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THE DYNAMICS OF CARBON, NITROGEN AND SOIL ORGANIC MATTER IN *POPULUS* PLANTATIONS

By

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ABSTRACT

THE DYNAMICS OF CARBON, NITROGEN AND SOIL ORGANIC MATTER IN *POPULUS* PLANTATIONS

By

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Short-rotation intensive-culture forestry is similar to agricultural systems requiring increased nutrient input and management. The expense and environmental concerns associated with fertilizers have raised questions about the sustainability of these ecosystems. Sustainability of production oriented ecosystems can be aided by understanding the mineralization-immobilization potential of the soil microbial biomass. The soil microbial biomass is central to a complex system of soil organic fractions that control soil fertility, production and environmental contamination.

The lack of root turnover studies has led to a inadequate understanding of the role of root turnover as substrate soil microbial biomass and organic matter formation. The current study was designed to determine: (i) the role of below-ground production and turnover in nutrient cycling processes; (ii) the contribution of leaf and root litter as substrate for the maintenance of soil organic matter; and (iii) relate soil microbial biomass and organic matter dynamics to plant carbon and nitrogen allocation patterns.

Two-year-old *Populus euramericana* cv. Eugenei trees were labeled with ^{14}C and ^{15}N in the field. The ^{15}N was injected into the stem to label leaves and roots without labeling the soil. The ^{14}C labeling was done in a Plexiglas chamber under ambient conditions. The tree-soil system was

sampled for one year. Labeled leaf litter was placed onto unlabeled tree plots to differentiate the contribution of leaf and root derived carbon and nitrogen to soil over a two year period.

The ^{14}C required two weeks to stabilize in the root system and averaged 20% of soil respiration. One year latter, 32% of applied ^{14}C and 33% of the injected ^{15}N was recovered. Reserves in the root system were sufficient to replace fine roots one and one half times. This represented significantly less C than leaf litter, yet similar amounts of ^{14}C were found in soil from both litters. Leaf litter appeared to dominate N cycling since ^{15}N was not detected in root labeled soil. Kinetic analysis of incubated soil showed a greater contribution of C and N to soil organic fractions from leaf litter. The turnover of labile soil ^{14}C in both leaf and root litter labeled soil was 14-64 days. The ^{14}C in pools of intermediate resistance had a turnover time of 2-16 years and increased with soil depth.

Theresa Marie

and

Derek Ryan

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Chapter 1

INTRODUCTION

Rationale and Justification

High production-short rotation plantation forestry has been suggested as a solution to limited fossil fuels and demand for pulp close to processing facilities. These plantations often contain hybrids, such as *Populus*, requiring nutrient supplies that are in excess of traditionally managed forests and even higher than some intensively managed agricultural systems (Ingestad and Ågren, 1984). The sustainability of such high production systems, especially successive rotations, will depend on management techniques based on understanding litter and soil organic matter turnover.

The initial decomposition of fine roots and leaf litter involves microbial immobilization of nitrogen (N) and phosphorous (P). The resulting detritus, composed of fine root necromass, leaf litter, microbial populations, and secondary microbial products constitute pools of N and P of major significance to soil nutrient dynamics. The study of the magnitude and dynamics of litter turnover, especially fine roots, and its effect on nutrient cycling has become an important consideration in ecosystem productivity. The processes involved in litter decomposition, however, have just begun to be understood.

We now realize that roots account for a large proportion of the carbon (C), N and P budgets of terrestrial plant communities (Harris *et al.,* 1977; Vogt *et al.,* 1986; Santantonio, 1989), however, we still do not understand how important death and decay of roots are to the functioning of the soil

community. The dynamic nature of fine root turnover has not been addressed, especially the role of turnover in regulating microbial populations and soil organic matter dynamics. The general requirements of high yield *Populus* systems are known (Dickmann *et al.,* 1988). To sustain this level of production it is normally necessary to use substantial amounts of fertilizer (Baker, 1977). By understanding the mineralization-immobilization potential of the soil biomass and formation and decay of soil organic matter we may be better able to sustain high production ecosystems without major fertilizer inputs and minimal losses of nutrients to the environment.

Fine Root Turnover

Fine root turnover has been measured in fewer than 30 forest stands world wide (Santantonio and Grace, 1987). The inherently difficult task of gathering and identifying fine roots, along with inadequate methods of estimating turnover, are reasons for this dearth of information (Gholz *et al.,* 1986; Santantonio and Grace, 1987; Raich and Nadelhoffer, 1989; Santantonio, 1989; Fogel, 1990). Since no standard method exists, researchers have used a variety of indirect and direct ways to estimate fine root turnover. Direct methods of coring and excavation include measuring changes in the live standing crop of roots (Harris *et al.,* 1977; McClaugherty *et al.,* 1982) or measuring both live and dead roots (Persson, 1979; Keyes and Grier, 1981; Gholz *et al.,* 1986). Indirect methods have been based on N balance and mineralization budgets (Nadelhoffer *et al.,* 1985) and carbohydrate concentrations of fine roots (Marshal and Waring, 1985).

Root biomass production in coniferous forests was historically estimated as about half that of leaf litter production (Gray and Williams,

1971; Whittaker and Marks, 1975). Fine root production and turnover are rarely included in these data. Recent investigations have shown that fine root production in hardwood and coniferous forests account for 1.5 to 5 times as much detritus as leaf litter (Edwards and Harris, 1977; Grier *et al.,* 1981; Nadelhoffer *et al.,* 1985). However, other authors report that the contribution of fine roots to detritus is only 3-29% of total detrital input (Keyes and Grier, 1981; Joslin and Henderson, 1987; Santantonio, 1989, Pregitzer *et al.,* 1990). Site quality and methodology must be taken into account when comparing data on fine root production. Fine root turnover and associated decomposition processes will have significant impact on net primary production and nutrient cycling in hybrid poplar systems regardless of the uncertainty associated with measuring below ground production.

The decomposition of fine roots and leaf litter exhibit a distinct pattern encompassing three phases (Fogel and Cromack, 1977; Berg and Staaf, 1981; McClaugherty *et al.,* 1984; Berg, 1988). During the first phase of decay, nutrients are mineralized through the decomposition of narrow C:N cytoplasmic constituents. The first phase is short in sequence (days to months) and leads to the second phase of immobilization. The second phase lasts up to 3 years and is characterized by slow weight loss attributed to wide C:N substrates such as lignocellulose, secondary microbial products and the accumulation of polymerized polyphenols. Following the second phase, a third phase of mineralization begins and continues until the recalcitrant components are incorporated into stabilized organic matter (Aber and Melillo, 1980; McClaugherty *et al.,* 1984; Berg, 1988).

Retranslocation of leaf nutrients is a mechanism whereby trees conserve nutrients and bypass the root uptake pathway and soil nutrient

cycling processes. Poplars can retranslocate up to 70% of N and 50% of P from leaves prior to senescence (Baker and Blackmon, 1977). However, little is known of what happens to nutrients in senescing fine roots. Evidence from fine root studies of *Pinus radiata* suggests that no conservation of nutrients occurs (Nambiar, 1987). Similar results were obtained for hybrid poplars (Horwath *et al.,* 1992). Nutrients lost through root turnover must be recycled through decomposition and subsequent uptake. Studies have shown that 22-75% of N uptake by trees was allocated to fine root production (McClaugherty *et al., 1982*; Meier *et al.,* 1985; Nadelhoffer *et al.,* 1985). Potentially large amounts of nutrients are involved in fine root mortality and decomposition.

Litter decomposition and subsequent microbial immobilization is an important means of conserving nutrients in forest systems (Aber and Melillo, 1982). This can be especially crucial in preventing leaching losses following disturbances such as whole tree harvesting, a method employed in many intensively-cultured forest plantations (Borman *et al.,* 1979; Vitousek and Melillo, 1979; Vitousek and Matson, 1985). Litter turnover is a continuing process taking years to complete. As a result, essential nutrients will be immobilized during stand establishment and regeneration, a time when nutrients are in great demand. Fertilization will be required to compensate for nutrient demand until large detrital and organic matter pools are established. When these pools are large enough, they should be able to maintain and buffer soil fertility with minimal fertilizer inputs. "Organic farming" is based on this concept and recent evidence demonstrates how successful management of soil organic matter can result in high agricultural yield with little or no fertilizer input (Doran *et al.,* 1988).

Microbial Biomass and Soil Organic Matter Dynamics

Controls on the mechanisms of microbial mineralization of organic matter and associated nutrients are being investigated from a genetic and cellular perspective (Payne, 1980; North, 1982; Dashman and Stotzky, 1986). But it has proven difficult to translate *in vitro* information at the enzyme, cellular and organismal level into an ecosystem context. Mechanisms controlling short-term nutrient dynamics cannot be understood or predicted by summing or averaging the characteristics of heterogeneous systems. Therefore, conceptual models have been used to simulate C, N and P dynamics and to divide soil organic matter components into biologically meaningful fractions.

Jansson (1958) proposed two decomposable organic fractions which he called active and passive. The active fraction is the most biologically significant pool in soil nutrition while the passive fraction is composed of recalcitrant and physically protected organic matter. We now consider that the active fraction contains the soil microbial biomass and easily mineralizable organic matter. Paul and Juma (1981) further advanced the concept of organic matter compartmentalization into five soil N fractions consisting of microbial biomass N, active N, metabolite N, stabilized N and old N. The investigators based these pools on decay rates determined by using tracers and isotopic dilution techniques. The stabilized N and old N turnover rates were based on carbon dating information (Martel and Paul, 1974).

Models which predict the rates and fluxes of soil nutrients are coupled to the need of the soil microbial biomass to obtain energy from the C-H and C-C bond of organic compounds (McGill and Cole, 1981; Frissel

and van Veen, 1980; van Veen *et al.*, 1981). The mineralization of N is a consequence of the microbial metabolism of organic compounds containing N. The C:N ratio of the substrate is used as an indicator to determine the potential mineralizable amounts of N. These models imply that the need for C ultimately controls the fate of N in soil. Other researchers have suggested that the quality of the organic matter (Bosatta and Staaf, 1982) and clay effects (Dashman and Stotzky, 1986), rather than C to N ratios, control N availability. Melillo *et al.* (1982) suggested this concept for forest systems when they related the decomposition of leaf litter to lignin/N ratio.

Objectives and Goals

The current study was designed to address and enhance the understanding of organic matter dynamics in ecosystems managed for high biomass output such as short-rotation intensive-culture forestry. This requires information on leaf and fine root litter turnover, plant-soil interactions, nutrient cycling and soil organic matter formation and decomposition. Carbon entering the soil from the plant biomass is the primary ecosystem process driving the cycling of nutrients. The production and turnover of fine roots is an important C input to soil, however, the magnitude and importance of root derived C to nutrient cycling processes is vaguely understood.

The amount and quality of plant C and nutrients returned to soil will determine the mineralization potential of the soil biomass. It is therefore important to establish the role of fine root necromass in regulating the cycling of nutrients and the formation of soil organic matter. The understanding of these ecosystem processes will enable us to manipulate

the dynamic nature of soil organic matter formation in a way that sustains productivity and minimizes chemical fertilizer inputs.

The current study was designed to address the uncertainties associated with the production and decay of fine roots and the potential impact on nutrient cycling and long-term soil organic matter maintenance.

The general objectives of this study were the following:

(i) To evaluate the relative role of fine root production and decomposition in the nutrient cycling process.

(ii) Determine the C and N flux through leaf litter, fine root litter, soil microbial biomass and soil organic matter fractions of hybrid poplar plantations.

(iii) Determine the impact of litter decomposition and the simultaneous mineralization-immobilization of N on a short-term and long-term basis.

(iv) Relate soil biomass activity and substrate availability to patterns of plant allocation.

The rationale for these objectives lies in the premise that fine root necromass will decompose at a slower rate than leaf litter and will exhibit minimal retranslocation of cytoplasmic constituents before death. This will lead to less immobilization of nutrients than leaf litter during the decomposition process. As a result, decomposing fine roots will have less associated microbial biomass and form less stabilized organic matter than is formed by leaf litter decomposition.

The determination of plant residue turnover, microbial biomass activity and soil organic matter formation was accomplished using tracers

(^{14}C and ^{15}N). The use of tracers provided a quantitative estimate of above- and below-ground production and its contribution to nutrient cycling and soil organic matter formation.

Overview of Chapters

Chapter 2

Chapter 2 reports the results of a pilot study detailing the development of a method to label *Populus* trees with ^{15}N without physically disturbing the soil. The labeling of root and shoot tissue by injecting N into the vessel elements of the stem was useful in measuring the flux of N from these litters into soil organic matter fractions. The ^{15}N was preferentially distributed among age classes of leaves and size classes of roots. The results from this study proved interesting in that the sink strength of the different tree tissues could be established. The movement of ^{15}N into fine root necromass was established by sorting live and dead roots over time. These results demonstrated the ability to measure the contribution of root litter to N cycling processes *in situ*.

Chapter 3

Chapter 3 describes the method used to radiolabel two-year-old hybrid poplars with $^{14}CO_2$ in the field. The combination of ^{15}N injection and ^{14}C labeling provided a dual tracer approach for studying the fate of C and N in this system. Trees were labeled on July 19 and September 5, 1990 to encompass distinct seasonal growth and storage patterns exhibited by this clone. The specific objectives addressed in this chapter include: (i) the measurement of the initial fate of tracers (^{14}C and ^{15}N) two weeks after the dual labeling; (ii) characterization of ^{14}C respiration from the root system;

(iii) description of sampling protocols for tree components and soil in the field; (iv) discussion of the laboratory sample preparation and analytical determinations.

Chapter 4

Chapter 4 examines the results from all samplings of trees labeled on July 19 and September 5, 1990. The trees were sampled two weeks after labeling, in November of 1990 and one year after labeling. Labeled leaf litter was placed onto unlabeled tree plots in a litter exchange experiment to determine the role of leaf decomposition and root turnover in nutrient cycling processes. The litter exchange plots were sampled over the next two years. Specific results addressed in this chapter include: (i) estimates of root turnover by the dilution of labeled C with unlabeled C; (ii) the long-term fate of ^{14}C and ^{15}N within tree components from the second to third year of growth; (iii) the potential magnitude of leaf and root derived C input to soil; (iv) assessing the role of leaf and root derived C in the maintenance of soil organic matter fractions.

Chapter 5

Chapter 5 examines the partitioning of ^{14}C-photosynthate within the different chemical fractions of the tree. The chemical fractions included lipids, sugars, protein, starch and structural residues. Structural residues were considered to include lignin and the various polysaccharides forming pectin, hemi-cellulose and cellulose. Specific results addressed in this chapter include: (i) characterizing the long-term fate of radiolabeled C within the different chemical fractions; (ii) exploring the relationships of translocation and storage of C during different periods of growth and

dormancy; and (iii) assessing the importance of reserve C to the subsequent growth of roots during the next growing season.

Chapter 6

Chapter 6 examines the dynamics of C and N in the soil microbial biomass and soil organic matter fractions of this hybrid poplar plantation. Long-term laboratory soil incubations were done on the last two samplings of labeled trees and first two samplings of leaf litter plots to determine the movement of C and N into the microbial biomass, labile soil C and organic matter pools of intermediate resistance. Potentially mineralizable C and turnover time of soil C pools were derived by applying mathematical models to C mineralization data. Specific objectives of this chapter include: (i) determining the flux of C and N to microbial biomass from leaf and root turnover; (ii) assess the flux of C and N from tree components labeled at different times of the growing season; (iii) quantify the stabilization of C into various fractions of soil organic matter.

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Chapter 2

THE INJECTION OF NITROGEN-15 INTO TREES TO STUDY NITROGEN CYCLING IN SOIL

ABSTRACT

A method was developed to introduce ^{15}N directly into trees to study internal N translocation and litter turnover. Leaf and root biomass were labeled by injection of ^{15}N directly into the vessel elements of hybrid *Populus* trees during their second growing season. The ^{15}N was uniformly distributed throughout the canopy and root system. The rate and amount of ^{15}N turnover from plant tissue can be determined by pool transfer or through differences in plant ^{15}N concentrations. The ^{15}N was detected in the dead root pool 8 weeks following injection, indicating root turnover. Results demonstrate the ability to measure the contribution of fine root litter to N cycling processes without disturbing the soil environment.

INTRODUCTION

The application of ^{15}N either as fertilizer or plant residue to soil has made it possible to study nitrogen transformations and processes in both agricultural and forest systems (Jansson, 1958; Amato and Ladd, 1980). By following ^{15}N through plant-soil N pools and then calculating isotopic dilution, a relatively accurate picture of the fate of N added as fertilizer has been determined (Vitousek and Andariese, 1986; Voroney *et al.,* 1989). However, there is a lack of information concerning the direct contribution of N from plant roots to soil N pools. Further, most dilution techniques disturb either the soil (by introducing labeled residues) or the N pool size.

Successful injection of ^{15}N directly into the plant allows for the study of N flux from plant to soil without introducing the label into the soil. Thus, ^{15}N can be followed from plant to litter and finally to soil N pools. The rate of N cycling from an *in situ* plant pool to various soil pools could then be directly calculated, avoiding some of the problems associated with other ^{15}N methodologies. However, discriminating N flux and plant uptake of mineralized ^{15}N from previously labeled tissues continues to be a problem associated with all ^{15}N techniques.

The injection of ^{15}N into trees was based on methods used for the systemic introduction of nutrients, growth regulators, and pesticides (Morris, 1951; Graham, 1954; Schreiber, 1969; Filer, 1973; Sterrett and Creager, 1977; Vreeland *et al.,* 1981; Schulert *et al.,* 1988). Applications of these methods have included the study of root distribution, interspecific plant nutrient transfers, metabolism of growth regulators and pesticides, and bioavailability and sink-strength of nutrients (Auerbach *et al.,* 1964;

Russell and Ellis, 1968; Woods and Brock, 1964; Sterrett and Hipkins, 1980; Domir, 1980; Schulert *et al.,* 1988).

The objective of this study was to examine the feasibility of labeling tree biomass *in situ* for the study of soil N cycling associated with leaf and fine root litter turnover. Tracer ^{15}N was injected into trees and carried in the transpiration stream to the leaves. About two weeks to a month following application the N was distributed throughout the plant. I report the development of a method for studying the direct flux of labeled N from trees to the soil.

MATERIAL AND METHODS

Injection Procedure

Trees of a single *Populus* clone (*P.* x *euramericana* cv. Eugenei) in their second season of growth were injected with ^{15}N. The N was dissolved in an artificial sap solution consisting of 5.0 mM KCl and 0.4 mM malic acid adjusted to pH 5.4 with KOH (Dickson *et al.,* 1985). The N was added as ^{15}N-$(NH_4)_2SO_4$ at levels equivalent to 5-10% of the total tree N. The volume of the ^{15}N solution injected was 100 mL. Unlabeled sap solution was used to chase and flush the labeled N from the severed vessel elements until uptake had ceased. The solutions were sterilized by autoclaving at 120° C for 15 minutes or filtering through a 0.2 μm Millipore filter to avoid introducing pathogens directly into the tree.

A centered 6 mm diameter hole was drilled 75% of the way through a 5.0 cm diameter stem at 8.0 cm above ground level using a brad point wood drill bit and rechargeable drill. The configuration was found to be ideal for trees this size, but may have to be adjusted according to the age and size of different trees. Hole diameters of 2 mm have also been tested on 3-5 cm

diameter trees with success. The injection method relies on an active transpiration stream and, therefore, irrigation may be necessary when soil matrix potential is low. With species other than *Populus* the ideal protocol may have to be obtained through experimentation. *Populus* is diffuse porous, the injection method may have only limited value in ring porous taxa.

The hole was fitted with a modified Tygon tubing connector with an inserted septum to create a tight seal (Figure 2.1). The cavity was then flushed to exclude air by connecting a syringe body (30 mL) filled with artificial sap to one of two syringe needles inserted into the septum. Sap was forced into the cavity until a steady stream of liquid was seen coming from the second needle. The syringe needles were removed following the flushing procedure. A gravity-fed reservoir containing the ^{15}N solution was then connected by inserting the tracer delivery tube tipped with a syringe needle into the septum (Figure 2.1). The sap reservoir was suspended 1 m above the hole. Care must be exercised when switching from the ^{15}N solution to the chase solution to avoid introducing air into the injection line. The entire operation from drilling the hole to connecting the labeled N solution took no more than 45 seconds.

Field Protocol and Sampling

Each replicate tree was centered in a 1 m^2 soil monolith by trenching to a depth of 45 cm and wrapping the soil column with two layers of plastic sheeting two months prior to ^{15}N injection. Tree Set 1 contained 4 replicate trees and they were injected on July 13, 1988 with 500 mg of 39.0 atom % ^{15}N-$(NH_4)_2SO_4$ which amounted to approximately 5% of the total tree N based on previous work by Pregitzer *et al.* (1990). Tree Set 2 also

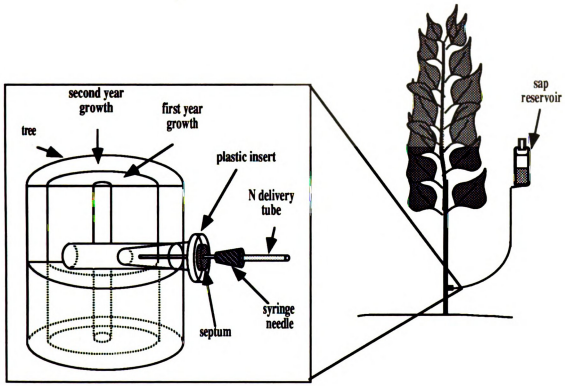

Figure 2.1. Diagram of the injection technique.

contained 4 replicate trees and they were injected on September 12, 1988 with 500 mg of 98.0 atom % ^{15}N-$(NH_4)_2SO_4$. Soil cores to a depth of 45 cm were taken from the 1 m^2 soil monoliths surrounding each tree at two and eight weeks after injection. Four cores were taken randomly from each of the soil monoliths two weeks following injection. Five additional cores were taken from each monolith eight weeks following injection. In order to avoid dead roots created by extracting the first set of cores, the cores extracted at eight weeks were all randomly located near the periphery of each monolith. Each tree was encased in commercial bird netting to trap falling leaves. Abscised leaves were recovered daily from the trees throughout the growing season until all leaves had fallen.

