, 2007, Kavanová and Gloser 2005), drawing conclusions of N

remobilization based on decreases of N in one tissue versus increase of N in another. However, changes in N concentration or content of different plant segments do not discriminate between remobilized N and N from root uptake (Jonasson and Chapin 1985, Thornton and Millard 1993), and conclusions of N remobilization could be erroneous. As a result, labeling, such as using the stable isotope ^{15}N as a tracer, has been used to determine the sources of N (De Visser et al. 1997).

The use of ^{15}N is common for N-remobilization studies of grasses in order to discriminate N reserves within the plant versus N uptake. The label can be introduced by feeding roots ^{15}N-enriched sources at a particular time point of treatment. Alternatively, endogenous plant N can be labeled with ^{15}N by growing plants first in a medium containing ^{15}N, then transplanting the plants to a medium with natural abundance ^{15}N at the time of treatment. This results in N reserves being labeled in the plant.

Many studies precisely regulate ^{15}N feeding by growing plants in solution culture in controlled environments. By labeling the solution with ^{15}N, N uptake from the solution can be quantified by the amount of label present in plant tissues. Some studies use this solution culture technique to determine the source of N utilized for new growth after a defoliation event (Clement et al. 1978, Ourry et al. 1988, 1989, 1990a, 1990b, 1996, Jarvis and Macduff 1989, Macduff et al. 1989, Millard et al. 1990, Lefevre et al. 1991, Thornton et al. 1993a, 1994, Thornton and Millard 1996, 1997, Louahlia et al. 1999, Lestienne et al. 2006).

Such methods allow for precise quantification of N from different sources. However, plants grown in nutrient solution have differences in physiology and root morphology than plants grown in soil or other types of media (Bowling 1976) and do not necessarily reflect plant growth and development in the field. Also, plants grown in controlled environments are not subjected to the environmental fluctuations found in the field, nor are the density and age of the plants accurately reflected as in field-grown plants in a pasture. Therefore, morphological and physiological responses can be different between plants cultured in controlled environments versus those grown in field conditions (Louahlia et al. 2000).

Other studies have used ^{15}N tracer techniques to study seasonal N remobilization in grasses using different growing conditions to circumvent the issues of controlled environments. Bausenwein et al. (2001a) grew red fescue and colonial bentgrass, and Thornton and Millard (1993) and Thornton and Bausenwein (2000) grew purple moor grass, in pots of inorganic media in a greenhouse that was exposed to external temperatures but supplemented with heat to avoid frost. These studies enriched the N reserves by supplying ^{15}N during the first year of growth, and supplying the plants with a non-labeled N source the second year. However, these plants were not exposed to cyclic ambient weather conditions (rain, snow, temperatures, etc.) and the physiological responses to winter weather may not have been accurately reflected as plants in the field. Another problem of such studies is the age of the plants. These studies used two year old plants that may not have older belowground structures,

such as old roots and rhizomes, present in an established stand. In order to study the involvement of such tissues, older and mature plants must be used.

Few N remobilization studies in grasses with ^{15}N labeling have used soil-grown plants in field conditions. In general, it is more difficult to get uniform labeling in field conditions, as well as to take into account the numerous soil N pools (Thornton and Macduff 2002). In some studies, soil was topdressed with ^{15}N-labeled fertilizers the first year in order to monitor N uptake and retention in the plants in subsequent years, such as with blue grama (Clark 1977) and reed canary grass (Partala et al. 2001).

Perennial ryegrass is an extensively studied grass in terms of N dynamics. However, only two field experiments have been reported to supplement the growth chamber studies on N dynamics. Louahlia et al. (1999) reported the only seasonal protein profile for VSP determination on field-grown plants and Louahlia et al. (2000) used topdressed ^{15}N to monitor N uptake after a defoliation event. However, both studies used perennial ryegrass plants that had been established only one year.

In order to assess seasonal growth and N dynamics between different parts of tall fescue, plant morphology must be similar to those plants found in established pastures, containing both new and older tissues, and be exposed to seasonal environmental changes. Therefore, established tall fescue plants must be studied in field conditions. In order to discriminate leaf N from other N sources, ^{15}N label can be incorporated into the leaf tissues by foliar application.

The ^{15}N label can also be used to determine leaf N remobilization within the tall fescue plants over time. Several considerations must be made when using foliar applied ^{15}N in tracer studies.

Estimate of ^{15}N Natural Abundance

Natural abundance of ^{15}N in the atmosphere is 0.3663 atom% ^{15}N (Junk and Svec 1958). Variations in natural abundance occur between N-containing compounds, with biological compounds ranging from 0.0037 atom% ^{15}N greater to 0.0018 atom% ^{15}N less than atmospheric ^{15}N (Shearer and Kohl 1986). Microbes discriminate against ^{15}N over ^{14}N, resulting in products lower in ^{15}N than atmospheric atom% ^{15}N (Mariotti et al. 1981, Shearer and Kohl 1986). Discrimination against ^{15}N occurs during mineralization and decomposition, nitrification (Nadelhoffer and Fry 1994), and denitrification (Piccolo et al. 1996) of soil N by microbes and results in the remaining organic forms of N being enriched in ^{15}N, while the inorganic products are depleted in ^{15}N. As a result, soil N is in generally more abundant (~0.3697 atom% ^{15}N) than atmospheric ^{15}N (Shearer et al. 1978, Karamanos et al. 1981, Steele and Wilson 1981). Since plants assimilate inorganic N, plant tissues generally have lower ^{15}N than soils (Fry 1991, Nadelhoffer and Fry 1994, Högberg 1997); however, the atom% ^{15}N of plants is dependant on the source of N (Nadelhoffer and Fry 1994). In general, N-fixing plants that derive a portion of N from the atmosphere have lower atom%

^{15}N than non-fixing plants that primarily derive N from the soil (Shearer and Kohl 1986).

Further changes of atom% ^{15}N can occur during N transformations within plants, resulting in the natural abundance of ^{15}N differing in different plant parts (Yoneyama and Kaneko 1989, Handley and Raven 1992, Evans et al. 1996, and Templer et al. 2007). Therefore, the natural abundance of ^{15}N in different plant parts at different times must be considered when calculating ^{15}N enrichment in tracer studies.

Foliar Uptake and Assimilation of ^{15}N

Foliar ^{15}N treatment applications offer a direct addition of ^{15}N to leaf tissue and subsequent assimilation in plant metabolism (Sparks 2009). Treatment solutions can contain a surfactant, such as Tween 80, to serve as a wetting agent. This reduces the surface tension of droplets, and increases the leaf surface area exposed to the solution (Neumann 1988). In ammonium nitrate applications, the ions of NH_4^+ and NO_3^- enter the leaf apoplast by diffusion, primarily through cuticular transport (Peuke et al. 1998). Although the cuticle often acts to protect against water and solute loss from the leaf tissue, pores throughout the cuticle layer allow solutes to permeate. The density of cuticular pores is greater between guard cells and subsidiary cells (Maier-Maercker 1999), thus leaf surfaces with more stomates have been found to take up more nutrients from foliar sprays (Levy and Horesh 1984). Although debated as a major site of

solute penetration (Marschner 1995), solutes may also enter through open stomatal pores, where ions diffuse through the water layers connecting the leaf surface to the mesophyll cells (Burkhardt and Eiden 1994, Peuke et al. 1998).

Once in the leaf apoplast, NH_4^+ and NO_3^- are transported into the cell. Nitrate is reduced to NO_2^- by nitrate reductase (Evans and Nason 1953) in the cytoplasm, and further reduced to NH_4^+ by nitrite reductase in chloroplasts (Dalling et al. 1972). Ammonium is directly assimilated by glutamine synthetase and glutamate synthase (Lea and Miflin 1974, Tischner 1987, Wallsgrove 1987) and subsequently incorporated into other organic compounds, such as amino acids and proteins (Lea et al. 1974, Raven 1986, Blevins 1989).

In several grass species, including perennial ryegrass, Andrews et al. (1992) and Scheurwater et al. (2002) found leaves to be the predominant site of NO_3^- reduction rather than in roots. Therefore, it is likely that foliar absorbed $^{15}NO_3^-$ is reduced in the cytoplasm and further assimilated in leaf tissues.

Foliar absorbed $^{15}NO_3^-$ may also be stored in vacuoles before being assimilated into organic compounds. It has been suggested that under a high influx of NO_3^- and NH_4^+, storage of NO_3^- and NH_4^+ can occur in the apoplast or vacuoles before being assimilated (Bowman and Paul 1992, Grundmann et al. 1993, Qiao and Murray 1997, Sparks 2009). In perennial ryegrass, Bowman and Paul (1992) noted that, following ammonium and urea applications, there was an increase in reduced-N in leaf tissues. However, following foliar nitrate applications, there was no increase of reduced N in leaf tissues and about 50%

of the nitrate was not reduced after 48 h. The authors suggested that the absorbed $^{15}NO_3^-$ was stored in the vacuole and released slowly as demanded by growth. To my knowledge, the fate of foliar applied NH_4^+ and NO_3^- have not been determined in tall fescue.

Recovery of Foliar Applied ^{15}N

Overall uptake efficiency of foliar applied ^{15}N is dependent on several factors, including species, leaf age, and application rate. For example, uptake efficiency ranged from 31-57% over a three week application period of KNO_3 and $(NH_4)_2SO_4$ to leaves of gray alder (*Alnus glutinosa*), with nitrate incorporation exceeding ammonium incorporation (Gonzalez-Prieto et al. 1995). While Garten et al. (1998) found no difference between nitrate and ammonium ^{15}N uptake in a mixture of various understory tree saplings, retention rates averaged up to 26% of foliar ^{15}N applications. Additionally, 33.7% of foliar nitrate was assimilated two d after application in tomato (*Lycopersicon esculentum* Mill.) (Wen et al. 1999).

The foliar uptake of $^{15}NO_3^-$ in tall fescue can be estimated from previous labeling studies of grasses. In a controlled environment, about 53% of foliar applied urea-^{15}N was recovered in tall fescue after 72 h from application (Bowman and Paul 1990). They also found similar recovery rates of foliar applied urea, ammonium, and nitrate in perennial ryegrass, with about 40% of the foliar applied N incorporating into the plants after 48 h (Bowman and Paul 1992). In addition, about 15% of the incorporated ^{15}N was found in the roots of

nitrate-applied perennial ryegrass after 48 h (Bowman and Paul 1992) and urea-applied Kentucky bluegrass (Bowman and Paul 1989) and tall fescue (Bowman and Paul 1990) after 72 h. Therefore, in tall fescue, remobilization of incorporated ^{15}N in leaves to other plant parts could be reasonably estimated to be about 15% within three d following application.

A field study by Stiegler et al. (2009) found foliar nitrate applications to creeping bentgrass (*Agrostis palustris*) led to 27% and 37% absorption of applied ^{15}N within one and eight h, respectively, after application. In addition, foliar ammonium applications on the bunchgrass, little bluestem (*Schizachyrium scoparium* Hubb.), in controlled conditions resulted in 93-99% of the applied ^{15}N remaining in the treated tiller after five d (Welker et al. 1991). They suggested that most of the N absorbed was incorporated into metabolic compounds, and eventual catabolism, perhaps through leaf senescence, and remobilization of these compounds. Welker et al. (1991) also found ^{15}N in small amounts in all plant tissues, including attached tillers, after 24 h. These studies suggest that a majority of foliar applied ^{15}N could be incorporated into tall fescue leaf tissues within the first few days following application and much of the absorption could occur within hours after application.

Objectives

To my knowledge, there has been no previous research on seasonal N remobilization dynamics in tall fescue. The overall objective of this study was to determine the fate of N from tall fescue leaves during late autumn, winter, and spring. Specific objectives included determining a) the growth habit of tall fescue throughout the stockpiling period, b) if ^{15}N-labeled leaf N is being remobilized during the stockpiling period, c) if ^{15}N-labeled leaf N is accumulated or stored in plant segments, and d) to identify specific forms of remobilized N.

Foliar applications of the stable isotope ^{15}N were used to distinguish remobilized leaf N from other N sources. The timing of ^{15}N incorporation in leaves during autumn allows leaf N to be traced throughout the plant during the winter months and throughout the period of spring regrowth. In order to study both younger and older tissues of tall fescue and the seasonal response of N remobilization, this study was conducted on established tall fescue stands in field conditions.

Chapter 2

Materials and Methods

Season 1 (2006-2007 Stockpiling Period)

An established tall fescue ('Kentucky-31', endophyte-infected) stand was selected at the University of Missouri Bradford Research and Extension Center (BREC) near Columbia, Missouri. The tall fescue was growing on a Leonard silt loam (fine, smectitic, mesic Veric Epiaqualf), with pHs = 6, NA = 1.5 meq/100g, OM = 4.6%, Bray I P = 8 kg/ha, Bray II P = 32 kg/ha, Ca = 4013 kg/ha, Mg = 353 kg/ha, K = 184 kg/ha, and CEC = 11.9 meq/100 g, , as determined to a 15 cm depth by the University of Missouri Soil Testing Laboratory (Columbia, MO). Kentucky bluegrass (*Poa pratensis* L.) was also prevalent in this tall fescue stand.

The plot area was mowed to a height of about 10 cm on August 31, 2006. On September 1, 81 plots were established by driving 25.4 cm diameter, 20 cm long, and 1.25 cm thick polyvinylchloride (PVC) pipes into the ground. The pipe segments were placed over clumps of tall fescue, soaked with water, then driven into the ground using a weighted front-end loader until the top of the pipe was about 1.25 cm above ground level (Figure 2-1A-C). Each plot was 506.7 cm^2 in surface area, reached ~20 cm in depth, and encompassed ~10,134 cm^3 of soil. Within each pipe, the aboveground material was trimmed by hand to a height of about 5 cm above ground level and excess debris was removed. Vegetation outside of the pipe was trimmed in order to avoid shading of the plots and 112 kg N/ha as ammonium nitrate granular fertilizer (33.5% N) was broadcasted over the entire plot area.

The tall fescue plots were allowed to regrow for 18 d before 72 plots were selected based on the uniformity of tall fescue tillers within the plot and treatments were randomly assigned. Thirty-six plots received a foliar ^{15}N application, at the equivalent of 2.24 kg ^{15}N/ha (5 lbs ^{15}N/acre) (rate modified from Glasener et al. 1998). Treatment consisted of applying 1.5 ml of 0.1565 g ≥ 98 atom% $NH_4{}^{15}NO_3$ (Cambridge Isotope Laboratories, Inc., Andover, MA) in 0.1% (v/v) Tween 80 per plot (= 28.98 mg ^{15}N applied to each plot). The solution was evenly brushed on all surfaces of the aboveground green leaf tissues (Figure 2-1D) with a small paint brush. The other 36 plots (controls) were sprayed with an equivalent solution of ammonium nitrate without the ^{15}N label in 0.1% (v/v) Tween 80. Tillers in all plots were tagged at the base using color tags and coded according to how many collared and expanding leaves were present at the time of treatment application and tillers were counted (Figure 2-1E).

The first harvest was two weeks after the ^{15}N application on October 4, 2006. Subsequent harvests were conducted through April 2007 on the following dates: October 30, November 29, December 20, March 12, April 2, and April 23. During each harvest, four ^{15}N-treated plots and four control plots were harvested. Harvests consisted of removing the 25.4 cm diameter pipe from the ground, removing only the tagged tillers at the soil surface, then extracting the soil and plant column from the pipe (Figure 2-1F). Soil was washed from the plants at the field site with cold water (Figure 2-1G), and the plant parts were packed in plastic bags, packed on ice, and transported to the laboratory for separation.

Non-fescue material and dead parts not connected to a live portion were removed from the underground samples. The plant material was washed free of soil, rinsed in deionized water, and kept over ice during the separation process. The belowground portions were separated into roots (brown and thin), new roots (larger, white and fleshy), old rhizomes (brown, lignified rhizomes with no tiller directly emerging from it), new rhizomes (emerging from the base of a live tiller, including new tillers below the surface that have not started to turn green), crown (the rhizomatous base of a tiller, just below the apical meristem region), and treated leaves (remaining sheaths and apical meristem region of tagged tillers that were below the soil line). Aerial parts were separated into treated leaves (leaves that received treatment application), new leaves (had formed on treated tiller after treatment), and new tillers (had formed after the time of treatment application and did not have a tag) (Figure 2-2). The number of recovered tagged tillers and new tillers were recorded. The plant parts were placed in aluminum foil packets, weighed for fresh weight determination, then frozen at -20 ^{o}C after separation, and stored at -70 ^{o}C until freeze-dried.

Samples were freeze-dried for at least 72 h until a steady dry weight was achieved. The samples were then weighed, ground, and stored in plastic Whirl-pak® bags (Nasco, Fort Atkinson, WI). Samples were double-bagged in plastic bags containing desiccant and stored at -70 ^{o}C.

Soil samples from the top 1 cm were randomly collected within each sampled plot. Collections were made on four ^{15}N-treated plots, each sampled

before the application of ^{15}N treatment, two h after ^{15}N treatment, seven d after ^{15}N treatment, and then on each harvest date. Also, a soil sample was collected from each plot just prior to being harvested on each harvest date. Soil samples were air-dried, ground by hand to a fine powder, then stored in plastic Whirl-pak® bags at room temperature.

Figure 2-1. Establishment, treatment of foliar applied ^{15}N, and harvest of

stockpiled tall fescue plots for a leaf N remobilization study at BREC. (A) Pipe segments were driven into the ground using weighted front-end loader. (B) Pipe segment placed over tall fescue tillers before being driven. (C) Pipe segment driven to about 1.25 cm above ground level. (D) Hand-brushed foliar ^{15}N application of tall fescue. (E) Plot of tall fescue tagged at the base of each tiller. (F) Pipe removed from field and soil core removed from pipe at harvest. (G) Soil core washed at field site, revealing tall fescue belowground segments.

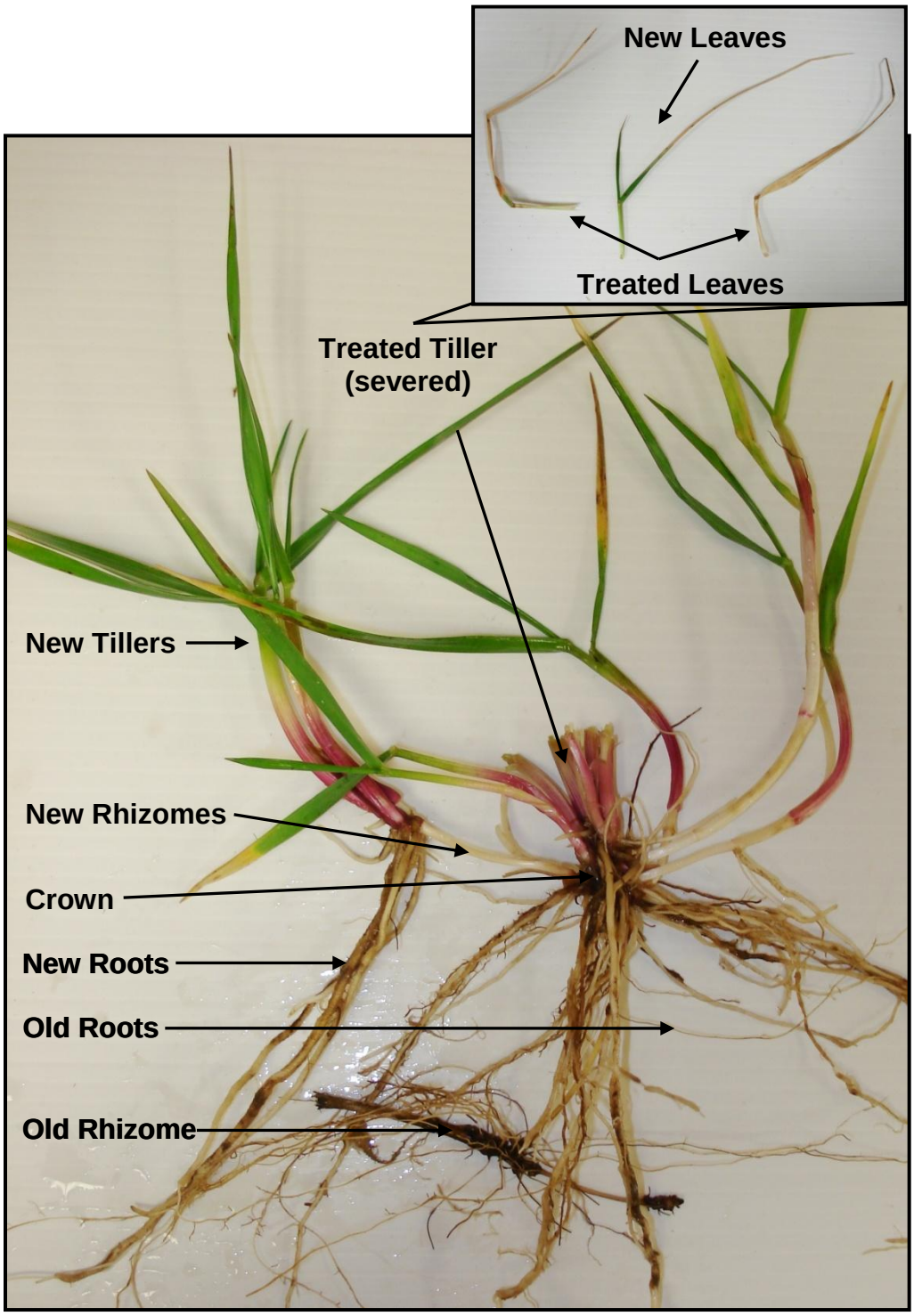

Figure 2-2. ^{15}N-treated tall fescue plants were separated into the identified plant segments at each harvest. Shoot portions included treated leaves (on treated

tillers), new leaves (have formed on treated tillers after treatment), and new tillers (formed after treatment). Belowground portions included new rhizomes (emerging from the base of the treated tiller), crowns (the rhizomatous base of a tiller, just below the apical meristem region), new roots (larger, white and fleshy), old roots (brown and thin), and old rhizomes (brown, lignified, no tiller directly emerging from it).

Season 2 (2007-2008 Stockpiling Period)

The tall fescue stand for this season was at a different location at BREC on a Leonard silt loam (fine, smectitic, mesic Veric Epiaqualf), with pHs = 6, NA = 2.5 meq/100g, OM = 5.6%, Bray I P = 81 kg/ha, Bray II P = 327 kg/ha, Ca = 4807 kg/ha, Mg = 471 kg/ha, K = 362 kg/ha, and CEC = 15.3 meq/100 g, as determined to a 15 cm depth by the University of Missouri Soil Testing Laboratory (Columbia, MO). Also at this location, Kentucky bluegrass was present throughout the tall fescue stand.

The area was mowed to about a 10 cm height on September 14, 2007. Sixty-six plots were established using the same method as in Season 1, driving the pipe on September 17. The equivalent of 112 kg N/ha in the form of ammonium nitrate (33.5% N) was applied by hand within each plot at this time.

The tall fescue plots were allowed to regrow for 17 d before 56 plots were selected for treatments. On October 1, the foliar applications of an equivalent of 2.24 kg ^{15}N/ha (1.5 ml of 0.1565 g ≥ 98 atom% $NH_4{}^{15}NO_3$ in 0.01% (v/v) Tween 80 /plot) were applied to 28 plots as in the previous season. The other 36 plots were sprayed with an equivalent solution without the ^{15}N label to serve as control plots. Tillers in all plots were tagged as in Season 1.

Soil samples were collected as in Season 1 on four ^{15}N-treated plots, each sampled before ^{15}N treatment application, two h after ^{15}N treatment, 10 d after ^{15}N treatment, and then on each harvest date. Also, a soil sample was collected from each plot prior to harvest.

The first harvest was four weeks after the ^{15}N application (October 29, 2007), and subsequent harvests were conducted through April on the following dates: November 29, January 4, January 28, February 25, April 7, and April 28. Plots were harvested, separated, and processed as in Season 1. When the soil was frozen, plots were removed from the field about 12 h in advance and stored at approximately 13 ^{o}C to thaw before processing.

Total N and ^{15}N Determination

Plant and soil samples were weighted in 5x9 mm tin capsules, 5.00 ± 0.20 mg for plant tissues and 40.00 ± 3.00 mg for soil samples. Sample total N and atom% ^{15}N were measured using continuous flow isotope ratio mass spectrometry (PDZ Europa 20-20, Sercon Ltd., Cheshire, UK) by the University of California-Davis Stable Isotope Facility.

Soluble N Pool Determination

All plant samples were analyzed for soluble N pools of protein, nitrate, and free amino acids. About 30 mg of freeze-dried, ground plant sample was extracted in 1.5 ml of 100 mM potassium phosphate buffer, pH 7.0 (adapted from Barber et al. 1996, Gloser 2002). Extraction consisted of hand-grinding plant tissue with washed sand in a mortar and pestle until all tissue was pulverized into a slurry. The sample was then centrifuged at 10,000 g for 20 min. The supernatant (buffer-soluble N fraction) was decanted and the pellet (buffer-

insoluble N fraction) was discarded. Soluble protein and nitrate contents of the buffer soluble N fraction were determined colorimetrically. Nitrate was determined with the salicylic acid method using sodium nitrate as a standard (Cataldo et al. 1975) and soluble protein was determined by protein-dye binding using the Bradford method (1976) with bovine serum albumin (BSA) as a standard. In order to determine free amino acids in the buffer-soluble N fraction, protein was first precipitated to avoid interference. Using 0.25 ml of the buffer-soluble N fraction, trichloroacetic acid was added to a final concentration of 20%, the solution was placed in 4 ^{o}C for 1 h, then centrifuged at 10,000 g for 15 min (adapted from Meuriot 2004, Peterson 1983). The resulting protein pellet was discarded and the supernatant containing the low molecular weight N fractions and was used to determine total free amino acids by the ninhydrin assay (Moore 1968, Kesner and Kirschenbaum 1970) which requires a 2% ninhydrin reagent solution (Product No. N7285, Sigma-Aldrich, Inc. St. Louis, MO) and L-leucine as a standard. The remainder of the buffer-soluble N fraction was frozen and stored at -20 ^{o}C.

Free Amino Acid Profiles

Free amino acid profiles were determined on crown and new root samples of control plots harvested on October 4, March 12, and April 23 of Season 1 and October 29, February 25, and April 28 of Season 2. Profiles were determined by the University of Missouri Agriculture Experiment Station using AOAC official

method 982.30 (AOAC 2006). Protein was precipitated from the buffer-soluble N fractions using sulfosalicylic acid, and the amino acid profile was determined using a Hitachi L8800 Amino Acid Analyzer with norleucine as a standard.

Calculations

The number of tillers present at the time of treatment application (number of initial tillers) varied between plots because plots were randomly selected in field conditions. Therefore, measurements could not be compared between plots based on the total plot, total plant, or total plant part. Measurements were normalized to compensate for the different number of tillers per plot and thus the different amount of total dry weight of plant segments per plot.

Dry Weight

Dry weights are given in units per initial tiller in order to estimate the growth of the individual tillers over the course of the season.

dry weight = g of dry weight in plot / # initial tillers in a plot

Recovered ^{15}N

Natural abundance atom% ^{15}N was determined for each plant segment at each harvest in control plots (plots not treated with ^{15}N). The mean value (n=4) of natural abundance ^{15}N was subtracted from the atom% ^{15}N of ^{15}N-treated samples to find the enriched atom% ^{15}N. The amount of ^{15}N in each plant

segment was calculated as a percentage of ^{15}N applied to the leaves (% of applied ^{15}N) in order to normalize values across plots.

atom% ^{15}N of plant part – natural abundance atom% ^{15}N of plant part
= enriched atom% ^{15}N

enriched atom% ^{15}N x total mg N in plant part
= total mg enriched ^{15}N in plant part

total mg enriched ^{15}N in plant part / 28.98 mg ^{15}N applied x 100
= % of applied ^{15}N

Total Plant N

The allocation of total plant N to different plant segments is calculated as a percent of total plant N.

total tissue dry wt x N concentration of plant part
= total N in plant part

total N in plant part / total N in all plant parts x 100
= % of total plant N

N Concentration

The concentration of N in each plant part was calculated as a percent of dry weight.

mg N / g dry wt x 100 = % N

Soluble Protein, Free Amino Acids, and Nitrate Concentration

The concentration of each N pool was calculated on a dry weight basis. Soluble protein is given as the equivalent mg BSA/g dry weight. Free amino

acids are given as the equivalent mg Leu/g dry weight, and nitrate as mg NO_3^-/g dry weight.

Tillering

The amount of tillering was estimated from tiller counts per plot at the time of harvest. Initial tillers visible aboveground were tagged at the time of treatment, while new tillers had no tag and had emerged since the time of treatment. Tillering is given as a ratio of the number of new tillers per number of initial tillers.

Initial Tiller Recovery

The recovery of treated tillers is estimated by the percentage of tagged tillers recovered at each harvest date.

> # tagged tillers at harvest date / # tagged tillers at treatment x 100 = % of tagged tillers

Leaf N Remobilization

Estimates of N remobilization can be calculated based on changes in ^{15}N content in treated leaves and other tissues. One method of estimating the amount of N remobilized from treated leaves is by calculating the % decline of ^{15}N in treated leaves from the time of treatment to the last harvest. The amount of ^{15}N incorporated in treated leaves at the time of treatment was not determined, therefore it is estimated based from the total ^{15}N recovered at the first harvest

from both treated leaves and other plant parts (all plant parts except treated leaves) (equation a).

(a) % of applied ^{15}N in other plant parts at last harvest /
total % of applied ^{15}N recovered at first harvest x 100
= % of treated leaf N remobilized to other plant parts

A more conservative estimate of remobilized N from treated leaves is calculated from ^{15}N recovered from first harvest (subtracting the ^{15}N remobilized between the time of treatment and first harvest) to last harvest in April (equation b).

(b) (% of applied ^{15}N in other tissues at last harvest – % of applied ^{15}N in other tissues at first harvest) /
% of applied ^{15}N in treated leaves at first harvest x 100
= % of treated leaf N remobilized to other plant parts

Another method to estimate the percentage of remobilized old leaf N in stockpiled tall fescue is based on the initial N:^{15}N ratio in treated leaves. This method estimates the amount of leaf N in different plant parts at each harvest based on the initial N:^{15}N ratio (after ^{15}N treatment and incorporation) in treated leaves and assumes that the labeling of leaf N represented all leaf N pools (equation c, plant part = PP, treated leaves = TL, time of treatment = trt, time of harvest = har).

(c) total N in TL at trt / total ^{15}N in TL at trt
= total N PP har / total ^{15}N PP har

Because the amount of plant material differs between plots, total N and total ^{15}N values cannot be used; normalized values of N per dry weight (or N concentration), and % of applied ^{15}N are used (equation d). Since N

concentration was not measured for the leaves at the time of ^{15}N treatment, this method of estimation is limited to making comparisons from the first harvest date, and any ^{15}N remobilized to plant parts before the first harvest must be subtracted. Both the contribution of N in a plant part originating from treated leaves (equation e) and the amount of treated leaf N remobilized to a particular plant part (equation f) from the first harvest date can be estimated .

(d) (N conc. TL first har / % applied ^{15}N TL first har) x (% applied ^{15}N PP har - % applied 15N PP first har)
= **N conc. PP har from TL**

(e) **N conc. PP har from TL** / N conc. PP har x 100
= % of N in PP from TL since first har

(f) **N conc. PP har from TL** / N conc.TL first har x 100
= % of TL N remobilized to PP by har

Weather

Weather data were collected from the Commercial Agriculture Automated Weather Station Network and compiled by the Missouri Historical Agricultural Weather Database. Air temperature and total precipitation data for the thirty-year monthly averages (1971-2000) were recorded at the Columbia Regional Airport (Columbia, MO). Daily average soil temperatures were recorded at 5 and 10 cm depths in sod-covered conditions at the University of Missouri South Farm Agricultural Experiment Station in Boone County, Missouri (~7 km west of tall fescue field plots). Daily average air temperatures and daily total precipitation were measured at BREC.