Roots were hand picked from soil cores sieved through a 3 mm mesh screen. Roots were classified under magnification according to size and age class. Sizes were determined on fresh roots with a microcaliper. The classification criteria included root color, texture, and tensile strength. Fresh dead roots were characterized by a dark stele and fragile cortex. New white fine roots were characteristically light (white) in color with a smooth cortex indistinguishable from the stele. Older brown roots had a brownish fibrous cortex and light stele that easily dissociated from the cortex.

Tissue Analysis

Leaf and root samples were dried in a convection oven at 60°C for 48 hours. Leaves were subsampled and ground in a household blender. The ground leaf tissue was then ball milled using a rolling glass jar and steel rod technique (Harris and Paul, 1989). Root samples were homogenized by cutting into small fragments with a dissecting blade. Samples of known mass were combusted in a biological sample converter (Roboprep, Europa

Scientific, Crewe, UK) to yield N_2 and CO_2. The gases were then analyzed with a continuous-flow stable isotope ratio mass spectrometer for N and ^{15}N (ANCA-MS, Europa Scientific, Crewe, UK) and thermal conductivity detector for CO_2 (Carle, Fullerton, CA). Urea-^{15}N (0.60571 atom % ^{15}N) and 44% carbon (as cellulose) Whatman #1 filter paper were used for reference standards (Harris and Paul, 1989).

RESULTS AND DISCUSSION

The correct drill configuration is necessary to avoid killing branches and leaves as a result of severing too many vessel elements. Also, accessing too few vessel elements can lead to N toxicity by delivering the N solution to limited parts of the canopy. In a correct drill configuration, the injected N will ascend in the transpiration stream and accumulate in the leaf biomass before equilibrating throughout the entire plant. Table 2.1, shows no significant aspect differences of the injected N or ^{15}N 72 hours after injection using Tukey's procedure for comparison of means at ∂=0.05. Following leaf fall, the leaves from Tree Sets 1 and 2 contained only 17% and 18% of the applied ^{15}N, respectively (Table 2.2). Most of the canopy N was apparently conserved as has been shown before for this genotype (Pregitzer *et al.*, 1990).

The amount of ^{15}N injected into a tree will depend on the plant or soil pool of interest. We believe the maximum amount of ^{15}N-$(NH_4)_2SO_4$ that can be injected into a tree is approximately 5-10% of the total tree N content. Our experience indicates that greater concentrations can be toxic to the canopy. Also, the level of soil N availability may influence the amount of N that can be injected. Existing high soil N levels followed by injection of ^{15}N

Table 2.1. Mean leaf N concentrations and the distribution of ^{15}N in the most recently mature leaf of each branch according to crown aspect 3 days following injection of tree set 1. Standard error of the mean given in parentheses.

Crown Aspect	g N kg^{-1}		Atom % ^{15}N Excess§	
N	3.64a	(0.18)	0.93a	(0.17)
NE	3.91a	(0.21)	1.22a	(0.24)
E	4.11a	(0.22)	0.78a	(0.18)
SE	3.93a	(0.15)	0.85a	(0.23)
S	4.28a	(0.33)	1.05a	(0.22)
SW	4.20a	(0.17)	0.80a	(0.24)
W	3.49a	(0.14)	0.66a	(0.17)
NW	3.41a	(0.20)	0.89a	(0.22)

§ Atom % ^{15}N excess is defined as ^{15}N above background (0.3663%).

Table 2.2. Summary of N and C concentrations and the recovery of ^{15}N in senescent leaves. The ^{15}N recovered is based on the amount injected. Standard error of the mean given in parentheses.

	g N kg^{-1}	g C kg^{-1}	Atom % ^{15}N Excess§	% ^{15}N Recovered
Tree Set 1	1.38 (0.13)	36.70 (0.25)	0.39 (0.05)	17.2
Tree Set 2	1.70 (0.05)	37.70 (0.32)	1.07 (0.11)	18.2

§ Atom % ^{15}N excess is defined as ^{15}N above background (0.3663%).

can lead to premature leaf abscission by a mechanism we do not understand. This imposes limitations on the detection limits of ^{15}N in soil organic matter or microbial biomass pools, especially in young trees with low total N contents. However, the use of several injections of ^{15}N may overcome limitations of a single injection.

The results of our initial experiments indicate that the enrichment of the root biomass occurred within 5 days (data not shown) and was stabilized about 2 weeks following injection, regardless of the time of addition (Table 2.3). The increased enrichment of the dead roots in Tree Set 2 between 2 to 8 weeks indicates that some turnover of the live root pool occurred during this period. Both tree sets exhibited a decrease in enrichment of new white and older brown roots with time. The decrease in enrichment was assumed to be associated with dilution from the uptake of unlabeled soil N, growth of new roots, and turnover.

Using data from Table 2.3 for the dead root pool and current field studies on root production (Chapter 4), the potential atom % ^{15}N excess of soil microbial biomass N is 0.022%. The standard deviation of analysis of our mass spectrometer is 0.0004 atom % ^{15}N. Detectability of ^{15}N will depend on the rate of litter turnover and retention time of N in the microbial biomass and soil organic matter, but appears feasible.

An alternative way to measure the transfer of ^{15}N from plant to soil is to determine the change in the percent of N in the plant biomass derived from ^{15}N over time (Table 2.4). This calculation is independent of the amount and enrichment of the injected N (Rennie and Rennie, 1983). The precision of this method can be increased by a complete harvest of the plant biomass (root and shoot). The assumption of this method is that all ^{15}N lost from the plant through time zero is due to litter turnover and that all ^{15}N

Table 2.3. Mean labeled N and N concentrations of different classes of roots collected over time from tree sets 1 and 2. Standard error of the mean given in parentheses.

Tree Set 1 (Injected 7/13/1988)

Root Class	Root Size	Atom % ^{15}N Excess§ 2 wk†	8 wk	g N kg^{-1} 2 wk	8 wk
New White	< 0.5	0.21 (0.03)	0.08 (0.01)	1.8 (0.0)	2.8 (0.2)
	0.5-1.0	0.38 (0.10)	0.18 (0.04)	2.0 (0.3)	1.5 (0.1)
Older Brown	< 0.5	0.19 (0.03)	0.12 (0.02)	1.7 (0.0)	2.8 (0.1)
	0.5-1.0	0.29 (0.03)	0.19 (0.02)	1.0 (0.1)	1.7 (0.1)
	1.0-3.0	0.30 (0.04)	0.21 (0.02)	1.0 (0.1)	1.4 (0.1)
	> 3.0	0.28 (0.02)	0.23 (0.3)	0.8 (0.1)	1.0 (0.3)
Dead	< 0.5	na na	0.07 (0.01)	na na	2.3 (0.1)
	0.5-1.0	na na	0.07 (0.02)	na na	1.3 (0.1)
	1.0-3.0	na na	0.18 (0.08)	na na	1.6 (0.1)

Tree Set 2 (Injected 9/12/1988)

Root Class	Root Size	Atom % ^{15}N Excess 2 wk	8 wk	g N kg^{-1} 2 wk	8 wk
New White	< 0.5	0.19 (0.09)	0.17 (0.06)	2.0 (0.1)	1.6 (0.1)
	0.5-1.0	0.55 (0.13)	0.30 (0.10)	1.6 (0.2)	1.5 (0.1)
Older Brown	< 0.5	0.15 (0.04)	0.14 (0.04)	1.6 (0.1)	1.6 (0.1)
	0.5-1.0	0.54 (0.11)	0.21 (0.05)	1.1 (0.1)	1.4 (0.1)
	1.0-3.0	0.93 (0.21)	0.22 (0.22)	0.1 (1.8)	0.2 (0.2)
	> 3.0	0.70 (0.20)	0.55 (0.19)	1.0 (0.1)	1.3 (0.1)
Dead	< 0.5	0.04 (0.02)	0.21 (0.09)	1.6 (0.1)	1.6 (0.1)
	0.5-1.0	0.01 (0.01)	0.51 (0.17)	na na	1.4 (0.2)

§ Atom % ^{15}N excess is defined as ^{15}N above background (0.3663%).

† Time since injection of ^{15}N.

na not available

Table 2.4. Percentage of N in various root classes derived from ^{15}N two and eight weeks after injection. †

Time Since Injection	2 weeks		8 weeks		
	Tree Set 1	Tree Set 2	Tree Set 1	Tree Set 2	Change ¥
New White Roots					
<0.5 mm	0.51	0.19	0.21	0.17	-0.18
0.5-1	0.97	0.56	0.46	0.31	-0.35
Older Brown Roots					
<0.5	0.49	0.15	0.31	0.14	-0.10
0.5-1	0.74	0.55	0.49	0.21	-0.29
1.0-3.0	0.77	0.71	0.54	0.45	-0.25
> 3.0	0.72	0.71	0.59	0.56	-0.14
Dead Roots					
<0.5	na	0.01	0.18	0.21	+0.18
0.5-1	na	na	0.46	0.52	+0.49
1.0-3.0	na	0.04	0.18	0.17	+0.18

† Calculated according to Rennie and Rennie (1983).
¥ Change calculated from the average of tree sets one and two at 8 weeks
na not available

lost has entered the soil N pool. Obviously, this assumption has limitations in a field situation.

Nitrogen derived from the ^{15}N is similar for both sets of trees 2 and 8 weeks following injection, with the exception that more label was found in the new white root pool of Tree Set 1 (Table 2.4). The dead root pool for both tree sets at 8 weeks contains similar amounts of N derived from the injected ^{15}N. Based on the July and September injections, the data indicate the flux of ^{15}N to the root system is independent of the time of injection. It may be that ^{15}N can be injected at different times during the growing season to label the root system. This notion, currently under study, may be useful in studying the temporal aspects of N cycling. The data also demonstrate the movement of label from live to dead roots and, therefore, the potential to measure root turnover.

Analysis of the total root biomass by excavation would be necessary to calculate total ^{15}N distribution and to obtain the greatest reproducibility (Waremburg and Paul, 1973; Harris and Paul, 1989). Though the degree of sampling was limited in this study, it aptly demonstrates the potential of tracing ^{15}N injected into the stem throughout the tree and into litter pools. The injection of tracer N provides a relatively easy means to transfer ^{15}N into the tree and promises to be an effective way to study N transformations and cycling in undisturbed tree-soil systems. The most powerful aspect of the injection method is the ability to measure the contribution of a functioning root system to soil N processes. The potential to label other soil N pools clearly exists.

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Chapter 3

^{14}C AND ^{15}N PARTITIONING IN TREE-SOIL SYSTEMS

ABSTRACT

Hybrid poplars were labeled with ^{14}C and ^{15}N to determine the partitioning of C and N during the second year of growth in the field. Eight trees were pulsed-labeled in a large Plexiglas chamber in July and September, 1990. Levels of $^{14}CO_2$ and climate within the chamber were controlled. Nitrogen-15 was stem injected into the trees. Three trees were harvested two weeks following each labeling period. Carbon dioxide assimilation values for whole-tree canopies averaged 3.8 μmol CO_2 m^{-2} s^{-1} in July and 6.2 μmol CO_2 m^{-2} s^{-1} in September. Above-ground dark respiration amounted to 18% and 24% of net assimilation in July and September. Carbon-14 root respiration peaked 2 days after labeling and after 2 weeks ^{14}C had stabilized below-ground, demonstrating that these trees primarily depend on current photosynthate for growth and maintenance during the growing season. Root respiration averaged 20% of total soil respiration in both July and September. In July, 80% of the recovered ^{14}C was located above-ground and closely resembled the weight distribution of the growing shoot. By September, 38% of the recovered ^{14}C was found in the root system and closely resembled the weight distribution of the different size classes of roots. The root system contained 8% of the injected ^{15}N in July and 20% in September.

INTRODUCTION

The productivity of trees is influenced by the partitioning of carbon and nitrogen. Allocation of C and N in above- and below-ground tree components affects harvest index and the performance of a species in a forest stand (Isebrands, 1982). In intensively cultured plantations the measurement of photosynthesis, dry matter production and respiration are important in interpreting how stands function. However, the majority of harvest and CO_2 exchange studies have been conducted on the shoot system, while the below-ground plant-soil system has received little attention (Tranquillini, 1963; Kinerson *et al.,* 1977; Ågren *et al.,* 1980; Linder and Troeng, 1981; Friend *et al.,* 1991) Nonetheless, below-ground production can account for greater than 50% of primary production in forests (Harris et al., 1975; Olsen, 1975; Persson, 1979; Keyes and Grier, 1981; Fogel, 1990, Hendrick and Pregitzer, 1993). The flux of C below-ground represents a considerable cost to trees and should be considered when managing forests (Dickson, 1989).

Detailed studies of above- and below-ground C flow and N distribution within the tree-soil system are possible with the use of isotopes (Dickson, 1989; Horwath *et al.,* 1992). Previous ^{14}C studies have estimated whole-tree C allocation by pulse-labeling potted trees, individual leaves or branches (Schier, 1970; Isebrands and Nelson, 1983). The advantage of isotope methods over harvest and CO_2 exchange methods is that growth and decomposition which occur simultaneously can be isolated to some extent by following long-term isotope flux and dilution below-ground (Warembourg and Paul, 1973). The general objectives of my study were to label whole-trees in the field with ^{14}C and ^{15}N and to quantify whole-tree C

and N allocation. The experimental system was designed to maintain CO_2 concentration and climate at ambient levels.

I was specifically interested in determining seasonal differences in: (1) the respiratory cost of the shoots and roots; (2) the fate of ^{14}C and ^{15}N in whole trees. In particular, I was interested in evaluating the use of ^{14}C to measure root-soil respiration. Seasonal rhythms in growth and the allocation of carbohydrates and nitrogen reserves have been documented in *Populus* (Isebrands and Nelson, 1983; Pregitzer *et al.*, 1990; Nguyen *et al.*, 1990), but budgets developed by labeling whole-trees greater than 3 m tall have never been reported. It is my belief that patterns of C and N allocation have ecological significance and that, if ignored, seasonal changes in source-sink relationships can greatly confound the interpretation of research and the application of cultural practices. This chapter represents my first attempt to study whole tree gas-exchange and C and N allocation in *Populus*-soil systems using a dual isotope approach in the field.

MATERIALS AND METHODS

Trees were grown at the Kellogg Biological Station, ("Long-Term Ecological Research" National Science Foundation), Michigan State University, in southwest Michigan. The soil series is Kalamazoo (fine loamy, mixed, mesic, Typic Hapudalf). The site was prepared by moldbroad plowing and disking. Cuttings of *Populus euramericana* cv. Eugenei 10 cm in length were planted on a 2 x 1 m spacing in the spring of 1989. Later in the first growing season, groups of eight trees were selected for uniform height and diameter characteristics. An undisturbed soil block (1 m^3) surrounding each tree was trenched using a narrow ditching

machine (Ditchwitch, Howell, MI). Plywood dividers were inserted into the trenches and the vertical faces of the monoliths were wrapped in 0.18 mm vinyl before back-filling.

The following summer (1990), injection of ^{15}N into trees was initiated five days prior to ^{14}C exposure. The ^{15}N (100 mg of 98% enriched $(^{15}NH_4)2SO_4$ was injected as an artificial sap solution into the vessel elements of the stem tissue (Horwath *et al.*, 1992). Following the ^{15}N injection, the vinyl sheeting of each soil block was sealed against the plywood structure with foam sealant (Insta-Foam Products, Inc., Marietta, GA). Polyvinyl Chloride sheets (3.2 mm) were sealed to the top of the plywood structure with silicone caulking to create a headspace (15 cm tall x 1 m x 1 m) above the soil surface of each soil block. Tree stems were wrapped with modeling clay, polyethylene plastic and duct tape before being sealed to the PVC plates with silicone caulking. Tygon tubing (1.3 cm I.D.) was routed from each headspace to a CO_2 trap containing 500 mL 4.0 M NaOH. The headspace atmosphere was drawn at a rate of 12 L min^{-1} with a diaphragm pump (Cole Parmer Inst. Co., Chicago, IL) through an aeration stone in each base trap to remove CO_2. Base traps were sampled daily to determine below-ground CO_2 and ^{14}C respiration during and following the radiolabeling procedure. Subsamples of 1 mL were titrated to a phenolthalein endpoint (pH 7.0) using excess $BaCl_2$ and 0.1 M HCl to determine total CO_2-C. Scintillation cocktail (10 mL of Safety Solve, Research Products International Corp., Mount Prospect, IL) was added to 1.0 mL subsamples from the base traps and analyzed for ^{14}C on a liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL).

A Plexiglas chamber (3.2 m tall x 3 m wide x 4 m long) was assembled over the eight trees and below-ground partitions (Figure 3.1).

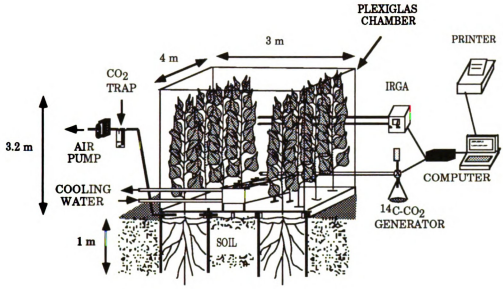


Figure 3.1. Schematic diagram of the ^{14}C exposure system used to label eight, two-year-old whole poplar trees. The trees were approximately three meters tall when labeled.

The chamber was sealed to the plywood structure and PVC sheets with silicone caulking. A $^{14}CO_2$ generator was connected to a diaphragm pump (GAST, Benton Harbor, MI) to circulate $^{14}CO_2$ into the chamber. The $^{14}CO_2$ generator consisted of a vessel containing 1.0 L of concentrated H_2SO_4 to which was attached a reservoir containing 6.0 moles of $Na_2{}^{14}CO_3$ solution. The carbonate solution was dispensed into the acid via a solenoid valve (Skinner, New Brunswick, CT) as required to maintain CO_2 levels equivalent to the ambient atmosphere outside the chamber. The acid-carbonate mixture was continuously agitated using a magnetic stirrer to facilitate the liberation of $^{14}CO_2$.

Chamber CO_2 level was monitored with an Infra Red Gas Analyzer, IRGA (Analytical Development Co. Ltd., Hoddesdon, England). Temperature sensors (Omega Engineering, Inc., Stamford, CT) were installed to monitor the soil surface, chamber air, and ambient air temperatures. Photon flux density was measured during the labeling periods with a radiometer/photometer (LI-COR, Inc., Lincoln, NE). The IRGA, solenoid valve and temperature sensors were connected to a data acquisition and control device (Remote Measuring Systems, Inc., Seattle, WA) that was interfaced by serial connection to a portable computer. Computer software was written in Quick Basic (Microsoft, Corp. Redmond, WA) to control the level of CO_2, monitor temperatures and collect data. The chamber temperature was controlled by forcing air through a water cooled heat exchanger (self customized). The air turbulence created by the heat exchanger was sufficient to mix the $^{14}CO_2$ gas uniformly.

Eight trees were labeled July 19, 1990 and September 5, 1990. A total of 3.04×10^8 Bq of $^{14}CO_2$ with a specific activity of 4215 Bq mg^{-1} C was added to the labeling chamber in July. In September, a total of 2.85×10^8 BQ of

$^{14}CO_2$ with a specific activity of 4300 Bq mg^{-1} C was added. Each tree received on average 1.0 mCi of ^{14}C. Chamber CO_2 levels were compared to ambient levels and maintained at 290-350 ppm. Night respiration was monitored until the following morning to determine above-ground dark respiration. The following morning CO_2 from night respiration was allowed to reassimilate before the Plexiglas chamber was removed.

Two weeks after the addition of ^{14}C, 3 randomly-selected trees were sampled. The leaves, stem and branches were composited according to the following designations: first year stem, bottom two-thirds (including stem, branches and leaves); first year stem, top one-third (including stem, branches and leaves); second year branches (including branches and leaves); and, second year leader (including stem and leaves). The soil block was excavated by depth (0-25 cm, 25-60 cm and 60-100 cm) into wheel barrows. The soil from each wheel barrow was weighed and sieved through a 8 mm sieve to remove coarse roots. The soil was then placed into a cement mixer, thoroughly mixed and a subsample taken. The sampled plant material and soils were stored on ice in the field and then transferred to a 4°C cold room.

In the laboratory, roots were sorted by hand into the following size classes; <0.5 mm, 0.5-1 mm, 1-3 mm, >3 mm, >10 mm. Leaf area was measured using a Delta-T meter (Decagon Devices, Pullman, WA). Plant material was dried at 80°C for 48- 96 hr, coarse roots and stems were dried for longer periods. The unrecovered fine roots (<0.5 to 1 mm) from the field-sieved soil sample composites were recovered by hydronuematic elutriation (Smucker *et al.,* 1982) to determine the total fine root biomass for each soil block.