Statistics

Each experiment was a completely randomized design with four replications. During each harvest (seven harvests each season), four control plots and four ^{15}N-treated plots were randomly selected and harvested for a total of 56 plots harvested each season (2 treatments x 7 harvest dates x 4 replications = 56 plots each season). Analysis of variance was conducted to test for the effects of ^{15}N treatment, harvest date, and the interaction of treatment and harvest date. Significant differences ($P<0.05$) were separated using Fisher's protected least significant differences ($\alpha=0.05$, $LSD_{0.05}$). When there was no interaction between treatment and harvest date, samples from both treatments were combined to give one mean value (n=8). The PROC MIXED procedure of Statistical Analysis Systems software version 9.2 (SAS Institute Inc., Cary, NC) was used. Statistical analyses were conducted for each season separately.

Chapter 3

Results

Weather

Weather in Season 1 was generally average, with changes in air temperatures associated with similar fluctuations in soil temperatures. A long period of below-freezing air temperatures from January through February (Figure 3-1A) resulted in the soil cores freezing to a 10 cm depth in February. The plots were also snow covered during most of this period (Figure 3-1B). Due to the plot area location, snow, and frozen soil, the plot area was not accessible for harvests. Therefore, no harvests were conducted in January and February due to these weather-related complications.

Season 2 had a similar period of time when air temperatures were below-freezing resulting in frozen soil to at least a 5 cm depth (Figure 3-2A). However, the location of the site during this season allowed access to the plots and harvests were conducted at near monthly intervals.

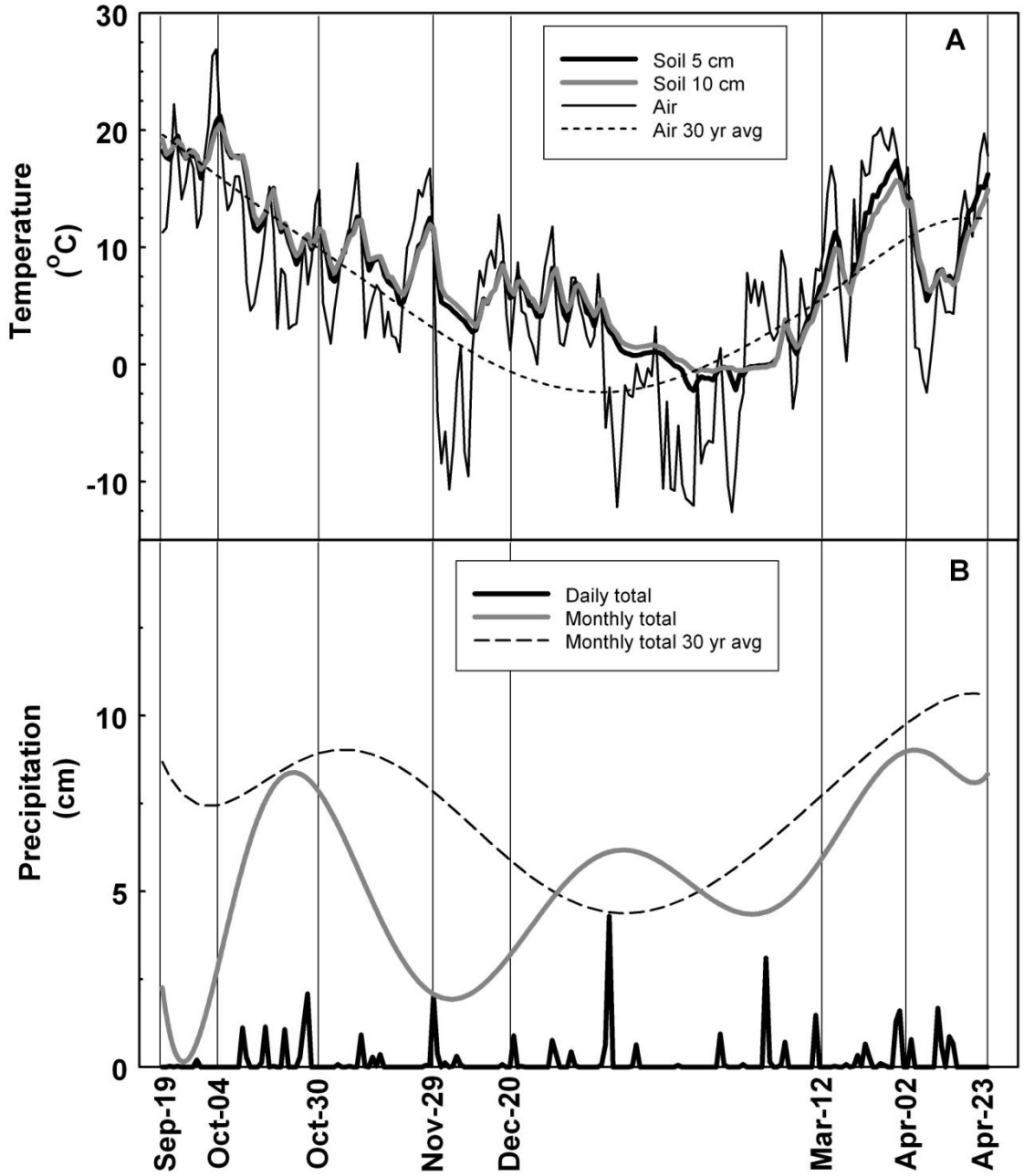

Figure 3-1. Average daily air and soil temperatures and daily precipitation totals for Season 1 (2006-2007) tall fescue stand at the Bradford Research and

Extension Center near Columbia, MO. (A) Average daily temperature of air and sod-covered soil at 5 and 10 cm depths with monthly thirty-year averages. (B) Precipitation daily totals, monthly totals, and monthly thirty-year averages. Vertical lines indicate treatment date (Sept-19) and harvest dates.

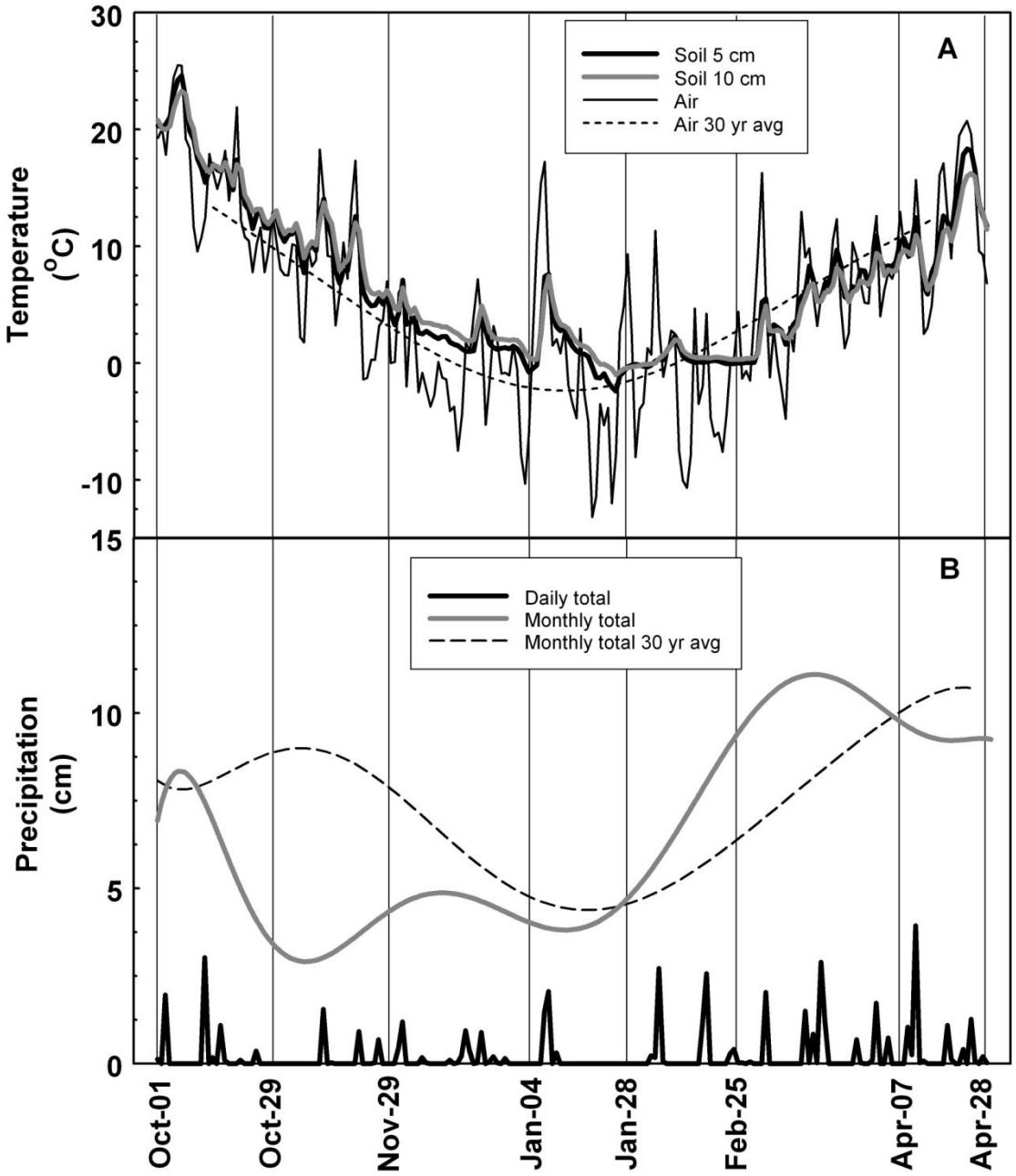

Figure 3-2. Average daily air and soil temperatures and daily precipitation totals for Season 2 (2007-2008) tall fescue stand at the Bradford Research and Extension Center near Columbia, MO. (A) Average daily temperature of air and

sod-covered soil at 5 and 10 cm depths with monthly thirty-year averages. (B) Precipitation daily totals, monthly totals, and monthly thirty-year averages. Vertical lines indicate treatment date (Oct-01) and harvest dates.

Soil ^{15}N

Surface soil samples collected before ^{15}N applications and on control plots had consistent ^{15}N natural abundance levels throughout both seasons, ranging between 0.3677-0.3682 atom% ^{15}N in Season 1 (Figure 3-3) and between 0.3684-0.3695 atom% ^{15}N in Season 2 (Figure 3-4). Post-^{15}N treatment, soil samples increased to more than 0.5 atom% ^{15}N in Season 1 (Figure 3-3) and slightly above 0.4 atom% ^{15}N in Season 2 (Figure 3-4). Soil atom% ^{15}N remained elevated in the ^{15}N-treated plots that were sampled throughout the season, as well as in the ^{15}N-treated plots harvested on each harvest date (Figures 3-3 and 3-4). The recovery of ^{15}N in the top one cm of soil, as % of applied ^{15}N, was estimated to be about 6% after the ^{15}N treatment applications in both Seasons (see Appendix B, Figures B-3 and B-4). Except for recovery after the first harvest in Season 1of about 15% of applied ^{15}N in the top one cm of soil, recovery was ≤ 4% of applied ^{15}N throughout the winter harvests in both seasons. However, in spring the recovery of ^{15}N increased to about about 4-7% of applied ^{15}N.

Initial Tiller Recovery

Tillers that received the treatment of ^{15}N foliar applications were tagged at the time of treatment. Recovery of the tagged tillers neared 100% and did not significantly change over time during both Season 1 (Figure 3-5) and Season 2 (Figure 3-6).

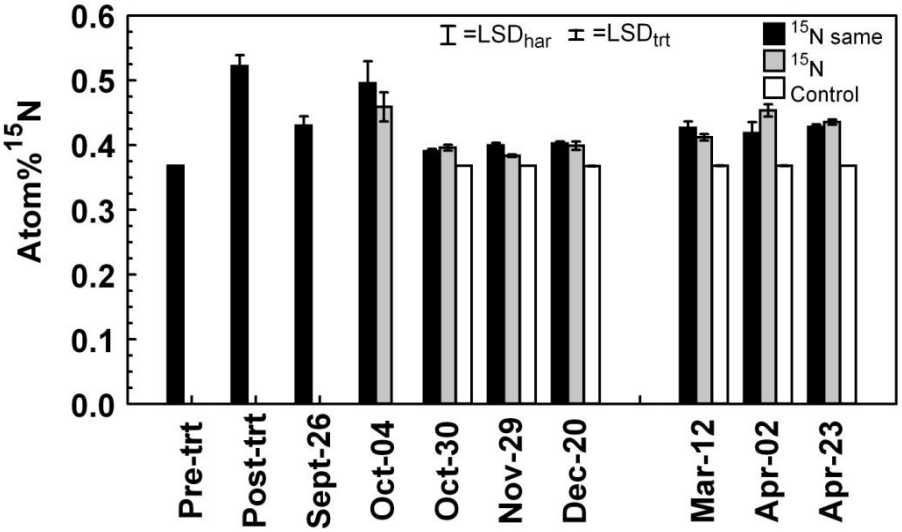

Figure 3-3. Atom % ^{15}N of soil samples (top 1 cm of soil surface) taken just before ^{15}N treatment (pre-trt), just after ^{15}N treatment on September 19, 2006, (post-trt), then on each harvest date indicated of Season 1 (2006-2007). Samples were collected on the same ^{15}N-treated plots over time (black-closed bars labeled ^{15}N same), ^{15}N-treated plots harvested on the indicated date (gray-closed bars labeled ^{15}N) and control plots (open bars) harvested on the indicated date. Values are the mean±SE (error bars are visible when larger than the symbol size, n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between treatments (LSD_{trt}).

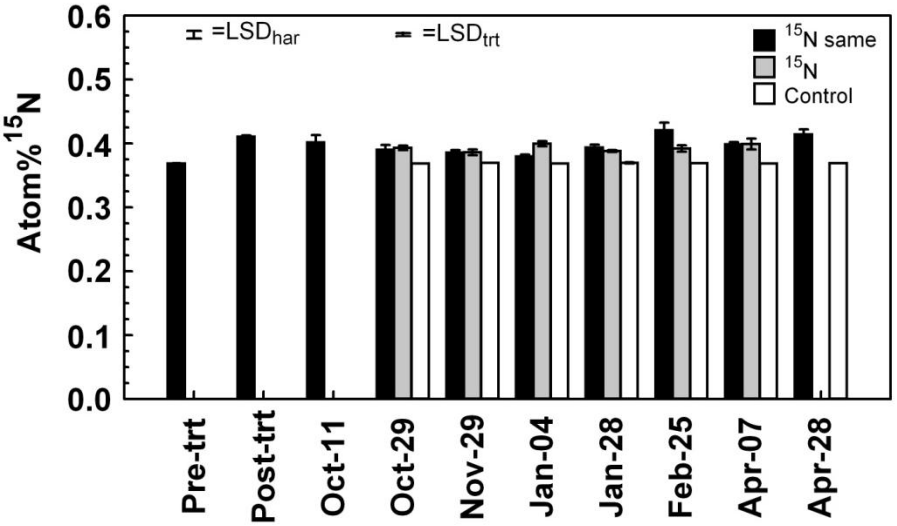

Figure 3-4. Atom % ^{15}N of soil samples (top 1 cm of soil surface) taken just before ^{15}N treatment (pre-trt), just after ^{15}N treatment on October 01, 2007, (post-trt), then on each harvest date indicated of Season 2 (2007-2008). Samples were collected on the same ^{15}N-treated plots over time (black-closed bars labeled ^{15}N same), ^{15}N-treated plots harvested on the indicated date (gray-closed bars labeled ^{15}N) and control plots (open bars) harvested on the indicated date. Values are the mean±SE (error bars are visible when larger than the symbol size, n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between treatments (LSD_{trt}).

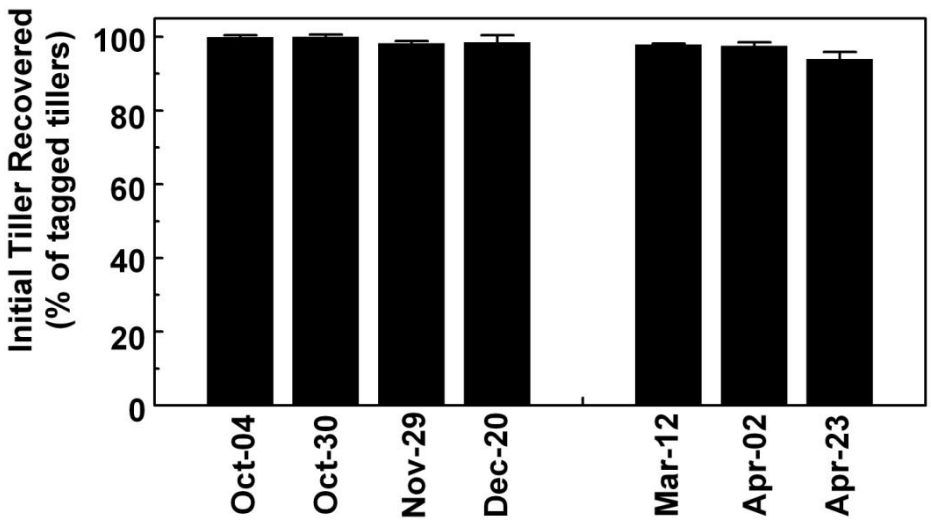

Figure 3-5. The percent recovery of tagged tall fescue tillers during Season 1 (2006-2007) harvests (values are mean±SE, error bars are visible when larger than the symbol size, n=4). Tillers were tagged at the time of treatment application on September 19, 2006.

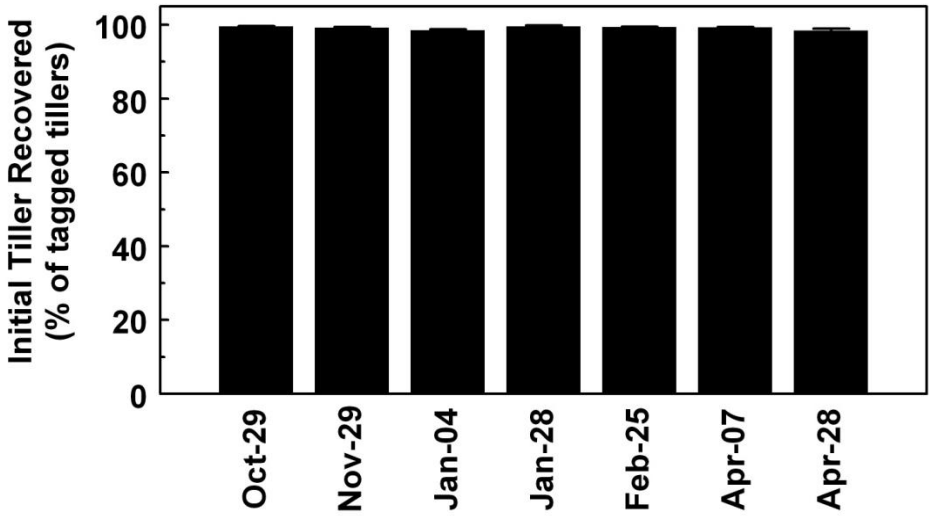

Figure 3-6. The percent recovery of tagged tall fescue tillers during Season 2 (2007-2008) harvests (values are mean±SE, error bars are visible when larger than the symbol size, n=4). Tillers were tagged at the time of treatment application on October 01, 2007.

Growth

Estimated by changes in dry weight of different plant parts, tall fescue exhibited growth throughout the stockpiling seasons. Overall, tall fescue in Season 1 had more dry matter portioned to shoots (Figure 3-7A-C) than belowground structures (Figures 3-8A-C and 3-9A-C). In early autumn, treated leaves and new leaves on the treated tillers increased in mass (Figure 3-7A,B). This was accompanied by an increase in belowground structures of crowns (three-fold increase), new rhizomes (eight-fold increase), and new roots (11-fold increase) through December (Figure 3-8A-C). By spring, growth of new tillers dominated (Figure 3-7C), with each parent tiller forming three to four new tillers (Figure 3-13). Overall, old roots and old rhizomes did not change throughout the stockpiling periods (Figure 3-9).

Season 2 tall fescue plants were more robust than the plants at the location in Season 1, however relative growth patterns were similar (Figures 3-10, 3-11, and 3-12). Although little change occurred in the dry weights of shoot sections through winter (Figure 3-10A-C), new tiller growth again dominated in April in both mass (Figure 3-10C) and in number of tillers formed from each parent tiller (Figure 3-14). Belowground, crowns and new roots increased 1.5-fold and 3.4-fold, respectively, through January (Figure 3-11A,C).

The growth of tall fescue throughout Season 2 is depicted in Figures 3-15 and 3-16. Treated leaves turned brown through winter (Figure 3-15A-E), while new green tillers emerged in April (Figure 3-15F-G). The increase in the number

of new tillers and tiller development was seen throughout the season, resulting in large, fully developed tillers by April-28 (Figure 3-16). Belowground, crowns increased in size throughout the season, and new root growth proliferated through February (Figure 3-16A-E).

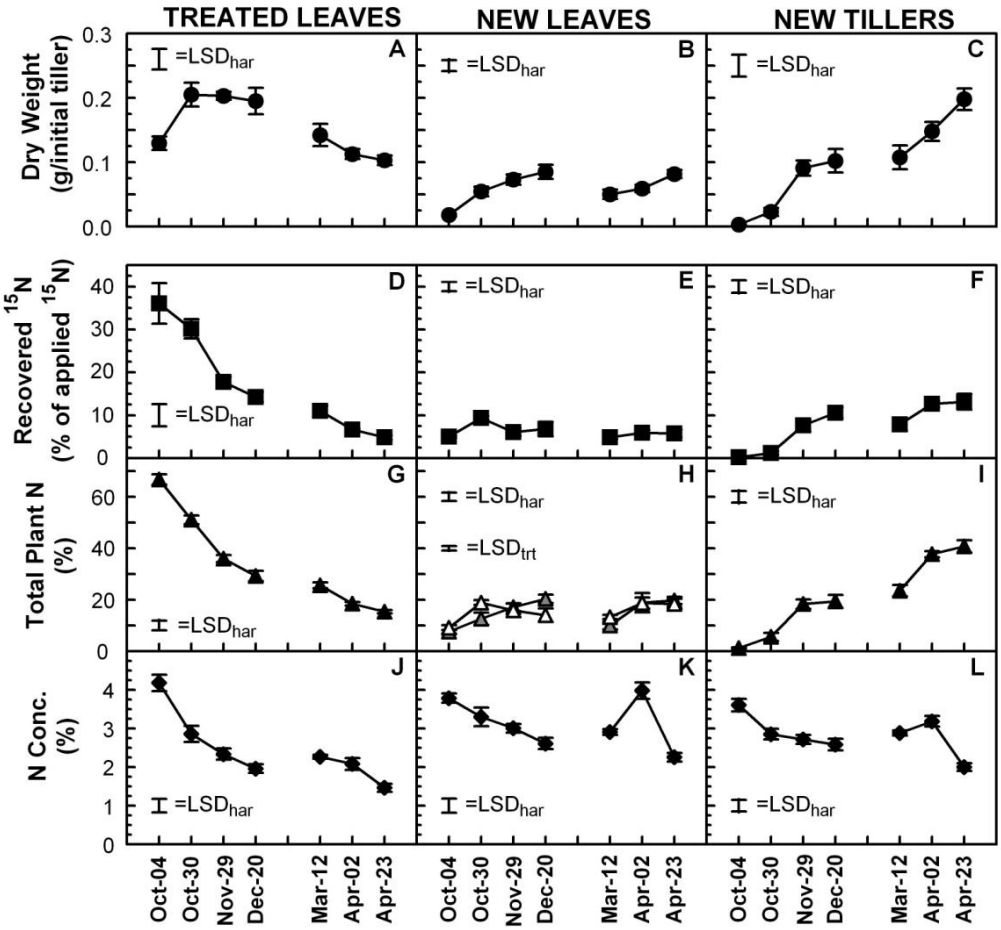

Figure 3-7. Season 1 (2006-2007) tall fescue shoot sections dry weight, recovered ^{15}N, percent of total plant N, and N concentrations. Each value is the mean±SE (error bars are visible when larger than the symbol size). Black-closed symbols are all samples combined (n=8, except Recovered ^{15}N values are n=4), gray-closed symbols are control samples (n=4), open symbols are ^{15}N-treated samples (n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between treatments (LSD_{trt}).

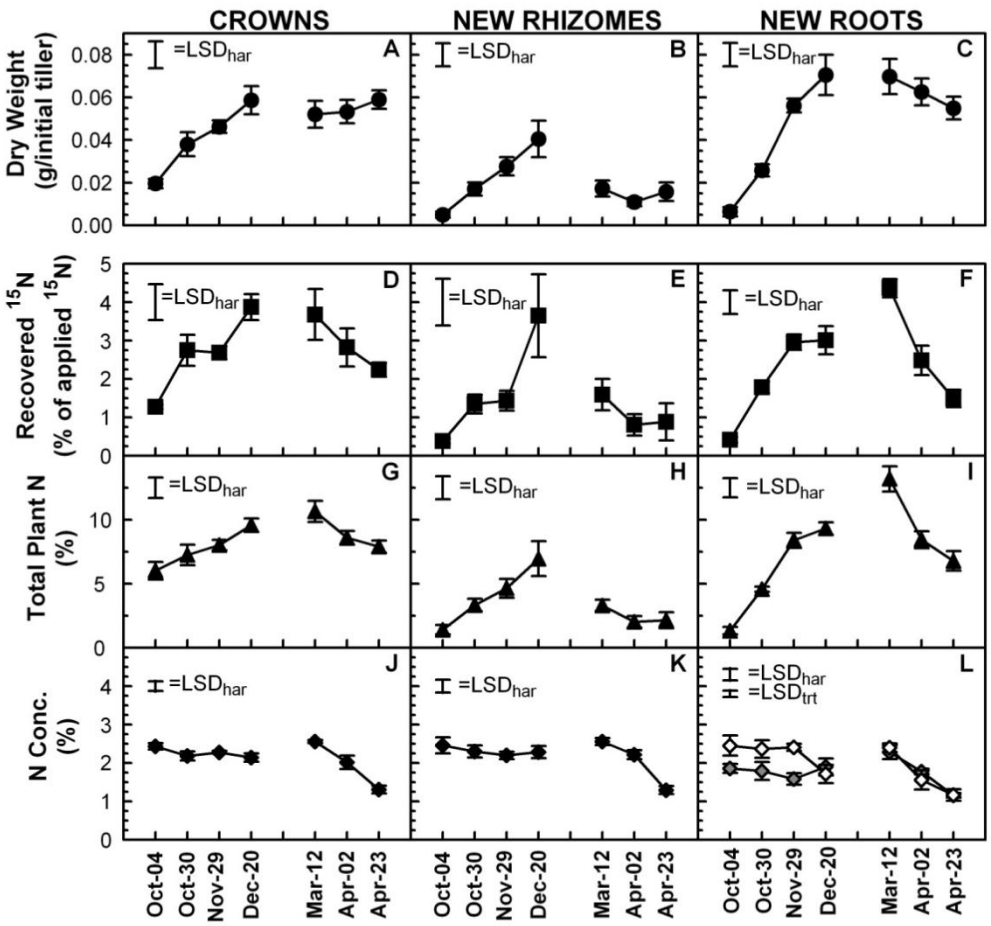

Figure 3-8. Season 1 (2006-2007) tall fescue belowground sections dry weight, recovered ^{15}N, percent of total plant N, and N concentrations. Each value is the mean±SE (error bars are visible when larger than the symbol size). Black-closed symbols are all samples combined (n=8, except Recovered ^{15}N values are n=4), gray-closed symbols are control samples (n=4), open symbols are ^{15}N-treated samples (n=4). Fisher's protected LSD (P<0.05) given between harvest dates (LSD_{har}) and between treatments (LSD_{trt}).

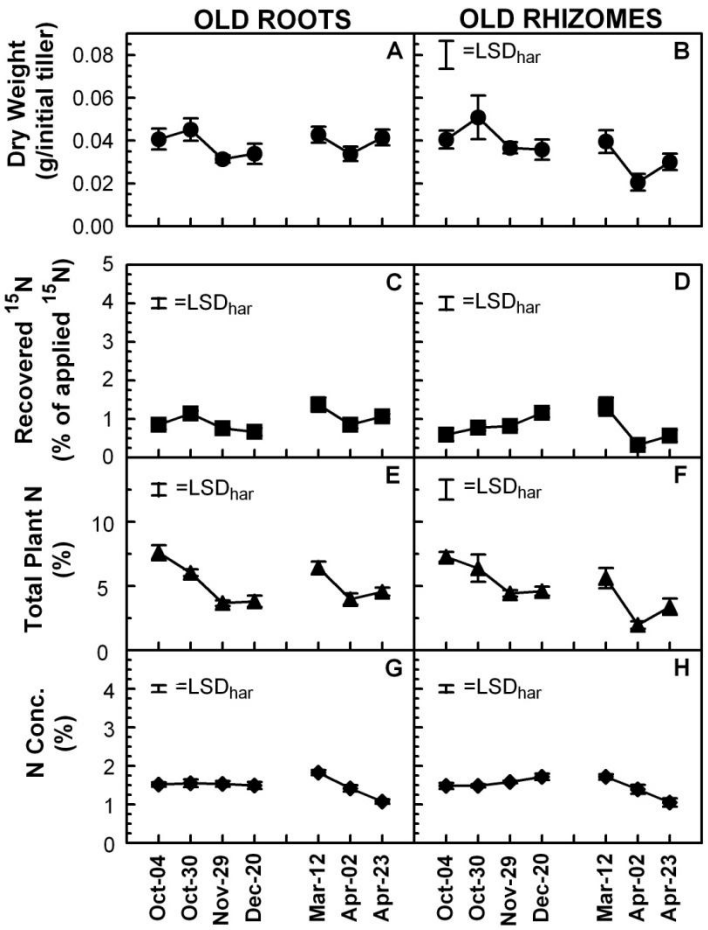

Figure 3-9. Season 1 (2006-2007) tall fescue old belowground sections dry weight, recovered ^{15}N, percent of total plant N, and N concentrations. Each value is the mean±SE (n=8, except Recovered ^{15}N values are n=4). Fisher's protected LSD (P<0.05) given between harvest dates (LSD_{har}).

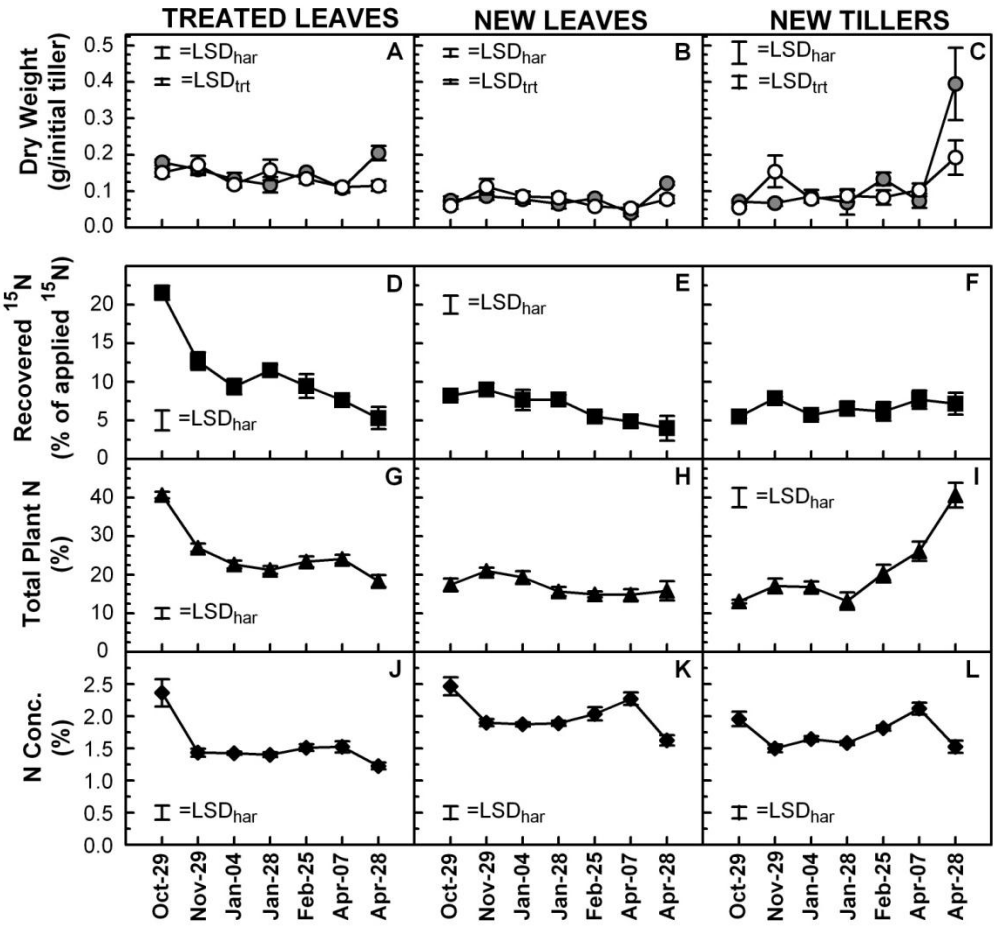

Figure 3-10. Season 2 (2007-2008) tall fescue shoot sections dry weight, recovered ^{15}N, percent of total plant N, and N concentrations. Each value is the mean±SE (error bars are visible when larger than the symbol size). Black-closed symbols are all samples combined (n=8, except Recovered ^{15}N values are n=4), gray-closed symbols are control samples (n=4), open symbols are ^{15}N-treated samples (n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between treatments (LSD_{trt}).

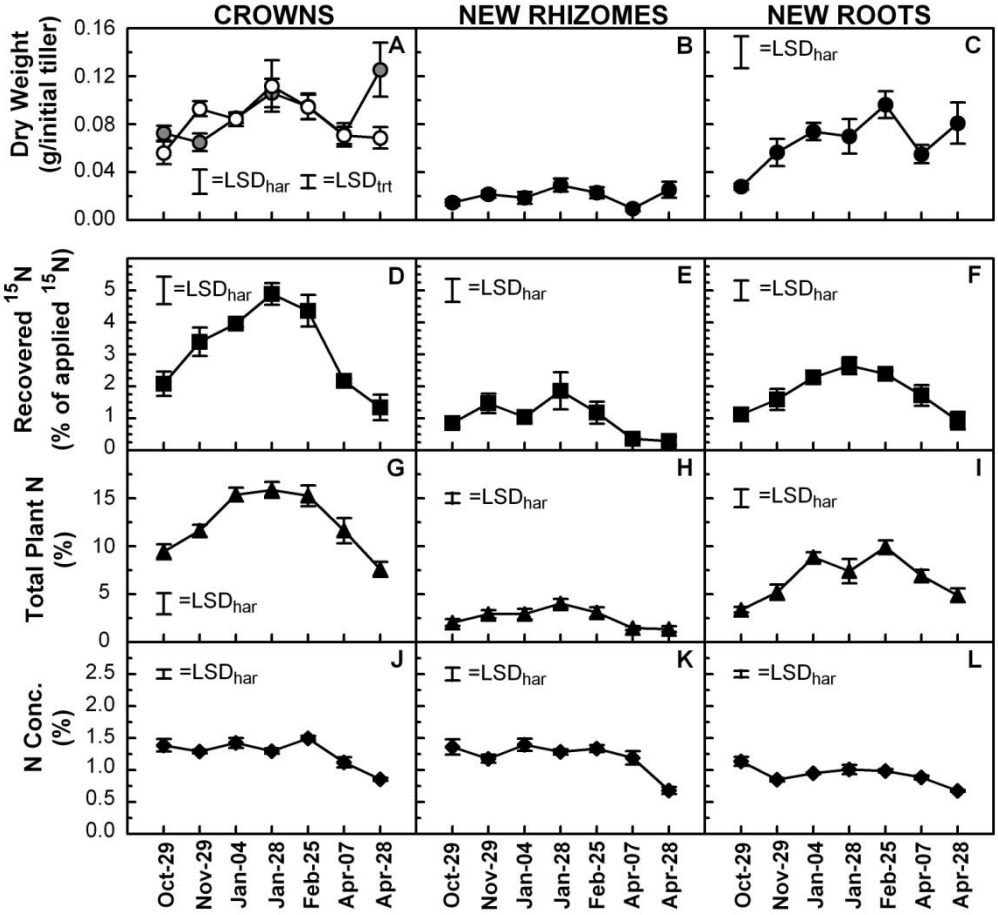

Figure 3-11. Season 2 (2007-2008) tall fescue belowground sections dry weight, recovered ^{15}N, percent of total plant N, and N concentrations. Each value is the mean±SE (error bars are visible when larger than the symbol size). Black-closed symbols are all samples combined (n=8, except Recovered ^{15}N values are n=4), gray-closed symbols are control samples (n=4), open symbols are ^{15}N-treated samples (n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between treatments (LSD_{trt}).

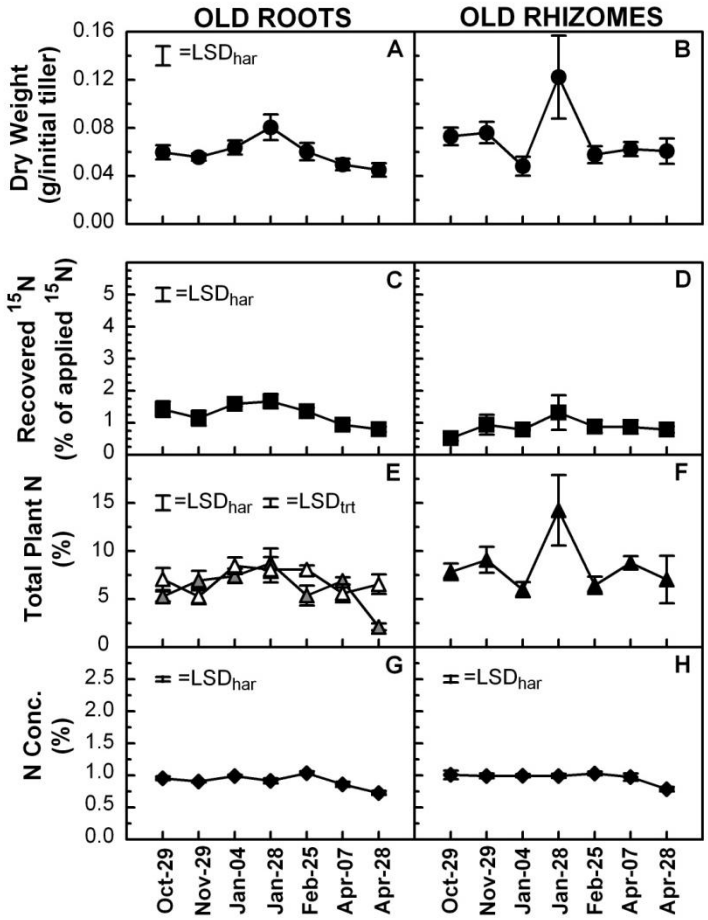

Figure 3-12. Season 2 (2007-2008) tall fescue old belowground sections dry weight, recovered ^{15}N, percent of total plant N, and N concentrations. Black-closed symbols are all samples combined (n=8, except Recovered ^{15}N values are n=4), gray-closed symbols are control samples (n=4), open symbols are ^{15}N-treated samples (n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between treatments (LSD_{trt}).

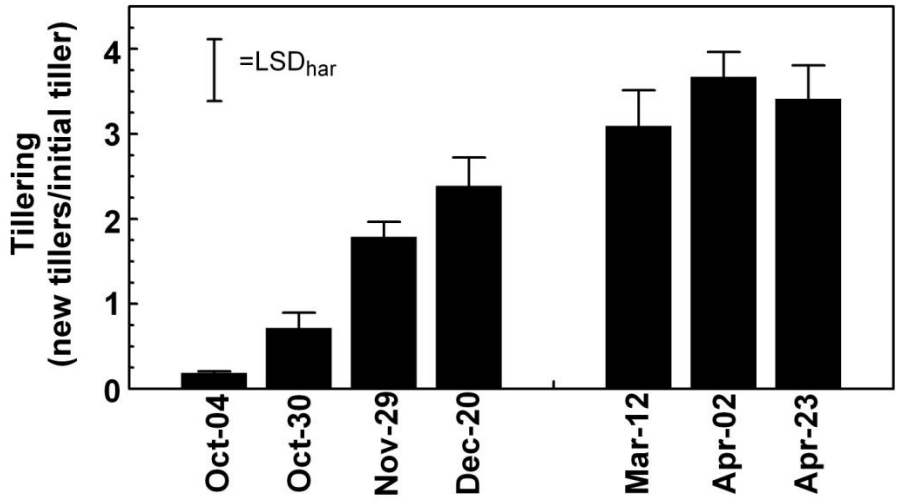

Figure 3-13. Tillering of tall fescue during Season 1 (2006-2007) harvests (values are mean±SE, error bars are visible when larger than the symbol size, n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}).

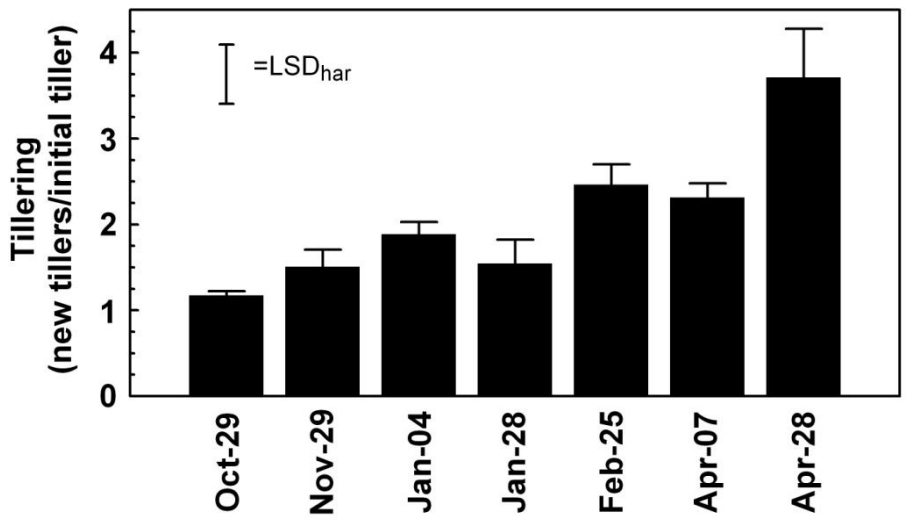

Figure 3-14. Tillering of tall fescue during Season 2 (2007-2008) harvests (values are mean±SE, error bars are visible when larger than the symbol size, n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}).

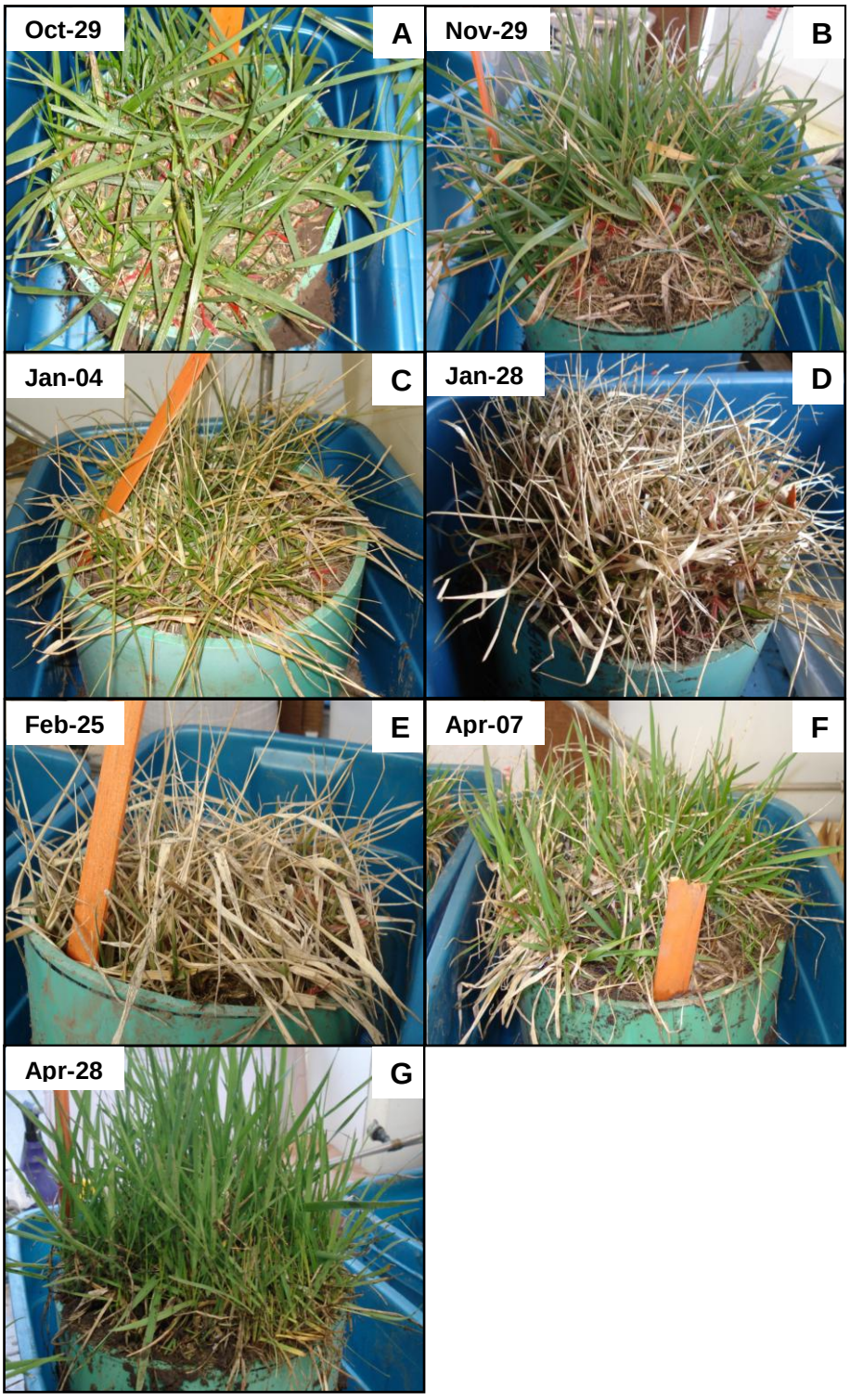

Figure 3-15. Tall fescue plots (in 25.4 cm diameter, 20 cm in depth PVC pipes) harvested throughout Season 2 (2007-2008).

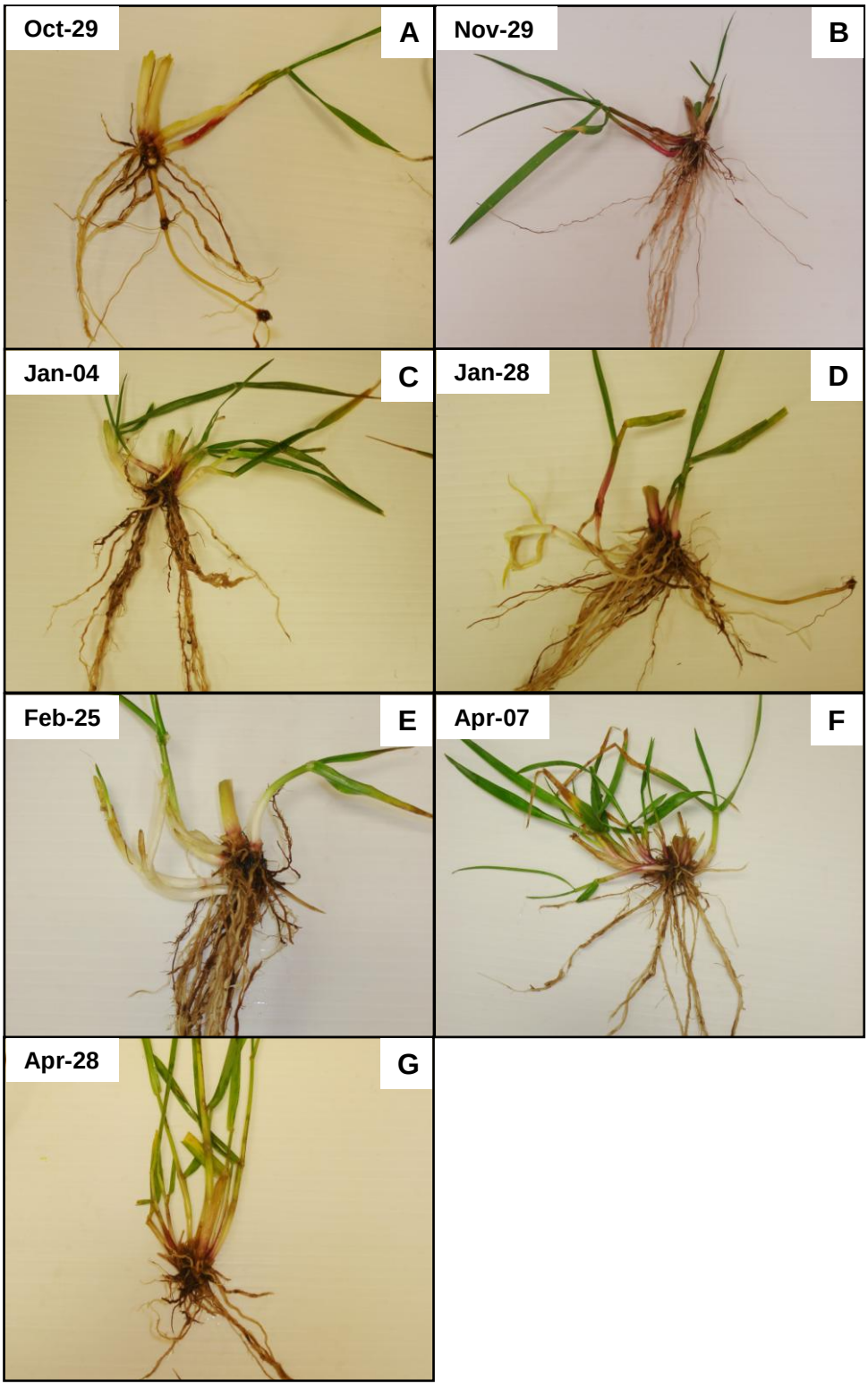

Figure 3-16. Tall fescue tillers harvested throughout Season 2 (2007-2008).

Recovered ^{15}N

More than 45% of the applied ^{15}N was recovered in the first harvest in Season 1 (Figure 3-17). The amount of ^{15}N recovered did not change through the winter harvests, but declined slightly in spring harvests. At first harvest on Oct-04, 80% of recovered ^{15}N was found in treated leaves. Throughout the season, ^{15}N declined in treated leaves and concurrently increased in other plant tissues (Figure 3-17). In Season 2, the initial ^{15}N recovery at first harvest (Oct-29) was about 40% of the applied ^{15}N and followed a similar decline in recovery as Season 1 (Figure 3-18). Again in Season 2, treated leaves initially had a majority of the applied ^{15}N, about 52% of the ^{15}N recovered, and this declined throughout the season as ^{15}N increased in other plant parts.

All plant segments contained ^{15}N at each harvest. In Season 1, the majority of ^{15}N was recovered in shoot sections (Figure 3-7D-F) compared to belowground sections (Figure 3-8D-F), while old roots and old rhizomes contained the least amount of ^{15}N of any plant segment (Figure 3-9C,D). At the first harvest, treated leaves contained the greatest amount of applied ^{15}N but declined by 87% throughout the season (Figure 3-7D). The ^{15}N label increased in new leaves by Oct-30 (Figure 3-7E) and increased in belowground sections of crowns (three-fold), new rhizomes (over nine-fold), and new roots (seven-fold) by Dec-20 (Figure 3-8D-F). New tillers were the greatest sink for ^{15}N from Nov-29 harvest through spring (Figure 3-7F), and by the final harvest on Apr-28, represented 52% of the ^{15}N recovered in plant parts other than treated leaves

(Figure 3-17). All belowground sections, including crowns, new rhizomes, new roots, old roots, and old rhizomes, declined in ^{15}N throughout the spring harvests (Mar-12 through Apr-23) (Figure 3-8D-F and 3-9C,D).

During Season 2, ^{15}N recovery followed similar patterns as Season 1, including a 75% decline of ^{15}N in treated leaves throughout the season with a majority of ^{15}N recovered in shoot sections (Figure 3-10D-F). Belowground segments of crowns, new rhizomes, and new roots increased more than two-fold in ^{15}N through Jan-28 followed by a decline throughout the spring (Figure 3-11D-F). By the last spring harvest on Apr-28, new tillers were again the largest ^{15}N sink, and had 46% of the recovered ^{15}N in parts other than treated leaves (Figure 3-18).

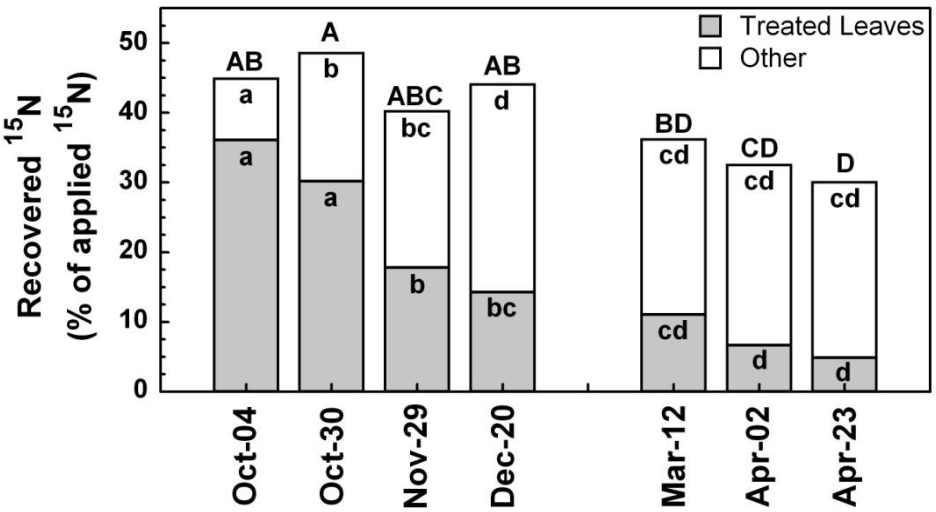

Figure 3-17. The percent recovery of applied ^{15}N in treated leaves and all other plant parts of tall fescue harvested throughout Season 1 (2006-2007). Values are means (n=4). Across harvest dates, different uppercase letters indicate different total values and different lowercase letters indicate different values within each plant segment according to Fisher's protected LSD when $P<0.05$.

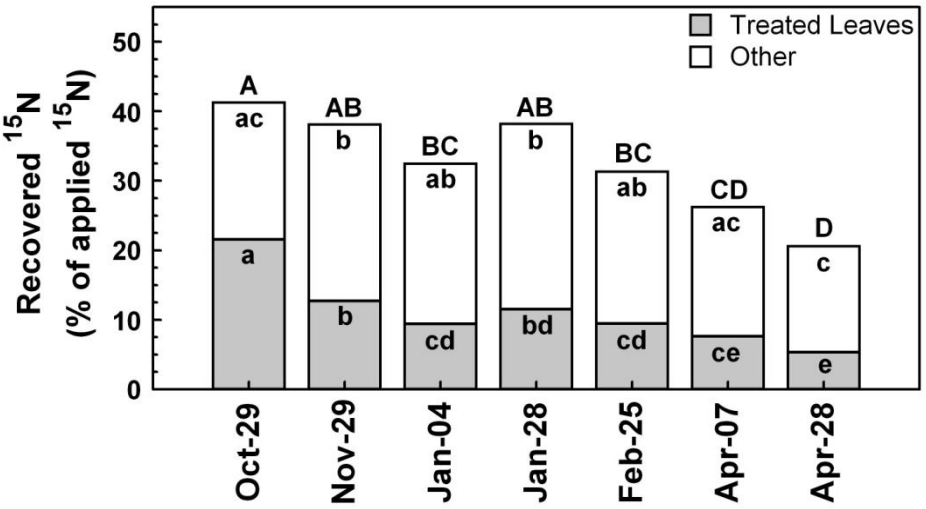

Figure 3-18. The percent recovery of applied ^{15}N in treated leaves and all other plant parts of tall fescue harvested throughout Season 2 (2007-2008). Values are means (n=4). Across harvest dates, different uppercase letters indicate different total values and different lowercase letters indicate different values within each plant segment according to Fisher's protected LSD when $P<0.05$.

Total Plant N

The allocation of N to plant segments in relation to the total plant N (% of total plant N) followed similar patterns as the recovered ^{15}N, declining in treated leaves while increasing in new tillers throughout Season 1 (Figure 3-7G,I) and Season 2 (Figure 3-10G,I). More N was allocated to crowns, new rhizomes, and new roots through the winter, but N in these plant parts declined in spring (Figures 3-8G-I and 3-11G-I). Also, allocation of N to old roots and old rhizomes declined throughout autumn and spring in Season 1 (Figure 3-9E,F).

N Concentration

The concentration of N in shoot segments generally declined through autumn and winter in Season 1 (Figure 3-7J-L) and through autumn in Season 2 (Figure 3-10J-L). Compared to all other plant parts, treated leaves showed the greatest decline in N concentration over the entire season, declining 65% through Season 1, and 48% in Season 2. Unlike shoot segments, belowground structures did not exhibit a change in N concentration through autumn and winter (Figures 3-8J-L; 3-9G,H; 3-11J-L; 3-12G,H). However, the N concentration of all segments declined in spring during both seasons (Figures 3-7J-L; 3-8J-L; 3-9G,H; 3-10J-L; 3-11J-L; 3-12G,H).

Soluble Protein, Free Amino Acids, and Nitrate Concentration

The soluble protein concentration of treated leaves declined sharply from the initial autumn harvest until the end of November, 56 and 60%, in Season 1 and 2, respectively (Figures 3-19A and 3-22A). New leaves and new tillers also exhibited a decline in soluble protein throughout the seasons with the exception of a brief spike in concentration in early April (Figures 3-19B,C and 3-22B,C). Belowground sections did not change in soluble protein concentration from autumn through winter (Figures 3-20A-C; 3-21A,B; 3-23A-C; 3-24A,B) except for an increase in new rhizomes and new roots in Season 1 Oct-30 harvest (Figure 3-20B,C). In general, soluble protein of all belowground sections declined during spring (Figures 3-20A-C; 3-21A,B; 3-23A-C; 3-24A,B). Soluble protein concentrations decreased in crowns, new rhizomes, and new roots 45, 39, and 28%, respectively in Season 1, and 45, 50, and 40% in Season 2, during the last three harvests in spring (Figures 3-20A-C and 3-23A-C).

Overall, free amino acid concentrations were greater in Season 1 plant segments than in Season 2, but the seasonal profiles were similar (Figures 3-19A-C; 3-20D-F; 3-21C,D; 3-22A-C; 3-23D-F; 3-24C,D). The free amino acid concentration of shoot sections generally remained unchanged until the spring months when a significant decline occurred (Figures 3-19D-F and 3-22D-F). However, crowns, new rhizomes, and new roots gradually increased in free amino acid concentration 28, 28, and 82%, respectively, in Season 1, and 21, 88, and 85%, respectively, in Season 2, throughout autumn and winter before

declining dramatically in the spring 69, 67, and 80% in Season 1 and 61, 53,and 68% in Season 2 (Figures 3-20D-F and 3-22D-F).

All plant segments showed little to no change in nitrate concentrations during autumn and winter months in Season 1 before declining in spring (Figures 3-19G-H; 3-20G-H; 3-21E,F), while in Season 2 nitrate concentrations declined steadily from Nov-29 through Apr-07 (Figures 3-22G-I; 3-23G-I; 3-24E,F). During spring of both seasons, nitrate concentrations of the belowground segments crowns and new rhizomes decreased about 30% while new roots decreased about 50% (Figures 3-20D-F and 3-22D-F). In both seasons, shoot section nitrate concentrations decreased slightly in early April (Figures 3-19G-I and 3-22G-I).

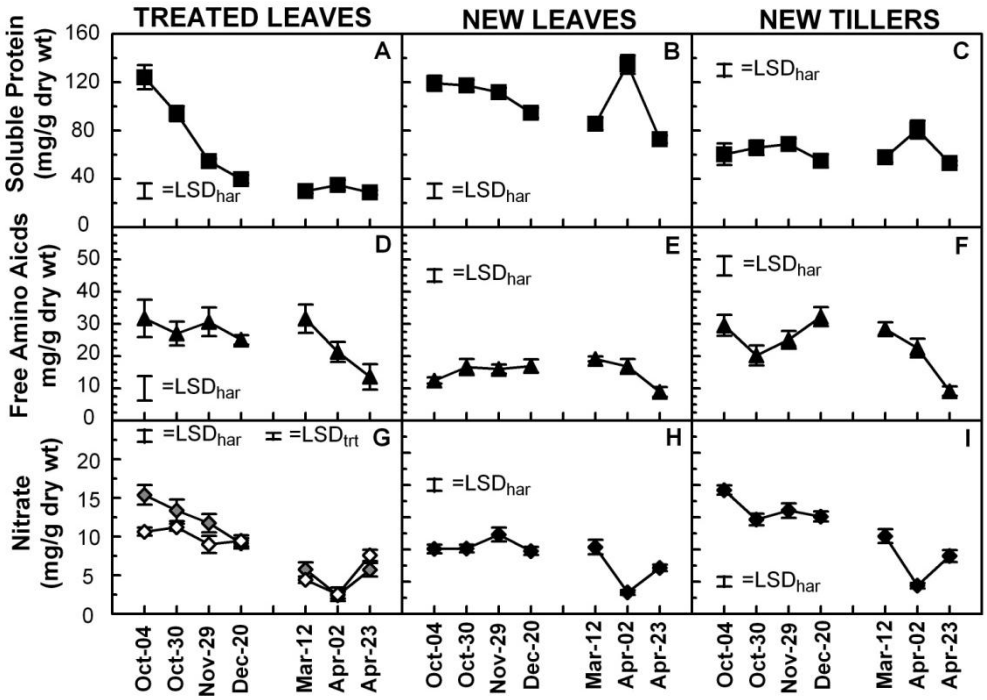

Figure 3-19. Soluble protein, free amino acids, and nitrate concentrations of tall fescue shoot sections in Season 1 (2006-2007). Each value is the mean±SE (error bars are visible when larger than the symbol size). Black-closed symbols are all samples combined (n=8), gray-closed symbols are control samples (n=4), open symbols are ^{15}N-treated samples (n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between treatments (LSD_{trt}).