Plant tissue was ground in a Wiley mill to pass a 60 mesh screen.

Plant and soil samples were analyzed for C, N and ^{15}N using a gas chromatography-mass spectrometer (ANCA-MS, Europa Scientific, Crewe, U. K.). Ammonium sulfate (0.3663 atom % ^{15}N) and forty-four percent C (as cellulose) Whatman no. 1 filter paper were used as a reference standards. Radiolabeled C from the gas chromatograph-mass spectrometer exhaust was collected in a CO_2 absorbing cocktail (J. R. Harvey Instrument Corp., Hillsdale, NJ) and analyzed for ^{14}C in a Liquid Scintillation Analyzer according to the procedure of Harris and Paul (1989).

Expression of Results

The ^{14}C results are expressed as specific activity (Bq mg^{-1} C). The ^{15}N results are expressed as atom percent ^{15}N excess above natural ^{15}N level and N derived from ^{15}N ($Ndf^{15}N$). The N derived from ^{15}N is the ratio of atom percent excess of tissue/atom percent excess applied. Standard error of the mean is shown to demonstrate the usefulness of our labeling techniques in the field.

RESULTS AND DISCUSSION

Tree and Environmental Variables

The Eugenei clones were growing rapidly in height at a rate of 35 mm d^{-1} for the 3 week period prior to the July labeling. By September, height growth had ceased as a result of budset. Trees labeled during July had an average diameter of 4.4 cm (at 10 cm) and a height of 310 cm. Trees labeled in September were 4.5 cm in diameter and 337 cm in height. The total leaf area of trees was 6.1 m^2 in July and 3.1 m^2 in September. A leaf area decrease in the autumn is not uncommon for this genotype. It is

attributable to late season drought stress and *Melampsora* rust and *Marsonnina* leaf spot disease which cause loss of leaves in the lower portion the canopy. A reduction in leaf biomass and increase in root mass was characteristic of trees harvested in September (Table 3.1). The increase in coarse root mass (> 3 mm) is associated with late season translocation and storage of photosynthate (Pregitzer *et al.,* 1990; Nguyen *et al.,* 1990). As a result of translocation and partial leaf loss, the root to shoot ratio increased from 0.34 to 0.62 from July to September.

The maximum light intensity inside the chamber during the labeling periods was 1250 μmol m^{-2} s^{-1} in July and 1060 μmol m^{-2} s^{-1} in September (Table 3.2). Net photosynthesis averaged over a photoperiod and tree canopy was 3.8 μmol CO_2 m^{-2} s^{-1} in July and 6.2 μmol CO_2 m^{-2} s^{-1} in September. Incident radiation levels inside the labeling chamber were typical of solar days and reached saturating light levels during midday. Saturating light levels of 800-1000 μmol m^{-2} s^{-1} are typical for field grown hybrid poplars (Ceulemans *et al.,* 1980). Under saturating light conditions, net photosynthesis values of 9.5-20.7 μmol CO_2 m^{-2} s^{-1} have been reported for hybrid poplars (Ceulemans *et al.,* 1980; Nelson *et al.,* 1982; Bassman and Zwier, 1991). The fact that the average rate of photosynthesis was somewhat lower than other values reported in the literature is probably attributable to whole-tree measurements compared with the single leaf or branch determinations of other studies. During autumn, an increases in photosynthesis has been observed in mature leaves of hybrid poplars as determined by $^{14}CO_2$ uptake (Nelson and Isebrands, 1983; Friend *et al.,* 1991), and I observed this phenomena as well (Table 3.2). Since significant leaf drop had occurred by September, the autumn canopies were composed of a relatively greater proportion of sun leaves (leaf drop is acropetal in

Table 3.1. The dry weight of tree components in July and September. Standard error of the mean shown in parentheses.

	July 17- August 1, 1990		September 5-19, 1990	
	weight (g)	% weight distribution	weight (g)	% weight distribution
stem	781 (122)	33	770 (125)	30
branches	432 (96)	18	519 (28)	20
leaves	528 (52)	23	303 (14)	12
total above-ground	1742 (270)	74	1592 (158)	62
roots				
<.5 mm	185 (15)	8	213 (4)	8
.5-1 mm	16 (2)	1	24 (2)	1
1-3 mm	49 (2)	2	53 (3)	2
3-10 mm	136 (36)	6	230 (32)	9
>10 mm	116 (38)	5	273 (53)	11
cutting	98 (18)	4	191 (10)	7
total below-ground	599 (104)	26	984 (25)	38
total	2341 (370)		2576 (182)	

Table 3.2. Gas exchange and environmental characteristics during labeling periods in July and September.

	July	September
Photon Flux Density (max.) μmol m-2 s-1	1250	1060
Net Assimilation μmol CO_2 m^{-2} s^{-1}	3.8	6.2
Above-ground Dark Respiration μmol CO_2 m^{-2} s^{-1}	0.67	1.47
Dark Respiration (% of ^{14}C added)	18	24
Chamber air °C (min.-max.)	19-33	19-34
Ambient air °C (min.-max.)	19-36	18-34
Soil surface °C (min.-max.)	18-24	18-24

Populus). This may have influenced average rates of photosynthesis.

Dark respiration measurements included leaves, branches and stem. Dark respiration expressed on the basis of leaf area was 0.67 μmol CO_2 m^{-2} s^{-1} in July and 1.47 μmol CO_2 m^{-2} s^{-1} September, or 18 to 24% of net assimilation (Table 3.2). Dark respiration measurements for hybrid poplars have been estimated at 1.1-1.6 μmol CO_2 m^{-2} s^{-1} on portions of intact leaves (Bassman and Zwier, 1991). The data demonstrate that night respiration during warm periods can be significant and should be addressed when calculating the C balance of field grown poplars.

Chamber air temperature was maintained between 19 to 34°C and it closely tracked ambient air temperature fluctuations of 18 to 36°C (Table 3.2). Surface soil temperature in the below-ground headspace ranged from 20 to 24°C for both labelings. To avoid water condensation and unnaturally dry air inside the chamber, the temperature of the heat exchanger was maintained at approximately the dew point of ambient air.

Root-Soil Respiration

Below-ground respiration measurements were recorded for each tree for two weeks following the labeling period (Figure 3.2). The soil surface respiration rate per tree averaged 240 mg CO_2-C m^{-2} hr^{-1} in July and 218 mg CO_2-C m^{-2} hr^{-1} in September. On average, between 5.2 and 5.8 g of CO_2-C m^{-2} d^{-1} were respired from the soil surface of each soil block (1 m^3 of soil). This measurement includes both root, associated rhizosphere organisms and soil microbial biomass respiration.

The specific activity (Bq mg^{-1} CO_2-C) traces of the below-ground respiration indicate that a period of approximately 2 weeks was required to metabolize the translocated ^{14}C-photosynthate in the root-soil system

Figure 3.2. Root-soil respiration averaged over eight trees for the period following labeling in July and September. Values are expressed as mg CO_2-C m^{-2} hr^{-1}. Standard error of the mean depicted as line bars.

Figure 3.3. Average specific activity of below-ground respiration. Each symbol represents the mean of eight root-soil microcosms.

Figure 3.2

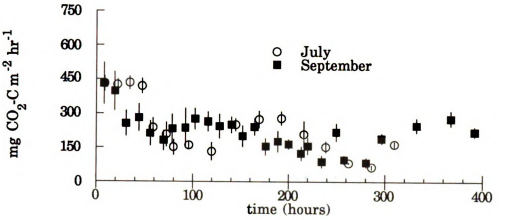
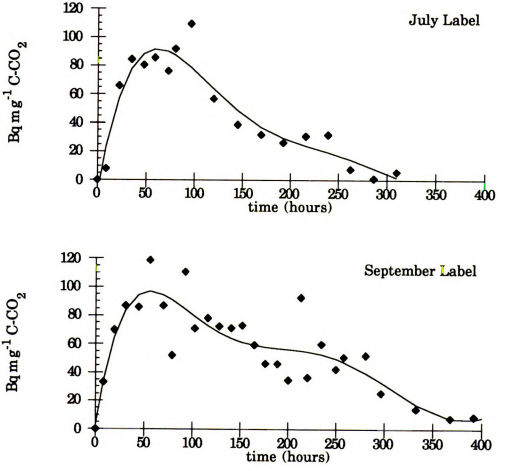


Figure 3.3.



(Figure 3.3). During both labeling periods, maximum below-ground specific activity (90-100 Bq mg^{-1} CO_2-C) occurred approximately 2 days after labeling. This represents the time needed to translocate radiolabeled photosynthate for growth and maintenance of the root system. The secondary rise occurring in the September specific activity curve may be attributable to mycorrhizal symbionts sequestering remobilized starch reserves, microbial utilization of root exudates or root decomposition (Harris and Paul, 1992). When the specific activity trace dropped to about 5% of its maximum I assumed that the translocated ^{14}C was no longer being metabolized and was now in either storage or structural forms (Harris and Paul, 1992). At this time, 14 days after labeling, the tree-soil system was harvested.

The ^{14}C recovered from below-ground respiration expressed as a percent of that applied (Bq/Bq added) is an index of the quantity of assimilated ^{14}C lost to root respiration. The amount of respired ^{14}C from roots and associated soil microorganisms was 8.1% in July and 9.8% in September (Figure 3.4). The exponential equation:

$$\frac{Bq_{root}}{Bq_{applied}} = Q(1-e^{-kt}) \qquad (1)$$

where Bq_{root} is the Bq in root-soil respiration, $Bq_{applied}$ is the total Bq added during labeling, Q is the accumulation of Bq in the root-soil respiration component, k is the rate constant for Bq accumulation and t is time, closely simulated the below-ground specific activity trace. The calculated accumulation of ^{14}C in the respired root-soil C pool was 9.1% in July and 10.8% in September using values extrapolated to 1000 hr. The specific activity trace of the September trees required a longer period to

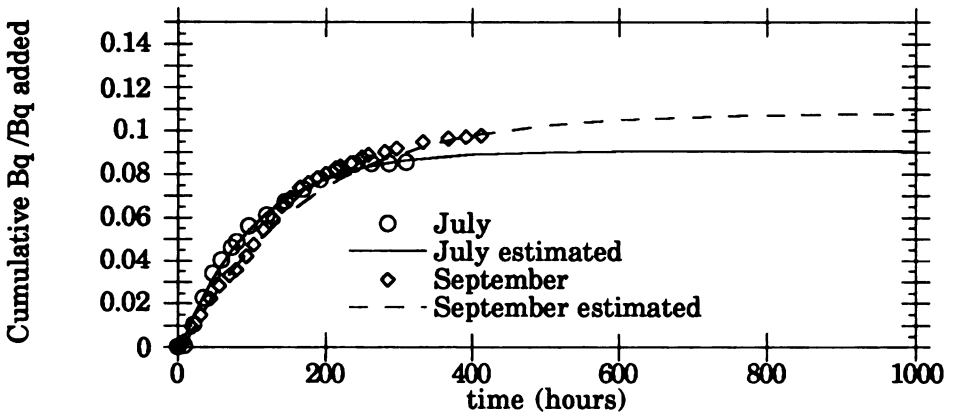


Figure 3.4. Carbon-14 respired from the root-soil system.

reach an asymptote than in July. This indicates that it took longer for ^{14}C to stabilize in September. The turnover rate of the translocated ^{14}C in the roots can be calculated from the rate constant for Bq accumulation in equation 1. The half-life of respired ^{14}C in the below-ground system was 69 hours in July and 99 hours in September.

Root respiration, as measured, represented the fraction of ^{14}C sustaining the metabolic activities of both the root system and associated soil organisms. In both the July and September labelings root-soil respiration accounted for 9 to 12% of the recovered ^{14}C. The majority of the respired ^{14}C was probably due to the metabolic activity of the root system since the microbial biomass contained only a small amount of the ^{14}C two weeks following the labelings (Horwath, unpublished data). As with our net photosynthesis measurements of the canopy, these values represent the entire root system and must be taken in light of the 81 to 89% total recovery of ^{14}C. The assumption concerning incomplete recovery of ^{14}C is that experimental error was spread randomly through the labeling and sampling protocols. If the majority of the loss occurred from the bottom of the unsealed soil block or leaks in the below-ground containment system, root respiration values could possibly double. However, it is more likely that the unrecovered ^{14}C was lost through above-ground respiration shortly after the Plexiglas chamber was removed.

The separate contributions of root and soil biomass respiration to CO_2 flux from soil is difficult to discriminate. In our study, the proportion of ^{14}C respired from the roots was related to net assimilation of ^{14}C during the labeling period. If we assume that all of the ^{14}C respired over 500 hours was due to root respiration (Figure 3.4), then the contribution of root respiration to total soil respiration was 19% in July and 21% in September.

Cropper and Gholz (1991) estimated that two thirds of soil respiration was from the fine root fraction in mature slash pine trees. Edwards and Harris (1977) calculated that 35% of forest floor respiration in a deciduous forest evolved from the metabolic activity of roots. Our values for root respiration are lower and represent a direct determination of the portion of gross assimilation used for the maintenance of the root system over a two week period following labeling. Figure 3.3 demonstrates that little ^{14}C was utilized for root respiration and growth two-weeks following labeling, i. e. it is at least temporarily stabilized in structural or nonstructural compounds. This clearly shows that these trees primarily depend upon current photosynthate for growth and maintenance during the growing season.

Carbon Partitioning

Whole-tree ^{14}C distribution varied according to clonal phenology. During active growth in July, 80% of the ^{14}C was found in above-ground components compared to 49% after budset in September (Table 3.3). The ^{14}C recovered in July correlates strongly to the weight of the most actively growing portions of the tree. In September, more ^{14}C was allocated to roots and recovery closely resembled the biomass distribution in the different root size classes. The amount of recovered ^{14}C in the below-ground components increased 3.5 fold to 38% in September (Table 3.3). The fine and coarse root fractions become significantly enriched with ^{14}C in September as compared to July. In July, the specific activity of the fine roots was about 25-50% of specific activity of the actively growing above-ground tissues. By September, the root system became a more active sink for ^{14}C with only the leaves having a higher specific activity. The total recovery of ^{14}C ranged from 81% in September to 89% in July and is among the highest recoveries we have

Table 3.3. Carbon concentration (%), specific activity (Bq mg-1 C), % of ^{14}C recovered of total applied (Bq/Bq added), and % ^{14}C of the total amount of ^{14}C recovered for the different tree components. Standard error of the mean in parentheses.

	%C	Bq mg^{-1} C	Bq/Bq added	% ^{14}C of total ^{14}C recovered
July 15- August 1, 1990				
stem	43.0 (0.6)	35.6 (2.5)	31.3 (4.8)	34.7 (2.0)
branches	42.5 (0.5)	36.6 (4.8)	16.8 (1.9)	18.8 (0.5)
leaves	40.4 (0.3)	42.8 (0.7)	23.9 (1.7)	26.9 (0.8)
total above-ground			72.0 (8.3)	80.4 (1.8)
roots				
<.5 mm	28.1 (2.4)	12.9 (1.6)	1.7 (0.0)	2.0 (0.2)
.5-1 mm	36.1 (2.0)	13.3 (1.8)	0.2 (0.0)	0.2 (0.0)
1-3 mm	38.2 (1.7)	15.9 (3.9)	0.8 (0.1)	0.9 (0.2)
3-10 mm	38.7 (1.0)	21.3 (5.5)	2.6 (0.3)	3.2 (0.5)
>10 mm	40.7 (0.0)	21.8 (5.4)	2.1 (0.2)	2.3 (0.2)
cutting	41.2 (1.2)	20.3 (3.9)	2.0 (0.1)	2.3 (0.1)
soil respiration		45.4 (1.8)	7.7 (0.2)	8.8 (0.9)
total below-ground			17.1 (0.2)	19.6 (1.8)
total tree			89.3 (8.1)	
September 5-19, 1990				
stem	40.8 (1.0)	17.3 (1.9)	15.6 (3.7)	19.2 (4.5)
branches	42.0 (2.0)	12.4 (1.8)	7.5 (1.0)	9.3 (1.4)
leaves	39.5 (2.2)	50.8 (4.6)	17.0 (1.3)	20.9 (1.3)
total above-ground			40.1 (3.9)	49.4 (4.4)
roots				
<.5 mm	28.7 (1.3)	31.5 (6.3)	5.5 (1.3)	6.8 (1.8)
.5-1 mm	31.9 (2.0)	35.2 (4.4)	0.8 (0.1)	0.9 (0.2)
1-3 mm	34.0 (1.2)	32.0 (5.7)	1.6 (0.3)	2.0 (0.3)
3-10 mm	36.6 (0.5)	38.9 (7.3)	8.8 (1.0)	10.9 (1.4)
>10 mm	37.3 (1.2)	38.1 (5.7)	11.1 (3.2)	13.5 (3.5)
cutting	43.0 (0.6)	25.9 (9.2)	3.4 (0.7)	4.3 (1.0)
soil respiration		39.0 (3.5)	9.0 (1.2)	12.2 (1.8)
total below-ground			37.7 (4.2)	50.6 (4.4)
total tree			81.1 (2.2)	

achieved in the field.

The C allocation characteristics of this clone are closely related to the termination of shoot growth (Michael *et al.,* 1988). After budset, source-sink relations change and roots became a major sink. Budset and leaf senescence on branches occurs acropetally in this clone. The onset of budset in both the terminal and branch shoots shifts the C allocation pattern from acropetal to basipetal (Isebrands, 1982). Hybrid poplars gain root sink strength as autumn approaches (Isebrands and Nelson, 1983; Friend *et al.,* 1991). Similar conclusions have been derived for apple trees (Quinlan, 1969). During our labeling studies, translocation of radiolabeled C to the root system nearly quadrupled from July to September.

The specific activity of labeled tissue proved useful in estimating sink strength and growth rates. The leaves were significant C sinks containing 21-27% of the recovered ^{14}C in July and September. The specific activity of the leaves remained higher than all other tissues regardless of the time of labeling. Similar results for conifer species have been obtained using different methods of pulse-labeling (Smith and Paul, 1989; Kuhns and Gjerstad, 1991). A significant amount of C exchange must occur in metabolic pools associated with C fixation and respiratory maintenance in leaves. This C exchange occurs even after budset when shoot growth has ceased.

Nitrogen Distribution

The above-ground components of the tree were a major sink for N in both July and September. In July, the leaves contained 57%, stem and branches 34% and roots 8% of the recovered ^{15}N (Table 3.4). During September, the leaves contained 16% of the injected N while stem and

Table 3.4. Nitrogen concentration (%), atom % ^{15}N excess, nitrogen derived from applied ^{15}N (%Ndf^{15}N) and % ^{15}N recovered of the total amount applied for the various tree components. Standard error of the mean in parentheses.

	%N	Atom % excess	%Ndf^{15}N	% ^{15}N of total applied
July 15- August 1, 1990				
stem	0.43 (0.03)	0.51 (0.08)	0.52	19.2 (0.7)
branches	0.59 (0.04)	0.53 (0.08)	0.54	15.3 (0.7)
leaves	2.41 (0.09)	0.46 (0.05)	0.47	57.3 (1.0)
total above-ground				91.7 (0.8)
roots				
<.5 mm	0.72 (0.06)	0.06 (0.00)	0.06	0.8 (0.0)
.5-1 mm	0.76 (0.06)	0.09 (0.01)	0.09	0.1 (0.0)
1-3 mm	0.73 (0.08)	0.17 (0.03)	0.17	0.6 (0.0)
3-10 mm	0.76 (0.06)	0.26 (0.04)	0.26	2.4 (0.3)
>10 mm	0.62 (0.05)	0.34 (0.07)	0.34	2.1 (0.6)
cutting	0.47 (0.02)	0.48 (0.06)	0.49	2.1 (0.1)
total below-ground				8.2 (0.9)
total tree				99.1 (2.2)
August 31-September 19, 1990				
stem	0.48 (0.01)	0.70 (0.06)	0.71	26.4 (2.1)
branches	0.87 (0.04)	0.78 (0.12)	0.80	36.1 (2.6)
leaves	2.44 (0.03)	0.22 (0.04)	0.23	17.4 (3.6)
total above-ground				79.8 (2.0)
roots				
<.5 mm	0.71 (0.03)	0.12 (0.02)	0.12	1.9 (0.3)
.5-1 mm	0.82 (0.03)	0.15 (0.02)	0.15	0.3 (0.1)
1-3 mm	0.93 (0.04)	0.21 (0.02)	0.22	1.1 (0.1)
3-10 mm	1.07 (0.06)	0.24 (0.03)	0.25	6.2 (1.0)
>10 mm	0.90 (0.02)	0.27 (0.03)	0.27	6.9 (1.4)
cutting	0.63 (0.05)	0.31 (0.03)	0.31	3.8 (0.4)
total below-ground				20.1 (2.0)
total tree				95.4 (1.8)

branches were now the strongest sink for N, containing 60%. The woody shoot of poplars have been shown to store proteins in ray cells during dormancy (Sauter *et al.*, 1988, 1989).

The root system contained twice as much ^{15}N in September compared to July, demonstrating the downward translocation of N typical of this clone in autumn (Pregitzer *et al.*, 1990). The majority of the additional ^{15}N found in the root system in September was located in the coarse root fraction. Fine roots were also more enriched with injected ^{15}N late in the growing season.

The plant nitrogen derived from the labeled nitrogen ($Ndf^{15}N$) expressed as a percentage of atom percent excess of each tree component to atom percent excess of injected ^{15}N indicates the amount of labeled N in tree tissue. The $Ndf^{15}N$ of all the tree components is less than 1% for both labelings (Table 3.4). This shows that the injected N was a minor component of the total N in all tissues. The injected ^{15}N was meant only to tag tree N pools. Recovery of labeled N was greater than 95% of that applied (both labelings).