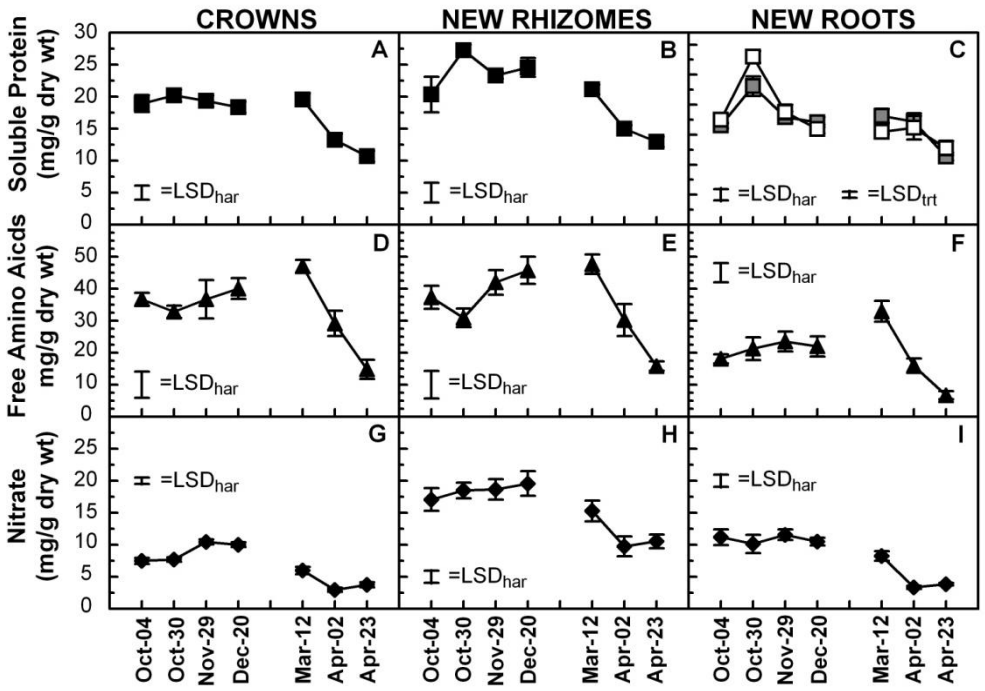

Figure 3-20. Soluble protein, free amino acids, and nitrate concentrations of tall fescue belowground sections in Season 1 (2006-2007). Each value is the mean±SE (error bars are visible when larger than the symbol size). Black-closed symbols are all samples combined (n=8), gray-closed symbols are control samples (n=4), open symbols are ^{15}N-treated samples (n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between treatments (LSD_{trt}).

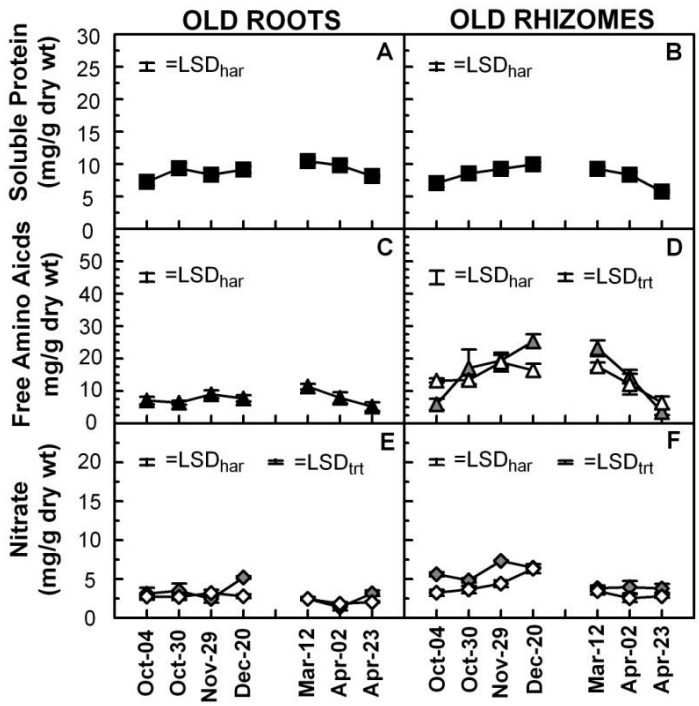

Figure 3-21. Soluble protein, free amino acids, and nitrate concentrations of tall fescue old belowground sections in Season 1 (2006-2007). Each value is the mean±SE (error bars are visible when larger than the symbol size). Black-closed symbols are all samples combined (n=8), gray-closed symbols are control samples (n=4), open symbols are ^{15}N-treated samples (n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between treatments (LSD_{trt}).

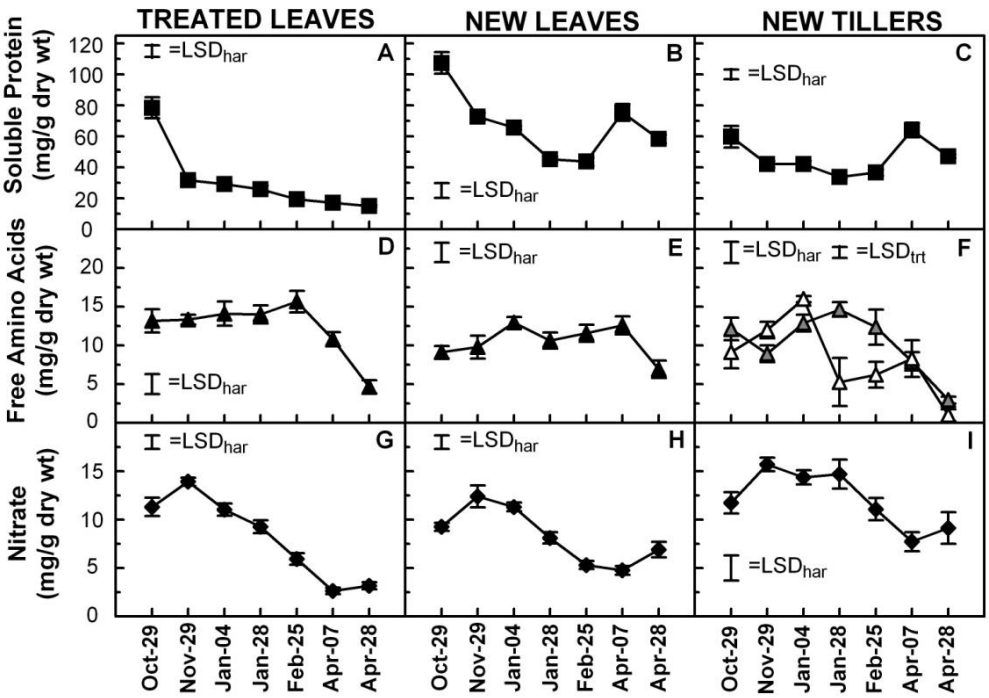

Figure 3-22. Soluble protein, free amino acids, and nitrate concentrations of tall fescue shoot sections in Season 2 (2007-2008). Each value is the mean±SE (error bars are visible when larger than the symbol size). Black-closed symbols are all samples combined (n=8), gray-closed symbols are control samples (n=4), open symbols are ^{15}N-treated samples (n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between treatments (LSD_{trt}).

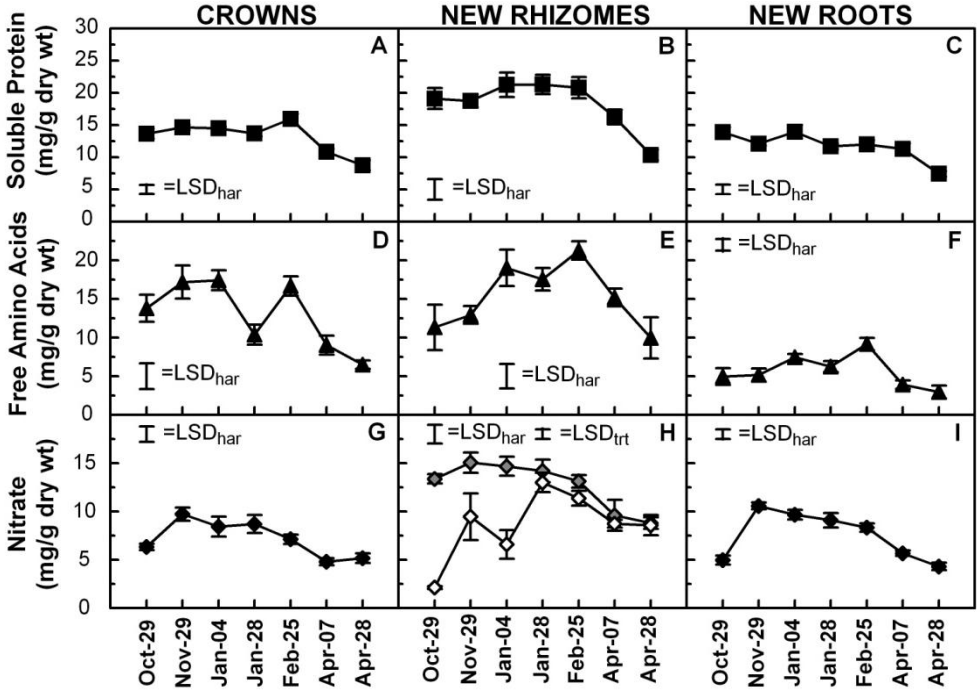

Figure 3-23. Soluble protein, free amino acids, and nitrate concentrations of tall fescue belowground sections in Season 2 (2007-2008). Each value is the mean±SE (error bars are visible when larger than the symbol size). Black-closed symbols are all samples combined (n=8), gray-closed symbols are control samples (n=4), open symbols are ^{15}N-treated samples (n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between treatments (LSD_{trt}).

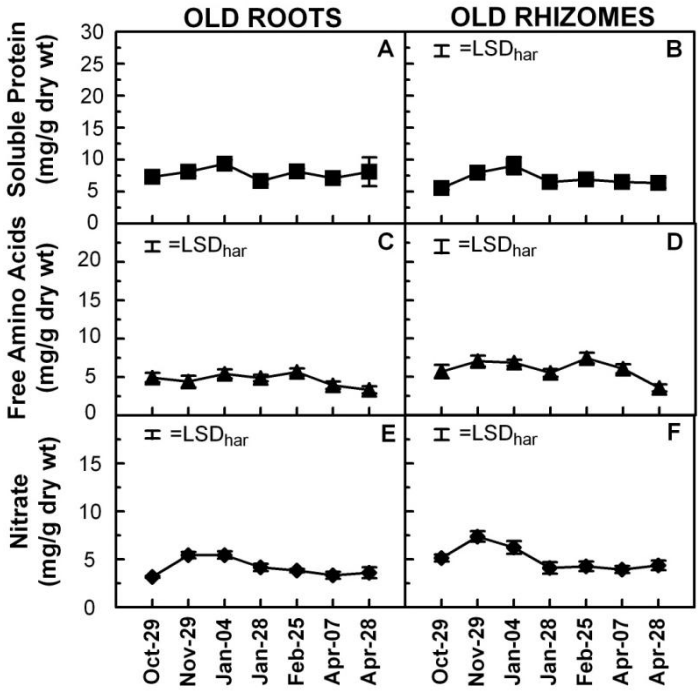

Figure 3-24. Soluble protein, free amino acids, and nitrate concentrations of tall fescue old belowground sections in Season 2 (2007-2008). Each value is the mean±SE (n=8, error bars are visible when larger than the symbol size). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}).

Free Amino Acid Profiles

The free amino acid profile of both crown and new root samples were analyzed from three harvests: the first harvest, the harvest with the greatest free amino acid concentration, and the last harvest in April (Oct-04, Mar-12, and Apr-23 in Season 1, and Oct-29, Feb-25, and Apr-28 in Season 2). In crowns, asparagine was the most prevalent amino acid at the first harvest in Oct, representing 47 and 41% of the free amino acids in Season 1 and 2, respectively (Tables 3-1 and 3-3). Other prevalent amino acids in crowns were glutamine, at 18 and 13%, and arginine, at 11 and 17%, in Season 1 and 2, respectively (Tables 3-1 and 3-3). The total amount of free amino acids in crowns increased through the winter months. By Mar-12 in Season 1, asparagine and glutamine were the most abundant at 58 and 17%, respectively (Table 3-1), and by Feb-25 in Season 2 represented 40 and 23%, respectively (Table 3-3). Following these harvest dates, the concentration of total free amino acids decreased in crowns by the end of April, to values only 37% and 9% of the greatest concentrations in Season 1 and 2, respectively (Tables 3-1 and 3-3). In both seasons, asparagine represented 41-47% of this decline in total amino acids, while glutamine represented about 25%.

Asparagine and glutamine also dominated the free amino acid pool in new roots, representing 39 and 17%, respectively, in October of Season 1. The total amount of free amino acids increased more than 2-fold by Mar-12, with asparagine and glutamine concentrations increasing to 53 and 23%, respectively

(Table 3-2). Season 2 new roots showed similar profiles with a >2.5-fold increase in free amino acids from October to Feb-25, resulting in asparagine comprising 29% and glutamine 18% of the total amino acids (Table 3-4). As with crowns, by the end of April in both seasons, the total free amino acid concentrations decreased in new roots to values of about 10% of the greatest concentrations, with asparagine representing 53 and 32% and glutamine 24 and 20% of this decline in Season 1 and 2, respectively (Tables 3-2 and 3-4).

Table 3-1. Free amino acid concentrations in crowns of tall fescue during Season 1, from control plots harvested on Oct-04, Mar-12, and Apr-23 (2006-2007). Fisher's protected LSD ($P<0.05$, $LSD_{0.05}$) given between and within harvest dates, NS=not significant. Values are means (n=3).

CROWNS (2006-2007)							
	Oct-04		**Mar-12**		**Apr-23**		**$LSD_{0.05}$**
	----------mg/g dry weight (fraction of total)----------						
Alanine	0.89	(0.02)	0.78	(0.01)	0.35	(0.02)	0.20
Arginine	5.23	(0.11)	0.89	(0.02)	0.14	(0.01)	1.34
Asparagine	22.86	(0.47)	32.87	(0.58)	16.12	(0.78)	7.38
Aspartic Acid	0.70	(0.01)	0.70	(0.01)	0.42	(0.02)	0.17
Glutamic Acid	2.32	(0.05)	4.04	(0.07)	1.08	(0.05)	1.46
Glutamine	8.67	(0.18)	9.83	(0.17)	0.85	(0.04)	3.28
Proline	2.21	(0.05)	1.28	(0.02)	0.02	(0.00)	0.50
Serine	0.43	(0.01)	0.82	(0.01)	0.14	(0.01)	0.15
γ-amino-butyric acid	1.12	(0.02)	1.23	(0.02)	0.53	(0.03)	0.29
1-methyl-histidine	0.18	(0.00)	0.13	(0.00)	0.02	(0.00)	0.05
3-methyl-histine	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	NS
Anserine	-	-	-	-	-	-	-
Carnosine	-	-	-	-	-	-	-
Citrulline	-	-	-	-	-	-	-
Cystathionine/allocystathionine	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	NS
Cystine	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	NS
Ethanolamine	0.11	(0.00)	0.10	(0.00)	0.06	(0.00)	0.02
Glycine	0.05	(0.00)	0.06	(0.00)	0.03	(0.00)	0.01
Histidine	0.46	(0.01)	0.47	(0.01)	0.08	(0.00)	0.11
Homocystine	-	-	-	-	-	-	-
Hydroxylysine	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	NS
Hydroxyproline	-	-	-	-	-	-	-
Isoleucine	0.26	(0.01)	0.26	(0.00)	0.05	(0.00)	0.08
Leucine	0.30	(0.01)	0.23	(0.00)	0.09	(0.00)	0.05
Lysine	0.56	(0.01)	0.24	(0.00)	0.09	(0.00)	0.07
Methionine	0.03	(0.00)	0.02	(0.00)	0.01	(0.00)	0.01
Ornithine	0.04	(0.00)	0.02	(0.00)	0.02	(0.00)	0.01
Phenylalanine	0.05	(0.00)	0.08	(0.00)	0.03	(0.00)	0.02
Phosphoethanolamine	0.07	(0.00)	0.04	(0.00)	0.05	(0.00)	NS
Phosphoserine	0.14	(0.00)	0.12	(0.00)	0.08	(0.00)	0.03
Sarcosine	0.41	(0.01)	0.38	(0.01)	0.03	(0.00)	0.15
Taurine	0.05	(0.00)	0.09	(0.00)	0.02	(0.00)	NS
Threonine	0.64	(0.01)	0.71	(0.01)	0.16	(0.01)	0.19
Tryptophan	0.13	(0.00)	0.33	(0.01)	0.12	(0.01)	0.15
Tyrosine	0.05	(0.00)	0.09	(0.00)	0.03	(0.00)	0.02
Urea	0.41	(0.01)	0.14	(0.00)	0.00	(0.00)	0.13
Valine	0.44	(0.01)	0.48	(0.01)	0.10	(0.00)	0.14
α-amino-adipic acid	0.08	(0.00)	0.03	(0.00)	0.01	(0.00)	0.02
α-amino-n-butyric acid	0.06	(0.00)	0.04	(0.00)	0.02	(0.00)	0.01
β-alanine	0.02	(0.00)	0.00	(0.00)	0.00	(0.00)	0.01
β-amino-isobutyric acid	0.02	(0.00)	0.02	(0.00)	0.01	(0.00)	0.00
TOTAL	48.98		56.53		20.75		9.73
$LSD_{0.05}$	0.52		0.91		1.34		

Table 3-2. Free amino acid concentrations in new roots of tall fescue during Season 1, from control plots harvested on Oct-04, Mar-12, and Apr-23 (2006-2007). Fisher's protected LSD ($P<0.05$, $LSD_{0.05}$) given between and within harvest dates, NS= not significant. Values are means (n=3).

NEW ROOTS (2006-2007)							
	Oct-04		**Mar-12**		**Apr-23**		**$LSD_{0.05}$**
	----------mg/g dry weight (fraction of total)----------						
Alanine	0.43	(0.02)	1.19	(0.02)	0.14	(0.03)	0.17
Arginine	0.58	(0.02)	0.42	(0.01)	0.09	(0.02)	0.17
Asparagine	10.24	(0.39)	29.07	(0.53)	2.66	(0.55)	7.66
Aspartic Acid	0.75	(0.03)	1.42	(0.03)	0.24	(0.05)	0.47
Glutamic Acid	2.37	(0.09)	2.74	(0.05)	0.39	(0.08)	NS
Glutamine	4.48	(0.17)	12.44	(0.23)	0.35	(0.07)	4.69
Proline	2.63	(0.10)	0.52	(0.01)	0.00	(0.00)	0.17
Serine	0.36	(0.01)	1.45	(0.03)	0.10	(0.02)	0.23
γ-amino-butyric acid	0.71	(0.03)	0.78	(0.01)	0.26	(0.06)	0.32
1-methyl-histidine	0.09	(0.00)	0.09	(0.00)	0.00	(0.00)	0.04
3-methyl-histine	-	-	-	-	-	-	-
Anserine	0.00	(0.00)	0.02	(0.00)	0.00	(0.00)	NS
Carnosine	-	-	-	-	-	-	-
Citrulline	-	-	-	-	-	-	-
Cystathionine/allocystathionine	-	-	-	-	-	-	-
Cystine	0.00	(0.00)	0.01	(0.00)	0.00	(0.00)	NS
Ethanolamine	0.07	(0.00)	0.08	(0.00)	0.05	(0.01)	NS
Glycine	0.07	(0.00)	0.13	(0.00)	0.02	(0.00)	0.03
Histidine	0.23	(0.01)	0.39	(0.01)	0.02	(0.00)	0.11
Homocystine	-	-	-	-	-	-	-
Hydroxylysine	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	NS
Hydroxyproline	-	-	-	-	-	-	-
Isoleucine	0.24	(0.01)	0.39	(0.01)	0.03	(0.01)	0.12
Leucine	0.29	(0.01)	0.24	(0.00)	0.05	(0.01)	0.09
Lysine	0.23	(0.01)	0.18	(0.00)	0.04	(0.01)	0.07
Methionine	0.02	(0.00	0.01	(0.00)	0.00	(0.00)	0.01
Ornithine	0.02	(0.00)	0.03	(0.00)	0.01	(0.00)	NS
Phenylalanine	0.04	(0.00)	0.07	(0.00)	0.02	(0.00)	NS
Phosphoethanolamine	0.06	(0.00)	0.05	(0.00)	0.05	(0.01)	NS
Phosphoserine	0.12	(0.00)	0.23	(0.00)	0.09	(0.02)	0.06
Sarcosine	0.34	(0.01)	0.68	(0.01)	0.02	(0.00)	0.09
Taurine	-	-	-	-	-	-	-
Threonine	0.51	(0.02)	0.76	(0.01)	0.06	(0.01)	0.23
Tryptophan	0.07	(0.00)	0.20	(0.00)	0.00	(0.00)	0.07
Tyrosine	0.06	(0.00)	0.14	(0.00)	0.00	(0.00)	NS
Urea	0.60	(0.02)	0.38	(0.01)	0.00	(0.00)	0.18
Valine	0.43	(0.02)	0.69	(0.01)	0.06	(0.01)	0.17
α-amino-adipic acid	0.06	(0.00)	0.01	(0.00)	0.01	(0.00)	0.02
α-amino-n-butyric acid	0.04	(0.00)	0.04	(0.00)	0.02	(0.00)	0.01
β-alanine	0.03	(0.00)	0.00	(0.00)	0.00	(0.00)	0.01
β-amino-isobutyric acid	0.02	(0.00)	0.02	(0.00)	0.00	(0.00)	0.01
TOTAL	26.20		54.90		4.80		15.04
$LSD_{0.05}$	0.43		1.96		0.71		

Table 3-3. Free amino acid concentrations in crowns of tall fescue during Season 2, from control plots harvested on Oct-29, Feb-26, and Apr-28 (2007-2008). Fisher's protected LSD ($P<0.05$, $LSD_{0.05}$) given between and within harvest dates, NS= not significant. Values are means (n=3).

CROWNS (2007-2008)							
	Oct-29		**Feb-25**		**Apr-28**		**$LSD_{0.05}$**
	----------mg/g dry weight (fraction of total)----------						
Alanine	0.44	(0.04)	0.91	(0.04)	0.20	(0.09)	0.19
Arginine	2.12	(0.17)	1.76	(0.07)	0.06	(0.03)	NS
Asparagine	5.01	(0.41)	10.17	(0.40)	0.59	(0.26)	2.85
Aspartic Acid	0.23	(0.02)	0.45	(0.02)	0.09	(0.04)	0.08
Glutamic Acid	0.45	(0.04)	0.79	(0.03)	0.24	(0.10)	0.21
Glutamine	1.59	(0.13)	5.85	(0.23)	0.15	(0.07)	1.53
Proline	0.22	(0.02)	0.94	(0.04)	0.00	(0.00)	0.16
Serine	0.23	(0.02)	0.91	(0.04)	0.08	(0.04)	0.21
γ-amino-butyric acid	0.72	(0.06)	1.20	(0.05)	0.38	(0.17)	0.24
1-methyl-histidine	0.03	(0.00)	0.11	(0.00)	0.00	(0.00)	0.03
3-methyl-histine	-	-	-	-	-	-	-
Anserine	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	-
Carnosine	-	-	-	-	-	-	-
Citrulline	-	-	-	-	-	-	-
Cystathionine/allocystathionine	-	-	-	-	-	-	-
Cystine	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	NS
Ethanolamine	0.09	(0.01)	0.09	(0.00)	0.06	(0.03)	NS
Glycine	0.02	(0.00)	0.04	(0.00)	0.02	(0.01)	0.01
Histidine	0.10	(0.01)	0.27	(0.01)	0.02	(0.01)	0.08
Homocystine	-	-	-	-	-	-	-
Hydroxylysine	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	NS
Hydroxyproline	-	-	-	-	-	-	-
Isoleucine	0.05	(0.00)	0.16	(0.01)	0.02	(0.01)	0.03
Leucine	0.09	(0.01)	0.14	(0.01)	0.04	(0.02)	0.02
Lysine	0.16	(0.01)	0.26	(0.01)	0.04	(0.02)	0.10
Methionine	0.02	(0.00)	0.01	(0.00)	0.01	(0.00)	NS
Ornithine	0.02	(0.00)	0.02	(0.00)	0.01	(0.01)	0.00
Phenylalanine	0.03	(0.00)	0.06	(0.00)	0.02	(0.01)	0.01
Phosphoethanolamine	0.07	(0.01)	0.08	(0.00)	0.05	(0.02)	0.01
Phosphoserine	0.09	(0.01)	0.10	(0.00)	0.06	(0.03)	0.02
Sarcosine	0.10	(0.01)	0.17	(0.01)	0.00	(0.00)	NS
Taurine	0.03	(0.00)	0.05	(0.00)	0.02	(0.01)	NS
Threonine	0.14	(0.01)	0.33	(0.01)	0.05	(0.02)	0.06
Tryptophan	0.03	(0.00)	0.33	(0.01)	0.00	(0.00)	0.10
Tyrosine	0.03	(0.00)	0.06	(0.00)	0.01	(0.01)	0.01
Urea	-	-	-	-	-	-	-
Valine	0.10	(0.01)	0.31	(0.01)	0.04	(0.02)	0.05
α-amino-adipic acid	0.02	(0.00)	0.02	(0.00)	0.00	(0.00)	0.01
α-amino-n-butyric acid	0.03	(0.00)	0.03	(0.00)	0.01	(0.00)	0.01
β-alanine	-	-	-	-	-	-	-
β-amino-isobutyric acid	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	0.00
TOTAL	12.22		25.61		2.29		4.79
$LSD_{0.05}$	0.67		0.49		0.11		

Table 3-4. Free amino acid concentrations in new roots of tall fescue during Season 2, from control plots harvested on Oct-29, Feb-26, and Apr-28 (2007-2008). Fisher's protected LSD ($P<0.05$, $LSD_{0.05}$) given between and within harvest dates, NS= not significant. Values are means (n=3).

NEW ROOTS (2007-2008)							
	Oct-29		**Feb-25**		**Apr-28**		**$LSD_{0.05}$**
	----------mg/g dry weight (fraction of total)----------						
Alanine	0.18	(0.04)	0.93	(0.09)	0.09	(0.08)	0.31
Arginine	0.16	(0.04)	0.17	(0.02)	0.06	(0.05)	0.07
Asparagine	1.07	(0.27)	2.96	(0.29)	0.06	(0.05)	0.97
Aspartic Acid	0.19	(0.05)	0.58	(0.06)	0.06	(0.05)	0.14
Glutamic Acid	0.57	(0.14)	0.89	(0.09)	0.19	(0.17)	0.30
Glutamine	0.32	(0.08)	1.89	(0.18)	0.08	(0.07)	0.38
Proline	0.19	(0.05)	0.12	(0.01)	0.00	(0.00)	NS
Serine	0.14	(0.03)	0.84	(0.08)	0.06	(0.05)	0.12
γ-amino-butyric acid	0.31	(0.08)	0.39	(0.04)	0.15	(0.13)	0.10
1-methyl-histidine	0.01	(0.00)	0.03	(0.00)	0.00	(0.00)	0.00
3-methyl-histine	-	-	-	-	-	-	-
Anserine	-	-	-	-	-	-	-
Carnosine	-	-	-	-	-	-	-
Citrulline	-	-	-	-	-	-	-
Cystathionine/allocystathionine	-	-	-	-	-	-	-
Cystine	0.01	(0.00)	0.00	(0.00)	0.00	(0.00)	NS
Ethanolamine	0.10	(0.02)	0.07	(0.01)	0.05	(0.04)	0.01
Glycine	0.03	(0.01)	0.06	(0.01)	0.01	(0.01)	0.02
Histidine	0.03	(0.01)	0.09	(0.01)	0.01	(0.01)	0.01
Homocystine	-	-	-	-	-	-	-
Hydroxylysine	-	-	-	-	-	-	-
Hydroxyproline	-	-	-	-	-	-	-
Isoleucine	0.05	(0.01)	0.14	(0.01)	0.02	(0.01)	0.02
Leucine	0.08	(0.02)	0.10	(0.01)	0.03	(0.03)	0.02
Lysine	0.05	(0.01)	0.07	(0.01)	0.03	(0.02)	0.01
Methionine	0.01	(0.00)	0.01	(0.00)	0.00	(0.00)	0.01
Ornithine	0.02	(0.00)	0.02	(0.00)	0.01	(0.01)	NS
Phenylalanine	0.02	(0.00)	0.03	(0.00)	0.01	(0.01)	0.01
Phosphoethanolamine	0.06	(0.01)	0.08	(0.01)	0.04	(0.03)	NS
Phosphoserine	0.08	(0.02)	0.12	(0.01)	0.06	(0.05)	0.02
Sarcosine	0.11	(0.03)	0.16	(0.02)	0.00	(0.00)	NS
Taurine	0.01	(0.00)	0.00	(0.00)	0.00	(0.00)	NS
Threonine	0.07	(0.02)	0.19	(0.02)	0.03	(0.02)	0.03
Tryptophan	-	-	-	-	-	-	-
Tyrosine	0.02	(0.00)	0.06	(0.01)	0.00	(0.00)	0.02
Urea	0.05	(0.01)	0.00	(0.00)	0.04	(0.03)	NS
Valine	0.09	(0.02)	0.27	(0.03)	0.04	(0.03)	0.03
α-amino-adipic acid	0.02	(0.00)	0.01	(0.00)	0.00	(0.00)	NS
α-amino-n-butyric acid	0.02	(0.01)	0.02	(0.00)	0.01	(0.01)	NS
β-alanine	-	-	-	-	-	-	-
β-amino-isobutyric acid	-	-	-	-	-	-	-
TOTAL	4.03		10.31		1.16		2.22
$LSD_{0.05}$	0.21		0.16		0.02		

Leaf N Remobilization

In Season 1, the N concentration of treated leaves decreased 65% from the first harvest on Oct-04 through the last harvest on Apr-23. The 65% decrease includes all N losses from the leaf tissue, including leaching and remobilization. Estimates of N remobilization based on ^{15}N changes in treated leaves and other tissues from ^{15}N treatment to the last harvest were that 56% of treated leaf N remobilized to other plant tissues from the time of ^{15}N treatment application (Sept-19) to the last harvest (Apr-23). The more conservative estimate from first harvest to last harvest in April was 45% of the treated leaf N was remobilized to other plant treatments from the first harvest (Oct-04) to the last harvest (Apr-23).

In Season 2, the N concentration of treated leaves decreased 48% from the first harvest on Oct-29 through the last harvest on Apr-28. When estimated from the time of treatment, 37% of treated leaf N was remobilized from Oct-01 through Apr-28 in Season 2. Estimates cannot be based on values from first harvest to last harvest because there was no difference of ^{15}N recovery in other tissues between these harvests.

Estimates based on the initial N:^{15}N ratio in treated leaves are also limited to only Season 1 because no further ^{15}N remobilization from treated leaves to other plant parts took place after the first harvest in Season 2. In Season 1, more than 60% of treated leaf N was remobilized within the tall fescue plants from the first harvest on Oct-04 through December (Figure 3-25). About half of

the remobilized N from treated leaves was found in new tillers, while new roots, new rhizomes, and crowns each contained about 15% of the remobilized N in December. Old root and old rhizomes were the smallest sink for leaf N throughout the entire stockpiling period. New roots were the major belowground sink of remobilized leaf N in March. By spring, about 47% of the treated leaf N was accounted for in other plant parts. All belowground segments in spring were reduced in treated leaf N compared to winter levels. By the end of April, new tillers contained about 75% of the remobilized N.

Remobilized N from treated leaves was estimated to supply more than 40% of N in new tiller growth by Dec-20, and about 70% by Apr-23 in Season 1 (Figure 3-26). The N content in new roots was about 20% from treated leaf N by December and March, while it decreased in spring to about 10%. Up to 20% of new rhizome N was remobilized from treated leaves by Dec-20, but sharply declined by March. The percent of leaf N in crowns remained fairly stable at about 5-10% through then entire season, while leaf N supply to old roots and old rhizomes was negligible.

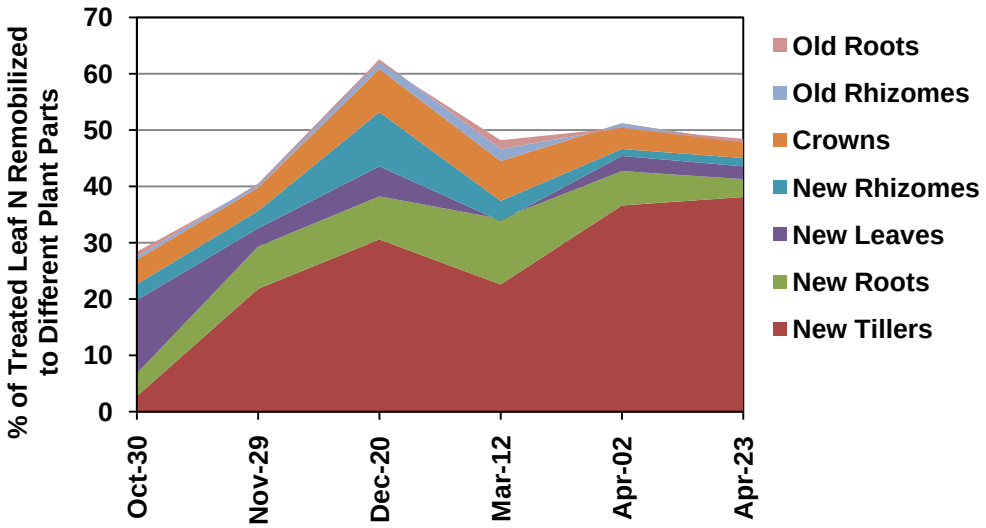

Figure 3-25. The percent of treated leaf N remobilized to different plant parts since the first harvest (Oct-04) in Season 1 (2006-2007) stockpiled tall fescue plants grown at BREC, near Columbia, MO. Values are estimated from the ^{15}N recovery and N concentrations of different plant parts of ^{15}N-treated plants (values are means, n=4).

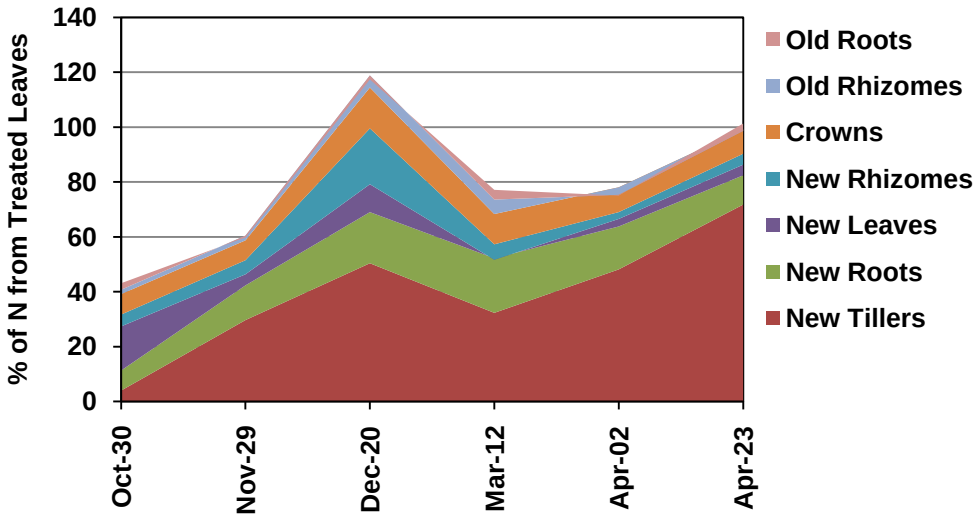

Figure 3-26. The percent of N in different plant parts that was remobilized from treated leaves since the first harvest (Oct-04) in Season 1 (2006-2007) in stockpiled tall fescue plants grown at BREC, near Columbia, MO. Values are estimated from the ^{15}N recovery and N concentrations of different plant parts of ^{15}N-treated plants (values are means, n=4).

Chapter 4

Discussion

Growth of Tall Fescue

From autumn through early winter in Season 1 (Oct-04 through Dec-20), air temperatures generally remained above 0 ^{o}C and were suitable for growth of tall fescue (Figure 3-1). New shoot and belowground tissues dramatically increased in mass during this period (Figures 3-7A-C; 3-8A-C, 3-9 A,B). Air and soil temperatures declined below levels for growth in tall fescue between Dec-20 and Mar-12 harvests in Season 1 (Figure 3-1A) and plant segments exhibited no change or a slight decline in dry weight (Figures 3-7A-C; 3-8A-C, 3-9 A,B) during this time.

In autumn of Season 2, shoot segments did not increase in mass, while belowground structures of crowns and new roots proliferated. The reduced growth response of shoot segments in early harvests during Season 2 could be due to cooler temperatures and later harvest dates (starting Oct-29 in Season 2 compared to Oct-04 in Season 1). Temperatures declined to levels that would slow tall fescue growth between Nov-29 and Feb-25 harvests in Season 2 (Figure 3-2A), however some plant segments, such as crowns and new roots, increased in dry weight during these winter months (Figure 3-11A-C). Tall fescue could have utilized the short time periods of increased air temperatures to increase belowground biomass (Figure 3-2A). My results suggest tall fescue developed belowground structures, such as crowns and new roots, and initiated new tiller development during winter when temperatures were favored for growth. In spring, however, new tiller growth predominated in both seasons, while

belowground segments generally declined in mass, indicating tall fescue does not produce new belowground structures during spring regrowth. This is, to my knowledge, the first report of tall fescue belowground growth over the stockpiling period.

Unlike tall fescue, many grasses have been found to produce the majority of new roots in the autumn and early winter, slow growth during colder soil temperatures in winter, and resume active growth of roots along with shoot regrowth in spring (Stuckey 1941). This seasonal growth habit of roots was reported in bushgrass (Gloser 2005), red fescue, and colonial bentgrass (Bausenwein et al. 2001a). In my study, tall fescue exhibited a decrease in root mass during spring regrowth, instead of increasing root mass like many other grasses. It is my hypothesis that tall fescue increases belowground biomass during winter as a strategy to sequester nutrients underground to be utilized in spring regrowth.

The proliferation of new root growth in tall fescue during winter months implied a major turnover cycle of root mass. New roots were initiated in autumn, and the new root mass increased to about twice the mass of old roots in mid-winter (Figures 3-8C, 3-9A, 3-11C, 3-12A). In my study, the classification of "new" versus "old" roots in tall fescue does not discriminate for alive versus dead tissue. Similarly, Stuckey (1941) found it difficult to distinguish alive versus dead roots because brown, brittle roots that seemed dead actually sustained normal growth in Kentucky bluegrass. In my study, root lifespan would be overestimated

since not all dead/decomposed roots would be retrieved during harvests. Therefore, the exact duration and rate of root turnover in tall fescue could not be determined.

Recovered ^{15}N

The recovery of applied ^{15}N in tall fescue tissues at first harvest was 45% in Season 1 (two weeks after ^{15}N application) and 40% in Season 2 (four weeks after ^{15}N application) (Figures 3-17 and 3-18). Similar recovery values of 40-53% were reported after 72 h in foliar-labeling studies in controlled environment chambers of tall fescue (Bowman and Paul 1990), Kentucky bluegrass (Bowman and Paul 1989), and perennial ryegrass (Bowman and Paul 1992). Therefore, the methods of foliar ^{15}N application in my field study resulted in comparable absorption rates as grasses that were grown in a controlled environment, suggesting weather related conditions in the field did not significantly affect ^{15}N foliar absorption.

Of the incorporated ^{15}N in the tall fescue at first harvest, about 22% and 48% were found in plant parts other than treated leaves in Seasons 1 and 2, respectively (Figures 3-17 and 3-18). These results were also consistent with the recovery of foliar ^{15}N in other grass species grown in growth chambers (Bowman and Paul 1989, 1990, 1992), where about 15% of the incorporated ^{15}N was found in roots after 72 h. Therefore, about 15% of the foliar absorbed ^{15}N in field-grown

tall fescue could have been remobilized to other plant parts within the first few days following treatment application.

Loss of Applied ^{15}N

The 40-45% recovery of ^{15}N at first harvest indicates there was a substantial loss of ^{15}N within the soil-plant-air system. The greatest amount of ^{15}N loss was most likely due to a lack of absorption into the leaves. Several steps were made in order to maximize uptake of the ^{15}N into the leaf. Hand-brushing of the solution ensured even distribution over all abaxial and adaxial leaf surfaces and eliminated loss from drift that would have been made by a spray application. Foliar applications were scheduled to be applied on sunny days followed by several days without precipitation, with the intention of avoiding removal of ^{15}N from the leaf surface by precipitation. After application, the solution dried quickly on the leaf. It is possible that the quick drying created residues on the leaf surface which inhibited N penetration (Neumann 1988). In the days following application, dew formed on the leaf surfaces and some ^{15}N residue may have been lost by dripping of dew. However, comparable absorption rates of foliar ^{15}N labeling of grasses in growth chambers (Bowman and Paul 1989, 1990, 1992) suggest that loss from dew in my field grown plants could be negligible.

Other possible opportunities for N loss are volatilization or denitrification of the nitrate from the leaf surface. However, unlike urea applications that lose an

estimated 30% by volatilization, Bowman and Paul (1992) found no losses due to volatilization or denitrification from ammonium or nitrate foliar applications on perennial ryegrass. Therefore, it is unlikely that substantial losses of ammonium nitrate from tall fescue leaves occurred from these processes in my field study.

The hand-brushing foliar application of the ^{15}N treatment solution was implemented to prevent soil contamination of ^{15}N. Some contamination of the soil was inevitable in the field setting, either from accidental dripping during application, or from subsequent washing of ^{15}N residues from the leaves into the soil surface from dew dripping or precipitation. The soil samples could contain plant material including ^{15}N-enriched tall fescue fine roots and other plant parts that would increase the estimate of soil ^{15}N contamination. However, soil sample N was not separated into organic and inorganic fractions and possible contamination from plant matter was not excluded. Even though soil samples indicated some initial ^{15}N contamination of the soil surface (Figures 3-3 and 3-4), the total ^{15}N in the plants did not increase over time, suggesting that additional input of ^{15}N into the plant through root uptake from soil was trivial.

The recovery of ^{15}N in plant parts declined over time in both seasons (Figure 3-17, 3-18). As treated leaves senesced, leaching of the ^{15}N from these tissues and other litter, as well as the release of mineral N from decomposition may have accumulated in the surface of the soil, leading to increased ^{15}N in the soil samples and a decline in ^{15}N recovery in plant parts. Erroneous labeling of other species, such as Kentucky bluegrass, and subsequent removal of non-

fescue tissues could have also reduced ^{15}N recovery over time. In addition, any roots not connected to a live tiller were discarded, therefore retrieval of decomposing roots and fine roots could have been reduced.

Remobilized Leaf N Used for Growth

Even though the total recovery of applied ^{15}N declined over time, a simultaneous decline of ^{15}N in treated leaves occurred as recovery of ^{15}N increased in other plant parts (Figures 3-17 and 3-18). In treated leaves, the decline of ^{15}N content (Figures 3-7D and 3-10D) and associated decline in N concentration (Figures 3-7G and 3-10G) strongly indicated that the ^{15}N was incorporated into the leaf N fraction and removed over time. The concurrent increase in recovery of ^{15}N in other plant parts as ^{15}N and N concentration of treated leaves declined confirms N remobilization from treated leaves to other plant segments did occur.

Interestingly, excluding treated leaves, greater ^{15}N recovery was found in plant segments that exhibited increases in dry weight. For example in Season 1, new leaves increased in both dry weight and ^{15}N content by Oct-30 (Figure 3-7B,E) and new tillers exhibited the greatest dry weight and ^{15}N content of any other plant part by Nov-29 (Figure3-7C.F). By Dec-20, crowns had a three-fold increase in both dry weight and ^{15}N, while new rhizomes had an eight and nine-fold increase and new roots had an 11 and seven-fold increase in growth and ^{15}N, respectively, (Figure 3-8A-F). Similarly in Season 2, crowns and new roots

exhibited a 1.5-fold and 3.4-fold increase in dry weight, while ^{15}N content more than doubled through January (Figure3-11A,C,D,F). These results show that leaf N was remobilized to support new growth of underground structures from autumn through winter months. Furthermore, N concentrations of these belowground segments did not change during this same time period of increased dry weight and ^{15}N content (Figures 3-8J-L and 3-11J,L). The stability of N concentration further suggests that leaf N was used to support the development of these tissues.

Changes in ^{15}N content of different plant parts over time indicated further remobilization of leaf N within the plant. In spring, new tillers were the greatest sink for ^{15}N and total plant N, and exhibited the greatest amount of growth. On the other hand, from Mar-12 through Apr-23 in Season 1 and Feb-25 through Apr-28 in Season 2, the ^{15}N content, % of total plant N, and N concentration dramatically declined and little or no growth occurred in all belowground plant segments (Figures 3-7, 3-8, 3-9, 3-10, 3-11, 3-12). This inverse relationship of N dynamics between new tillers and other segments suggests belowground plant segments acted as sources of N and remobilized N to support the sink of new tiller growth in spring.

Overall, the seasonal pattern of ^{15}N remobilization was similar to that of total plant N (Figures 3-7D-I; 3-8D-I; 3-9C-F; 3-10D-I; 3-11D-I; 3-12C-F). This suggests that leaf N is remobilized to support the greatest N sink(s). Nitrogen concentration, however, did not follow the pattern of ^{15}N accumulation in plant

parts nor total plant N dynamics (Figures 3-7D-L; 3-8D-L; 3-9C-H; 3-10D-L; 3-11D-L; 3-12C-H). These results further support that leaf N was used for growth and development of different plant segments, rather than in the concentration of N in a particular tissue. Similar conclusions were drawn in overwintering roots in red fescue and colonial bentgrass (Bausenwein et al. 2001a), however my results are the first evidence of leaf N remobilization to support overwinter growth in tall fescue.

Nitrogen Storage Forms in Tall Fescue

Soluble Protein

At first harvest in autumn, the soluble protein fraction of treated leaves was more than four-fold greater in concentration than free amino acids and nitrate (Figures 3-19 and 3-22). Following the first harvest, protein concentrations dramatically declined in treated leaves through winter months (56-60%). The concurrent decline of soluble protein, ^{15}N, and N concentration in treated leaves during autumn harvests suggests that leaf soluble protein is a major source of the remobilized N. The breakdown of protein is also an indication of leaf senescence and Rubisco would likely be a major constituent of this degraded protein fraction (Wittenbach 1979, Callow 1974, Feller and Fischer 1994, Hörtensteiner and Feller 2002).

Through winter, soluble protein concentrations remained steady as dry weight increased in belowground segments of crowns, new rhizomes, and new roots (Figures 3-8A-C; 3-9A,B; 3-11A-C; 3-12A,B; 3-20A-C; 3-21A,B; 3-23A-C; 3-24A,B). During spring regrowth of tall fescue, the soluble protein concentration declined in all plant segments, suggesting that the protein pool was utilized as an N store to support tiller growth. Similar declines in protein pools were found in bushgrass stubble, roots, and rhizomes during spring growth (Gloser (2002, 2005). Soluble protein in leaf sheaths from perennial ryegrass has also been reported to decline 82% during spring regrowth (Louahlia et al. 1999). They suggested that the protein pools are degraded and remobilized to support the greater sink strength of new tiller growth in spring. However, no VSPs were identified in these grasses (Louahlia et al. 1999, Kavanová and Gloser 2005). Instead, it has been hypothesized that the decline in soluble proteins in grasses is a result of the breakdown of proteins involved in leaf senescence, or as a result of an inhibition of root processes, rather than a preferential degradation of specific storage proteins (Louahlia et al. 1999, Gloser 2002, 2005, Kavanová and Gloser 2005). In tall fescue, the stable concentrations of protein in belowground segments indicated that preferential accumulation of protein was not occurring through winter. Therefore, it is my hypothesis that tall fescue did not concentrate proteins in overwintering tissues and accumulation of specific VSPs was unlikely. As an alternative, spring tiller growth was partially supported by the breakdown and remobilization of the soluble protein fraction of many senescing tissues.

Free Amino Acids

In belowground segments of tall fescue, the free amino acid pool exhibited a greater seasonal fluctuation than protein or nitrate, including an accumulation in autumn through winter followed by a dramatic decline in spring. The seasonal dynamics of free amino acid accumulation in tall fescue were similar to those of overwintering tissues of other perennial species including woody perennials (Sagisaka 1974, 1983), herbaceous perennials (Rosnitschek-Schimmel 1985b, Chapin et al. 1986, Cyr and Bewley 1989, Cyr et al. 1990, Hendershot and Volenec 1993, Volenec et al. 1996, Nordin and Näsholm 1997, Bausenwein et al. 2001b), and grasses (Nordin and Näsholm 1997, Bausenwein 2001a, Gloser 2002, 2005). In my tall fescue study, changes in concentrations indicated an accumulation of free amino acids more than two-fold from autumn through winter in crowns, new rhizomes, and new roots. Further analyses clearly identified that asparagine and glutamine were the most abundant amino acids, representing 58 and 17%, respectively, in crowns, and 53 and 23%, respectively, in new roots by early spring. Both asparagine and glutamine were found to accumulate as major overwinter N storage forms in other perennial grasses (Sagisaka 1987, Nordin and Näsholm 1997, Gloser 2002, 2005), but had not been reported for tall fescue. Asparagine has been found to be the most common form of transported N from senescing leaves, roots, and stubble, to support growth in other tissues in perennial ryegrass (Bigot et al. 1991, Lefevre et al. 1991, Thornton and Macduff 2002). In addition, asparagine and glutamine have been found to dominate the

free amino acid pool in perennial ryegrass stubble and roots, and were remobilized to support regrowth after defoliation (Lefevre et al. 1991). Documented for the first time in tall fescue, my study found that both asparagine and glutamine preferentially accumulated in crowns and new roots during winter and were used during spring growth, suggesting both amino acids were predominate storage forms of N in overwintering tall fescue.

During both stockpiling seasons, the concentration of free amino acids was similar to that of soluble protein in underground plant parts of tall fescue just before spring regrowth. Both pools were subsequently reduced during spring regrowth, however in underground plant parts, the changes in free amino acid concentrations were more dynamic than the protein pool. Similar dynamics were found in bushgrass roots, where Gloser (2002, 2005) determined that the total N available from free amino acids was greater in both concentration and total N than the protein-N fraction. Therefore, in my study, it is likely that the amino acid fraction contributed more N to storage in underground plant parts in tall fescue than the protein fraction. This may be an advantageous N storage strategy for grass growth, as proteins would require energy for catabolism and conversion into transportable forms of N before remobilization, while free amino acids are readily accessible for N transport.

The remobilization of amino acids may contribute more than just N to regrowing plant parts (Lattanzi et al. 2005). It has been estimated by Schnyder and de Visser (1999) that 75% of remobilized carbon (C) used for shoot regrowth

two d after a defoliation event in perennial ryegrass was as amino-C. Similar contributions from old leaf N and subsequent amino acid N stores in overwintering tall fescue could also contribute as a major C source for new growth.

Nitrate

The time lapse between sample removal from the field and freezing of the tall fescue tissues may have allowed nitrate reductase activity to significantly reduce nitrate levels in plant tissues by the time the tissues were frozen. Therefore, my reported values of nitrate may not accurately reflect concentrations at the time of harvest. However, nitrate concentrations reported in my study for tall fescue segments are similar to those found in a study on overwintering bushgrass (Gloser 2002), in which tissues were frozen immediately following harvest. In my study, the reported nitrate concentrations were the lowest of all N pools, suggesting that it is not a major storage form of N in tall fescue. In addition, nitrate concentrations peaked in early to mid-winter in many belowground segments of tall fescue. This has been reported in other perennial species (Rosnitschek-Schimmel 1985a, Cyr and Bewley 1989, Cyr et al. 1990, Gloser 2002). It has been suggested that during early winter, nitrate availability from the soil is greater while senescing shoots have a lowered ability for nitrate reduction. Since roots have a reduced capacity for nitrate reduction compared to

shoots, the result would be an accumulation of nitrate in belowground tissues in early winter (Millard 1988, Gloser 2002, 2005).

In conjunction with the N pools of soluble proteins and free amino acids, nitrate concentrations declined to levels less than autumn concentrations in all plant segments during spring regrowth of tall fescue. This suggests that many plant segments were sources of all three N pools and remobilized these pools to support new tiller growth in spring.

Nitrogen Remobilization Dynamics in Tall Fescue

Strategies for seasonal N dynamics differ across species. In general, N compounds are stored in overwintering tissues, such as bark of woody perennials, and in large underground storage organs in herbaceous perennials, such as tubers, taproots, stolons, and rhizomes. Nitrogen compounds of mainly free amino acids and storage proteins (BSPs and VSPs) are accumulated in autumn as remobilization from senescing tissue occurs, remain dormant in winter, then are utilized for spring regrowth. However, in perennial species that do not have large overwintering storage organs, and species that do not go completely dormant in winter (“evergreen”), such as tall fescue in Missouri, N storage strategies may be different.

Herbaceous grasses that go dormant in winter with relatively larger diameter root systems, such as purple moor grass, may remobilize and store more N from senescing aboveground tissues in autumn, store more N in

belowground segments, and remobilize N predominately from belowground structures in spring (Thornton and Millard 1993). Evergreen grasses, or grasses with relatively smaller diameter root systems, such as perennial ryegrass, bushgrass, and tall fescue, may utilize remobilized leaf N directly for new growth during winter and spring, rather than concentrating N in a tissue for storage. In addition, grasses with smaller root systems may have greater root turnover than grasses with larger roots. Roots with greater turnover may not be as suitable as a long-term storage organ (Bausenwein et al. 2001a).

From my study, N remobilization in tall fescue seemed to be a dynamic process, supporting the plant's "evergreen" growth habit through winter. Nitrogen from senescing leaves of tall fescue was remobilized to support new growth sinks of both new shoot formation and belowground crown and new root development from autumn through winter. The N dynamic continued in spring, when N from all plant parts was again remobilized to support the strong sink of new tiller growth.

The cycling of N between plant parts of tall fescue was continuous throughout the stockpiling season. The usefulness of the ^{15}N tracer to discriminate sources of N decreased over time as ^{15}N cycles between plant parts (Santos et al. 2002). Unlabeled N in the plant from N uptake, as well as endogenous N that was unlabeled, may be transported and remobilized. Consequently, an exact determination of the total amount of N remobilized from one plant part to another cannot be concluded from my ^{15}N labeling experiment.

However, the contribution of leaf N from different plant parts to support spring regrowth can be estimated based on changes in ^{15}N.

In Season 1, leaf N concentrations declined 69% (Figure 3-7J) from Oct-04 through Apr-23, and based on ^{15}N differences, 45-60% of leaf N was remobilized to other plant parts from September through April. Values based on ^{15}N recovery in April are most likely underestimated because ^{15}N recovery declined over time in this experiment. The largest recovery of ^{15}N occurred on the Dec-20 harvest. When calculated from the total amount of ^{15}N recovered in Dec-20, estimates of remobilization increased to 58-66% of treated leaf N. All estimates of tall fescue leaf N remobilization coincided with the results of Aerts (1996), who calculated a 59% resorption efficiency of leaf N in graminoids across multiple studies.

In Season 2, the N concentration of treated leaves decreased 48% from the first harvest on Oct-29 through the last harvest on Apr-28. When estimated from the time of treatment, 37% of treated leaf N was remobilized from Oct-01 through Apr-28 in Season 2. Estimates cannot be determined based on values from first harvest to last harvest because there was no difference of ^{15}N recovery in other tissues between these harvests. Due to the later treatment and harvest dates in autumn in Season 2 than in Season 1, it could be postulated that leaf N could have been remobilized before the first harvest in autumn. Therefore, estimates for leaf N remobilization in Season 2 would not include the majority of leaf N remobilized before first harvest.

In winter months in Season 1, about half of the remobilized leaf N was found to support new tiller growth, while the other half was used to support new growth sinks of crowns, new rhizomes, and new roots (Figure 3-25). New roots were the largest belowground sink of remobilized leaf N in winter. About 10% of autumn leaf N was remobilized to supply 20% of N in new root growth in winter (Figures 2-25 and 3-26). By spring, new tiller growth contained 75% of the remobilized leaf N. Almost 40% of the autumn leaf N was remobilized to new tiller growth the following spring (Figure 3-25), supplying 70% of the N in new tiller growth (Figure 3-26).

The amount of stored N used for growth versus N from root uptake was estimated in experiments that had labeled endogenous N by growing plants in ^{15}N. Bausenwein et al. (2001a) found 70% (red fescue) and 82% (colonial bentgrass) of new spring growth to have been remobilized from old, senescing leaves on overwintering tillers. However, the contribution of remobilized N to new growth was dependent on the timing of harvest, since N from root uptake during spring would reduce the relative amount of total N from remobilization. Additionally, the efficiency of leaf N remobilization on intact tillers before leaf death had been estimated to be 70% in perennial ryegrass (Robson and Deacon 1978), and 75% in purple moor grass (Morton 1977). Results of my study suggest tall fescue remobilization efficiency of old leaf N through winter (45-66%) and subsequent contribution to spring growth (old leaf N supplied 70% of N used for new growth in spring) is similar to that of other grasses.

Implications of N Remobilization for Forage Management of Tall Fescue

Estimates of the contribution of old leaf N (from treated leaves) to new growth in stockpiled tall fescue during winter suggest a majority of N is remobilized from senescing tissues to support the initiation of new shoots and underground growth, including a proliferation of new root growth during winter. In spring, the N contributions from all segments, including new underground growth and senescing leaves, suggests the remobilized N is further utilized for spring tiller growth.

Many studies have found a positive relationship between plant N reserves and regrowth potential, such as in alfalfa after defoliation (Ourry et al. 1994, Barber et al. 1996, Avice et al. 1997) and in spring regrowth (Justes et al. 2002, Dhont et al. 2003). Increased N stores positively affected regrowth after defolation in perennial ryegrass (Ourry et al. 1990b, Louahlia et al. 1999, 2000, Morvan-Bertrand et al. 1999) and bushgrass (Gloser et al. 2007). Therefore, a reduction of N reserves in tall fescue could reduce the ability for winter root growth and shoot initiation, and in turn impact internal N sources for spring regrowth. It has been suggested that relatively moderate differences in N reserves in grasses may have a large impact on forage regrowth yields (Louahlia et al. 2000). Nitrogen reserves can be impacted by removal of N storage tissues, such as the removal of old leaf N by forage removal, and N supply, both of which are implicated by management practices of stockpiled tall fescue pastures.

Forage Removal

Red fescue, colonial bentrgrass (Bausenwein et al. 2001a), and bushgrass (Gloser 2005) have been found to remobilize most of the N used for new spring tiller from old leaf N, while roots contributed little N. However, research in grasses suggests that the relative contributions of remobilized N to new growth are dependent on the amount of N available from each plant segment. For example, when the N pools from old leaves are reduced, more N from roots may be remobilized to support new shoot growth (Li et al. 1992, Bausenwein et al. 2001a, Santos et al. 2002). Therefore, defoliation stresses, such as mechanical forage harvesting or grazing, may severely reduce the old leaf N pool for remobilization, alter the N remobilization dynamics in tall fescue, and may result in more N remobilized from crowns and roots to support regrowth.

In addition, defoliation has been shown to reduce root mass in grasses (Louahlia et al. 2000), and is further reduced under repeated defoliations and more severe defoliation intensity and frequency (Thornton and Millard 1996, 1997, Lestienne et al. 2006). The further reduction in root mass also affected N stores available for regrowth. For example, after a single defoliation, mobilization of stored N supplied 50 and 41% of new leaf N in perennial ryegrass and red fescue, respectively. After repeated defoliations, regrowth rates were reduced and the contribution from N stores was reduced to 4 and 16% of new leaf N (Thornton and Millard 1997).

Although, to my knowledge, no studies have focused on the impacts of defoliation on N remobilization dynamics in stockpiled tall fescue, many studies have examined the effects of stockpiling initiation dates (date of last herbage removal) on autumn and early winter forage yields. In general, later initiation dates resulted in decreased yields in autumn and early winter. Burns and Chamblee (2000a) reported decreasing November yields as stockpiling was delayed from June through September. However, the stockpiling initiation date had no effect on spring yield. Similarly, Rayburn et al. (1979) in Virginia found decreased December yields as initiation dates were delayed from June through September with no effect on spring yields, and Fribourg and Bell (1984) reported decreased yields in October through December as initiation was delayed from July through September in Tennessee. Stockpiled tall fescue yields as late as February were reported by Collins and Balasko (1981a), who found decreased yields from December through February as initiation dates were delayed from September through early October in West Virginia.

The effects of stockpiling initiation date are attributed to the amount of time the plants have to regrow before the harvest dates, with longer regrowth periods resulting in greater yields (Rayburn et al. 1979). In relation to N remobilization dynamics, a longer regrowth period in autumn would increase the amount of leaf N available for remobilization to support growth through autumn and winter. However, Collins and Balasko (1981a) found that a delay of the last herbage removal from September 1 to October 3 had a negative effect on winter

yields, but increased spring yields in one out of two years. By shortening the regrowth period in autumn, smaller winter and spring yields would be expected. However, when taking into account N dynamics in tall fescue, it could be speculated that the delayed shoot removal could have allowed time for remobilization of N from older leaves to support growth of underground segments and new tiller starts, resulting in greater spring growth.

Although plant N status is dependent on many factors, including past management and environmental conditions, the timing of N remobilization in tall fescue could explain regrowth dynamics in stockpiling systems. Tall fescue is often stockpiled in autumn for winter grazing, however winter grazing could affect spring yields. Taylor and Templeton (1976) found that removal of forage in November and December reduced spring (May) dry matter yields significantly more than when forage was removed in October, February, or March. Depending on the status of the plants, leaf removal in early winter could have deleterious effects on the amount of N remobilized. For example, if the forage was still green with high N content (crude protein, CP) in mid-winter, grazing would remove leaf N before remobilization and could affect spring growth. In Taylor and Templeton's study (1976), forage removal later in winter did not affect spring regrowth, suggesting that by late winter all the N was remobilized.

Nitrogen Fertilization

The amount of N supplied to plants affects the internal cycling of N and the available N stores (Millard et al. 1990). In grasses, increased N availability has been shown to increase all N pools, with preferential accumulation of asparagine and glutamine in the free amino acid fraction (Gloser 2005). When N supply is limited, overall N uptake is decreased and storage N pools were also decreased (Ourry et al. 1990b, Li et al. 1992, Thornton et al. 1993a, 1994, Thornton and Millard 1993, Louahlia et al. 1999, Morvan-Bertrand et al. 1999 Gloser et al. 2007). Therefore, an increased supply of N can increase N stores, favoring regrowth after a defoliation event or during spring regrowth. Increased N supply has been shown to positively affect N stores and regrowth after defoliation in perennial ryegrass (Ourry et al. 1990b, Louahlia et al. 1999, Morvan-Bertrand et al. 1999), purple moor grass (Thornton and Millard 1993), and bushgrass (Gloser et al. 2007). Although the proportion of N remobilized from older leaves to new leaves did not change with a 10-fold increase in N supply in perennial wheatgrass (Li et al. 1992), nor with a 50-fold increase in N supply in purple moor grass (Thornton and Millard 1993), the net contribution of N from remobilization was greater in plants receiving greater N supply. Similarly, Ourry et al. (1990b) reported that in defoliated perennial ryegrass, 69% of new leaf regrowth was from remobilization in plants supplied with adequate N, while in N-stressed plants, remobilization accounted for 40% of N used for new leaf growth.