The ^{15}N labeling of poplar trees by injection has been shown to enrich individual tree components relatively uniformly (Horwath *et al.*, 1992). The method is useful to determine N sink strength and to preferentially label leaves and roots for long-term turnover studies. Cooper and Clarkson (1989) have proposed that N utilization is regulated by a common pool of N. This would explain the uniform labeling of tissues and preferential loading of stronger N sinks.

The clonal response to senescence is to store N in woody tissue for subsequent growth the following growing season (Pregitzer *et al.*, 1990). The September increase of ^{15}N in coarse roots and branches illustrates this

point. The ^{15}N distribution increased in the root pool along with total N. However, the distribution is not equivalent in different root classes. Uptake of soil N, or a diminishing sink for N in the fine roots could explain the preferential enrichment of the woody coarse roots. However, preferential storage in coarse roots is a more likely answer. The above-ground Ndf^{15}N values for the July and September illustrate that the stem and branches are also a strong sink for the injected ^{15}N during the latter part of the growing season.

The comparison of the interrelationships between N distribution and C flow during the growing season are made possible using the dual labeling approach. Table 3.5 adapted from the ^{15}N and ^{14}C distribution in Tables 3.3 and 3.4 shows that N and C do not move similarly. The high recovery of ^{15}N to ^{14}C in leaves during July is not surprising. The ratio of 4.8 in branches in September and general retention of ^{15}N in the above-ground portion is noteworthy. The root system gains an additional 10% of the injected ^{15}N by September, but its distribution is masked by the additional accumulation of ^{14}C changing the ratio of the isotopes little. The ^{15}N injection technique preferentially loads the aboveground tree components and may require a longer equilibration period to distribute the N throughout the entire tree. This is possible since the unlabeled N distribution above-ground is 82% in July and 65% in September (of the total tree N). The ^{15}N injection technique demonstrates marked changes in N distribution between the labeling dates and this may reflect the storage pattern of tree components for N at specific times during the growing season.

The pronounced seasonal pattern of C and N allocation in *Populus* was first elucidated by Dickson and Nelson (1982) and Isebrands and

Table 3.5. The interrelationships between the distribution of ^{15}N and ^{14}C of the various tree components from the July and September labelings. The values are expressed as the ratio of the distribution of ^{15}N to ^{14}C recovered in the tree components.

Tree Component	July	September
stem	0.61	1.69
branches	0.91	4.80
leaves	2.41	1.02
total aboveground	1.27	1.99
roots		
<.5 mm	0.47	0.35
.5-1 mm	0.45	0.38
1-3 mm	0.75	0.69
3-10 mm	0.92	0.70
>10 mm	1.00	0.62
cutting	1.05	1.12
total below-ground	0.87	0.70

Nelson (1983). Later, Pregitzer *et al.* (1990) and Nguyen *et al.* (1990) demonstrated that the root system of Eugenei is a major site for the storage of nonstructural carbohydrates during the dormant season and that N allocation, like C allocation, exhibits a pronounced seasonal pattern of distribution and storage that reflects relative sink strength. This whole-tree dual tracer study confirms these earlier studies in rich detail. It is clear that this genotype allocates C and N to the different components of the tree preferentially, depending on time of year. These inherent patterns of C and N partitioning can have a major impact on ecosystem studies attempting to understand the role of the root system in providing substrate (C) for microbial metabolism and litter decomposition. This topic will be discussed in subsequent communications, but it is clear that C and N flux to the roots varies significantly over the course of the growing season depending on relative sink strength.

My results also point to the importance of developing decent estimates of tree respiration. Above-ground dark respiration accounted for 18-24% of net daily assimilation and root respiration accounted for 19-21% of soil respiration. Total respiration estimates are useful in determining gross production and the impact of forest ecosystems on global C budgets. Finally, I believe this study demonstrates the power of using tracers to study whole-tree C and N allocation. Clearly, the pronounced seasonal patterns documented in this chapter need to be tested by working with other genotypes and species.

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Chapter 4

THE FATE OF ^{14}C AND ^{15}N IN HYBRID POPLAR PLANTATIONS.

ABSTRACT

The growth of plants is often described as a series of competing sinks for carbon and nutrients. Carbon and nutrient requirements of fine roots are vaguely understood. The intent of this research was to determine the role of fine roots in the cycling of C and nutrients in a *Populus*-soil system. Two-year-old hybrid poplars were radiolabeled with $^{14}CO_2$ and stem injected with ^{15}N. The tree-soil system was sampled during the following year. Labeled leaf litter was placed on unlabeled tree plots in a leaf litter exchange designed to differentiate C and N flux from leaf litter and root turnover. The microbial biomass contained from 0.4-1.2% of the photoassimilated C and no ^{15}N two weeks after labeling in root labeled soil. This amount declined steadily during the following year. Conversely, the leaf litter labeled soils contained more ^{14}C and ^{15}N than root labeled soils and maintained the isotopic C and N levels throughout the first year. During the initial years of plantation establishment, leaf litter plays a more significant role in supplying substrate for microbial biomass activity and soil organic matter maintenance than does root turnover.

INTRODUCTION

The growth of plants is often generalized as a series of competing sinks for C and nutrients (Wardlaw, 1989). Bloom *et al.* (1985) maintain that the allocation of resources will favor an optimal balance between the shoot and roots eliminating resource limitation. The plasticity of the shoot to root ratio ultimately will lead to changes in nutrient cycling and soil organic matter conservation by altering the amount of leaf and root litter entering the soil. The practice of fertilizing forests to increase production is becoming more common, especially in short-rotation intensively-cultured systems (Miller, 1984). The prospect of altering nutrient cycling processes exists by changing the quality and amount of litter in high production plantation forests.

Carbon allocation and nutrient requirements of fine root systems in forests are vaguely understood (Gholz *et al.,* 1986; Santantonio and Grace, 1987; Raich and Naddlehoffer, 1989; Santantonio, 1989; Fogel, 1990). Fine roots generally account for only 5% of the standing biomass, but often account for a major proportion of stand production (Santantonio, 1989). The variability of fine root production estimates is often attributed to methodology and assumptions for production and mortality (Kurz and Kimmins, 1987; Vogt *et al.,* 1986; Singh *et al.,* 1984). Fine root production expressed as a proportion of total net primary production ranged from 3-6% for fast growing plantations on fertile sites (Santantonio and Grace, 1987; Pregitzer *et al.,* 1990) to 5-73% for various conifer systems (Ågren *et al.,* 1980; Keyes and Grier, 1981; 1980; Fogel, 1983; Vogt, 1991). Fine root production and turnover may have a substantial influence on the amount of

litter returned to soil and the rate of nutrient cycling (Wollum and Davey, 1975; Henderson and Harris, 1975; Fogel, 1990).

Carbon and N allocation between the shoot and root will be dependent on species and site. Modern forest practice selects for species that preferentially allocate to shoot production to increase harvestable yield (Nambiar and Fife, 1991). The change in allocation does not affect branches, coarse roots or leaves, rather, a tradeoff between stem and fine roots occurs (Linder and Axelsson, 1982; Axelsson and Axelsson, 1986; Santantonio and Santantonio, 1987; Santantonio, 1989). This fundamental change in C and N allocation to fine roots, a potential litter pool, may alter soil N processes by changing the diversity and amount of litter input. An understanding of soil N processes is crucial since N often limits growth of trees (Keeny, 1980; Nommik and Larsson, 1989). The nitrogen cycle is generally tightly conserved in aggrading natural forest systems (Cole and Rapp, 1981; Gholz, 1981). In plantations, the change in root litter input may alter N cycling, especially if root decomposition is a continuous process rather than temporal (Miller, 1984). Nitrogen availability has been shown to alter shoot/root allocation in trees (Linder and Troeng, 1981; Axelsson and Axelsson, 1986; Gower and Vitousek, 1989; Pregitzer *et al.*, 1990).

Nutrient uptake in a forest system is at its maximum during the establishment period (Miller, 1989). The rotation length of intensively-cultured forests is sufficiently brief to perpetuate the cycle of maximum nutrient uptake. The consequence of perpetuating this cycle is to maintain high concentrations of nutrients within the tree. Nutrient uptake may exceed requirements in young trees (Nambiar and Fife, 1991). In forests where rotation lengths are longer, the net accumulation of nutrients declines after canopy closure (Miller, 1984; Meier *et al.*, 1985). At this time,

maximum wood production occurs through the retranslocation of nutrients from older tissue. The importance of retranslocated nutrients in fast-growing plantations from roots is not well characterized (Nambiar and Fife, 1991). Horwath *et al.* (1992) found only small differences in nitrogen concentration of dead and live roots in hybrid poplar. Nambiar (1987) found no significant evidence of nutrient retranslocation from roots of *Pinus radiata*. Similarly, woody heathland plants demonstrated no root nutrient retranslocation (Aerts, 1990). In contrast, Meier *et al.* (1985) suggested that the internal redistribution of N and P from fine roots of various age *Abies amabilis* stands significantly met the requirements for the production of fine roots. The high nutrient requirements of plantation forestry may alter soil N processes through changes in rotation length, intensity of culture, season of harvest and loss of nutrient rich biomass through harvest. It is also important to realize that changes in shoot/root to maximize production may alter litter input to soil. The change in litter replenishment can significantly alter processes conserving the labile or active N and soil organic matter pools.

The change in litter input will alter the quality of plant residues, a major source of energy and nutrients for heterotrophic microorganisms in forest ecosystems. The subsistence of soil organisms on residues will alter concurrent immobilization, mineralization and stabilization reactions that maintain nutrient cycling and conserve soil organic matter (Voroney *et al.*, 1989). Therefore, the understanding of fine root production, mortality and turnover is essential to understanding the affects of forest management practices on soil quality and long term nutrient cycling.

In the present study, I investigated the role of root and leaf litter in nutrient cycling and soil organic matter dynamics. The introduction of

dual tracers, ^{14}C and ^{15}N, into trees at different times in the growing season encompassed different patterns of C and N allocation. The objective was to determine the fate of C and N within the tree and compare the potential nutrient return and soil organic matter production from the two different litters. Root turnover and production were estimated using pool dilution of labeled ^{14}C roots with unlabeled C. Measurement of ^{14}C and ^{15}N flux into soil and microbial biomass provided a means to determine the turnover rate of the two litter types. The fate of C and N in the tree was also determined. This report describes the long term fate (1-2 years) of dual labeled trees and litter in an intensively-cultured short-rotation hybrid poplar plantation.

MATERIALS AND METHODS

A short rotation-intensively cultured hybrid poplar plantation was established on a 0.2 hectare plot at the Kellogg Biological Station, LTER site, Michigan State University, in southwest Michigan. Cuttings of *Populus euramericana* cv. Eugenei, 10 cm in length, were planted on a 2 by 1 m spacing in the spring of 1989. The Kalamazoo soil series is a fine loamy, mixed, mesic, Typic Hapudalf. The site was prepared by moldbroad plowing and disking a field previously used for agricultural crops. During the establishment year, two sets of 8 trees consisting of 4 trees in adjacent rows were selected for uniformity of growth characteristics. An undisturbed 1 m^3 soil block was trenched around each tree to 1 m depth (Horwath, Chapter 3). Plywood partitions were inserted into the trenches and the vertical faces of the soil pedon were wrapped with vinyl sheeting

before backfilling the slots. The partition served both as a gas tight seal for root respiration studies and for the quantitative recovery of tracers.

^{15}N Injection

The two sets of trees were stem injected with ^{15}N, one set July 13, 1990 and the other set September 1, 1990. Each tree received 100 mg of 98 Atom % enriched $(^{15}NH_4)_2SO_4$ in an artificial sap solution (Horwath, Chapter 2). Leaves were collected daily from the time of labeling and until the finish of leaf fall in autumn. Upon collection leaves were placed in a 60°C forced air drying oven. Leaves from each tree were pooled for subsequent analysis.

^{14}C Labeling

Trees were exposed to $^{14}CO_2$ for an entire photoperiod on July 19, 1990 (July labeling) and September 5, 1990 (September labeling). Ambient levels of $^{14}CO_2$ (290-350 ppm) and temperature were maintained during the assimilation period (Horwath, Chapter 3). The level of CO_2 was monitored by an Infra Red Gas Analyzer and controlled by a computer operated $^{14}CO_2$ generator. In July, a total of 3.04×10^8 Bq for an average 3.79×10^7 Bq tree^{-1} and specific activity of 4215 Bq mg^{-1} CO_2-C was assimilated in the labeling chamber. During the September labeling, 2.85×10^8 Bq was added to the chamber for an average 3.56×10^7 Bq tree^{-1} and specific activity of 4300 Bq mg^{-1} CO_2-C.

Sampling Procedure

Three trees of eight were randomly selected and destructively sampled two weeks after each labeling. In November, an additional three

trees were sampled from the July (November 2, 1990) and the September labelings (November 4, 1990). The remaining two trees from each set were sampled one year later on July 25, 1991 and September 5, 1991. The sampling included the entire tree located above and within the 1 m^3 soil block. The sampling of leaves, branches and stem was described previously (Chapter 3). The soil block was excavated by depth (0-25 cm, 25-60 cm, 60-100 cm) and sieved through an 8 mm screen to recover coarse roots. Subsamples of soil from each depth were hydronuematically elutriated to recover and estimate fine root biomass. The washed roots were stored in 80% ethanol.

The washed roots were separated from organic residues by hand and the length determined using a modified Newman's line intercept method (Newman, 1966). The weight of the washed roots from each soil sample was determined after drying at 80°C for 24 hours. The root length and the oven dry weight of < 0.5 mm roots was used to develop a regression equation that estimated the relationship between root length and weight [(weight of washed roots < 0.5 mm, mg) = 0.984 (root length, cm) + 0.076, R^2=0.97, standard error of estimate = 6.5%)]. The equation was used to derive the weight of roots (< 0.5 mm) from trees sampled 1 year after the dual labelings. Roots taken from the field sieved soil were hand sorted according to size classes; < 0.5 mm, 0.5-1 mm, 1-3 mm, 3-10 mm, > 10 mm and cutting.

Plant Tissue and Soil Analysis

Leaf area was determined using a video imaging system (Delta-T meter, Decagon Devices, Pullman, WA). Plant material was dried at 80°C for 48-96 hours. Plant tissue was ground in a Wiley mill to pass a 60 mesh

screen. Plant and soil samples were combusted in a biological sample converter (Roboprep, Europa Scientific, Crewe, England) to yield N_2 and CO_2. The gases were analyzed on a continuous-flow dual isotope ratio mass spectrometer (ANCA-MS Europa Scientific, Crewe, England) for N, C, ^{15}N. Reference standards consisted of natural abundance 0.3663 atom % $(^{15}NH_4)_2SO_4$ and 44% C, as cellulose (Whatman no. 1 filter paper, Harris and Paul, 1989). The CO_2 from the combusted plant tissue was trapped in a CO_2 absorbing scintillation cocktail (Harvey Instrument Co. Hillsdale, N.J., USA) and the ^{14}C determined on a liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL). Radiolabeled glucose containing 120 Bq 5 μl^{-1} was pipetted onto the cellulose filter paper and dried at 80° C to determine the efficiency of $^{14}CO_2$ trapping.

Soil ^{14}C was determined using a dichromate digestion and trapping of CO_2. Soil (1 g, oven dry) was digested with 1g $K_2Cr_2O_8$ and 12 mL of H_2SO_4: H_3PO_4 (3:2, v/v) in a modified 25 X 200 mm culture tube. A 15 x 45 mm vial containing 3 mL of CO_2 trapping scintillation cocktail was inserted and rested on a restriction placed into the glass wall of the culture tube (Smith *et al.,* 1989). The tube was promptly capped (Poly seal cap, Fisher Scientific, Fairlawn, N.J,) and placed on a digestion block at 120°C for 2 hours. Following digestion, the tubes were allowed to stand overnight. The vials were capped and enclosed in 10 x 22 mm glass scintillation vial and the ^{14}C activity determined on a liquid scintillation spectrometer.

Microbial Biomass Determinations

Soil microbial biomass was estimated following each sampling of all plots. Composite soil samples from each tree plot were resieved through a 4 mm screen. Soil moisture was determined gravimetrically following

drying at 105°C for 24 hours. Initial inorganic N levels were determined on 2 M KCl (5:1, v/w) soil (25 g, wet weight) extracts. Inorganic N was analyzed on an auto-flow analyzer (Lachet Chemicals Co., Mequin, WI). Soil (50 g) was fumigated with ethanol-free chloroform in an evacuated desiccator for 18-24 hours (Horwath and Paul, 1992). After the removal of the chloroform, the soil was brought to 55% of water holding capacity and placed into 1 L Mason jars. A vial containing 5 mL of 0.6 M NaOH was placed into the mason jar to consume respired CO_2. The jars were closed and allowed to incubate for 10 days at 25°C. An unfumigated set of controls was incubated under the same conditions. After 10 days, the soil was extracted for inorganic N as described above. Half the contents of the CO_2 trap were titrated to a phenolthalein endpoint (pH 7) to determine respired CO_2. The remaining NaOH was combined with 10 mL of scintillation cocktail (Safety solve, Research products Int. Corp., Mount Prospect, IL) and ^{14}C activity determined on a liquid scintillation counter.

Microbial biomass C was calculated according to Voroney and Paul (1984) using the following equation,

$$\text{Biomass C} = C_f / 0.41$$

where, C_f indicates the amount of CO_2 respired from the fumigated soil and 0.41 is the efficiency of the microbial biomass C determination. The amount of ^{14}C within the microbial biomass was determined by dividing the ^{14}C released upon fumigation incubation by 0.41 after correction for background. Microbial biomass N was calculated by subtracting the initial soil NH_4^+ level from the fumigated N flush and correcting for efficiency using a variable efficiency factor (k_n) (Voroney and Paul, 1984).

$$k_n = (1.86 * (C_f / N_f) - 0.879$$

and

$$\text{Biomass N} = (N_f - N_i) / k_n$$

N_f is the amount of NH_4^+ mineralized in the fumigated soil, N_i initial soil NH_4^+ level and k_n is the efficiency of the microbial biomass N determination.

Litter Exchange Experiment

Leaf litter from the two labelings was collected from each tree until late autumn when all leaves had abscised. Litter from each tree was analyzed separately to complete whole tree ^{14}C and ^{15}N budgets and then composited by labeling. The litter was placed on plots that were trenched to a 25 cm depth and lined with plywood and Visqueen plastic. Each of these plots contained a tree planted at the same time as the labeled trees simulating the same design as those used in the dual labeling procedures. Unlabeled leaf litter was placed on plots with labeled trees to complete the litter exchange experiment. The amount of litter collected varied from each labeling due to differences in the collection period. The amount of leaf litter placed on the plots receiving September dual labeled leaves was less than plots with July labeled leaves. The September plots contained 268 g and July plots contained 601 g of leaf litter. These values were averages of the total amount of litter that fell from the different trees labeled in July and September. The litter on each plot was covered with plastic netting (garden netting, 1.8 cm mesh) at a height of approximately 2 cm by attaching the netting to the 15 cm tall plywood plot lining.

At approximately 6 month intervals a 50 by 50 cm area was sampled from each plot. Remaining litter was collected from the soil surface by carefully removing petioles and lamella structures. The soil was excavated in two depths, 0-10 cm and 10-25 cm. The soil was sieved through a 6 mm

sieve, homogenized in a cement mixer and a subsample taken. The litter material was dried at 60°C for 24 hours, coarsely ground in a Waring blender and ball milled (Harris and Paul, 1989). The litter, soil and microbial biomass were analyzed for ^{14}C and ^{15}N as described previously.

Expression of Results

Data are expressed as means with standard errors of the mean on replicate components of the tree-soil system. During the first year of labeling and harvest, each mean is composed of 3 replicates (n = 3). The harvests done one year later were composed of 2 replicates (n = 2). The first two harvests of the litter exchange experiment consist of 3 replicates (n=3) for September labeled leaves and 4 replicates (n=4) for July labeled leaves. The last harvest consisted of 2 replicates (n=2) for each litter. The ^{14}C results are expressed as specific activity (SA, Bq mg^{-1} C), labeled C (total Bq found in a component/SA of added $^{14}CO2$) and % of ^{14}C applied. The ^{15}N results are expressed as atom percent ^{15}N excess (atom percent ^{15}N of component - natural abundance, 0.3663%), labeled N (atom percent excess x % N x sample wt) and % of ^{15}N applied. Standard errors of the mean are shown to demonstrate the reproducibility of dual isotopic labeling in the field.

RESULTS

Tree Characteristics

The trees grew rapidly, doubling in height every growing season. By the end of the third summer, the trees were approximately 5 m tall (Table 4.1). Leaf area determined on July harvested trees remained constant

Table 4.1. Height and leaf area measurements taken from samplings done during the growing season from trees labeled July 19, 1990 and September 5, 1990. Standard error of the mean shown in parentheses.