Seasonal N dynamics may also be altered by N availability. High rates of autumn N have been shown to increase storage N pools in bushgrass (Gloser 2005) and purple moor grass (Thornton and Millard 1993). Tiller initiation and development increased in autumn with increased N supply, and in turn increased spring regrowth, mainly as leaf area development (Gloser 2005).

The impacts of available N on N dynamics and regrowth of grasses is supported in N management strategies in stockpiled tall fescue systems. Late summer to early autumn N applications are commonly implemented to support the fall growth in tall fescue pastures for stockpiling. In general, late summer or early autumn N fertilizer applications increased both dry matter yield and CP of stockpiled tall fescue. Archer and Decker (1977a) in Maryland reported late September N applications increased yield and CP in harvests from October through December. While several studies have shown August N applications increased yield and CP in tall fescue harvested from December through February in West Virginia (Balasko 1977), Virginia (Teutsch 2005), Tennessee (Ross and Reynolds 1981), and West Virginia (Collins and Balasko 1981a, b), others have reported similar effects even in later harvest dates such as March in Tennessee (Taylor and Templeton 1976), and spring harvests (Collins and Balasko 1981a, b). The effect of August N fertilization on winter-harvested plots has also been found to carry over to increase the spring regrowth (Ross and Reynolds 1981).

Taylor and Templeton (1976), Balasko (1977), and Rayburn et al (1979) have attributed N applications to increase the amount of “green”, frost-resistant

leaves of stockpiled tall fescue. The increase of green leaves in N fertilized plots could be a result of a greater abundance of newer leaves due to increased growth from greater N availability. However, the explanation could be expanded to include a reduction of leaf senescence. When adequate N is supplied in autumn, more N is available for uptake, and increased N stores could accumulate within plant tissues. Increased N stores and N availability could be utilized to support new growth through winter months, reducing the demand of remobilized N from old leaves, and thus reducing leaf senescence.

Grasses with a greater ability to store N may be better adapted to survive in environments of low or fluctuating N availability (Thornton and Millard 1996). In nutrient-limited conditions, grass species with greater ability to remobilize internal N reserves have a competitive advantage over species that rely relatively more on N uptake to regrow shoots (Thornton et al. 1993a, 1994, Thornton and Millard 1996). Storage and remobilization of N also results in better N conservation with grass systems, by reducing loss through root exudation, leaching, and tissue death (de Aldana and Berendse 1997). Nitrogen is a substantial and costly input in stockpiling forage systems (Poore et al. 2000). Knowledge about N use, remobilization, and storage in tall fescue can support the development of more efficient management strategies to reduce N inputs (Santos et al. 2002) and improve productivity in low input farming systems.

The results of my study emphasize the importance of remobilized autumn leaf N for winter and spring growth of stockpiled tall fescue. Such information

offers insight into the regrowth dynamics of tall fescue in winter and spring as well as regrowth after grazing in stockpiling systems. Current recommendations of late-summer N applications to stockpiled pasture systems support the N status of the plant and stores for winter and spring growth. However, winter grazing in stockpiling systems may affect the N pools available for remobilization and the regrowth potential of the plants. Further studies on the effects of management practices on N remobilization dynamics in stockpiled tall fescue systems may offer more insight into N management recommendations.

Summary

Foliar applied ^{15}N was used to determine the fate of leaf N from field-grown tall fescue during winter and spring. Results indicate that autumn senescing leaves remobilized about 45-60% of leaf N to growth sinks through winter and spring. About half of the remobilized leaf N was found to support the initiation of new tillers, while the other half was used to support new growth sinks of crowns, new rhizomes, and new roots. The new root mass increased to more than twice the amount of old roots by December, and remobilized leaf N supported 20% of N used for this new root growth. Free amino acids were a predominant storage form of N in overwintering tall fescue. Asparagine and glutamine preferentially accumulated in crowns and new roots, representing about 50 and 20%, respectively, of the amino acid profile. In spring, N from old leaves and belowground plant segments was remobilized to support the sink of

new tiller growth. Remobilization of old leaf N accounted for 70% of the N in new tiller growth by the end of April. Nitrogen remobilization dynamics can be used to develop more efficient management practices of N fertilization and grazing strategies in tall fescue stockpiling systems.

Future Directions

Many nitrogen remobilization studies have used ^{15}N to discriminate N reserves within the plant versus N uptake. A constant supply of ^{15}N was required and growing conditions usually involved solution culture or inorganic media. These methods allowed precise quantification of different N sources. However, plants that are cultured in these growing environments did not accurately reflect the plant age, morphology, soil environment, and seasonal weather conditions found in the field. My experiment avoided these complications by using tall fescue plants in the field environment with minimal disturbance to normal conditions of an established Missouri tall fescue pasture.

In my study, new methods were developed to expand N remobilization studies to established stands of grass in the field. The methods of plot establishment minimally disturbed plant structures and allowed the tall fescue plants to remain in natural environmental conditions. The use of PVC pipes allowed quick establishment of both an above and belowground barrier. Pipes were also easily removed, even during winter months when the soil was frozen. In addition, the size of the pipes resulted in representative sampling of

underground tissues. The method of tagging tillers was an effective system with near 100% tag recovery, and was effective to distinguish treated tillers from new tillers.

Conducting this experiment in the field offered some challenges in ^{15}N labeling. In the field environment, ^{15}N is often topdressed on the soil surface and used to quantify fertilizer uptake by plants and can give an estimate on the amount of N uptake from the soil during a period of time. However, soil applied ^{15}N does not ensure even availability of ^{15}N in the different N pools in the soil system, and would not ensure even availability of ^{15}N over time in this long-term seasonal experiment. In addition, labeling of endogenous N to discriminate N from root uptake in tall fescue would not be feasible in field-grown plants. However, the current methods of ^{15}N incorporation into leaves allowed discrimination of leaf N from different sources, albeit precise quantification of remobilized N from other plant parts over time was not possible.

Overall, the methodology employed in my study was effective at deciphering leaf N remobilization in an overwintering tall fescue and is well suited to be used for future experiments of N remobilization in grasses. Labeling leaf tissue by hand with ^{15}N ensured even distribution over all leaf surfaces and minimized ^{15}N loss and soil contamination. However, in future experiments the solution should be applied in the same manner on ^{15}N treated plots as on control plots to avoid treatment differences. In addition, harvests should be conducted at the time of treatment application to determine the initial N concentration of leaf

tissues. In my study, harvests were not conducted until 2-4 weeks following treatment. The lack of initial N concentration values of tissues inhibited calculations of N remobilization from the time of treatment.

Studies on N remobilization in stockpiled tall fescue can be further expanded to explore the effects of the developmental stage on N remobilization dynamics. Labeling of leaf N earlier in autumn will help decipher the state of development leaf N begins to remobilize to support new growth, and later harvests in spring can be made to include reproductive N sinks. Quantification of N content in the different N pools will also allow more insight into the relative importance of different N storage pools in tall fescue. Further studies on the effects of environmental conditions and management practices, including N fertilization and winter grazing strategies, on the N remobilization dynamics in stockpiled tall fescue will be useful to improve tall fescue as stockpiled forage.

Chapter 5

Literature Cited

Aerts R (1996) Nutrient resorption from senescing leaves of perennials: are there general patterns? J Ecol **84**: 597-608

Andrews DL, Beames B, Summers MD, Park WD (1988) Characterization of the lipid acyl hydrolase activity of the major potato (*Solanum tuberosum*) tuber protein, patatin, by cloning and abundant expression in a baculovirus vector. Biochem J **252**: 199-206

AOAC (2006) Official methods of analysis of the Association of Official Analytical Chemists (18th edn). Association of Official Analytical Chemists, Washington, DC, USA

Archer KA, Decker AM (1977a) Autumn-accumulated tall fescue and orchardgrass. I. Growth and quality as influenced by nitrogen and soil temperature. Agron J **69**: 601-605

Archer KA, Decker AM (1977b) Autumn-accumulated tall fescue and orchardgrass. II. Effects of leaf death on fiber components and quality parameters. Agron J **69**: 605-609

Arnon DI, Stout PR (1939) The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant Physiol **14**: 371-375

Avice JC, Ourry A, Lemaire G, Volenec JJ, and Boucaud J (1997) Root protein and vegetative storage protein are key organic nutrients for alfalfa shoot regrowth. Crop Sci **37**: 1187-1193

Avice JC, Ourry A, Volenec JJ, Lemaire G, Boucaud J (1996) Defoliation-induced changes in abundance and immuno-localization of vegetative storage proteins in taproots of *Medicago sativa*. Plant Physiol **34**: 561-570

Balasko JA (1977) Effects of N, P, and K fertilization on yield and quality of tall fescue forage in winter. Agron J **69**: 425-428

Barber LD, Joern BC, Volenec JJ, Cunningham SM (1996) Supplemental nitrogen effects on alfalfa regrowth and nitrogen remobilization from roots. Crop Sci **36**: 1217-1223

Bausenwein U, Millard P, Raven JA (2001a) Remobilized old-leaf nitrogen predominates for spring growth in two temperate grasses. New Phytol **152**: 283-290

Bausenwein U, Millard P, Thornton B, Raven JA (2001b) Seasonal nitrogen storage and remobilization in the forb *Rumex acetosa*. Func Ecol **15**: 370-377

Bewley JD (2002) Root storage proteins, with particular reference to taproots. Can J Bot **80**: 321-329

Bigot J, Lefevre J, Boucaud J (1991) Changes in the amide and amino acid composition of xylem exudates from perennial ryegrass (*Lolium perenne* L.) during regrowth after defoliation. Plant Soil **136**: 59-64

Blevins DG (1989) An overview of nitrogen metabolism in higher plants. In JE Poulton, JT Romeo, EE Conn, eds, Recent advances in phytochemistry: plant nitrogen metabolism, Vol 23. Plenum Press, New York, pp 1-41

Bowling DJF (1976) Uptake of ions by plant roots. Chapman and Hill, London

Bowman DC, Paul JL (1989) The foliar absorption of urea-N by Kentucky bluegrass turf. J Plant Nutr **12**: 659-673

Bowman DC, Paul JL (1990) The foliar absorption of urea-N by tall fescue and creeping bentgrass turf. J Plant Nutr **13**: 1095-1113

Bowman DC, Paul JL (1992) Foliar absorption of urea, ammonium, and nitrate by perennial ryegrass turf. J Am Soc Hortic Sci **117**: 75-79

Bouchart V, Macduff JH, Ourry A, Svenning MM, Gay AP, Simon JC, Boucaud J (1998) Seasonal pattern of accumulation and effects of low temperatures on storage compounds in *Trifolium repens*. Physiol Plant **104**: 65-74

Boudet AM, Canut H, Alibert G (1981) Isolation and characterization of vacuoles from *Melilotus alba* mesophyll. Plant Physiol **68**: 1354-1358

Boyce PJ, Volenec JJ (1992) β-amylase from tap roots of alfalfa. Phytochemistry **31**: 427-431

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72**: 248-254

Burkhardt J, Eiden R (1994) Thin water films on coniferous needles. Atmos Envrion **28**: 2001-2017

Burns JC, Chamblee DS (1979) Adaptation. In RC Buckner, LP Bush, eds, Tall fescue. Agron Monogr No 20. ASA, CSSA, and SSSA, Madison, WI, pp 9-30

Burns JC, Chamblee DS (2000a) Summer accumulation of tall fescue at low elevations in the humid piedmont: I. Fall yield and nutritive value. Agron J **92**: 211-216

Burns JC, Chamblee DS (2000b) Summer accumulation of tall fescue at low elevations in the humid piedmont: II. Fall and winter changes in nutritive value. Agron J **92**: 217-224

Callow JA (1974) Ribosomal RNA, fraction 1 protein synthesis, and ribulose diphosphate carboxylase activity in developing and senescing leaves of cucumber. New Phytol **73**: 13-20

Cataldo MH, Schrader LE, Youngs VL (1975) Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun Soil Sci Plant Anal **6**: 71-80

Chapin FS III, Schulze ED, Mooney HA (1990) The ecology and economics of storage in plants. Annu Rev Ecol Syst **21**:423-447

Chapin FS III, Shaver GR, Kedrowski RA (1986) Environmental controls over carbon, nitrogen and phosphorus fractions in *Eriophorum vaginatum* in Alaskan tussock tundra. J Ecol **74**: 167-195

Clark FE (1977) Internal cycling of ^{15}nitrogen in shortgrass prairie. Ecology **58**: 1322-1333

Clarkson DT, Jones LHP, Purves JV (1992) Absorption of nitrate and ammonium ions by *Lolium perenne* from flowing solution cultures at low root temperatures. Plant Cell Envrion **15**: 99-106

Clement CR, Hopper MJ, Jones LHP, Leafe EL (1978) The uptake of nitrate by *Lolium perenne* from flowing nutrient solution. J Ex Bot **29**: 1173-1183

Collins M, Balasko JA (1981a) Effects of N fertilization and cutting schedules on stockpiled tall fescue. I. Forage yield. Agron J **73**: 803-807

Collins M, Balasko JA (1981b) Effects of N fertilization and cutting schedules on stockpiled tall fescue. II. Forage quality. Agron J **73**: 821-826

Corbel G, Robin C, Frankow-Lindberg BE, Ourry A, Guckert A (1999) Regrowth of white clover after chilling: assimilate partitioning and vegetative storage proteins. Crop Sci **39**: 1756-1761

Corre N, Bouchart V, Ourry A, Boucaud J (1996) Mobilization of nitrogen reserves during regrowth of defoliated *Trifolium repens* L. and identification of potential vegetative storage proteins. J Ex Bot **47**: 1111-1118

Culvenor RA, Simpson RJ (1991) Mobilization of nitrogen in swards of *Trifolium subterraneum* L. during regrowth after defoliation. New Phytol **117**: 81-90

Cunningham SM, Volenec JJ (1996) Purification and characterization of vegetative storage proteins from alfalfa (*Medicago sativa* L.) taproots. J Plant Physiol **147**: 625-632

Cunningham SM, Volenec JJ (1998) Seasonal carbohydrate and nitrogen metabolism in roots of contrasting alfalfa (*Medicago sativa* L.) cultivars. J. Plant Physiol. **153**: 220-225

Cyr DR, Bewley JD (1989) Carbon and nitrogen reserves of leafy spurge (*Euphorbia esula*) roots as related to overwintering strategy. Physiol Plant **77**: 67-72

Cyr DR, Bewley JD (1990a) Proteins in the roots of the perennial weeds chicory (*Cichorium intybus* L.) and dandelion (*Taraxacum officinale* Weber) are associated with overwintering. Planta **182**: 370-374

Cyr DR, Bewley JD (1990b) Seasonal variation in nitrogen storage reserves in the roots of leafy spurge (*Euphorbia esula*) and responses to decapitation and defoliation. Physiol Plant **78**: 361-366

Cyr DR, Bewley JD, Dumbroff EB (1990) Seasonal dynamics of carbohydrate and nitrogenous compounds in the roots of perennial weeds. Plant Cell Environ **13**: 359-365

Dalling MJ, Tolbert NE, Hageman RH (1972) Intracellular location of nitrate reductase and nitrite reductase in spinach and tobacco leaves. Biochem Biophys Acta **283**: 505-512

De Aldana BRV, Berendse F (1997) Nitrogen-use efficiency in six perennial grasses from contrasting habitats. Func Ecol **11**: 619-626

De Visser R, Vianden H, Schnyder H (1997) Kinetics and relative significance of remobilized and current C and N incorporation in leaf and root growth zones of *Lolium perenne* after defoliation: assessment by ^{13}C and ^{15}N steady-state labeling. Plant Cell Environ **20**: 37-46

Dhont C, Castonguay Y, Nadeau P, Belanger G, Chalifour F (2003) Alfalfa root nitrogen reserves and regrowth potential in response to fall harvests. Crop Sci **43**: 181-194

Doehlert DC, Duke SH, Anderson L (1982) Beta-amylases from alfalfa (*Medicago sativa* L.) roots. Plant Physiol **69**: 1096-1102

Evans RD, Bloom AJ, Sukrapanna SS, Ehleringer JR (1996) Nitrogen isotope composition of tomato (*Lycopersicon esculentum* Mill, cv. T-5) grown under ammonium or nitrate nutrition. Plant Cell Environ **19**: 1317-1323

Evans HJ, Nason A (1953) Pyridine nucleotide nitrate reductase from extracts of higher plants. Plant Physiol **28**: 233-254

Feller U, Fischer A (1994) Nitrogen metabolism in senescing leaves. Crit Rev Plant Sci **13**: 241-273

Fischer A, Brouquisse R, Raymond P (1995) Organic nitrogen reserves and their mobilization during sprouting of purple nutsedge (*Cyperus rotundus* L.) tubers. J Ex Bot **46**: 1803-1808

Fleming GA, Murphy WE (1968) The uptake of some major and trace elements by grasses affected by season and stage of maturity. Grass Forage Sci **23**: 174-185

Franceschi VR, Giaquinta RT (1983) The paraveinal mesophyll of soybean leaves in relation to assimilate transfer and compartmentation II. Structural, metabolic and compartmental changes during reproductive growth. Planta **157**: 422-431

Franceschi VR, Wittenbach VA, Giaquinta RT (1983) Paraveinal mesophyll of soybean leaves in relation to assimilate transfer and compartmentation. III. Immunohistochemical localization of specific glycopeptides in the vacuole after depodding. Plant Physiol **72**: 586-589

Fribourg HA, Bell KW (1984) Yield and composition of tall fescue stockpiled for different periods. Agron J **76**: 929-934

Fry B (1991) Stable isotope diagrams of freshwater food webs. Ecology **72**: 2293-2297

Gana JA, Kalengamaliro NE, Cunningham SM, Volenec JJ (1998) Expression of β-amylase from alfalfa taproots. Plant Physiol **118**: 1495-1505

Garten CT, Schwab AB, Shirshac TL (1998) Foliar retention of ^{15}N tracers: implications for net canopy exchange in low- and high-elevation forest ecosystems. Forest Ecol Manage **103**: 211-216

Glasener KM, Wagger MG, MacKown CT, Volk RJ (1998) Nitrogen-15 labeling effectiveness of two tropical legumes. Plant Soil **200**: 149-156

Gloser V (2002) Seasonal changes of nitrogen storage compounds in a rhizomatous grass *Calamagrostis epigejos*. Biol Plant **45**: 563-568

Gloser V (2005) The consequences of lower nitrogen availability in autumn for internal nitrogen reserves and spring growth of *Calamagrostis epigejos*. Plant Ecol **179**: 119-126

Gloser V, Košvancová M, Gloser J (2004) Changes in growth parameters and content of N-storage compounds in roots and rhizomes of *Calamagrostis epigejos* after repeated defoliation. Biologia **59**: Suppl. 13: 179-184

Gloser V, Košvancová M, Gloser J (2007) Regrowth dynamics of *Calamagrostis epigejos* after defoliation as affected by nitrogen availability. Biol Plant **51**: 501-506

Gonzalez-Prieto SJ, Beaupied H, Moiroud A, Domenach AM (1995) Uniformity of labeling of alder leaves fertilized with NH_4^+-^{15}N and NO_3^--^{15}N by roots or leaves. Soil Biol Biochem **27**: 1559-1563

Goulas E, Le Dily F, Simon J, Ourry A (2002) Morphological pattern of development affects the contribution of nitrogen reserves to regrowth of defoliated white clover (*Trifolium repens* L.). J Ex Bot **53**: 1941-1948

Goulas E, Le Dily F, Teissedre L, Corbel G, Robin C, Ourry A (2001) Vegetative storage proteins in white clover (*Trifolium repens* L.): quantitative and qualitative features. Ann Bot **88**: 789-795

Goulas E, Richard-Molard C, Le Dily F, Le Dantec C, Ozouf J, Ourry A (2007) A cytosolic vegetative storage protein (TrVSP) from white clover is encoded by a cold-inducible gene. Physiol Plant **129**: 567-577

Grundmann GL, Lensi R, Chalamet A (1993) Delayed NH_3 and N_2O uptake by maize leaves. New Phytol **124**: 259-263

Handley LL, Raven JA (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant Cell Environ **15**: 965-985

Heilmeier H, Freund M, Steinlein T, Schulze ED, Monson RK (1994) The influence of nitrogen availability on carbon and nitrogen storage in the biennial *Cirsium vulgare* (Savi) Ten. I. Storage capacity in relation to resource acquisition, allocation and recycling. Plant Cell Environ **17**: 1125-1131

Hendershot KL, Volenec JJ (1992) Taproot nitrogen accumulation and use in overwintering alfalfa (*Medicago sativa* L.). J Plant Physiol **141**: 68-74

Hendershot KL, Volenec JJ (1993) Nitrogen pools in taproots of *Medicago sativa* L. after defoliation. J Plant Physiol **141**:129-135

Högberg P (1997) ^{15}N natural abundance in soil-plant systems. New Phytol **137**: 179-203

Hogg EH, Lieffers VJ (1991) Seasonal changes in shoot regrowth potential in *Calamagrostis Canadensis*. Oecologia **85**: 596-602

Hörtensteiner S, Feller U (2002) Nitrogen metabolism and remobilization during senescence. J Ex Bot **53**: 927-937

Huang JF, Bantroch DJ, Greenwood JS, Staswick PE (1991) Methyl jasmonate treatment eliminates cell-specific expression of vegetative storage protein genes in soybean leaves. Plant Physiol **97**: 1512-1520

Huffaker RC (1982) Biochemistry and physiology of leaf proteins. In D Boulter, B Parthier, eds, Nucleic acids and proteins in plants. 1. Encyclopedia of Plant Physiology, Vol 14A. Springer-Verlang, Berlin, pp 370-400

Jarvis SC, Macduff JH (1989) Nitrate nutrition of grasses from steady-state supplies in flowing solution culture following nitrate deprivation and/or defoliation. J Ex Bot **40**: 965-975

Jonasson S, Chapin FS (1985) Significance of sequential leaf development for nutrient balance of the cotton sedge *Eriophorum vaginatum* L. Oecologia **67**: 511-518

Junk G, Svec HV (1958) The absolute abundance of the nitrogen isotopes in the atmosphere and compressed gas from various sources. Geochim Cosmochim Acta **14**: 234-243

Justes E, Thiébeau P, Avice J, Lemaire G, Volenec JJ, Ourry A (2002) Influence of summer sowing dates, N fertilization and irrigation on autumn VSP accumulation and dynamics of spring regrowth in alfalfa (*Medicago sativa* L.). J Ex Bot **53**: 111-121

Kallenbach RL, Bishop-Hurley GJ, Massie MD, Rottinghaus GE, West CP (2003) Herbage mass, nutritive value, and ergovaline concentration of stockpiled tall fescue. Crop Sci **43**: 1001-1005

Kang S, Ko K, Titus JS (1982) Mobilization and metabolism of protein and soluble nitrogen during spring growth of apple trees. J Am Soc Hortic Sci **107**: 209-213

Karamanos RE, Voroney RP, Rennie DA (1981) Variation in natural ^{15}N abundance of central Saskatchewan soils. Soil Sci Soc Am J **45**: 826-828

Kavanová M, Gloser V (2005) The use of internal nitrogen stores in the rhizomatous grass *Calamagrostis epigejos* during regrowth after defoliation. Ann Bot **95**: 457-463

Kesner L, Kirschenbaum D (1970) Amino acids, polypeptides, and proteins. In CA Streuli, PR Averell, eds, The analytical chemistry of nitrogen and its compounds vol. 2, Wiley-Interscience Press, NY, pp 635-704

Kim TH, Bigot J, Ourry A, Boucaud J (1993a) Amino acid content in xylem sap of regrowting alfalfa (*Medicago sativa* L.): relations with N uptake, N_2 fixation and N remobilization. Plant Soil **149**: 167-174

Kim TH, Ourry A, Boucaud J, Lemaire G (1991) Changes in source-sink relationship for nitrogen during regrowth of Lucerne (*Medicago sativa* L.) following removal of shoots. Aust J Plant Physiol **18**: 593-602

Kim TH, Ourry A, Boucaud J, Lemaire G (1993b) Partitioning of nitrogen derived from N_2 fixation and reserves in nodulated *Medicago sativa* L. during regrowth. J Ex Bot **44**: 555-562

Ku SB, Schmitt MR, Edwards GE (1979) Quantitative determination of RuBP carboxylase-oxygenase protein in leaves of several C_3 and C_4 plants. J Ex Bot **30**: 246-250

Lähdesmäki P, Pakonen T, Saari E, Laine K, Tasanen L, Havas P (1990) Changes in total nitrogen, protein, amino acids and NH_4^+ in tissues of bilberry, *Vaccinium myrtillis*, during the growing season. Holarctic Ecol **13**: 31-38

Lattanzi FA, Schnyder H, Thornton B (2005) The sources of carbon and nitrogen supplying leaf growth. Assessment of the role of stores with compartmental models. Plant Physiol **137**: 383-395

Lawlor DW, Boyle FA, Kendall AC, Keys AJ (1987) Nitrate nutrition and temperature effects on wheat: enzyme composition, nitrate and total amino acid content of leaves. J Ex Bot **38**: 378-392

Lea PJ, Miflin BJ (1974) An alternative route for nitrogen assimilation in higher plants. Nature **251**: 614-616

Lefevre J, Bigot J, Boucaud J (1991) Origin of foliar nitrogen and changes in free amino-acid composition and content of leaves, stubble, and roots of perennial ryegrass during re-growth and defoliation. J Ex Bot **42**: 89-95

Lestienne F, Thornton B, Gastal F (2006) Impact of defoliation intensity and frequency on N uptake and mobilization in *Lolium perenne*. J Ex Bot **57**: 997-1006

Levy Y, Horesh I (1984) Importance of penetration through stomata in the correction of chlorosis with iron salts and low-surface-tension surfactants. J Plant Nutr **7**: 279-281

Li H, Ôba K (1985) Major soluble proteins of sweet potato roots and changes in proteins after cutting, infection, or storage. Agric Biol Chem **49**: 737-744

Li R, Volenec JJ, Joern BC, Cunningham SM (1996) Seasonal changes in nonstructural carbohydrates, protein, and macronutrients in roots of alfalfa, red clover, sweetclover, and birdsfoot trefoil. Crop Sci **36**: 617-623

Li YS, Redmann RE, van Kessel C (1992) Nitrogen budget and ^{15}N translocation in a perennial wheatgrass. Func Ecol **6**: 221-225

Limami MA, Dufosse C, Rhichard-Molard C, Fouldrin K, Roux L, Morot-Gaudry J (1996) Effect of exogenous nitrogen ($^{15}NO_3$) on utilization of vegetative storage proteins (VSP) during regrowth in chicory (*Cichorium intybus*). J Plant Physiol **149**: 564-572

Louahlia S, Lainé P, Thornton B, Ourry A, Boucaud J (2000) The role of N-remobilization and the uptake of NH_4^+ and NO_3^- by *Lolium perenne* L. in laminae growth following defoliation under field conditions. Plant Soil **220**: 175-187

Louahlia S, Macduff JH, Ourry A, Humphreys M, Boucaud J (1999) Nitrogen reserve status affects the dynamics of nitrogen remobilization and mineral nitrogen uptake during recovery of contrasting cultivars of *Lolium perenne* from defoliation. New Phytol **142**: 451-462

Macduff JH, Jackson SB (1992) Influx and efflux of nitrate and ammonium in Italian ryegrass and white clover roots: comparisons between effects of darkness and defoliation. J Ex Bot **43**: 525-535

Macduff JH, Jarvis SC, Mosquera A (1989) Nitrate nutrition of grasses from steady-state supplies in flowing solution culture following nitrate deprivation and/or defoliation. J Ex Bot **40**: 977-984

Maeshima M, Sasaki T, Asahi T (1985) Characterization of major proteins in sweet potato tuberous roots. Phytochemistry **24**: 1899-1902

Maier-Maercker U (1999) New light on the importance of peristomatal transpiration. Aust J Plant Physiol **26**: 9-16

Mariotti A, Germon JC, Hubert P, Kaiser P, Letolle R, Tardieux A, Tardieux P (1981) Experimental determination of nitrogen kinetic isotope fractionations: some principles; illustration for denitrification and nitrification processes. Plant Soil **62**: 413-430

Marschner H (1995) Mineral nutrition of higher plants. Academic Press, London

Mason HS, Mullet JE (1990) Expression of two soybean vegetative storage protein genes during development and in response to water deficit, wounding, and jasmonic acid. Plant Cell **2**: 569-579

Matches AG (1979) Management. In RC Buckner , LP Bush, eds, Tall Fescue. Agron Monogr No 20. ASA, CSSA, and SSSA, Madison, WI, pp 171-199

McClain WE, Blevins DG (2007) Phosphorus fertilization increased macronutrient concentrations in leaves of stockpiled tall fescue. Forage and Grazinglands doi: 10.1094/FG-2007-0302-01-RS

McClain WE, Blevins DG (2009) Poultry litter application caused low leaf calcium and magnesium, increasing the grass tetany potential of stockpiled tall fescue. Forage and Grazinglands doi: 10.1094/FG-2009-1022-01-RS

McKendrick JD, Owensby CL, Hyde RM (1975) Big bluestem and indiangrass vegetative reproduction and annual reserve carbohydrate and nitrogen cycles. Agro Ecosystems **2**: 75-93

Meuriot F, Noquet C, Avice JC, Volenec JJ, Cunningham SM, Sors TG, Caillot S, Ourry A (2004) Methyl jasmonate alters N partitioning, N reserves accumulation and induces gene expression of a 32-kDa vegetative storage protein that possesses chitinase activity in *Medicago sativa* taproots. Physiol Plant **120**: 113-123

Millard P (1988) The accumulation and storage of nitrogen by herbaceous plants. Plant Cell Environ **11**: 1-8

Millard P, Thomas RJ, Buckland ST (1990) Nitrogen supply affects the remobilization of nitrogen for the regrowth of defoliated *Lolium perenne* L. J Ex Bot **41**: 941-947

Moore S (1968) Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction. J Biol Chem **243**: 6281-6283

Morton AJ (1977) Mineral nutrient pathways in a Molinietum in autumn and winter. J Ecol **65**: 993-999

Morvan-Bertrand A, Boucaud J, Prdu'homme M (1999) Influence of initial levels of carbohydrates, fructans, nitrogen, and soluble proteins on regrowth of *Lolium perenne* L. cv. Bravo following defoliation. J Ex Bot **50**: 1817-1826

Mussigmann C and Ledoigt G (1989) Major storage proteins in Jerusalem artichoke tubers. Plant Physiol Biochem **27**: 81-86

Nadelhoffer KJ, Fry B (1994) Nitrogen isotope studies in forest ecosystems. In K Lajtha, RH Michener, eds, Stable isotopes in ecology and environmental science. Blackwell Scientific Publishers, Cambridge, UK, pp 22-44

Nelson CJ (1996) Physiology and developmental morphology. In LE Moser, Buxton DR, Casler MD, eds, Cool-season forage grasses. Agron Monogr No 34. ASA, CSSA, and SSSA, Madison, WI, pp 87-125

Neumann PM (1988) Agrochemicals: Plant physiological and agricultural perspectives. In PM Neumann, ed, Plant growth and leaf-applied chemicals. CRC Press, Inc., Boca Raton, FL, pp 2-10

Noquet C, Avice J, Rossato L, Beauclair P, Henry M, Ourry A (2004) Effects of altered source-sink relationships on N allocation and vegetative storage protein accumulation in *Brassica napus* L. Plant Sci **166**: 1007-1018