	Year		
Labeling	1	2	3
July labeled trees			
Height (current increment) (cm)	159.3 (10.7)	151.0 (5.2)	207.5 (27.5)
Total height (cm)	159.3 (10.7)	310.3 (11.9)	517.8 (30.0)
Leaf area (m^{-2} $tree^{-1}$)	NA	6.1 (0.6)	6.0 (1.4)
September labeled trees			
Height (current increment) (cm)	153.3 (5.7)	183.7 (32.8)	172.0 (28.0)
Total height (cm)	153.3 (5.7)	337.0 (33.3)	509.0 (43.5)
Leaf area (m^{-2} $tree^{-1}$)	NA	3.1 (0.6)	1.0 (0.3)

through the second and third year at approximately 6 m^2 $tree^{-1}$. Leaf area from September labeled trees was reduced from leaf diseases such as *Melampsora* rust and *Marsonnina* leaf spot . The dry weight of trees dual labeled in July, 1990 and sampled through July, 1991 increased two fold (Table 4.2). The September, 1990 dual labeled trees had less leaf biomass than July labeled trees as a result of leaf drop in both 1990 and 1991 (Table 4.3). Despite the leaf mass loss in the September dual labeled trees, these trees were still useful for the study of tracer dynamics and budgets. The trees assimilated ^{14}C according to leaf area and tree biomass thus achieving similar concentrations and turnover rates of tracers.

Distribution of C and N in the Tree-Soil System

Figure 4.1 shows the total C and N contained within the tree and soil microcosm of July labeled trees sampled August 2, 1990 and November 2, 1990. The soil contained 6.9 kg of C. Tree C represents 11% and the soil 74% of the total C in the tree-soil system. The remaining C in the system was composed of leaf litter, microbial biomass and soluble soil C. The amount of C in the microbial biomass was twice the amount found in fine roots (< 0.5 mm). Leaf litter C was approximately twice microbial biomass C and four times standing fine root biomass (< 0.5 mm) C in November. Fine roots contain less than 1% and leaf litter 3.4% of soil C. The root system nearly doubles in C content from July to November.

The soil within the 1 m^3 microcosm contained 608 g of N (Figure 4.1). The tree comprises 3% of the total N in the system. Leaves contain more than 50% of total tree N during the growing season. Fine roots comprised 5% of tree N during the growing season and dormancy. The N level in woody shoots and coarse roots (> 0.5 mm) doubles from July to November.

Table 4.2. The dry weight (g) of tree components from July 19, 1990 dual labeled trees sampled over the following year. Standard error of the mean shown in parentheses.

	Sampling date		
	2-Aug-90	2-Nov-90	25-Jul-91
stem	781 (122)	1053 (106)	1468 (59)
branches	432 (96)	534 (77)	702 (168)
leaves	528 (52)	619 (22)	597 (190)
total	1742 (270)	2206 (203)	2766 (81)
roots 0-25 cm			
< 0.5 mm	88 (8)	70 (8)	114 (4)
0.5-1 mm	4 (1)	10 (2)	21 (1)
1-3 mm	11 (1)	23 (1)	16 (3)
3-10 mm	53 (20)	80 (3)	60 (31)
> 10 mm	82 (42)	188 (32)	396 (192)
cutting	98 (18)	183 (11)	423 NA
total	336 (82)	554 (28)	1095 (225)
roots 25-60 cm			
< 0.5 mm	51 (4)	45 (5)	63 (4)
0.5-1 mm	7 (2)	9 (1)	2 (1)
1-3 mm	18 (2)	31 (5)	14 (0)
3-10 mm	55 (21)	96 (15)	76 (18)
> 10 mm	36 (11)	114 (46)	104 NA
total	155 (34)	295 (24)	208 (75)
roots 60-100 cm			
< 0.5 mm	46 (11)	51 (1)	48 (6)
0.5-1 mm	5 (1)	9 (1)	11 (0)
1-3 mm	20 (5)	36 (5)	22 (2)
3-10 mm	36 (13)	81 (11)	59 (14)
> 10 mm	3 NA	42 (7)	27 NA
total	108 (28)	205 (20)	154 (24)
Root total	599 (93)	1054 (42)	1456 (238)
Tree total	2341 (370)	3260 (239)	4223 (45)

Table 4.3. The dry weight (g) of tree components from September 5, 1990 dual labeled trees sampled over the following year. Standard error of the mean shown in parentheses.

	Sampling date		
	20-Sep-90	4-Nov-90	5-Sep-91
stem	770 (125)	654 (56)	1650 (255)
branches	519 (28)	375 (67)	470 (229)
leaves	303 (14)	281 (10)	106 (37)
total	1592 (158)	1310 (122)	2226 (522)
roots 0-25 cm			
< 0.5 mm	107 (11)	49 (9)	74 (2)
0.5-1 mm	10 (2)	7 (1)	8 (2)
1-3 mm	17 (3)	13 (1)	12 (2)
3-10 mm	65 (22)	69 (4)	40 (29)
> 10 mm	163 (38)	77 (24)	128 NA
cutting	191 (10)	133 (18)	233 (53)
total	552 (16)	348 (32)	431 (151)
roots 25-60 cm			
< 0.5 mm	55 (8)	36 (3)	48 (5)
0.5-1 mm	6 (0)	5 (0)	8 (1)
1-3 mm	16 (2)	15 (3)	19 (7)
3-10 mm	82 (11)	99 (16)	72 (2)
> 10 mm	95 (19)	71 (6)	37 (17)
total	255 (7)	226 (14)	184 (17)
roots 60-100 cm			
< 0.5 mm	51 (8)	49 (5)	40 (5)
0.5-1 mm	8 (0)	7 (1)	7 (1)
1-3 mm	20 (3)	26 (2)	11 (1)
3-10 mm	84 (6)	80 (13)	48 (26)
> 10 mm	14 (5)	9 (3)	4 NA
total	177 (13)	170 (10)	108 (33)
Root total	984 (21)	744 (36)	723 (156)
Tree total	2576 (182)	2054 (168)	2949 (723)

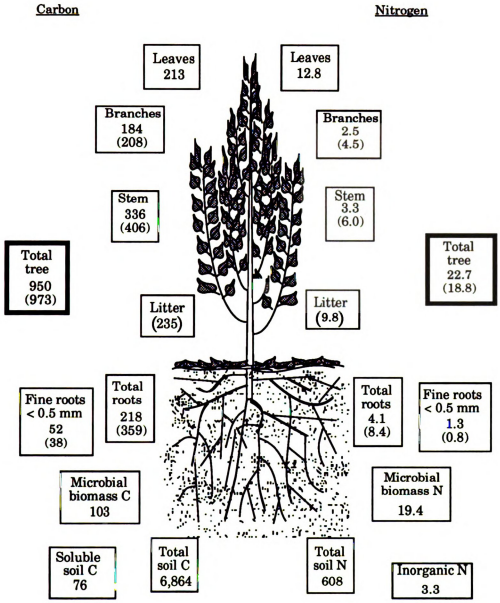


Figure 4.1. The distribution of C and N (g) in the *Populus*-soil system for July 19, 1990 labeled trees sampled August 2, 1990. The values in parentheses represent the amount of C and N for the November 2, 1990 sampling.

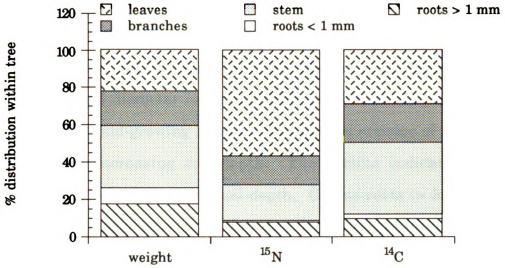
The apparent retranslocation of N from senescing leaves represents a third of total leaf N or 5.1 g on a mass basis. The level of microbial biomass N was equivalent to total tree N in the second year of growth. This indicates the importance of the microbial biomass as a source-sink for N and other nutrients in this system. Inorganic N (NO_3^- and NH_4^+) represents 15% of both tree and microbial N. Complete tree C and N concentrations for all labelings can be found in Appendix A (Tables A.1-A.4).

Distribution of ^{14}C and ^{15}N in the Tree

During mid-growing season, the shoot was actively growing and contained greater than 70% of the tree biomass and ^{14}C (Figure 4.2). Leaves retained greater than 50% of the injected ^{15}N 19 days following the dual labeling procedure. Roots contained approximately 10% of the ^{15}N and ^{14}C during July. The majority of the recovered ^{14}C was in the stem. The distribution of ^{14}C closely resembles the weight distribution of the tree in July. The distribution of ^{14}C represents the allocation of net primary production. The shoot was the strongest sink for C and N during mid-growing season.

The September dual labeling illustrates the basipetal movement of both C and N during autumn (Figure 4.2). The shoot received 80% and roots 20% of the injected ^{15}N. The major N sink in autumn was the woody shoot, especially branches. The root system contained approximately 45% of the assimilated ^{14}C 14 days after labeling. The root system received the majority of net primary production in September. The shift in C and N allocation from July to September exemplifies the phenological growth pattern of this hybrid poplar genotype.

A. July label



B. September Label

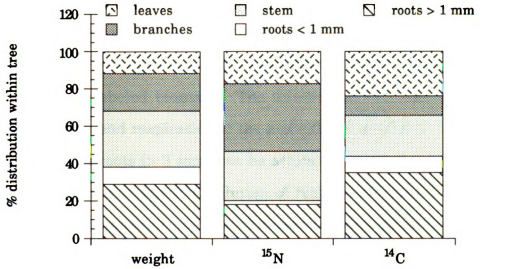


Figure 4.2. Relative distribution of weight (g), ^{15}N (mg) and ^{14}C (mg) within trees labeled on July 19, 1990 (A) and September 5, 1990 (B).

Specific Activity of ^{14}C

Tables 4.4 and 4.5 present the specific activity, Bq mg^{-1} C, of the different components of the tree-soil system. In July, the specific activity of the shoot components were twice that of the root system. The increased ^{14}C activity demonstrates the sink strength for C of the shoot during mid-growing season. The specific activity of roots was dependent on root diameter. Small diameter roots contained less ^{14}C than coarse roots or the cutting during mid-growing season. The specific activity of roots (< 3 mm) declines with increasing soil depth. The decline indicated lower sink strength for C with increasing soil depth. Coarse roots (> 3 mm) maintain similar specific activities at all soil depths. Microbial biomass C specific activity was low compared to tree components. The assay for microbial biomass encompassed the entire microbial population diluting any rhizosphere interactions.

The November sampling of the July labeled trees shows dilution of specific activity in all tree components and no change in the microbial biomass. The ^{14}C dilution in the woody shoot did not occur entirely from an increase in unlabeled biomass. The dilution of ^{14}C was also a result of retranslocation and respiration of the assimilated labeled C. The dilution of ^{14}C activity in roots (> 3 mm) can be accounted for through the addition of unlabeled biomass. The ^{14}C dilution of roots (< 3 mm) was dependent on soil depth. The constant biomass of these fine roots from July to November indicates either turnover of biomass and or, labile C.

One year following the July labeling, the specific activity of the shoot and roots (3-10 mm) continued to decline. This dilution did not occur from the addition of unlabeled biomass. This illustrates that C was still being retranslocated or used in maintenance respiration one year after

Table 4.4. The specific activity (Bq mg^{-1} C) for the different tree components sampled during the year following ^{14}C labeling on July 19, 1990. Standard error of the mean shown in parentheses.

	Sampling date		
	2-Aug-90	2-Nov-90	25-Jul-91
stem	35.6 (2.5)	23.4 (3.3)	12.3 (0.7)
branches	36.6 (4.8)	21.0 (3.5)	10.0 (0.3)
leaves	42.8 (0.7)	25.9 (1.7)	0.3 (0.1)
root 0-25 cm			
< 0.5 mm	16.3 (1.7)	9.5 (1.2)	9.8 (2.7)
.5-1 mm	18.7 (2.0)	9.1 (1.8)	12.2 (2.7)
1-3 mm	18.8 (3.0)	7.9 (1.5)	6.7 (2.5)
3-10 mm	22.0 (5.6)	8.6 (0.2)	5.7 (1.4)
> 10 mm	22.7 (6.2)	11.1 (0.7)	5.6 (0.1)
cutting	20.3 (3.9)	10.7 (0.5)	9.2 NA
root 25-60 cm			
< 0.5 mm	11.6 (1.6)	7.6 (0.6)	10.5 (0.7)
.5-1 mm	13.0 (2.8)	8.2 (0.4)	3.7 (2.5)
1-3 mm	15.9 (4.5)	8.0 (0.7)	9.1 (0.9)
3-10 mm	19.7 (5.8)	6.3 (0.6)	6.0 (1.4)
> 10 mm	21.3 (8.8)	7.3 (0.4)	4.8 NA
root 60-100 cm			
< 0.5 mm	7.9 (2.6)	6.7 (1.7)	6.9 (1.3)
.5-1 mm	8.8 (3.0)	7.2 (1.0)	8.2 (0.9)
1-3 mm	14.5 (3.7)	6.5 (1.2)	7.2 (0.5)
3-10 mm	21.4 (5.2)	6.7 (0.9)	5.1 (0.1)
> 10 mm	11.5 NA	7.5 (0.9)	4.0 NA
Microbial Biomass C	1.3 (0.2)	1.4 (0.2)	1.0 (0.2)

Table 4.5. The specific activity (Bq mg^{-1} C) for the different tree components sampled during the year following ^{14}C labeling on September 5, 1990. Standard error of the mean shown in parentheses.

	Sampling date		
	20-Sep-90	4-Nov-90	5-Sep-91
stem	17.3 (1.9)	13.6 (1.5)	4.8 (0.5)
branches	12.4 (1.8)	10.9 (0.5)	4.2 (0.4)
leaves	50.8 (4.6)	49.3 (3.5)	0.5 (0.2)
root 0-25 cm			
< 0.5 mm	35.7 (7.7)	27.4 (2.1)	21.2 (0.2)
.5-1 mm	38.7 (6.0)	36.1 (1.8)	27.7 (3.8)
1-3 mm	37.8 (12.0)	32.2 (5.6)	24.8 (1.8)
3-10 mm	41.6 (8.4)	30.5 (1.6)	19.6 (5.0)
> 10 mm	35.1 (5.5)	19.0 (1.9)	9.0 NA
cutting	14.7 (2.8)	6.8 (1.7)	8.6 (2.4)
root 25-60 cm			
< 0.5 mm	33.3 (3.5)	32.4 (4.3)	29.9 (3.8)
.5-1 mm	40.7 (2.9)	38.4 (6.0)	33.8 (4.9)
1-3 mm	38.7 (2.3)	36.3 (1.4)	29.8 (3.1)
3-10 mm	34.3 (7.6)	29.3 (4.7)	27.4 (6.6)
> 10 mm	41.1 (6.1)	24.5 (5.7)	19.0 (2.9)
root 60-100 cm			
< 0.5 mm	16.1 (3.2)	18.7 (2.0)	30.0 (3.7)
.5-1 mm	25.7 (6.6)	22.0 (1.2)	26.3 (0.9)
1-3 mm	20.8 (3.6)	22.3 (5.1)	18.4 (3.9)
3-10 mm	42.2 (8.2)	34.7 (3.7)	11.5 (3.5)
> 10 mm	59.3 NA	36.2 (4.4)	15.5 NA
Microbial Biomass C	4.5 (0.5)	2.6 (0.2)	1.1 (0.1)

assimilation. The ^{14}C dilution in roots > 10 mm can be accounted for through an increase in biomass. Some shoot ^{14}C may have also been translocated to the root system. Roots (< 3 mm) maintain the same levels specific activity from dormancy in November to one year following the labeling. The constant fine root ^{14}C activity indicated minimal pool turnover or synthesis of new roots from labeled reserves.

In the September labeling, the specific activity of roots exceeded that of the woody shoot (Table 4.5). The cutting behaved similarly to the woody shoot in ^{14}C activity. Roots in the 0-60 cm soil profile have become uniformly labeled with ^{14}C regardless of diameter. The microbial biomass became significantly labeled, containing 3.5 times more ^{14}C activity than in the July labeling. The increase in all below-ground components of the tree-soil system was typical of this clone and exemplifies the basipetal movement of C during autumn, some of which moves directly into microbial biomass.

The dilution of ^{14}C activity in shoot tissue was small from September to November compared to the July labeling ^{14}C dynamics. The specific activity of leaves and leaf litter remain unchanged. The lack of ^{14}C dilution in most of the shoot-root system illustrates that C storage was ending just after budset. The dilution of ^{14}C still occurred in coarse roots (> 3 mm) and fine roots (< 0.5 mm) in the 0-25 cm soil profile. The dilution of the ^{14}C activity in fine roots was similar to results of the July labeling and may indicate fine root turnover during the latter part of the growing season and the beginning of dormancy.

One year following the September, 1990 labeling, dilution of ^{14}C had occurred in the woody shoot and coarse roots. Leaves regain only a minor portion of the previous years assimilated ^{14}C. Little dilution of the ^{14}C

activity of fine roots (< 3 mm) occurs. This difference between July labeled and September labeled trees show the potential importance of late season reserves in the production of fine roots. The ^{14}C activity of the microbial biomass probably declines from the lack of root C input. The dilution of ^{14}C activity in coarse roots and lack of dilution in fine roots may indicate movement of reserve C to sustain the fine root system during the next growing season. Both the July and September labeling exemplify that fine root turnover was probably occurring during late autumn and early dormancy immediately after leaf fall.

Distribution of ^{15}N Activity

The largest atom % ^{15}N excess was found in the shoot and coarse roots (> 10 mm) from the stem injection of ^{15}N in July, 1990 (Table 4.6). Leaves and coarse roots contained similar concentrations of ^{15}N. The atom % ^{15}N excess of roots (< 10 mm) was dependent upon root diameter corroborating data obtained with ^{14}C. Small diameter roots contained the lowest level of ^{15}N, typically ranging from 10 and 20% of the ^{15}N excess found in large roots (> 10 mm). The concentration of ^{15}N within root size classes was not affected by depth of the soil profile as it was with ^{14}C activity. This indicates that C and N may be utilized via different mechanisms since the sink strength of the two tracers was different within the same root size classification.

Subsequent sampling of the July, 1990 ^{15}N injection revealed a decrease in the atom % in shoot and coarse roots (> 3 mm). Leaves of the subsequent growing season contained significant ^{15}N, having an atom % excess of 0.1%. The decline in atom percent of the stem, branches and coarse roots was probably associated with uptake of unlabeled soil N and

Table 4.6. Atom percent ^{15}N excess (atom % - natural abundance) is shown for the different tree components sampled the year following ^{15}N injection on July 14, 1990. Standard error of the mean shown in parentheses.

	Sampling date		
	2-Aug-90	2-Nov-90	25-Jul-91
stem	0.58 (0.07)	0.30 (0.04)	0.18 (0.00)
branches	0.63 (0.08)	0.35 (0.04)	0.13 (0.01)
leaves	0.46 (0.05)	0.37 (0.01)	0.10 (0.02)
roots 0-25 cm			
< 0.5 mm	0.06 (0.01)	0.09 (0.02)	0.09 (0.00)
.5-1 mm	0.11 (0.01)	0.11 (0.02)	0.11 (0.01)
1-3 mm	0.18 (0.03)	0.16 (0.04)	0.20 (0.07)
3-10 mm	0.26 (0.03)	0.20 (0.03)	0.14 (0.03)
> 10 mm	0.31 (0.04)	0.21 (0.03)	0.14 (0.02)
cutting	0.48 (0.06)	0.21 (0.01)	0.16 NA
roots 25-60 cm			
< 0.5 mm	0.07 (0.01)	0.09 (0.02)	0.08 (0.00)
.5-1 mm	0.10 (0.01)	0.12 (0.02)	0.09 (0.03)
1-3 mm	0.17 (0.04)	0.16 (0.03)	0.12 (0.01)
3-10 mm	0.25 (0.06)	0.18 (0.04)	0.13 (0.01)
> 10 mm	0.40 (0.12)	0.20 (0.04)	0.13 NA
roots 60-100 cm			
< 0.5 mm	0.05 (0.02)	0.07 (0.02)	0.08 (0.03)
.5-1 mm	0.07 (0.02)	0.11 (0.02)	0.11 (0.03)
1-3 mm	0.16 (0.03)	0.14 (0.02)	0.12 (0.02)
3-10 mm	0.23 (0.01)	0.16 (0.03)	0.11 (0.01)
> 10 mm	0.43 NA	0.14 (0.00)	0.11 NA

Table 4.7. Atom percent ^{15}N excess (atom % - natural abundance) for the different tree components sampled during the year following ^{15}N injection on August 31, 1990. Standard error of the mean shown in parentheses.

	Sampling date		
	20-Sep-90	4-Nov-90	5-Sep-91
stem	0.70 (0.06)	0.68 (0.06)	0.26 (0.01)
branches	0.78 (0.12)	0.91 (0.07)	0.29 (0.08)
leaves	0.22 (0.04)	0.22 (0.04)	0.21 (0.05)
roots 0-25 cm			
< 0.5 mm	0.12 (0.02)	0.09 (0.01)	0.12 (0.03)
.5-1 mm	0.16 (0.01)	0.14 (0.01)	0.16 (0.05)
1-3 mm	0.26 (0.03)	0.21 (0.03)	0.21 (0.07)
3-10 mm	0.30 (0.03)	0.27 (0.03)	0.28 (0.09)
> 10 mm	0.29 (0.02)	0.29 (0.02)	0.20 NA
cutting	0.31 (0.03)	0.26 (0.05)	0.25 (0.08)
roots 25-60 cm			
< 0.5 mm	0.13 (0.01)	0.12 (0.01)	0.15 (0.04)
.5-1 mm	0.17 (0.01)	0.17 (0.03)	0.20 (0.05)
1-3 mm	0.23 (0.00)	0.22 (0.03)	0.24 (0.07)
3-10 mm	0.22 (0.02)	0.25 (0.04)	0.31 (0.11)
> 10 mm	0.24 (0.03)	0.25 (0.02)	0.31 (0.11)
roots 60-100 cm			
< 0.5 mm	0.08 (0.02)	0.09 (0.01)	0.14 (0.03)
.5-1 mm	0.13 (0.02)	0.12 (0.01)	0.15 (0.03)
1-3 mm	0.16 (0.02)	0.18 (0.03)	0.16 (0.01)
3-10 mm	0.23 (0.05)	0.25 (0.02)	0.14 (0.01)
> 10 mm	0.26 (0.05)	0.21 (0.03)	0.18 NA

retranslocation of ^{15}N to new leaves. The decrease of atom % ^{15}N excess in the woody shoot and coarse roots may have also maintained fine roots. The atom % ^{15}N excess of roots (< 3 mm) remained constant into the next growing season. The constant level of ^{15}N in fine roots suggested that turnover of this biomass pool was minimal or that stored ^{15}N was used to construct new roots. These observations were in agreement with the ^{14}C results, mentioned earlier and implies the two tracers were behaving similarly over time.

In the September N injection, the largest atom % ^{15}N excess occurred in the woody shoot with branches containing 0.78% and stem 0.65% (Table 4.7). The remaining tissues of the tree contain 0.31% or less atom % ^{15}N excess. The atom % excess of roots was again dependent on diameter as it was in the July ^{15}N injection. Roots (> 1 mm) in the 0-25 cm soil profile contained slightly more atom % ^{15}N than roots at lower soil depths. The subsequent November sampling of the September injected trees showed slight dilution of labeled N throughout the tree. An increase of atom % ^{15}N excess occurred only in branches from 0.78 to 0.91% and was probably a result of the retranslocation of N from leaves before senescence.

No significant changes in the atom % excess of roots had occurred by September, 1991, one year following the ^{15}N injection. The shoot tissue ^{15}N enrichment decreased dramatically during the third growing season. New leaves acquired ^{15}N from stored N. The constant ^{15}N enrichment of roots corroborated with the July, 1990 ^{15}N injection results (Table 4.6). These results combined with the ^{14}C activity data (Tables 4.4 and 4.5) suggest that either minimal turnover or reliance on labeled reserves maintained the fine root system from dormancy to the next growing season. Nitrogen taken from the soil to support the third season leaf growth does not interact

with the existing N within the root system. The contrasting N results may provide evidence for the persistence of fine roots from the second to third growing season.

The Fate of Labeled C and N

The recovery of ^{14}C and ^{15}N from the July and September labelings declined during the following year (Table 4.8 and 4.9). The loss of the applied ^{14}C one year after labeling was 63% in July and 73% in September labeled trees. Similarly, the loss of injected ^{15}N was 28.2% in July and 45% in September labeled trees. A large portion of both tracers was lost with the fall of leaves during autumn. The majority of the ^{14}C loss from the trees, excluding loss from leaf and root turnover, was associated with respiratory activity. Stem, branches and roots (> 0.5 mm) from both labelings lost a significant amount of ^{14}C over the year. Fine roots (< 0.5 mm) from both sets of labeled trees lost about 50% of their ^{14}C from the time of labeling to the November sampling indicating rapid turnover of ^{14}C. A small increase of ^{14}C in fine roots one year following both labelings indicates that new growth of fine roots was dependent on reserve C. Little ^{14}C was found in new leaves, less than 0.2% of the applied, indicating new leaf growth does not rely heavily on stored reserves or that the reserves were mainly used to initiate the formation of new leaves.

The fate of ^{15}N in the shoot and roots of these hybrid poplars was similar to ^{14}C (Tables 4.10-4.11). Major movement of the injected ^{15}N to other areas occurred from leaf litter, woody shoot and coarse roots amounting to over 50% of the applied for both labelings. New leaf growth the year following injection contained 10.3% in July labeled trees and 4.2%

Table 4.8. Recovery of labeled C in mg* and percent of the amount applied from tree-soil components sampled the year following labeling July 19, 1990. Standard error of the mean shown in parentheses.

	Sample date					
	2-Aug-90		4-Nov-90		25-Jul-91	
	^{14}C mg	% of applied	^{14}C mg	% of applied	^{14}C mg	% of applied
Leaves/litter	2155 (157)	23.9	1446 (90)	16.1	19 (9)	0.2
Stem & branches	4328 (463)	48.1	3264 (385)	36.3	2342 (242)	26.0
Roots < 0.5 mm	155 (18)	1.7	74 (9)	0.8	120 (11)	1.3
Roots > 0.5 mm	732 (93)	8.1	696 (50)	7.7	753 (92)	8.4
Root soil respiration	694 (20)	7.7	NA		NA	
Microbial biomass	35 (3)	0.4	26 (1)	0.3	26 (0)	0.3
Soil-Microbial biomass	106 (26)	1.2	235 (23)	2.3	130 (11)	1.4
Total	8175 (718)	90.8	5705 (464)	63.4	3366 (69)	37.4

* 9000 mg $^{14}CO_2$-C applied

Table 4.9. Recovery of labeled C in mg* and percent of the total amount applied from tree-soil components sampled the year following labeling September 5, 1990. Standard error of the mean shown in parentheses.

	Sample date					
	20-Sep-90		4-Nov-90		5-Sep-90	
	^{14}C mg	% of applied	^{14}C mg	% of applied	^{14}C mg	% of applied
Leaves/litter	1406 (106)	17.0	1252 (58)	15.1	5 (0)	0.1
Stem & branches	1916 (316)	23.1	1214 (164)	14.7	921 (113)	11.1
Roots < 0.5 mm	454 (117)	5.5	201 (119)	2.4	315 (30)	3.8
Roots > 0.5 mm	2127 (220)	25.7	1191 (83)	14.4	756 (71)	9.1
Root soil respiration	813 (102)	9.8	NA		NA	
Microbial biomass	91 (12)	1.1	57 (1)	0.7	35 (6)	0.4
Soil-Microbial biomass	45 (33)	0.5	226 (46)	2.7	246 (153)	3.0
Total tree	6806 (201)	82.2	4140 (310)	50.0	2227 (134)	26.9

* 8300 mg $^{14}CO_2$-C applied

Table 4.10. Recovery of labeled N in mg and percent of the total amount applied the year following injection July 14, 1990. Standard error of the mean shown in parentheses.*

	Sample date					
	2-Aug-90		4-Nov-90		25-Jul-91	
	^{15}N mg	% of applied	^{15}N mg	% of applied	^{15}N mg	% of applied
Leaves/litter	57 (0.3)	57.9	36 (2.8)	36.7	10 (1.9)	10.3
Stem & branches	34 (1.4)	34.9	33 (1.2)	33.8	10 (1.3)	10.6
Roots < 0.5 mm	1 (0.1)	0.8	1 (0.1)	0.8	1 (0.2)	1.0
Roots > 0.5 mm	8 (1.0)	7.9	14 (1.0)	14.4	7 (0.6)	6.8
Total	99 (2.2)	101.1	84 (3.7)	86.0	28 (1.5)	28.2

* 98 mg $(^{15}NH_4)_2SO_4$ stem injected.

Table 4.11: Recovery of labeled Ni

Table 4.11. Recovery of labeled N in mg and percent of the total amount applied the year following injection August 31, 1990. Standard error of the mean shown in parentheses.*

	Sample date					
	20-Sep-90		4-Nov-90		5-Sep-90	
	^{15}N mg	% of applied	^{15}N mg	% of applied	^{15}N mg	% of applied
Leaves/litter	16 (3.2)	16.8	12 (2.4)	12.7	4 (1.3)	4.2
Stem & branches	60 (3.7)	60.9	58 (3.5)	59.0	27 (6.0)	27.8
Roots < 0.5 mm	2 (0.3)	1.8	1 (0.1)	0.8	2 (0.0)	1.7
Roots > 0.5 mm	17 (1.5)	17.8	17 (1.5)	17.5	12 (1.5)	12.7
Total	95 (1.8)	97.3	88 (3.3)	90.0	44 (11.9)	44.7

* 98 mg $(^{15}NH_4)_2SO_4$ stem injected.

in September injected trees. The recovery of ^{15}N in September injected trees was low due to early leaf drop. Fine roots (< 0.5 mm) maintained constant levels of ^{15}N exhibiting similar trends as with ^{14}C. The loss of ^{15}N from the woody shoot and coarse roots was difficult to explain since N was thought to be tightly conserved. Turnover of the fine root system can not explain this disappearance since the levels of ^{14}C do not increase in the soil (Tables 4.8- 4.9). However, C and N may not necessarily be linked. For example, N may preferentially lost through amino acid and protein exudation from roots.

Dynamics of ^{14}C and ^{15}N in Leaf Litter

The turnover of leaf litter was determined on plots containing unlabeled trees. This was done to compare the amount of C and N input to soil from the different tree litters. The decomposition of leaf litter ^{14}C proceeded rapidly regardless of when the leaves were labeled (Table 4.12). At 613 days, 29% of the July and 8% of the September leaf litter ^{14}C remained in the litter. Soil ^{14}C accumulated rapidly to 248 mg of labeled C or 17.1% of the ^{14}C originally present in July labeled leaves. In the September labeled litter, the amount of ^{14}C in soil declines from 212 mg at 168 days to 166 mg at 613 days or 13% of that originally present in the litter. The amount of ^{14}C found in the microbial biomass was always less than 2% of what was originally present for both leaf and root derived C.

The dynamics of leaf litter ^{15}N differs from that of ^{14}C. Movement of ^{15}N from leaf litter does not occur until 168 days for September and 613 days for July labeled litter. This difference can be attributed to the C:N ratio, the July labeled litter has a C:N ratio 2.5 times that of September labeled leaves. Secondly, the mass of the July litter lain on the plots was four times larger

Table 4.12. The fate of labeled C in leaf litter from both the July and September labelings. Labeled C in litter, soil and microbial biomass was sampled over 613 days. Total values represent soil minus microbial biomass. Standard error of the mean shown in parentheses were applicable.

	Sampled (days)							
	0		168		328		613	
	^{14}C (mg)	% of applied	^{14}C (mg)	% of applied	^{14}C (mg)	% of applied	^{14}C (mg)	% of applied
July labeling								
Litter	1446.0 NA	100	909.0 (35.5)	62.9	469.5 (12.3)	32.5	416.1 (17.6)	28.8
Soil	- -		118.1 (11.9)	8.2	185.9 (20.1)	12.9	247.6 (17.7)	17.1
Microbial biomass	- -		12.0 (1.4)	0.8	9.3 (0.4)	0.6	- -	-
Total	1446.0	100	1015.1	70.0	646.1	44.7	663.2	45.9
September labeling								
Litter	1333.5 NA	100	641.1 (29.7)	48.1	363.9 (16.6)	27.3	104.4 (1.6)	7.8
Soil	- -		212.2 (22.9)	15.9	223.8 (24.4)	16.8	166.0 (2.3)	12.5
Microbial biomass	- -		22.3 (2.1)	1.7	12.6 (0.7)	0.9	- -	-
Total	1333.5	100	831.0	62.3	575.1	43.1	270.4	20.3

Table 4.13. Fate of labeled N in leaf litter from both the July and September stem injections. Labeled N in litter, soil and microbial biomass sampled over 613 days. Total values represent soil minus microbial biomass. Standard error of the mean shown in parentheses were applicable.

	Sampled (days)							
	0		168		328		613	
	^{15}N (mg)	% of applied	^{15}N (mg)	% of applied	^{15}N (mg)	% of applied	^{15}N (mg)	% of applied
July injection								
Litter	25.5 NA	100	23.9 (1.1)	93.7	24.7 (2.8)	96.7	15.7 (5.6)	61.4
Soil	- -	-	2.8 (1.0)	10.9	1.4 (0.5)	5.4	1.5 (0.5)	5.7
Microbial biomass	- -	-	0.7 (0.1)	2.8	0.6 (0.1)	2.2	- -	-
Litter C/N	34		17		15		13	
Total	25.5	100	26.0	102	25.5	100	17.2	66
September injection								
Litter	14.4 NA	100	15.0 (2.7)	104.5	9.2 (1.5)	64	3.1 (0.5)	21.7
Soil	- -	-	4.2 (1.2)	29.3	2.0 (0.6)	13.6	0.7 (0.1)	4.8
Microbial biomass	- -	-	0.7 (0.1)	4.8	0.5 (0.1)	3.3	- -	-
Litter C/N	14		13		14		13	
Total	14.4	100	18.5	128	10.7	74	3.8	27

than the September litter. The July labeled litter mass was more typical of the amount of litter that would fall and decompose under the rotation conditions. Microbial biomass and soil ^{15}N begin to accumulate at 168 days. The lower C:N ratio litter (September litter) releases ^{15}N to the soil faster and earlier than the higher C:N ratio litter. The microbial biomass does not accumulate more than 5% of the applied ^{15}N. By day 613, 61% of July litter and 22% of the September litter ^{15}N remained in the litter.

DISCUSSION

Carbon and N Allocation

The late season transport of photosynthate to stem and roots sinks for storage is characteristic of hybrid poplars (Isebrands and Nelson, 1983). Our data demonstrate the shift in C allocation from the acropetal (up) to basipetal (down) orientation from July to September. The largest increase in biomass C occurred in the stem and coarse roots. Fine roots (< 0.5 mm) maintain a consistent standing biomass C of 5% of net primary production from mid-growing season to the onset of dormancy. This low proportion of net primary production in standing fine roots is typical of plantation forests (Santantonio and Grace, 1987; Pregitzer *et al.* , 1990).

In August, the above-ground tree components contained 82% of total tree N. Leaves contained 60% of the total tree N. The major site of N storage was in the woody shoot and coarse roots. Pregitzer *et al.* (1990) found similar seasonal N distributions in one year-old hybrid poplars. The two-year-old trees represented 3% of total N found in the plant-soil system. Updegraff *et al.* (1990) found a similar above-ground-soil N balance in three-year-old hybrid poplar plantations. These data exemplify the importance of

N distribution at centers of meristematic shoot activity during the growing season and at storage sites in woody shoot and coarse roots during dormancy.

In this study, microbial biomass N represented a large labile pool of N which was equivalent to total tree N. Microbial biomass N turnover is replenished by litter input and acts to conserve soil nutrients for plant uptake. The major standing crop of litter N was found in leaf litter and amounted to 50% of the N found in the microbial biomass. The standing crop of fine root N represented 5% of microbial biomass N. Significant fine root turnover must occur to be equivalent to leaf litter N. However, turnover of root biomass may not be the only means that N is returned to soil from roots. Root exudation and mycorrhizal turnover may contribute to N input to soil, but have yet to be studied carefully (Fahey, 1992).

The Fate of ^{14}C

The specific activity is defined as the ratio of labeled C to unlabeled C. Any change in the ratio of the two C isotopes indicates a change in pool size either through addition of unlabeled C or loss of labeled C. Caldwell and Camp (1974) used the ratio of $^{14}C/^{12}C$ in structural residue to evaluate below-ground production and turnover in cool desert communities. Milchunas *et al.,* (1985) found this relationship to be invalid in plants when stored reserves containing ^{14}C were used to construct new tissue. After estimating the amount of ^{14}C used to construct new tissue, the researchers found good agreement with actual production estimates. They suggested that sampling for ^{14}C dilution in the field should be performed the year after labeling to avoid problems with retranslocation.

The largest dilution of ^{14}C activity occurred in stem and branches of both labelings in the following year. A comparable loss of ^{14}C occurred in roots (> 10 mm) from the September labeling. The loss of ^{14}C in roots (> 10 mm) from the September labeling as compared to no loss in July labeled roots was probably associated with photosynthate storage. In July, the actively growing tree was depositing C into structural residues, while in September, non-structural C was being stored. Therefore, C assimilated in September was more labile and subject to retranslocation and respiratory loss. Loss of ^{14}C from fine roots (< 0.5 mm) occurred from labeling to the second harvest in November for both labelings. No significant change in fine root mass occurred during this period. Most of the dilution in $^{14}C/^{12}C$ occurred in the 0-25 cm soil profile and indicated that the production of fine roots or turnover of labile C accounted for approximately 50% of the standing crop. The fine root pool $^{14}C/^{12}C$ ratio remained unchanged from dormancy into the next growing season. The lack of the dilution of $^{14}C/^{12}C$ during this period indicated minimal root production, turnover and or the synthesis of new roots from labeled reserves. Milchunas *et al.,* (1992) suggested that the movement of labile C may also have been used to support new root growth in a short grass steppe. The use of reserve C in both fine root maintenance and construction has been demonstrated in seedlings of *Pseudotuga menziesii* (Mirlu.) Franco (van den Dressche, 1985).

Horwath (Chapter 5) found that the amount of stored nonstructural reserves (in glucose equivalents) in the root system was equivalent to the standing crop of fine roots. The reserves had double the $^{14}C/^{12}C$ ratio than the fine root residue C. Chung and Barnes (1977) estimated that it would take 1.5 g glucose to synthesize 1 g of *Pinus taeda* roots. Assuming the construction costs of roots between species are similar, the amount of root

reserves contained in these hybrid poplars was sufficient to replace the fine root system 1.3 times with the addition of current photosynthate to dilute the higher specific activity reserves. The cost of root construction does not include maintenance activity of the root system during dormancy. The reserves in coarse roots were not exhausted during the sampling period. This would reduce the estimates of fine root production unless coarse root C was replenished from retranslocated reserves from the shoot.

The movement of C from the woody shoot to roots during and after the dormant season would explain the low loss of labeled C from the root system. Most studies on reserves in fruit trees have shown acropetal movement of stored C to synthesize new leaves (Hansen, 1967; Quinlan, 1969; Kandiah, 1979). Lockwood and Sparks (1978) demonstrated the acropetal movement of labeled reserves to synthesize leaves followed by a basipetal shift back to the root system after leaves were fully expanded. In this study, only 0.2% of the applied ^{14}C was recovered in new leaves the following year. The amount of labeled C lost from the woody shoot was equivalent or less than the amount in the root system, and thus, would not change the magnitude of the estimate of fine root production from reserves and current photosynthate.

The Fate of ^{15}N

The activity and disposition of ^{15}N within the tree was similar to the fate of ^{14}C. The stem injected ^{15}N composed no more than 1% of tissue N in both July and September labelings (Horwath, Chapter 3). The atom % ^{15}N excess varied among different tree components. Nommik (1966) found similar differences in atom % ^{15}N excess in various organs and in organs of different ages from fertilizer ^{15}N in *Pinus silvestris* L. and *Picea abies*

karst. This ^{15}N distribution was attributed to the preferential loading of active tissue, such as meristem regions. The majority of the injected ^{15}N in both July and September remained in the shoot 20 days after injection. In September, the woody shoot receives the majority of ^{15}N. The root system in September accumulates twice as much ^{15}N as compared to mid growing season in July. However, the root system never receives more that 20% of the injected ^{15}N in July or September. Nommik *et al.* (1983) had also demonstrated lower recovery of soil applied ^{15}N in roots than shoots of *Pinus sylvestris* L. 2 years after application.

The temporal shift in N allocation was exhibited by the distribution of isotopically labeled N. During July, in mid-growing season, the majority of N was found in leaves and was being used to support growing meristematic regions in the shoot (Dickson, 1989). During autumn, N storage begins in the woody shoot and roots? Movement of ^{15}N to the root system from the shoot was observed in July trees. No movement of ^{15}N occurred after the September injection indicating that the storage of N occurs at or before budset. The constant atom % ^{15}N excess of dormant roots and woody shoots from the September labeled trees indicates no uptake of N occurred after budset. During the September labeling, leaf drop was proceeding rapidly. Weinbaum *et al.* (1978) found that $^{15}NO_3^-$ uptake in non-bearing prune trees was correlated to leaf retention. The authors attributed the presence of leaves in supplying labile photosynthate for the energy requiring process of NO_3^- uptake. Similarly, maximum uptake of $^{15}NO_3^-$ in *Malus* occurred in August and the least in October after leaf fall (Hill-Cottingham and Lloyd-Jones, 1975).

The loss of ^{15}N occurred from both July and September trees during the following year. Knowles (1975) reviewing ^{15}N studies in forest

ecosystems found that 23-104% of ^{15}N applied to the soil was recovered from the plant-soil system. Recovery of ^{15}N was dependent on the length of time after application. The loss of ^{15}N in this study from the tree-soil system was 35% in July and 43% in the September injections, including the amount recovered in leaf litter. The application of ^{15}N-urea to *Malus* leaves resulted in similar losses (Hill-Cottingham and Lloyd-Jones, 1975). The average accounted for loss of ^{15}N from the urea application was 32% during the following growing season. Some of the ^{15}N loss from this system may have occurred through leaf leaching on to adjacent plots along the canopy drip zone. Chapin and Moilanen (1991) estimated loss of N from leaching by autumn rains in *Betula papyrifera* to be 25% of leaf N. Gaseous loss of N from leaves has also been suspected (Luxmore *et al.*, 1981).

The significance of N turnover through the root system seems negligible since the standing crop of fine roots contained less than 2% of the injected ^{15}N. No significant dilution of ^{15}N occurs in the fine root pool from November into the following growing season for both July and September injections. The production of new roots from higher atom % ^{15}N enriched pools of N in coarse roots must be considered as it was with ^{14}C. The tracers ^{14}C and ^{15}N behaved similarly and there is no reason to believe they would be utilized differently in the construction of fine roots. Clark (1977) observed that the ^{15}N content of roots in a short grass prairie remained constant over a five year period. He attributed the constant level of ^{15}N in roots to efficient recycling and uptake of mineralized N from the decomposition of above-ground tissue. However, in our system the leaf litter was collected and replaced with unlabeled litter, eliminating ^{15}N recycling through leaf decomposition. The dilution of atom % ^{15}N excess occurred only in July labeled coarse roots and in the shoots from both

injections. Apparently, the uptake of N to dilute these pools does not interact with N contained in fine roots. The stability of the dual tracers in the fine root pool may suggest that this component is long-lived in hybrid poplars.