Nordin A, Näsholm T (1997) Nitrogen storage forms in nine boreal understorey plant species. Oecologia **110**: 487-492

Oaks A, Hirel B (1985) Nitrogen metabolism in roots. Annu Rev Plant Physiol **36**: 345-365

Ocumpaugh WR, Matches AG (1977) Autumn-winter yield and quality of tall fescue. Agron J **69**: 639-643

Opitz von Boberfeld W, Banzhaf K (2006) The effect of sward management on the mineral content of winter grazed herbage. J Agron Crop Sci **192**: 1-9

Ourry A, Bigot J, Boucaud J (1989) Protein mobilization from stubble and roots, and proteolytic activities during post-clipping re-growth of perennial ryegrass. J Plant Physiol **134**: 298-303

Ourry A, Boucaud J, Duyme M (1990a) Sink control of nitrogen uptake and assimilation during regrowth after cutting of ryegrass (*Lolium perenne* L.). Plant Cell Environ **13**: 185-189

Ourry A, Boucaud J, Salette J (1988) Nitrogen mobilization from stubble and roots during re-growth of defoliated perennial ryegrass. J Ex Bot **39**: 803-809

Ourry A, Boucaud J, Salette J (1990b) Partitioning and remobilization of nitrogen during regrowth in nitrogen-deficient ryegrass. Crop Sci **30**: 1251-1254

Ourry A, Kim TH, Boucaud J (1994) Nitrogen reserve mobilization during regrowth of *Medicago sativa* L.: relationships between availability and regrowth yield. Plant Physiol **105**: 831-837

Ourry A, Macduff JH, Ougham HJ (1996) The relationship between mobilization of N reserves and changes in translatable messages following defoliation in *Lolium temulentum* L. and *Lolium perenne* L. J Ex Bot **47**: 739-747

Ourry A, Macduff JH, Volenec JJ, Gaudillere JP (2001) Nitrogen traffic during plant growth and development. In PJ Lea, Morot-Gaudry JF, eds, Plant nitrogen. Springer-Verlag, Berlin, pp 255-273

Paiva E, Lister RM, Park WD (1983) Induction and accumulation of major tuber proteins of potato in stems and petioles. Plant Physiol **71**: 161-168

Park WD, Blackwood C, Mignery GA, Hermodson MA, Lister RM (1983) Analysis of the heterogeneity of the 40,000 molecular weight tuber glycoprotein of potatoes by immunological methods and by NH_2-terminal sequence analysis. Plant Physiol **71**: 156-160

Partala A, Mela T, Esala M, Ketoja E (2001) Plant recovery of ^{15}N-labelled nitrogen applied to reed canary grass grown for biomass. Nutr Cycling in Agroeco **61**: 273-281

Pate JS (1983) Patterns of nitrogen metabolism in higher plants and their ecological significance. In JA Lee, S McNeill, IH Rorison, eds, Nitrogen as an ecological factor, Blackwell, Oxford, pp 225-255

Peterson GL (1983) Determination of total protein. Meth Enzymol **91**: 95-119

Peuke AD, Jesche WD, Dietz KJ, Schreiber L, Hartung W (1998) Foliar application of nitrate and ammonium as sole nitrogen supply in *Ricinus communis*. I. Carbon and nitrogen uptake and inflows. New Phytol **138**: 675-687

Phillips DA, Center M, Jones MB (1983) Nitrogen turnover and assimilation during regrowth in *Trifolium subterraneum* L. and *Bromus mollis* L.. Plant Physiol **71**: 472-476

Piccolo MC, Neill C, Cerri CC (1996) ^{15}N natural abundance in forest and pasture soils of the Brazilian Amazon Basin. Oecologia **99**: 112-117

Poore MH, Benson GA, Scott ME, Green JT (2000) Production and use of stockpiled tall fescue to reduce beef cattle production costs. J Anim Sci **79**: 1-11

Power JF (1986) Nitrogen cycling in seven cool-season perennial grass species. Agron J **78**:681-687

Qiao Z, Murray F (1997) The effects of root nitrogen supplies on the absorption of atmospheric NO_2 by soybean leaves. New Phytol **136**: 239-243

Racusen D (1986) Esterase specificity of patatin from two potato cultivars. Can J Bot **64**: 2104-2106

Racusen D, Foote M (1980) A major soluble glycoprotein of potato tubers. J Food Biochem **4**: 43-52

Rayburn EB, Blaser RE, Wolf DD (1979) Winter tall fescue yield and quality with different accumulation periods and N rates. Agron J **71**: 959-963

Raven JA (1986) Biochemical disposal of excess H^+ in growing plants? New Phytol **104**: 175-206

Roberts CA (2004) Tall fescue toxicosis. MU Extension G4669

Roberts CA, Lacefield GD, Ball D, Bates G (2009) Management to optimize grazing performance in the northern hemisphere. In HA Fribourg, DB Hannaway, CP West, eds, Tall fescue for the twenty-first century. Agron Monogr No 53. ASA, CSSA, and SSSA, Madison, WI, pp 85-99

Robson MJ, Deacon MJ (1978) Nitrogen deficiency in small closed communities of S24 ryegrass. II. Changes in the weight and chemical composition of single leaves during their growth and death. Ann Bot **42**: 1199-1213

Rosnitschek-Schimmel (1985a) Seasonal dynamics of nitrogenous compounds in a nitrophilic weed I. Changes in inorganic and organic nitrogen fractions of the different plant parts of *Urtica dioica*. Plant Cell Physiol **26**: 169-176

Rosnitschek-Schimmel (1985b) Seasonal dynamics of nitrogenous compounds in a nitrophilic weed II. The role of free amino acids and proteins as nitrogen store in *Urtica dioica*. Plant Cell Physiol **26**: 177-183

Ross JP, Reynolds JH (1979) Nutritive value of fall-stockpiled tall fescue. Tenn Farm Home Sci **112**: 44-48

Ross JP, Reynolds JH (1981) Forage quality of fescue regrowth after removal of stockpiled forage. Tenn Farm and Home Sci **118**: 11-13

Rossato L, Le Dantec C, Laine P, Ourry A (2002) Nitrogen storage and remobilization in *Brassica napus* L. during the growth cycle: identification, characterization and immunolocalization of a putative taproot storage glycoprotein. J Ex Bot **53**: 265-275

Rossato L, Laine P, Ourry A (2001) Nitrogen storage and remobilization in *Brassica napus* L. during the growth cycle. I. Nitrogen fluxes within the plant and changes in soluble protein patterns. J Ex Bot **52**: 1655-1663

Sagisaka S (1974) Transition of metabolism in living poplar bark from growing to wintering stages and *vice versa*. Plant Physiol **54**: 544-549

Sagisaka S (1983) Amino acid pools in perennial plants at the wintering stage and at the beginning of growth. Plant Cell Physiol **24**: 479-494

Sagisaka S (1987) Amino acid pools in herbaceous plants at the wintering stage and at the beginning of growth. Plant Cell Physiol **28**: 171-178

Santos PM, Thornton B, Corsi M (2002) Nitrogen dynamics in the intact grasses *Poa trivialis* and *Panicum maximum* receiving contrasting supplies of nitrogen. J Ex Bot **53**: 2167-2176

Scheurwater I, Koren M, Lambers H, Atkin OK (2002) The contribution of roots and shoots to whole plant nitrate reduction in fast- and slow-growing grass species. J Ex Bot **53**: 1635-1642

Schmitt MR, Edwards GE (1981) Photosynthetic capacity and nitrogen use efficiency of maize, wheat and rice: a comparison between C_3 and C_4 photosynthesis. J Ex Bot **32**: 459-466

Schnyder H, de Visser R (1999) Fluxes of reserve-derived and currently assimilated carbon and nitrogen in perennial ryegrass recovering from defoliation. The regrowing tiller and its component functionally distinct zones. Plant Physiol **119**: 1423-1435

Shearer G, Kohl DH (1986) N_2-fixation in field settings: Estimations based on natural ^{15}N abundance. Aust J Plant Physiol **13**: 699-756

Shearer G, Kohl DH, Chien SH (1978) The nitrogen-15 abundance in a wide variety of soils. Soil Sci Soc Am J **42**: 899-902

Sleper DA, West CP (1996) Tall fescue. In LE Moser, Buxton DR, Casler MD, eds, Cool-season forage grasses. Agron Monogr No 34. ASA, CSSA, and SSSA, Madison, WI, pp 471-502

Smirnoff N, Stewart GR (1985) Nitrate assimilation and translocation by higher plants: comparative physiology and ecological consequences. Physiol Plant **64**: 133-140

Sonnewald U, Studer D, Rocha-Sosa M, Willmitzer L (1989) Immunocytochemical localization of patatin, the major glycoprotein in potato (*Solanum tuberoseum* L.) tubers. Planta **178**: 176-183

Sparks JP (2009) Ecological ramifications of the direct foliar uptake of nitrogen. Oecologia **159**: 1-13

Staswick PE (1989) Preferential loss of an abundant storage protein from soybean pods during seed development. Plant Physiol **90**: 1252-1255

Staswick PE (1994) Storage proteins of vegetative plant tissues. Annu Rev Plant Physiol Plant Mol Biol **45**: 303-22

Steele KW, Wilson AT (1981) Nitrogen isotope ratios in surface and sub-surface horizons of New Zealand improved grassland soil. NZ J Agric Res **24**: 167-170

Stiegler C, Richardson M, and McCalla J (2009) Foliar uptake of inorganic and organic nitrogen compounds by creeping bentgrass putting green turf. Arkansas Turfgrass Report 2008, Ark Ag Exp Stn Res Ser **568**: 116-120

Streeter JG (1979) Allantoin and allantoic acid in tissues and stem exudate from field-grown soybean plants. Plant Physiol **63**: 478-480

Stuckey IH (1941) Seasonal growth of grass roots. Am J Bot **28**:486-491

Ta TC, Macdowall FDH, Faris MA (1990) Utilization of carbon and nitrogen reserves of alfalfa roots in supporting N_2-fixation and shoot regrowth. Plant Soil **127**: 231-236

Taylor BR, Parkinson D, Parsons WFJ (1989) Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. Ecology **70**:97-104

Taylor TH, Templeton WC (1976) Stockpiling Kentucky bluegrass and tall fescue forage for winter pasturage. Agron J **68**: 235-239

Templer PH, Arthur MA, Lovett GM, Weathers KC (2007) Plant and soil natural abundance ^{15}N: indicators of relative rates of nitrogen cycling in temperate forest ecosystems. Oecologia **153**: 399-406

Teutsch CD, Fike JH, Groover GE, Aref S (2005) Nitrogen rate and source effects on yield and nutritive value of tall fescue stockpiled for winter grazing. Forages and Grazinglands doi:10.1094/FG-2005-1220-01-RS

Thomas H (1978) Enzymes of nitrogen mobilization in detached leaves of *Lolium temulentum* during senescence. Planta **142**: 161-169

Thomas H, Stoddart JL (1980) Leaf senescence. Ann Rev Plant Physiol **31**: 83-111

Thornton B, Bausenwein U (2000) Seasonal protease activity in storage tissue of the deciduous grass *Molina caerulea*. New Phytol **146**: 75-81

Thornton B, Macduff JH (2002) Short-term changes in xylem N compounds in *Lolium perenne* following defoliation. Ann Bot **89**: 715-722

Thornton B, Millard P (1993) The effects of nitrogen supply and defoliation on the seasonal internal cycling of nitrogen in *Molinia caerulea*. J Ex Bot **44**: 531-536

Thornton B, Millard P (1996) Effects of severity of defoliation on root functioning in grasses. J Range Manage **49**:443-447

Thornton B, Millard P (1997) Increased defoliation frequency depletes remobilization of nitrogen for leaf growth in grasses. Ann Bot **80**: 89-95

Thornton B, Millard P, Duff EI (1994) Effects of nitrogen supply on the source of nitrogen used for regrowth of laminae after defoliation of four grass species. New Phytol **128**: 615-620

Thornton B, Millard P, Duff EI, Buckland ST (1993a) The relative contribution of remobilization and root uptake in supplying nitrogen after defoliation for regrowth of laminae in four grass species. New Phytol **124**: 689-694

Thornton B, Millard P, Galloway S (1993b) The effects of temperature and form of nitrogen supply on the relative contribution of root uptake and remobilization in supplying nitrogen for laminae regrowth of *Lolium perenne* L. J Ex Bot **44**: 1601-1606

Thornton B, Paterson E, Kingston-Smith AH, Bollard AL, Pratt SM, Sim A (2002) Reduced atmospheric CO_2 inhibits nitrogen mobilization in *Festuca rubra*. Physiol Plant **116**: 62-72

Tischner R (1987) Regulation of ammonium incorporation. In WR Ullrich, PJ Aparicio, PJ Syrett, F Castillo, eds, Inorganic nitrogen metabolism. Springer, Berlin, pp 126-131

Tromp J, Ovaa JC (1973) Spring mobilization of protein nitrogen in apple bark. Physiol Plant **29**: 1-5

Turner LB, Pollock CJ (1998) Changes in stolon carbohydrates during the winter in four varieties of white clover (*Trifolium repens* L.) with contrasting hardiness. Ann Bot **81**: 97-107

USDA-NASS (2009) United States Department of Agriculture-National Agricultural Statistics Service, http://www.nass.usda.gov

Van Damme EJM, Hao Q, Barre A, Rougé P, Van Leuven F, Peumans WJ (2000) Major protein of resting rhizomes of *Calystegia sepium* (hedge bindweed) closely resembles plant RNases but has no enzymatic activity. Plant Physiol **122**: 433-445

Vance CP, Heichel GH (1981) Nitrate assimilation during vegetative regrowth of alfalfa. Plant Physiol **68**: 1052-1056

Vance CP, Heichel GH, Barnes DK, Bryan JW, Johnson LE (1979) Nitrogen fixation, nodule development, and vegetative regrowth of alfalfa (*Medicago sativa* L.) following harvest. **64**: 1-8

Volenec JJ, Boyce PJ, Hendershot KL (1991) Carbohydrate metabolism in taproots of *Medicago sativa* L. during winter adaptation and spring regrowth. Plant Physiol **96**: 786-793

Volenec JJ, Ourry A, Joern BC (1996) A role for nitrogen reserves in forage regrowth and stress tolerance. Physiol Plant **97**: 185-193

Vouillot MO, Devienne-Barret F (1999) Accumulation and remobilization of nitrogen in a vegetative winter wheat crop during or following nitrogen deficiency. Ann Bot **83**: 569-575

Wallsgrove RM (1987) The roles of glutamine synthetase and glutamate synthase in nitrogen metabolism of higher plants. In WR Ullrich, PJ Aparicio, PJ Syrett, F Castillo, eds, Inorganic nitrogen metabolism. Springer, Berlin, pp 137-141

Welker JM, Briske DD, Weaver RW (1991) Intraclonal nitrogen allocation in the bunchgrass *Schizachyrium scoparium* Hubb.: an assessment of the physiological individual. Func Ecol **5**: 433-440

Wen TX, Hideo I, Masayuki O (1999) Absorption, translocation, and assimilation of foliar-applied urea compared with nitrate and ammonium in tomato plants. Soil Sci Plant Nutr **45**: 609-616

Wetzel S, Demmers C, Greenwood JS (1989) Seasonally fluctuating bark proteins are a potential form of nitrogen storage in three temperate hardwoods. Planta **178**: 275-281

Williams RF (1955) Redistribution of mineral elements during development. Ann Rev Plant Physiol **33**: 263-302

Willms WE, King J, Dormaar JF (1998) Weathering losses of forage species on the fescue grassland in southwestern Alberta. Can J Plant Sci **78**: 265-272

Wittenbach VA (1979) Ribulose bisphosphate carboxylase and proteolytic activity in wheat leaves from anthesis through senescence. Plant Physiol **64**: 884-887

Wittenbach VA (1983) Purification and characterization of a soybean leaf storage glycoprotein. Plant Phyisiol **73**: 125-129

Wolf DD, Balaska JA, Ries RE (1996) Stand establishment. In LE Moser, DR Buxton, MD Casler, eds, Cool-season forage grasses, Agron Monogr No 34. ASA, CSSA, and SSSA, Madison, WI, pp 71-85

Xu X, Butler SM, Greenwood JS, Bewley JD (2000) Stress-mediated effects on the expression of a predominant 18-kDa *Taraxacum officinale* root protein. Plant Physiol Biochem **38**: 491-497

Yeh K, Chen J, Lin M, Chen Y, Lin C (1997) Functional activity of sporamin from sweet potato (*Ipomoea batatas* Lam.): a tuber storage protein with trypsin inhibitory activity. Plant Mol Biol **33**: 565-570

Yoneyman T, Kaneko A (1989) Variations in the natural abundance of ^{15}N in nitrogenous fractions of komatsuna plants supplied with nitrate. Plant Cell Physiol **30**: 957-962

Appendix A

Preliminary Field Experiment

During the 2005-2006 Stockpiling Season

Introduction

The overall objective of this study was to determine the fate of N from tall fescue leaves during winter and spring. A preliminary field study was conducted to test field-based methodology for ^{15}N foliar-labeling study of an established tall fescue stand. This study was conducted during the stockpiling period of 2005-2006.

Materials and Methods

An established tall fescue ('Kentucky-31', endophyte-infected) stand was selected at BREC. This pasture was on a Leonard silt loam (fine, smectitic, mesic Veric Epiaqualf), with pHs = 6.3, NA = 1.75 meq/100g, OM = 5.2%, Bray I P = 75 kg/ha, Bray II P = 288 kg/ha, Ca = 4628 kg/ha, Mg = 393 kg/ha, K = 283 kg/ha, and CEC = 15.3 meq/100 g, as determined to a 15 cm depth by the University of Missouri Soil Testing Laboratory (Columbia, MO). Kentucky bluegrass was present throughout the tall fescue stand.

The plot area was mowed to about a 10 cm height on September 16, 2005. Three weeks later, 100 plots, each 30.5 cm^2 and spaced evenly with 1.22 m centers, were established by spraying glyphosate (Round-up WeatherMax®) with ammonium sulfate at the rate of 2% active ingredient (1.5 oz/gal, 16 gal used for 602 m^2 area). A 30.5 cm^2 cardboard box was used to keep the glyphosate spray from drifting onto the plots. After 14 d, the dead debris was removed from around the plots and any non-fescue foliage and dead debris was

removed from within the plots (Figure A-1A,B). On November 2, 48 plots were selected based on the uniformity of tall fescue tillers within the plot and treatments were randomly assigned.

Twenty-four plots received a foliar ^{15}N application, at the equivalent of 2.24 kg ^{15}N/ha (5 lbs ^{15}N/acre) (rate modified from Glasener et al. 1998). Application consisted of brushing on 1.5 ml solution of 0.1453 g ≥ 98 atom% $^{15}NH_4{}^{15}NO_3$ (Cambridge Isotope Laboratories, Inc., Andover, MA) in 0.1% (v/v) Tween 80 /plot. The solution was evenly applied to all surfaces of aboveground green leaf tissues (Figure A-1C). Twenty-four control plots did not receive the ^{15}N application. In every plot, tillers were individually tagged at the base using a colored wire and coded according to how many collared and expanding leaves were present at the time of treatment application and the number of tillers were counted (Figure A-1D). Also at this time, ammonium nitrate granular fertilizer (33.5% N) was broadcasted over the entire plot area by hand at a rate of 112 kg N/ha. After four days, the entire plot area was covered in wheat straw and manually incorporated around the base of the tillers of each plot since most of the debris had been previously removed.

The first harvest was conducted four weeks after the ^{15}N application on December 1, then subsequent harvests occurred monthly through April on the following dates: January 4, February 6, March 6, and April 3. During each harvest, four plots treated with ^{15}N and four control plots were harvested. Harvesting consisted of removing each plot from the ground by digging a 0.6 m^2

area around each plot to about a 20 cm depth (Figure A-1E). All aboveground tillers were removed at ground level, and separated by tag color or no tag, and placed in plastic bags and packed in ice. Each sample was washed at the field site with cold water to remove the soil (Figure A-1F), packed in plastic bags, packed on ice, and transported to the laboratory for tissue separation.

Non-fescue tissue and dead tissue not connected to a live portion of tissue were removed from the underground samples. Tissues were washed free of soil, rinsed in deionized water, and kept over ice during the separation process.

The belowground portions were separated as in Season 1 and 2 (Chapter 2). Since all aboveground parts and tags were previously removed at the field site, all remaining sheaths and apical meristem region of tillers that were below the soil surface were indistinguishable as from treated tillers or new tillers. Therefore, all of these segments were included with the treated leaves.

After separation, tall fescue segments were placed in aluminum foil packets, weighed for fresh weight determination, frozen at -20 ^{o}C, and stored at -70 ^{o}C until being freeze-dried. Samples were freeze-dried for at least 72 h until a steady dry weight was achieved, weighed, ground, and stored in plastic Whirl-pak® bags (Nasco, Fort Atkinson, WI). Samples were double-bagged in plastic bags containing desiccant and stored at -70 ^{o}C.

Soil samples were collected from the top 1 cm within the 30.5 cm^2 plot. Collections were made on four ^{15}N-treated plots, each sampled before the application of ^{15}N treatment, two hours after ^{15}N treatment, 48 h after ^{15}N

treatment, and then on each harvest date. Soil samples were air-dried, ground by hand to a fine powder, then stored in plastic Whirl-pak® bags at room temperature.

Determination of total N and ^{15}N in samples, weather data collection, calculations, and statistics were conducted as described in Chapter 2.

Results

Weather

Temperatures were generally average during the 2005-2006 stockpiling season, however there were prolonged periods of below-freezing air temperatures in November and December (Figure A-2A). Precipitation was below average in autumn and again in February (Figure A-2B).

Soil ^{15}N

Surface soil samples collected before ^{15}N applications were about 0.38 atom% ^{15}N and remained at about 0.40 atom% ^{15}N through April (Figure A-3). There were no statistically significant differences in atom% ^{15}N of the soil samples over time. Recovered ^{15}N in the top one cm of soil was estimated to range from 1-3% of applied ^{15}N throughout the season (see Appendix B, Figure B-1).

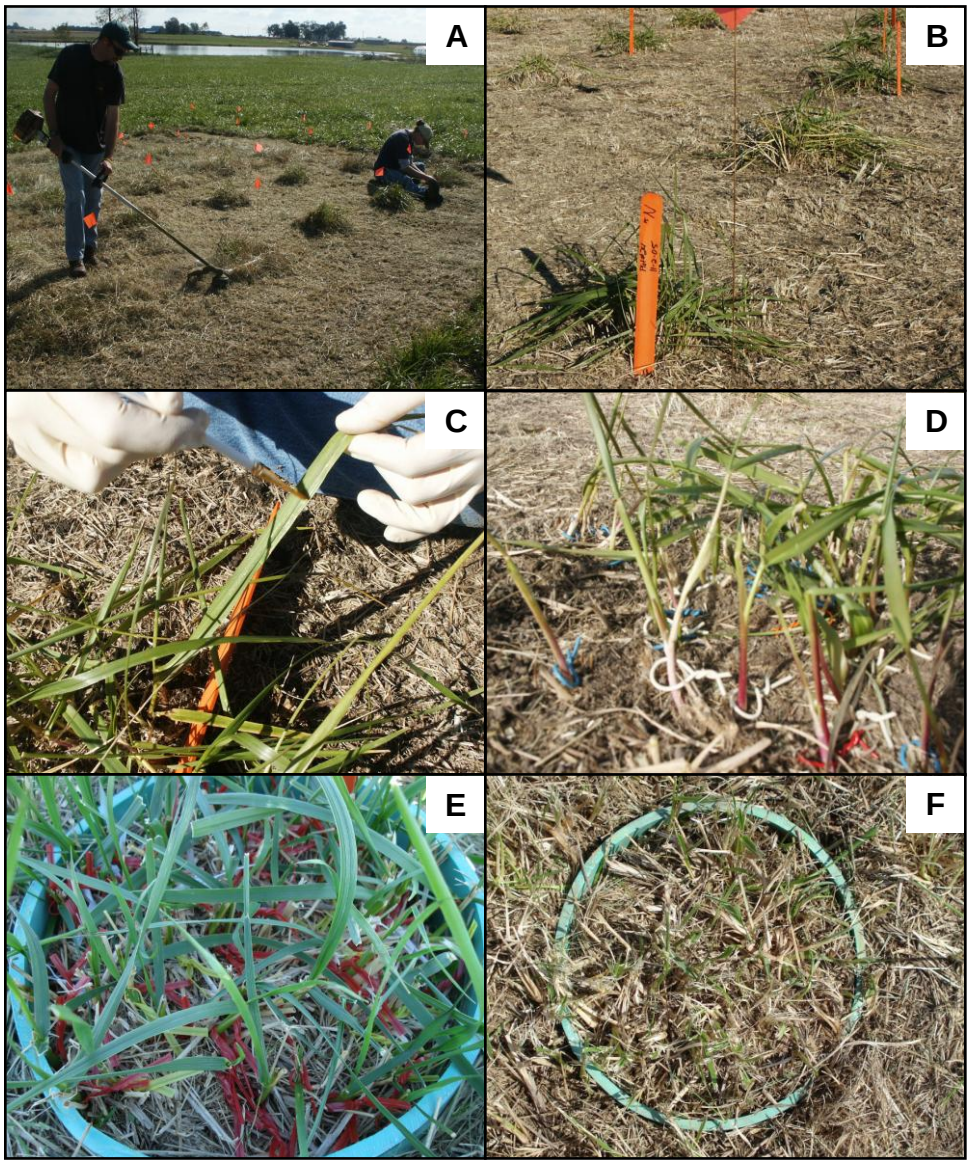

Figure A-1. Establishment, treatments of foliar applied ^{15}N, and harvest of stockpiled tall fescue plots for a leaf N remobilization study at BREC. (A) Debris being removed around 30.5 cm^2 tall fescue plots, 14 d after glyphosate application. (B) Tall fescue plots at the time of ^{15}N application. (C) Hand-brushed foliar ^{15}N application of tall fescue. (D) Plot of tall fescue tagged at the

base of each tiller. (E) Removal of tall fescue plots from field at harvest. (G) Samples washed at field site, revealing tall fescue belowground segments.

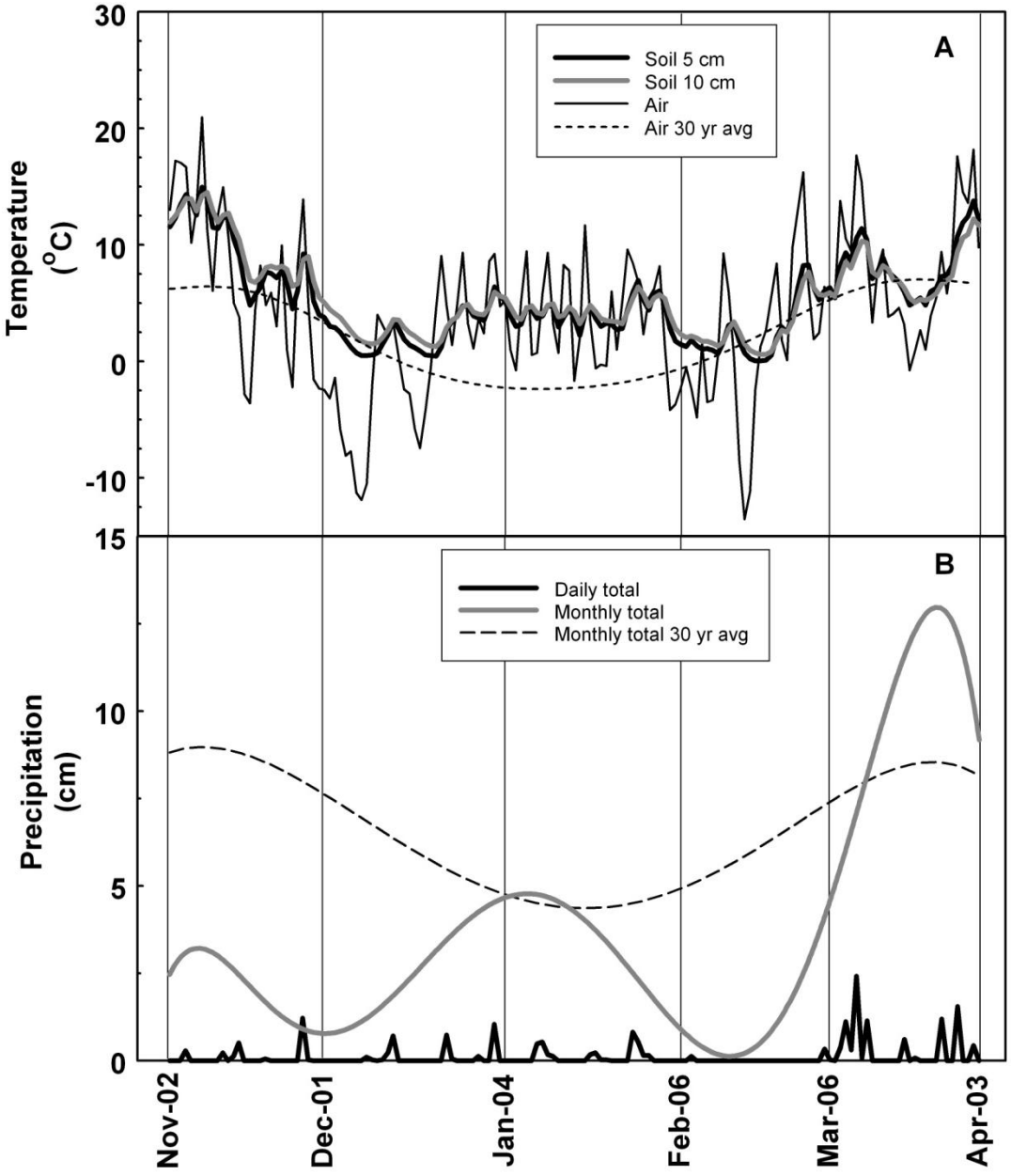

Figure A-2. Average daily air and soil temperatures and daily precipitation totals for the 2005-2006 stockpiling season of tall fescue at BREC. (A) Average daily temperature of air and sod-covered soil at 5 and 10 cm depths with monthly thirty-year averages. (B) Precipitation daily totals, monthly totals, and monthly

thirty-year averages. Vertical lines indicate treatment date (Nov-02) and harvest dates.

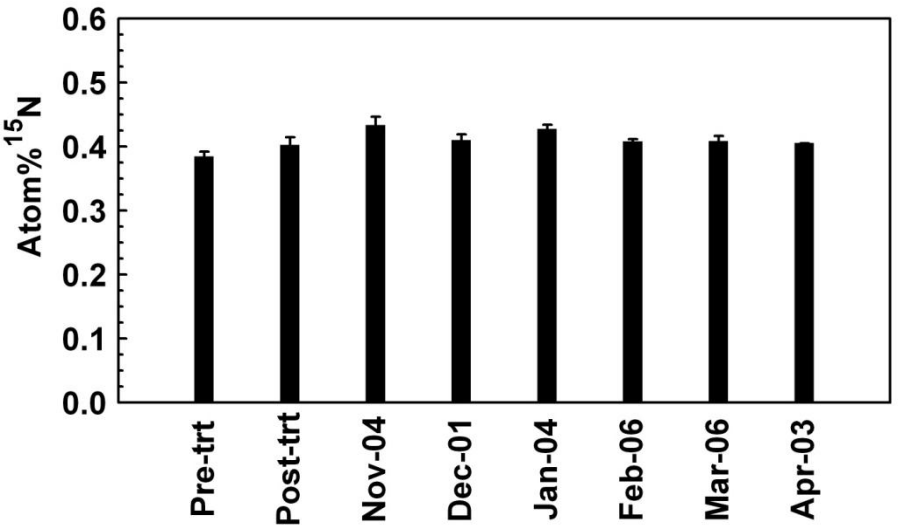

Figure A-3. Soil atom% ^{15}N of samples (top 1cm of soil surface) taken before ^{15}N treatment, just after ^{15}N treatment, then on each date indicated of the 2005-2006 stockpiling season at BREC. Samples were collected on the same ^{15}N-treated plots over time. Values are the mean±SE (error bars are visible when larger than the symbol size, n=4).