The Turnover of ^{14}C and ^{15}N

The intent of litter residue decomposition studies is to determine the amount of C and nutrients entering the active soil organic matter fraction and microbial biomass (Paul and Juma, 1981). The amount of C and N entering this labile fraction has been studied extensively with isotopically labeled residues in agronomic systems (Shields and Paul, 1973; Jenkinson and Rayner, 1977; Ladd *et al.*, 1981; Voroney *et al.*, 1989). These studies have used unconfined litter to determine the processes involved in conserving the labile active soil fraction and associated nutrients. These processes have been described as a sum of events occurring in two phases; the initial rapid mineralization of substrate and incorporation into microorganisms (Shield *et al.*, 1973; Amato *et al.*, 1984), followed by a slower mineralization of microbial products and resistant plant materials (Jenkinson and Rayner, 1977; Voroney *et al.*, 1989). Researchers studying these processes in forest systems describe the above process as a short period of nutrient accumulation and a period of slow release from decomposing litter confined in bags (Melillo and Aber, 1982; Berg, 1988).

The ^{14}C pool dilution technique to estimate below-ground production and turnover was found to work well on recalcitrant biomass components, such as lignocellulose (Caldwell and Camp, 1974). Milchunas and Laurenroth (1992) using this technique found anomalous root production and turnover estimates in short grass steppe and suggested that non-

uniform labeling and movement of labeled C were the cause. Estimating and correcting for the synthesis of new roots from reserves, root turnover was calculated to be 8 years in these perennial grasslands. Conversely, estimates of root production were zero in 2 of 4 years using the method of changes in standing crop. This slow rate of below-ground production was realistic since the added ^{14}C was only sufficient to tag biomass pools, and therefore, the absolute amount was limiting in the production of new biomass. The slow loss of ^{14}C from root system of trees has been observed in previous studies (Hansen, 1967; Schier, 1970; Lockwood and Sparks, 1978). Unfortunately, these tree labeling experiments have used potted plants and their comparison to natural systems is inappropriate.

In this study, the ^{14}C activity of structural residue of fine roots does not change over the year sampling period for both labelings (Horwath, Chapter 5). The approach of Caldwell and Camp (1974) would indicate no turnover of the fine root pool. However, our study shows an increase in soil ^{14}C and decrease in ^{14}C activity of the labile fine root pool. The possibility that the labile pool was turning over through root exudation and mycorrhizal maintenance must be considered. Vesicular-arbuscular mycorrhizal infection rates were observed to be 50% of the fine root surface in the 0-25 cm soil profile (Horwath, personal observation). Most of the ^{14}C dilution of fine roots occurred in the 0-25 cm soil profile where mycorrhizal infection was the greatest. The largest addition of ^{14}C to soil occurred at the same time ^{14}C dilution occurred in the fine root pool. No ^{14}C dilution occurred from November to the one year sampling for both labelings and no significant increase in soil ^{14}C was detected.

The stability of microbial material in soil has been studied extensively and has been suggested to be important in conserving soil organic matter

(Shields and Paul, 1973; Voroney *et al.*, 1989). The turnover of microbial material from mycorrhizal maintenance or microbial utilization of root exudates are possible sources of soil ^{14}C. The turnover of ^{14}C stabilized into the soil active organic matter from the root labeled plots amounts to 1-3% of the ^{14}C applied from both labelings. Though no significant fine root standing crop production or turnover could be demonstrated with the traditional ^{14}C pool dilution techniques, significant amounts of labeled C turnover occurred through synthesis of new roots from reserves, exudation and or mycorrhizal turnover. This C input was playing an active role in maintaining soil organic matter.

The decomposition of the isotopically labeled leaf litter proceeded at a rapid rate. The loss of ^{14}C from September labeled leaves occurred more rapidly than from July labeled leaves. This discrepancy can be attributed to the labeling of the leaf constituents. During July, the leaf growth was occurring, therefore, uniformly labeling the separate leaf constituents. Conversely, in September the leaves were mature and primarily synthesizing carbohydrates for export. The C/N of the September leaves was lower explaining the more rapid decomposition (Melillo and Aber, 1982). The difference in litter quality within the same stand and year has been observed in *Populus tremuloides* to affect rates of decomposition (Taylor and Parkinson, 1988).

The fate of ^{15}N differs substantially from that of ^{14}C in the dual labeled leaf litters. Significant ^{15}N movement from decomposing leaf litter does not occur until 328 days for September and 613 days for July labeled leaves. The lower C/N September leaves released labeled N at a faster rate the higher C/N July leaves. The release of ^{15}N in July leaves occurs when the C/N of the decomposing litter reaches a C/N of 13, similar to the

September litter. However, in both litters the accumulation of ^{15}N in soil and microbial biomass occurs at 168 days. The amounts of ^{15}N in soil, microbial biomass and litter exceeded the amount applied at day 168 for September labeled leaves. This error was partially explained in that the litter was lain uniformly on to a 1 m^2 area by distributing a known quantity on the soil surface. Subsequent samplings of one quarter of each plot may have been subject to error by non-uniform litter distribution across the plot. The high standard errors of the mean for soil ^{15}N indicate that the discrepancy lies in the ^{15}N recovered from soil. Conversely, the low amount of error and ^{15}N found in the microbial biomass does indicate movement of ^{15}N into soil. During the second year of decomposition at 613 days 40-80% of litter ^{15}N has transferred to soil. Since the amount of N in litter was 10 times the amount found in the standing crop of fine roots, the return of N to soil for future uptake was dominated by leaf litter N in this system during stand initiation.

The amount of ^{14}C entering the soil was 13-17% of the initial litter content. The ^{14}C specific activity of the microbial biomass under decomposing leaf liter was approximately 10 times that of microorganisms utilizing root derived labeled C (data not shown). Yet the amount of labeled C stabilizing in soil was similar between the two litters. The distribution of leaf ^{14}C in soil was mainly in the surface soil (0-10 cm) while root C input occurred over the entire soil profile (0-100 cm). These differences in C input to soil demonstrate the role of these litters in the spatial conservation and formation of soil organic matter. This would imply that the litters also have a spatial role in developing soil quality.

CONCLUSION

The amount of C entering the soil was similar for both leaves and roots. The low estimates of root production and turnover by ^{14}C pool dilution and potential synthesis from reserves could not account for this C input into soil. The C entering the soil from the labeled root system may have come from sloughing, exudates and or, mycorrhizal turnover. Nitrogen cycling through the root system to soil could not be demonstrated. The constant atom % ^{15}N and labeled N in the root system implies minimal turnover through root biomass loss. Soil microbial biomass obtains more substrate from leaf litter than it does from root C based on ^{14}C enrichment of microbial C. This may indicate that more stabilized organic matter will form from leaf litter derived C than from root C. Both litters play an active spatial role in conserving the soil organic matter with leaf litter primarily enriching surface soil with C and N. In this system, it is important to retain leaf litter on site to conserve available soil N to satisfy these fast-growing nutrient-demanding trees during the initial phases of stand generation.

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Chapter 5

THE DYNAMICS OF ASSIMILATED $^{14}CO_2$ IN THE CHEMICAL FRACTIONS OF THE SHOOTS AND ROOTS OF *POPULUS* PLANTATIONS

ABSTRACT

The allocation of carbon to the chemical fractions of perennating tissue is characterized by a series of competing centers of growth and storage influenced by seasonal growth patterns. This research was conducted to distinguish between the utilization of current and reserve photosynthate for the long-term maintenance of the shoot and root system of hybrid poplars. Trees were pulsed labeled with $^{14}CO2$ in the field during the growing season of their second year of growth to encompass seasonal growth patterns. Tree tissue was fractionated into nonstructural (lipids, sugars, protein and starch) and structural cell wall (cellulose and lignin) components. During the mid-growing season, between 0.5-2.7% of the assimilated ^{14}C was in sugars. In autumn, up to 4.6% of the radiolabeled C was in the sugar fraction. Less ^{14}C was found in structural components late in the growing season. The distribution of labeled chemical fractions was similar to the distribution of the unlabeled fractions indicating that assimilated ^{14}C was following normal translocation pathways. In both the mid-growing season and autumn labeling, the specific activity of the sugar, starch and residue fractions of fine roots changed little from dormancy to one year after the radiolabeling procedure. The lack of decline of ^{14}C activity in the fine root cell wall residue indicated a lack of fine root turnover or the utilization of reserves to maintain fine root during the next growing season.

INTRODUCTION

The allocation of carbon to perennating tree tissues is characterized by the distribution of photosynthate between competing sinks. Normally, leaves are considered sources of assimilate production, but when immature, leaves both produce and import photosynthate (Dickson, 1989). Mature leaves export photosynthate to competing centers of growth and storage. Carbon flow early in the growing season is predominately in the acropetal direction in perennial plants supporting the growth of the shoot (Donnelly, 1974; Isebrands and Nelson, 1983; Dickson, 1989). The amount of current photosynthate used for shoot growth will differ among species according to shoot type and growth pattern. Heterophyllous species, such as hybrid poplar, require more photosynthate for shoot growth because of continual growth and longer growing seasons (Kozlowski and Keller, 1966). As the season progresses, lower leaves of hybrid poplars export C basipetally to the stem and root system (Isebrands and Nelson, 1983). Late in the season large quantities of photosynthate move to the root system, bypassing branch and stem sinks. This seasonal distribution of photosynthate is common among many tree species with varying shoot and growth patterns. The basipetally exported assimilates are stored as carbohydrates, lipids and nitrogenous compounds (Dickson, 1989). These reserve C compounds are used for cold hardening of tissues, respiratory maintenance and for the initiation of growth the following season (Krammer and Kozlowski, 1979). The importance of reserve material can not be underestimated since the availability of these substances will determine the success of the tree during the next season.

The seasonal variation of storage compounds in trees has been the object of many studies (Ziegler, 1964; Kozlowski and Keller, 1966; Glerum, 1980; Loescher *et al.,* 1990). Deciduous trees must transpose seasons when no photosynthesis can occur and are, therefore, reliant on stored reserves from the previous season for the maintenance of life and resumption of growth in the spring (Priestly, 1970; Krammer and Kozlowski, 1979). Gymnosperms, on the other hand, utilize both current and stored photosynthate to maintain life and growth throughout the year (Glerum, 1980). The highest concentration of reserves is usually found in roots while the major quantity is found in the above-ground portion of the tree as a result of the accumulation of biomass in the stem (Krammer and Kozlowski, 1979). The use of reserves in respiration during dormancy, in leaf initiation and expansion, in cambial growth of woody tissue and root growth are not well characterized for most trees (Loescher *et al.,* 1990).

Patterns of carbohydrate, fat and protein distribution and turnover directly influence phenological expression and plasticity as a result of competition, abiotic interactions and cultural practices. However, it is extremely difficult to distinguish current and reserve photosynthate in order to resolve their origin, transformation and utilization. The radiolabeling of assimilates can distinguish between utilization of current and reserve photosynthate. Few radiolabeling studies have been done under field conditions to characterize the temporal fate of radiolabeled photosynthetic products (Dickson, 1989; Loescher *et al.,* 1990).

To characterize the fate of reserves in trees I radiolabeled hybrid poplars in their second season of growth. The radiolabeling procedure was done under ambient conditions of CO_2 and temperature on replicate trees for one photoperiod. The labelings were done to encompass two distinct

physiological growth patterns of rapid shoot elongation and the autumn basipetal movement of photosynthate. Sampling of the labeled whole trees was done for a period of one year. The intent of this research was to characterize the fate of radiolabeled chemical fractions, especially of nonstructural and structural carbohydrates and residue. I hoped to discern the origin and utilization of assimilates and to distinguish the usage of current from reserve in the maintenance of fine roots. This chapter describes the radiolabeling of rapidly-growing, intensively cultured hybrid poplars and the fate of radiolabeled photoassimilates over time.

MATERIAL AND METHODS

Plant Material

Cuttings of *Populus euramericana* cv. Eugenei, 10 cm in length, were planted on a 2 by 1 m spacing in the spring of 1989. The site was located at the Kellogg Biological Station, LTER site, Michigan State University, southwest Michigan. The Kalamazoo soil series is classified as a fine loamy, mixed, mesic, Typic Hapudalf. Eight different trees were exposed to $^{14}CO_2$ in a large Plexiglas chamber on July 19 and September 5, 1990. The radiolabeling procedure was described previously (Chapter 3). Ambient $^{14}CO_2$ concentration (290-350 ppm) and temperature were maintained for the entire photoperiod. The level of $^{14}CO_2$ was maintained with an Infra Red Gas Analyzer that was interfaced to a computer operated $^{14}CO_2$ generator. The chamber air temperature was controlled by forcing the enclosed air though a water-cooled heat-exchanger. In July, 3.04×10^8 Bq of $^{14}CO_2$-C with a specific activity of 4215 Bq mg^{-1} CO_2-C for an average of 3.79×10^7 Bq $tree^{-1}$ were added to the labeling chamber. During the

September labeling, 2.85×10^8 Bq of $^{14}CO_2$-C with a specific activity of 4300 Bq mg^{-1} CO_2-C for an average of 3.56×10^7 Bq tree -1 were assimilated.

Sampling Procedure

Three randomly selected trees were sampled two weeks after each labeling. An additional three trees from each labeling were sampled in November, 1990 and the remaining two trees were sampled one year latter. The sampling included all above- and below-ground tree material located within a 1 m^3 soil block. Leaves, branches and stem were separated by height and age (Chapter 3). The soil block was excavated by depth (0-25 cm, 25-60 cm and 60-100 cm) and the soil from each depth was thoroughly mixed and subsampled to recover roots (< 0.5 mm) by hydronuematic elutriation (Smucker *et al.,* 1982). Roots were sorted by hand into the following size classes; < 0.5 mm, 0.5-1 mm, 1-3 mm, >3 mm, >10 mm and cutting. Plant material was dried at 80°C for 48-96 hours and was ground in a Wiley mill to pass a 60 mesh screen. Tissue samples (leaves, branches, stem and all root classes) were composited by the proportion of weight found in all sources of that particular tissue.

Analysis of Chemical Fractions

Plant tissue was fractionated into chloroform solubles (lipids, cutin, suberin and pigments), sugars, protein, starch and structural residue (Figure 5.1). Homogenized plant material (25-30 mg) was weighed in triplicate into 50 mL centrifuge tubes (Sardstedt , Newton, NC). The tissue was extracted [modified Bligh and Dyer (1959)] at room temperature with 4 mL of methanol: chloroform: water (12:5:3, v:v). After the addition of the solvent, the sample was sonicated for 45 seconds in a water bath, allowed to

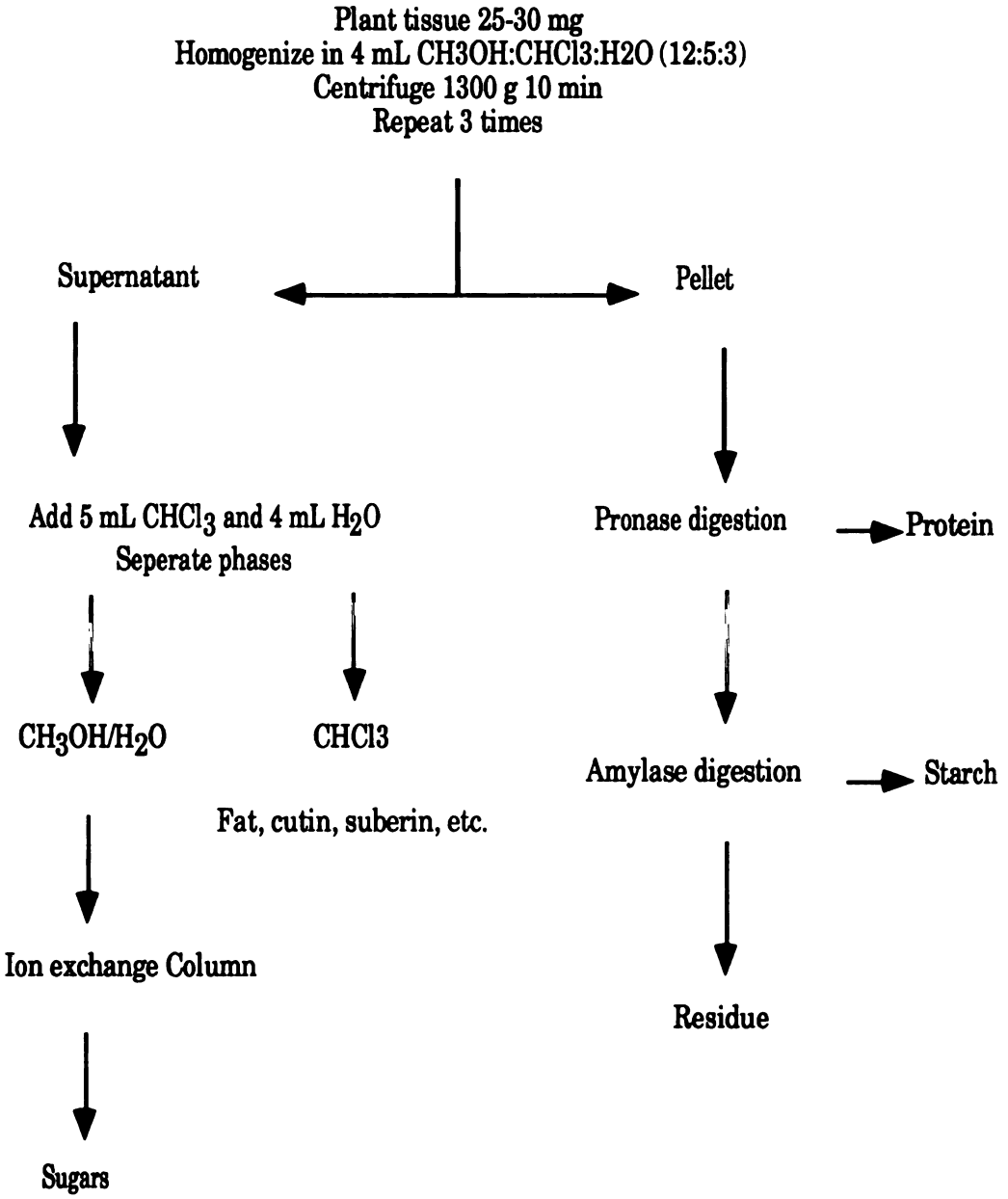


Figure 5.1. Diagram of the tissue fractionation protocol.

stand for 10 minutes, and centrifuged (1300 *g*, 0°C, 10 minutes) for a total of four extractions. The supernatants were combined into another centrifuge tube. The pellet was set aside for subsequent analysis. The supernatant was combined with 5 mL of $CHCl_3$ and 4 mL water, vortexed, and then centrifuged (1300 *g*, 0°C, 5 minutes). The chloroform and methanol-water phase (top) were separated and analyzed separately.

Analysis of the Chloroform phase

The chloroform phase was transferred to a dried (24 hours at 45°C) pretared scintillation vial and evaporated for 24 hours under a fume hood. The vial was then dried overnight at 45°C. The vial was reweighed to determine chloroform soluble residue. The residue was digested with 1 mL of methylbenzonium hydroxide (Sigma, St. Louis, MO) by wetting the sides of the vial, capping and incubating at 50°C for 24 hours. Hydrogen peroxide (100 μL) was added to highly colored samples (i.e. leaves) to remove color. Acetic acid (0.5 mL) was added to the digested residue to quench chemiluminescence. Finally, 10 mL of scintillation cocktail (Research Products International Corp., Mount Prospect, IL) were added, and the samples counted in a liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL). The $CHCl_3$ soluble residue was assumed to be 75% C by weight for ^{14}C calculations (calculated from Robertson, 1980).

Analysis for Sugars

Neutral sugars were separated from amino acids, organic acids and sugar phosphates by eluting the methanol-water phase through tandem cationic and anionic exchange columns. The columns were prepared from

cation exchange resin (DOWEX 50W; 50x8-400; 200-400 mesh, Sigma, St. Louis, MO) and anion exchange resin (DOWEX 1; 1x8-400; 200-400 mesh, Sigma, St. Louis, MO) washed in an equal volume of acetone overnight on a stir plate. The acetone was decanted and the resin washed in an equal volume of methanol for 8 hours. The methanol was decanted and the resin washed 3 times with purified water. The resin was then covered with 2 volumes of 1.0 M HCL and slowly heated on stir plate to 60°C and repeated. The resins were then washed with water to a neutral pH. The cationic exchange resin was washed two times in 80% ethanol and stored under 80% ethanol at room temperature. The anionic exchange resin was washed with 2 volumes of 1.0 M NaOH and then drawn through a #1 Whatman filter with a vacuum and sidearm Erlenmeyer flask. This procedure was repeated with fresh NaOH for a total of 20 washes. The anion exchange resin was then washed to a neutral pH with water and then washed twice with two volumes of 1.0 M Formic acid. The anion exchange resin was washed to a neutral pH with water, washed twice with 80% ethanol and stored under 80% ethanol.