Initial Tiller Recovery

Tillers that received ^{15}N foliar applications were tagged at the time of treatment. Recovery of the tagged tillers neared 100% and did not change over time (Figure A-4).

Growth of Tall Fescue

Overall, more dry matter was proportioned to shoots of tall fescue (Figure A-5A-C) than belowground structures (Figures A-6A-C and A-7A-C). Treated leaves decreased slightly in mass through February and greatly reduced in mass from March to April (Figure A-5A), while new tillers increased through the winter but most abundantly increased from March to April (Figure A-5C). Very few new leaves formed by January, and no leaves were present on the treated tillers in the subsequent harvests (Figure A-5B). In belowground structures, crowns and new roots showed little change (Figure A-6A,C) while new rhizomes decreased in spring (Figure A-6B). However, old roots and rhizomes declined after December and remained unchanged through spring (Figure A-7A,B).

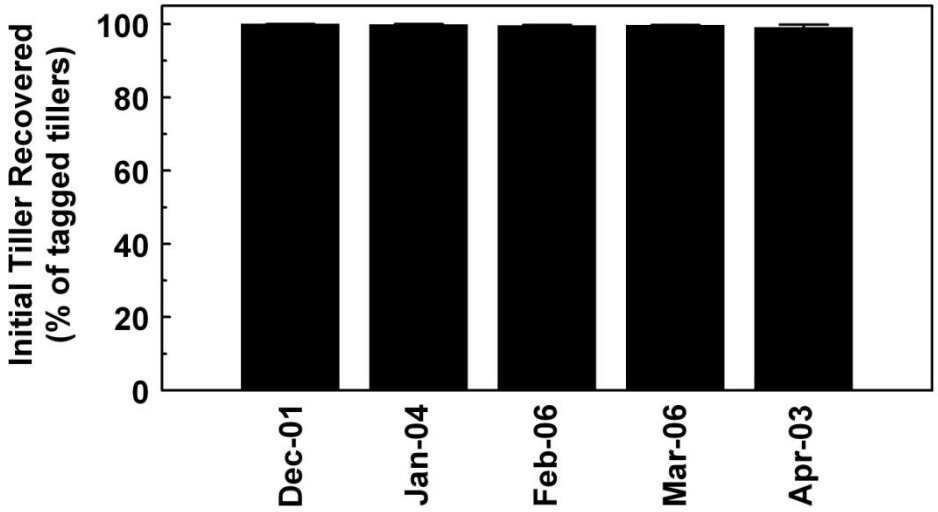

Figure A-4. The percent recovery of tagged tall fescue tillers during the 2005-2006 harvests (values are mean±SE, error bars are visible when larger than the symbol size, n=4). Tillers were tagged at the time of treatment application on November 2.

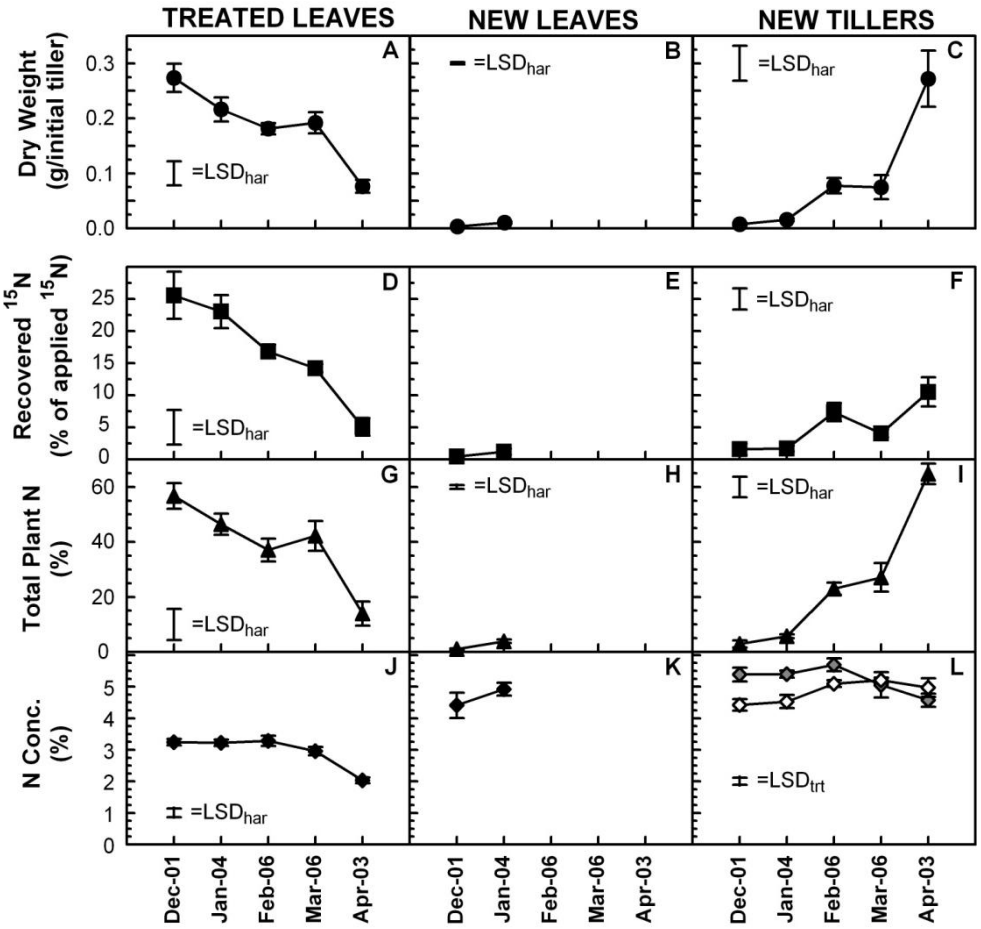

Figure A-5. Tall fescue shoot sections dry weight, recovered ^{15}N, percent of total plant N, and N concentrations during the 2005-2006 stockpiling season at BREC. Each value is the mean±SE (error bars are visible when larger than the symbol size). Black-closed symbols are all samples combined (n=8), gray-closed symbols are control samples (n=4), open symbols are ^{15}N-treated samples (n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between treatments (LSD_{trt}).

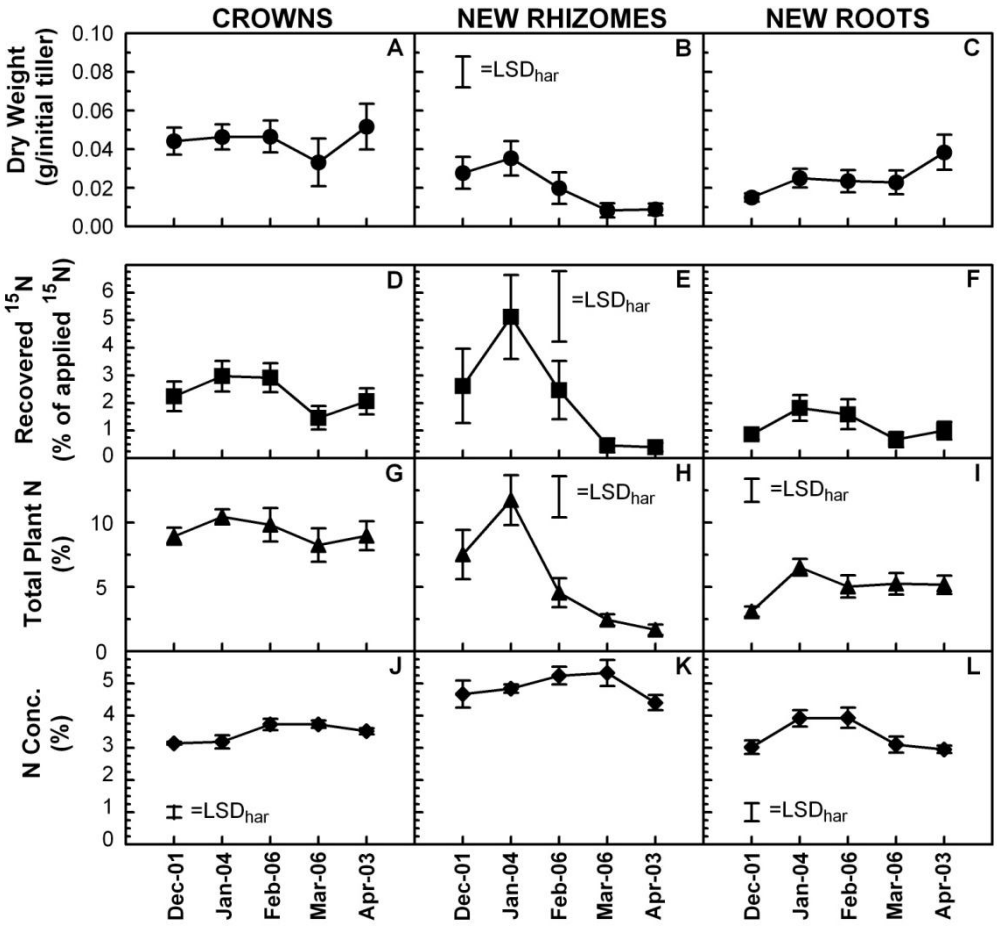

Figure A-6. Tall fescue belowground sections dry weight, recovered ^{15}N, percent of total plant N, and N concentrations during the 2005-2006 stockpiling season at BREC. Each value is the mean±SE (error bars are visible when larger than the symbol size, n=8). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}).

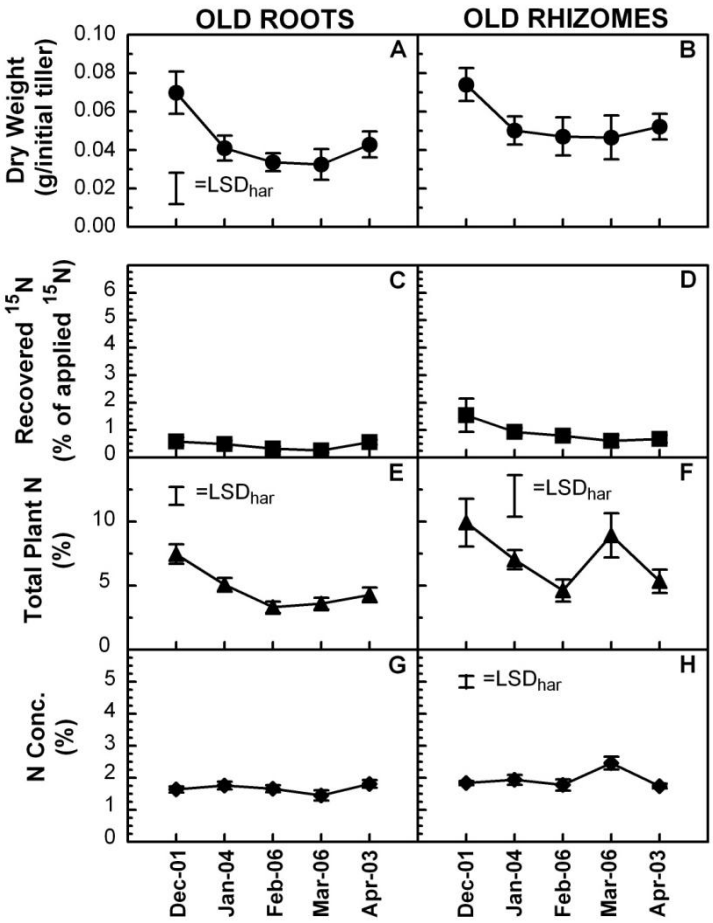

Figure A-7. Tall fescue old belowground sections dry weight, recovered ^{15}N, percent of total plant N, and N concentrations during the 2005-2006 stockpiling season at BREC. Each value is the mean±SE (error bars are visible when larger than the symbol size, n=8). Fisher's protected LSD (P<0.05) given between harvest dates (LSD_{har}).

Recovered ^{15}N

About 35% of the applied ^{15}N was recovered in the first harvest (Figure A-8). The amount of ^{15}N recovered did not change through the winter harvests, but declined to about 22% in March and April harvests. In December and January, a majority of ^{15}N was found in treated leaves. Throughout the rest of the season, ^{15}N declined in treated leaves, however total combined recovery in other tissues did not change over time (Figure A-8).

All tissue segments contained ^{15}N at each harvest. The majority of ^{15}N was recovered in shoot sections (Figure A-5D-F) compared to belowground sections (Figures A-6D-F and A-7C,D). At the first harvest, treated leaves contained the greatest amount of applied ^{15}N and this declined throughout the season (Figure A-5F). The ^{15}N label increased in crowns, new rhizomes, and new roots in January, but decreased in March and April (Figure A-6D-F). New tillers contained the most ^{15}N by April (Figure A-5F). No change in ^{15}N content occurred in old rhizomes and old roots over the season (Figure A-7C,D).

Total Plant N

The allocation of N to plant segments in relation to the total plant N (% of total plant N) followed similar patterns as the recovered ^{15}N, declining in treated leaves while increasing in new tillers (Figure A-5G, I). More N was allocated to new rhizomes and new roots through January, but declined in spring (Figure A-

6H,I). Also, N allocation to old roots and old rhizomes was reduced through February (Figure A-7E,F).

N Concentration

The concentration of N in tall fescue segments remained unchanged through autumn and winter with the exception of a slight increase in crowns and new roots during the winter (Figures A-5J-L; A-6J-L; A-7G,H). In spring, N concentrations declined slightly in treated leaves, new rhizomes, new roots and old rhizomes (Figures A-5J; A-6K,L; A-7H).

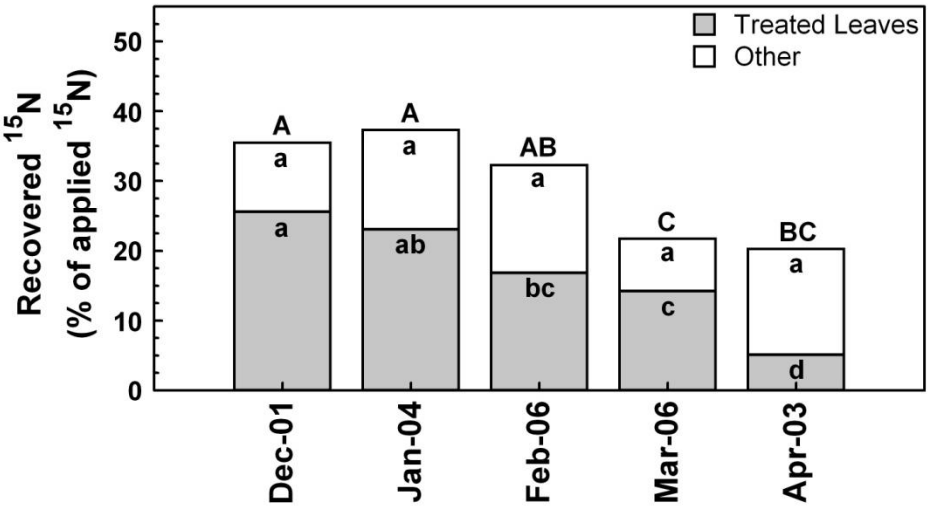

Figure A-8. The percent recovery of applied ^{15}N in treated leaves and all other plant parts of tall fescue harvested throughout the 2005-2006 season at BREC. Values are means (n=4). Across harvest dates, different uppercase letters indicate different total values and different lowercase letters indicate different values within each plant segment according to Fisher's protected LSD when $P<0.05$.

Discussion

Through visual observations, the glyphosate that was applied to the surrounding area of each plot negatively affected the growth of the tillers within the plot. This is most likely a result of the transport of glyphosate through the interconnecting rhizomes between tillers or contamination of glyphosate tillers within the plots. Also, the removal of all the debris surrounding the treated tillers may have mechanically damaged tissues, exposed them to colder temperatures, and stressed the plants despite the mulching of wheat straw. Much of the aboveground portions of the tall fescue tillers in the plots were necrotic by January. The tillers present at the time of application (treated leaves) did not produce new leaves (Figure A-5B) and eventually died. The recovery of the initial tillers remained near 100% (Figure A-4), however as the season progressed the dead leaves on the tagged tillers were detaching from the tillers. Thus, some of the treated leaves, which had senesced, were not recovered and this was observed to occur more frequently as the season progressed. The lack of treated leaf recovery may be a strong factor in the reduction of overall ^{15}N recovery in March and April, since the recovery of ^{15}N in other segments remained similar through this period (Figure A-8).

Belowground tissues seemed to remain viable despite the glyphosate treatment, with new tiller (Figure A-5C) and new root growth (Figure A-6C) occurring through the season. Overall, growth may have been suppressed due to the tiller dieback. Also to note, several plots harvested in March were poor in

terms of tiller health, and data for March may not accurately reflect healthy tiller growth.

At harvest, the aboveground portion of tillers and tags were removed at the soil line. The rest of the sample was then washed of soil and separated. The belowground portions of the tillers (including remaining sheaths and apical meristem regions) were indistinguishable as treated tillers or new tillers from that point on. All of this material was included with the treated leaves segment. This technique overestimated the weight and ^{15}N content in treated leaves and underestimated new tiller growth and ^{15}N content. Also, in early development, new tillers emerging from active rhizomes were indistinguishable from developing new rhizomes. These segments were deemed new rhizomes until they reached the soil surface and begun turning green, at which point they were deemed new tillers. Consequently, in early development, the new tillers were segmented to the new rhizomes, overestimating new rhizome development and ^{15}N content and underestimating new tillers. From observation, this was most prevalent in the earlier harvests (December and January) when early development of new tillers was mostly occurring. Therefore, the increased amount of ^{15}N and total plant N in new rhizomes in earlier harvests were most likely utilized for new tiller growth.

Surface soil samples were slightly greater than natural abundance levels of about 0.3697 atom% ^{15}N (Shearer et al. 1978, Karamanos et al. 1981, Steele and Wilson 1981) reading about 0.38 atom% ^{15}N and remained at about 0.40

atom% ^{15}N through April (Figure A-3). Even though the soil samples were slightly elevated in atom% ^{15}N, pre-^{15}N treatment levels were statistically similar and there were no differences in soil atom% ^{15}N over the course of the experiment (Figure A-3). Hence, there seemed to be no significant contamination of ^{15}N to the soil surface from the foliar ^{15}N applications. Any ^{15}N enrichment of plant parts, other than treated leaves, was likely due to remobilization of ^{15}N from other plant parts.

The initial ^{15}N recovery rate of about 35% was similar to foliar nitrate applications in other species, including a 31-57% recovery in gray alder (*Alnus glutinosa*) over a 3 week application period (Gonzalez-Prieto et al. 1995), 33.7% in tomato (*Lycopersicon esculentum* Mill.) two days after application (Wen et al. 1999), and 27 and 37% recovery after 1 and 8 hours, respectively, in creeping bentgrass (Stiegler et al. 2009). Also, the amount of ^{15}N applied successfully enriched tall fescue tissues to levels above detection limits for enriched samples.

The amount of ^{15}N declined in treated leaves, and it was found in other segments, suggesting that ^{15}N remobilization was occurring from treated leaves to other plant tissues. However, the amount of ^{15}N remobilized out of the treated leaves to other plant segments did not change over the course of the experiment (Figure A-8), indicating that most of the ^{15}N remobilization from treated leaves occurred before the first harvest on Dec-01. The lack of further ^{15}N remobilization could be due to the early senescence of the treated tillers from the previously mentioned stresses. Because there was no further ^{15}N remobilized

from the treated leaves, any changes in ^{15}N content of different plant segments after the first harvest was from the reallocation of ^{15}N within the plants. Interestingly, the ^{15}N was found more abundantly in actively growing segments. Also, the pattern of ^{15}N content in the segments over time follows that of total plant N (N allocation) and not N concentration. This suggests that ^{15}N was utilized for growth and development of these plant segments, rather than contributing to concentrating N in existing tissues. The extent of the contribution of remobilized leaf ^{15}N could not be accurately determined in this study based on the stress-induced premature death of the treated leaves.

In spring (March and April), the increase of new tillers dry weight and ^{15}N content coincided with a decrease of ^{15}N and N concentration in other segments. This implies that N from other segments was remobilized to support the abundant tiller growth in spring.

Methodology Adaptations in Future Experiments

As a result of this preliminary experiment, a new approach to plot establishment and several adaptations to treatment and harvest techniques were developed. The stress and subsequent dieback of tillers within the plots established chemically with glyphosate applications was reduced by establishing plots using a physical barrier. Also, in order to maintain natural insulation and avoid temperature stresses, debris was not removed from the base of the tillers within the plots. Overall, the plot establishment and ^{15}N treatments were made

earlier (treatments applied before Nov-02) in the autumn, so harvests can be made before Dec-01. This allows for determination of earlier ^{15}N remobilization from existing leaves before senescence begins. In order to account for any additional N and effects of foliar treatments, control plots received an equivalent application of ammonium nitrate (without the heavy isotope enrichment) in Tween 80. In addition, samples of soil were collected from control samples at each harvest date to account for variations in natural abundance of ^{15}N. At harvest, tagged tillers were removed before washing of the samples. However, new tillers that had formed after treatment (not tagged) were removed after washing, rather than before washing, in order to be able to distinguish new tillers from treated tillers at the time of plant segment separation. Also, the number of new tillers was counted at each harvest in order to calculate the amount of tillering of the initial tillers.

Appendix B

^{15}N Recovery in Soil

Calculations

Natural abundance atom% ^{15}N was determined for each soil sample that was collected before ^{15}N treatment and on control plots (plots not treated with ^{15}N) at each harvest date. During the 2005-2006 stockpiling season, soil samples were not collected from control plots, therefore the ^{15}N natural abuncance was estimated from samples taken before ^{15}N treatment. The mean value (n=4) of natural abundance ^{15}N was subtracted from the atom% ^{15}N of samples from ^{15}N-treated plots to find the enriched atom% ^{15}N.

atom% ^{15}N of soil – natural abundance atom% ^{15}N of soil
= enriched atom% ^{15}N

The bulk density was not measured on the soil samples, and an estimate of 1.35 g/cm^3 (personal communications, Randall Miles) was used to determine the total amount of N in the top 1 cm of soil in each plot (area= 506.7 cm^2).

%N of soil sample / 100 x 1.35 g/cm^3 x 506.7 cm^3
= total g N in top 1cm of soil

total g N in top 1cm of soil x enriched atom% ^{15}N / 100 x 1000
= total mg ^{15}N in top 1 cm of soil

The amount of ^{15}N in each soil sample was calculated as a percentage of ^{15}N applied to the leaves (% of applied ^{15}N) in order to normalize values across plots. Total mg of ^{15}N applied to each plot equaled 28.976712 mg ^{15}N for Seasons 1 and 2, and equaled 53.12 mg in the 2005-2006 season.

total mg ^{15}N in top 1 cm of soil / total mg of ^{15}N applied to each plot x 100 = % of applied ^{15}N in top 1 cm of soil

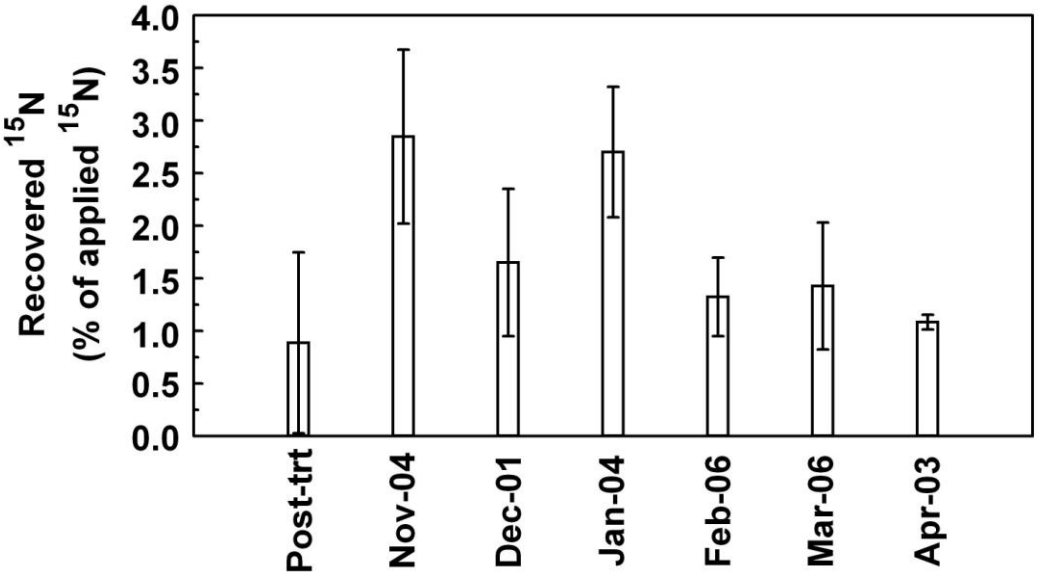

Figure B-1. Recovery of ^{15}N, as % of applied ^{15}N, in the top 1 cm of soil during the 2005-2006 stockpiling season. Soil samples were collected after ^{15}N treatment on November 2**,** 2005, (post-trt), then on each harvest date indicated of the 2005-2006 stockpiling season. Soil samples were collected on the same ^{15}N-treated plots over time. Values are the mean±SE (error bars are visible when larger than the symbol size, n=4).

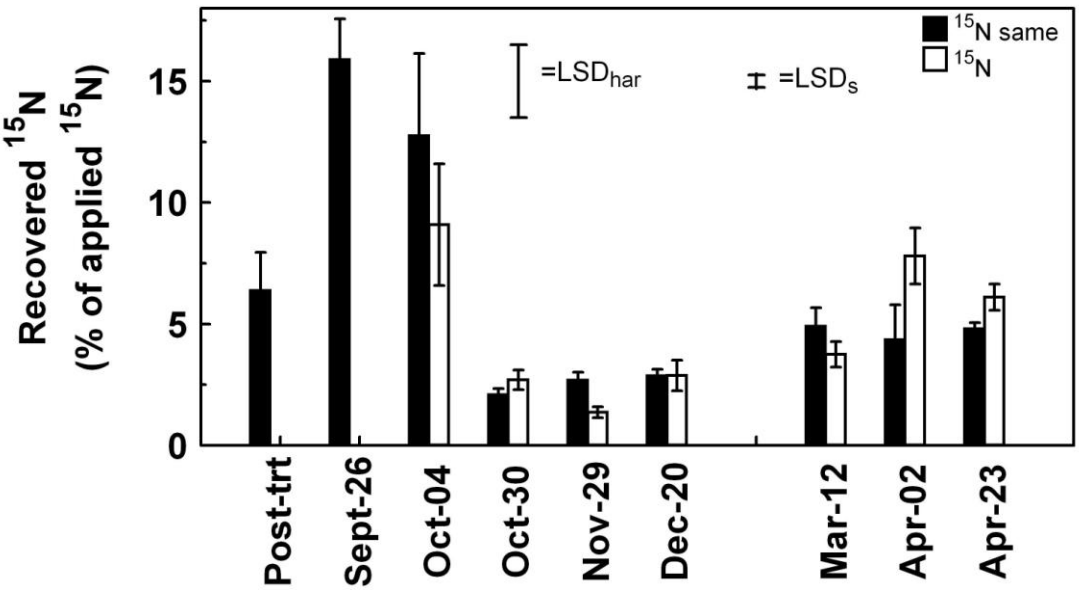

Figure B-2. Recovery of ^{15}N, as % of applied ^{15}N, in the top 1 cm of soil during Season 1 (2006-2007). Soil samples were collected just after ^{15}N treatment on September 19**,** 2006 (post-trt), then on each harvest date indicated of Season 1 (2006-2007). Soil samples were collected on two regimes; from the same ^{15}N-treated plots over time (black-closed bars labeled ^{15}N same), and from ^{15}N-treated plots harvested on the indicated date (open bars labeled ^{15}N). Values are the mean±SE (error bars are visible when larger than the symbol size, n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between different sampling regimes (LSD_s).

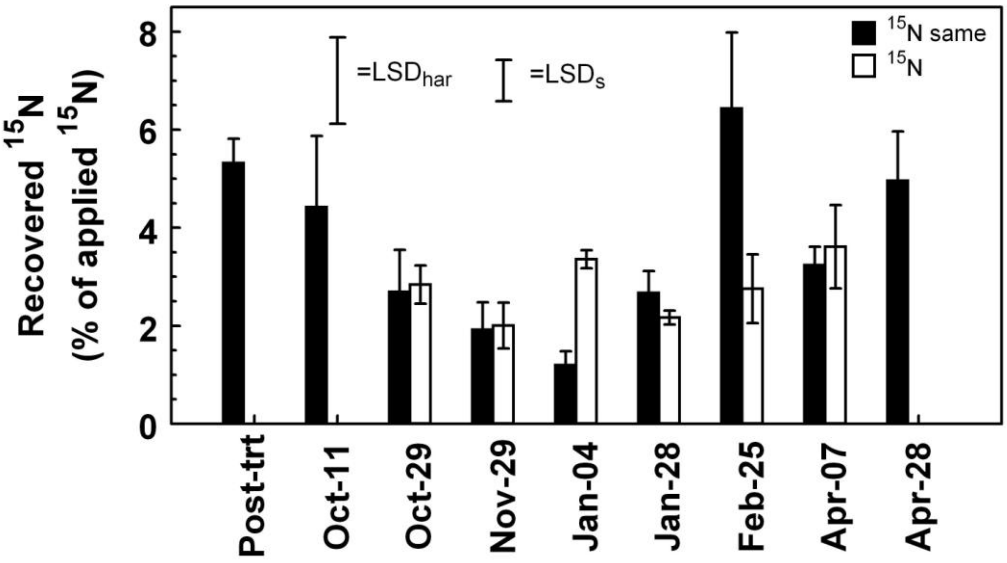

Figure B-3. Recovery of ^{15}N, as % of applied ^{15}N, in the top 1 cm of soil during Season 2 (2007-2008). Soil samples were collected just after ^{15}N treatment on October 1**,** 2007 (post-trt), then on each harvest date indicated of Season 2 (2007-2008). Soil samples were collected on two regimes; from the same ^{15}N-treated plots over time (black-closed bars labeled ^{15}N same), and from ^{15}N-treated plots harvested on the indicated date (open bars labeled ^{15}N). Values are the mean±SE (error bars are visible when larger than the symbol size, n=4). Fisher's protected LSD ($P<0.05$) given between harvest dates (LSD_{har}) and between different sampling regimes (LSD_s).

VITA

Melissa Ann Remley was born on March 2, 1980 in Silvis, IL, and was raised in East Moline, IL. She graduated from Riverdale Senior High, Port Byron, IL in 1998. After attending Truman State University in Kirksville, MO for one year, she transferred to Missouri State University (formerly Southwest Missouri State University) in Springfield, MO and earned a Bachelor's of Science in Horticulture in 2002, and a Master's of Plant Science in 2004, under the advisement of Dr. Frank Einhellig. In February 2010, she received her Doctorate of Philosophy degree at the University of Missouri-Columbia in Plant, Insect, and Microbial Sciences, under the advisement of Dr. Dale Blevins. Melissa is presently a Cotton Inc. Post-doctoral Fellow in the crop physiology lab of Dr. Felix Fritschi at the University of Missouri-Columbia.