Ion exchange columns were prepared from Pyrex tubing (15 cm long, 7 mm I.D.) for the cation exchange resin and 4 mL pasteur pipette (Fisher, Fairlawn, NJ) for the anion exchange resin. The tips of the columns were plugged with glass wool and loaded with a slurry of resin under 80% ethanol to a bed length of 4 cm. Columns were washed several times with 80% ethanol to a neutral pH and connected with Tygon tubing (cation over anion). A syringe needle (18 gauge) was inserted downwards into the Tygon tubing to avoid an air lock forming between the two columns. The tandem columns were placed with the tip of the pasteur pipette into a 50 mL volumetric flask. The methanol-water phase from the initial extraction

was applied to the column and eluted with 80% ethanol until the volumetric flask was nearly full. The volume of the flask was brought to 50 mL with 80% ethanol and the elutent analyzed for ^{14}C and total sugars.

Total sugars were measured colormetrically using the phenol-sulfuric acid technique (Dubois *et al.*, 1956). Glucose standards were prepared by drying dextrose (J. T. Baker Inc., Phillipsburg, NJ) at 80°C and preparing a standard series in methanol-water eluted through ion exchange columns. The elutent (5 mL) was transferred to a scintillation vial and dried at 50°C. Water (1 mL) was added to hydrate the dried sugar and the ^{14}C activity was determined in a liquid scintillation spectrometer after the addition of 10 mL of scintillation cocktail. The sugars were assumed to be 40% C by weight for ^{14}C calculations.

Analysis for Proteins

Protein was hydrolyzed and separated from the plant tissue by proteolytic digestion (Dickson, 1979). The pellet remaining after the methanol-chloroform-water extraction was incubated with 2 mL 0.4% Pronase (Calbiochem, La Jolla, CA) in 0.05 M Tris pH 7.4 and 0.1 mL 0.01 M sodium azide to prevent microbial contamination. The pellet-Pronase mixture was incubated at 30°C for 48 hours, with occasionally swirling. The pellet was then centrifuged (1300 *g*, 0°C, 10 minutes) and the supernatant drawn off with a pasteur pipette. The pellet was re-extracted with 2 mL water three more times. The supernatants were combined, freeze-dried and rehydrated with 1 mL of water. An aliquot (0.25 mL) was combined with 5 mL of scintillation cocktail and ^{14}C activity determined on a liquid scintillation spectrometer. Total protein was determined using a ninhydrin technique (Lee and Takahashi, 1966). For each set of samples,

1% albumin bovine (Sigma, St Louis, MO) was hydrolyzed and used to set up a series of standards. Protein was assumed to be 51% C by weight for ^{14}C calculations.

Analysis for Starch

Starch was hydrolyzed to glucose by enzymatic digestion with ∂-amylase (Dickson, 1979). The ∂-amylase enzyme Clarase 5000 (Miles Laboratory, Elkhart, IN) was dialyzed to remove contaminates. The dialysis tube (Spectrum Medical Industries Inc., Los Angeles, CA), molecular weight cutoff of 12,000-14,000, was prepared by autoclaving in 2% sodium bicarbonate and 1 mM EDTA for 10 minutes. A 0.5 % Clarase 5000 solution was dialyzed in water for 48 hours at 4°C, changing the water frequently. The volume of the enzyme solution was determined and combined with sodium acetate to achieve a 0.1 M (adjusted to pH 5.5 with acetic acid) enzyme-buffer solution. The pellet remaining after the protein hydrolysis was boiled with 1 mL 0.1 M sodium acetate buffer to gelatinize the starch. After the pellet was brought to room temperature, 1 mL of Clarase 5000 buffer and 0.1 mL 0.01 mM sodium azide were added to each sample. The samples were incubated for 48 hours at 50°C, swirling the samples occasionally. Starch in the samples was determined with peroxidase-glucose oxidase-o-dianisidine dihydrochloride reagent (Sigma Chemical Co., St. Louis, MO). A glucose standard series was developed from a standard glucose solution, 1 mg mL^{-1} (Sigma Chemical Co., St. Louis, MO). Starch was assumed to be 40% C by weight for ^{14}C calculations.

Analysis of the Structural Residues

The pellet remaining after the starch hydrolysis was transferred into a dried (50°C for 24 hours) and pretared glass vial with 100% ethanol. The samples were dried at 45°C and reweighed. Total C was determined on a Gas Chromatograph-Mass Spectrometer (Europa Scientific, Crewe, England) using 44% C (as cellulose) Whatman no. 1 filter paper as a reference standard (Harris and Paul, 1989). Radiolabeled C from the GC-MS exhaust was collected in CO_2 absorbing scintillation cocktail (Harvey Instrument Co., Hillsdale, NJ) and analyzed on a Liquid Scintillation Spectrometer.

RESULTS

Distribution of Chemical Fractions.

The concentration of biochemical fractions varied seasonally and among tree components. The concentration of sugars was 4-13% (dry weight) in July and September in above-ground tissue and 1-6% in the root system (Tables 5.1-5.2). Leaves had the highest concentrations of sugar ranging from 10-13% and was no doubt due to the fact that leaves were sites of sugar synthesis. The levels of sugar were 6% in branches and 4.1-4.8% in coarse roots (> 3 mm) in diameter during the growing season. During dormancy in November, the concentration of sugar in roots (> 3 mm) increased to 7-10% of the dry weight. Regardless of the season, the concentration of sugars in roots was proportional to root diameter and probably related to the amount of ray parenchyma cells available for storage. Leaf litter contained a significant amount of sugar, 3% by weight. The remaining sugar in leaves was probably a result of translocation

Table 5.1. *Concentration (mg g⁻¹)* of chemical fractions found in shoot and root components during growth

Table 5.1. Concentration (mg g^{-1}) of chemical fractions found in shoot and root components during growth and dormancy. July 19, 1990 labeling with subsequent sampling August 2, 1990 and November 2, 1990. Standard error of the mean in parentheses.

		Sugars	Starch	Protein	Lipids	Residue
Sampled 8/2/1990						
Branches		60.1 (2.5)	37.3 (3.2)	10.2 (0.4)	62.3 (2.4)	878.2 (9.8)
Leaves		128.9 (2.9)	12.5 (1.5)	45.8 (7.1)	95.4 (0.6)	484.8 (9.1)
Stem		37.9 (1.6)	14.9 (1.0)	25.9 (4.0)	68.0 (2.0)	842.7 (13.3)
Roots	< 0.5 mm	10.9 (2.2)	5.2 (0.4)	12.7 (2.5)	91.0 (7.7)	788.2 (47.2)
	0.5-1 mm	19.9 (0.7)	11.1 (2.0)	14.6 (3.0)	90.1 (6.4)	795.0 (6.6)
	1-3 mm	35.2 (2.9)	58.6 (10.8)	12.1 (1.3)	83.9 (7.9)	721.1 (11.7)
	3-10 mm	48.4 (2.6)	127.8 (18.4)	11.8 (3.8)	87.7 (15.5)	583.1 (25.9)
	> 10 mm	41.6 (0.8)	88.6 (3.3)	15.3 (0.7)	115.0 (4.5)	620.0 (24.8)
Cutting		25.7 (2.5)	47.3 (0.9)	14.6 (0.5)	78.6 (3.4)	833.3 (42.2)
Sampled 11/2/1990						
Branches		71.1 (5.0)	22.6 (1.6)	26.8 (1.5)	92.0 (1.6)	840.1 (18.8)
Leaf litter		30.8 (5.2)	6.9 (3.0)	33.8 (3.8)	136.8 (7.8)	635.9 (20.4)
Stem		47.9 (1.8)	33.1 (3.0)	41.3 (6.2)	60.6 (1.7)	810.3 (7.1)
Roots	< 0.5 mm	13.2 (1.3)	17.0 (2.8)	25.7 (4.4)	81.1 (2.8)	694.4 (25.1)
	0.5-1 mm	30.7 (1.6)	37.7 (12.0)	27.9 (7.8)	76.1 (4.1)	735.2 (16.1)
	1-3 mm	42.9 (3.3)	101.1 (12.6)	36.0 (4.1)	74.9 (3.3)	606.0 (37.4)
	3-10 mm	77.2 (3.2)	138.0 (19.9)	45.0 (1.1)	72.2 (7.5)	486.3 (19.1)
	> 10 mm	104.2 (8.2)	193.5 (16.8)	24.8 (0.6)	91.2 (1.7)	576.5 (27.3)
Cutting		46.7 (3.5)	46.7 (5.0)	20.8 (5.4)	69.1 (4.0)	731.8 (9.7)

Table 2. Concentration (mg g^{-1}) of chemical fractions found in shoot and root components during growth and dormancy. September 5, 1990 labeling with subsequent sampling September 20, 1990 and November 4, 1990. Standard error of the mean in parentheses.

		Sugars	Starch	Protein	Lipids	Residue
Sampled 9/20/2990						
Branches		64.0 (0.5)	47.5 (4.3)	25.7 (8.1)	82.6 (4.2)	831.1 (7.1)
Leaves		97.5 (12.8)	9.4 (0.7)	72.2 (3.7)	99.3 (1.7)	473.5 (10.0)
Stem		40.7 (3.1)	40.4 (1.0)	46.5 (0.8)	65.6 (0.9)	792.4 (9.2)
Roots	< 0.5 mm	10.2 (2.4)	28.6 (6.7)	5.4 (1.4)	124.8 (0.7)	734.7 (64.1)
	0.5-1 mm	21.4 (3.0)	41.1 (5.1)	9.5 (4.1)	125.5 (3.4)	726.9 (15.6)
	1-3 mm	35.1 (0.2)	97.3 (3.1)	11.9 (3.3)	128.9 (8.8)	652.3 (13.3)
	3-10 mm	53.9 (6.3)	184.3 (10.1)	13.1 (4.8)	106.1 (19.2)	512.2 (11.2)
	> 10 mm	67.4 (19.9)	175.5 (24.7)	37.9 (5.6)	119.6 (4.1)	531.2 (31.9)
Cutting		27.2 (2.4)	45.5 (4.0)	20.2 (1.7)	71.0 (12.8)	757.8 (15.7)
Sampled 11/4/1990						
Branches		70.8 (2.3)	32.5 (0.8)	35.2 (2.6)	107.6 (4.3)	779.5 (9.0)
Leaf litter		33.1 (4.8)	11.1 (2.3)	68.0 (1.8)	352.1 (27.8)	585.8 (1.1)
Stem		47.7 (1.2)	31.8 (1.3)	38.3 (1.6)	75.9 (6.2)	808.9 (1.5)
Roots	< 0.5 mm	16.0 (3.0)	18.7 (2.2)	20.2 (3.8)	49.2 (1.3)	679.4 (68.9)
	0.5-1 mm	28.6 (3.4)	47.7 (3.7)	35.5 (5.2)	62.9 (2.1)	680.5 (33.1)
	1-3 mm	42.4 (3.3)	77.7 (21.2)	42.1 (2.7)	65.8 (6.5)	602.5 (29.1)
	3-10 mm	62.9 (7.1)	133.1 (19.6)	48.4 (2.6)	55.4 (0.9)	442.3 (17.0)
	> 10 mm	69.8 (8.0)	181.3 (4.8)	36.6 (5.0)	124.8 (26.9)	514.7 (9.4)
Cutting		50.3 (5.2)	58.3 (3.5)	24.3 (5.1)	85.0 (14.9)	719.4 (4.5)

constraints from the unloading and loading of phloem cells with photosynthate during autumnal photosynthesis.

The concentration of starch in roots (> 1 mm) always exceeded starch levels found in the above-ground tissue (table 5.1-5.2). In July, the concentration of starch in fine roots (< 0.5 mm) was 0.5% and large roots contained up to 12.8%. Levels of starch increased in fine roots to 2-3% and up to 19% for large roots during September and November. The above-ground tissue contained between 1-5% starch from July to November. Leaf litter contained 1% starch. The concentration of starch in roots was proportional to diameter and was similar to the distribution of sugar in roots. The cutting always contained less starch and sugars than large structural roots and more closely resembled carbohydrate concentrations of the stem tissue.

Protein levels generally comprised 1-5% of tissue throughout the growing and dormant seasons. The protein concentration of leaves was 4.6% in July and 7.2% in September. The concentration of leaf litter protein changed little from that of green leaves. Root protein levels increased from about 1% in July to 5% in November. Coarse roots contained more hydrolyzable protein than finer roots and were similar to protein levels found in the stem. Protein content was proportional to root diameter except that roots (> 10 mm) and cutting contained less protein. The protein determination using Pronase is not effective in removing cell wall bound protein. The protein determined is indicative of cellular proteins not associated with the cell wall. There is no reason to suspect any changes in the concentration of cell wall structural protein in the woody shoot and roots throughout the season, consequently, the increase in tissue protein was probably associated with the accumulation of storage protein.

The chloroform-soluble fraction is composed of lipids, cutin, suberin, waxes and glycerated substances, such as phospholipids and glycolipids. This fraction is an integral component of cells involved in photosynthesis, storage and can itself be a form of C storage. The chloroform fraction varied in all tissues during the growing and dormant season. In July, this fraction composed approximately 6% of branch and stem tissue and 8-9% of leaves and roots. An increase in the chloroform fraction in roots (< 10 mm) occurred in September. Conversely, this fraction decreased in roots (< 10 mm) from July to November. An increase of chloroform soluble material occurred in branches from September to November. The increases found in these fraction can be associated with a storage function, but many of the constituents of this fraction are also involved in other cellular functions, such as cold acclimation.

The residue fraction is composed of transformed sugars (cellulose, hemicellulose and pectin), lignin and cell wall protein. The trees were composed of 50-88% residue throughout the growing and dormant seasons. The branches and stem contained the greatest concentration of residue by weight. Leaves generally contained 50% residue, while roots (3-10 mm) contained similar or less residue than leaves during November. Roots contain a higher proportion of ray parenchyma cells resulting in less residue than found in stem tissue. The percentage of storage cells in roots (3-10 mm) increased as food reserves accumulate during the dormant period. There was a general decline in residue content of woody tissue during dormancy.

Distribution of ^{14}C Within Chemical Fractions

The data in Tables 5.3 and 5.4 show the amount of ^{14}C (%) contained within the C component of each biochemical fraction. The data exemplifies the relative sink strength of storage, metabolic and structural pools of the different tissues over the growing season. In July, 1-1.5% of the radiolabeled C was found in the sugar pool throughout the tree. The similar levels of ^{14}C in the different tree components shows the uniformity of labeled C distribution. The quantity of ^{14}C derived from the labeled assimilates was inversely proportional to root diameter with the fine roots (< 1 mm) containing the highest amount of ^{14}C two weeks after the labeling. Labeled C in the starch pools ranged from 0.5-2.7% with leaves having the highest proportion and the cutting the least. The C derived from labeled C in root starch was slightly higher than in the sugar pool and was also inversely distributed according to root diameter. The increase of ^{14}C in the composition of the starch pool indicates that equilibrium distribution of ^{14}C was shifting to starch from sugar as a result of new unlabeled C diluting the sugar pools. This indicated that even after two weeks following exposure to ^{14}C the radiolabeled photosynthate in nonstructural carbohydrates was still equilibrating. Root respiration studies in Chapter 3 indicated that a minimal amount of ^{14}C was being respired after two weeks from the root system. Therefore, the sugar pool was in some way compartmentalized from current photosynthate since root respiration had been mostly diluted with unlabeled C. The compartmentalization of current assimilates from reserve sugar suggested that current photosynthate was the source of C for root respiration during mid growing season.

In autumn, sugars in roots (<10 mm) contained approximately 4% of the labeled C. Coarse roots (> 10 mm) and cutting contained approximately 2.5% of the ^{14}C. The above-ground woody tissue contained 1-1.5% labeled C and leaves possessed 2.1%. Root starch contained 1.4-1.9% ^{14}C and, therefore, contained less ^{14}C than the sugar pools in contrast to July results two weeks after labeling. The woody shoot tissue contained 0.7% ^{14}C in starch and leaves contained 3.7%. The ^{14}C activity of the starch pool was now proportional to root diameter, differing from the July results, while the sugar pool showed no relationship to root size. The labeled sugar pool was not contributing significantly to root respiration two weeks after labeling. Again, this indicated that the labeled sugar pool was separate from current photosynthate in supplying the substrate for root respiration.

In November, the ^{14}C comprising the sugar and starch pools from both labelings had decreased due to utilization and dilution with unlabeled C. The lone exception was the root starch pools from trees labeled in September. These showed little change except for a decline in roots (> 10 mm) and the cutting. The starch deposited in roots (< 10 mm) in September had not accumulated any additional unlabeled C (Table 5.4). This implied that the storage of photosynthate in the root starch pool had been completed in early September before photosynthesis had ceased. The ^{14}C in the root sugar pool was diluted from approximately 4% in September to 1% in November. The dilution of the root sugar pool occurred both from unlabeled C and utilization since the concentration and mass of sugar in roots declined only slightly. The utilization of the root sugar pool may have been indicative of the transition from current to storage photosynthate for root maintenance as dormancy approached.

Table 5.3. Amount of labeled C (%) found in the different chemical fractions expressed as the percentage of C derived from ^{14}C. July labeling with subsequent August 2, 1990 and November 2, 1990. Standard error of the mean in parentheses.

		Sugars	Starch	Protein	Lipids	Residue
Sampled 8/2/1990						
Branches		1.15 (0.12)	1.04 (0.14)	2.62 (0.35)	0.40 (0.06)	0.79 (0.12)
Leaves		1.35 (0.09)	2.70 (0.17)	1.68 (0.17)	0.62 (0.05)	0.90 (0.01)
Stem		1.16 (0.14)	1.22 (0.19)	0.49 (0.03)	0.19 (0.01)	0.97 (0.03)
Roots	< 0.5 mm	1.45 (0.34)	1.96 (0.15)	0.98 (0.23)	0.10 (0.01)	0.30 (0.03)
	0.5-1 mm	1.42 (0.12)	1.73 (0.24)	1.02 (0.25)	0.12 (0.01)	0.30 (0.05)
	1-3 mm	1.12 (0.20)	1.26 (0.36)	1.08 (0.13)	0.11 (0.02)	0.36 (0.09)
	3-10 mm	0.98 (0.14)	1.24 (0.35)	1.64 (0.40)	0.08 (0.01)	0.42 (0.14)
	> 10 mm	0.71 (0.16)	0.87 (0.17)	1.87 (0.24)	0.08 (0.02)	0.47 (0.13)
Cutting		1.11 (0.16)	0.53 (0.09)	1.00 (0.05)	0.10 (0.02)	0.45 (0.07)
Sampled 11/2/1990						
Branches		0.54 (0.05)	0.54 (0.02)	0.48 (0.02)	0.15 (0.01)	0.55 (0.07)
Leaf litter		1.70 (0.06)	4.04 (1.38)	1.49 (0.18)	0.20 (0.02)	0.45 (0.01)
Stem		0.35 (0.07)	0.37 (0.05)	0.28 (0.05)	0.13 (0.01)	0.62 (0.08)
Roots	< 0.5 mm	0.46 (0.12)	0.48 (0.19)	0.33 (0.23)	0.16 (0.03)	0.22 (0.01)
	0.5-1 mm	0.34 (0.09)	0.49 (0.14)	0.27 (0.13)	0.24 (0.03)	0.26 (0.06)
	1-3 mm	0.25 (0.07)	0.31 (0.06)	0.16 (0.04)	0.19 (0.02)	0.23 (0.03)
	3-10 mm	0.17 (0.03)	0.46 (0.06)	0.13 (0.02)	0.18 (0.03)	0.21 (0.04)
	> 10 mm	0.19 (0.01)	0.19 (0.00)	0.40 (0.01)	0.04 (0.01)	0.29 (0.04)
Cutting		0.36 (0.12)	0.35 (0.07)	0.81 (0.31)	0.05 (0.00)	0.24 (0.08)

Table 5.4. Amount of labeled C (%) found in the different chemical fractions expressed as the percentage of C derived from ^{14}C. September 5, 1990 labeling with subsequent sampling September 20, 1990 and November 4, 1990. Standard error of the mean in parentheses.

		Sugars	Starch	Protein	Lipids	Residue
Sampled 9/1/1990						
Branches		0.93 (0.10)	0.30 (0.06)	1.00 (0.38)	0.28 (0.03)	0.12 (0.02)
Leaves		2.11 (0.24)	3.66 (0.30)	2.11 (0.05)	0.79 (0.03)	0.78 (0.05)
Stem		1.56 (0.13)	0.70 (0.07)	0.72 (0.07)	0.17 (0.01)	0.34 (0.12)
Roots	< 0.5 mm	4.01 (1.27)	1.36 (0.20)	3.90 (1.37)	0.03 (0.00)	0.52 (0.06)
	0.5-1 mm	4.02 (0.51)	1.63 (0.11)	2.78 (1.35)	0.05 (0.01)	0.65 (0.15)
	1-3 mm	3.91 (0.48)	1.64 (0.15)	3.19 (1.58)	0.03 (0.01)	0.61 (0.12)
	3-10 mm	4.61 (0.45)	1.90 (0.11)	4.36 (1.58)	0.03 (0.00)	0.69 (0.17)
	> 10 mm	2.64 (0.17)	1.55 (0.50)	1.89 (0.73)	0.13 (0.05)	0.59 (0.11)
Cutting		2.34 (0.23)	0.87 (0.11)	1.19 (0.20)	0.08 (0.01)	0.19 (0.03)
Sampled 11/4/1992						
Branches		0.60 (0.09)	0.29 (0.05)	0.67 (0.04)	0.27 (0.03)	0.12 (0.02)
Leaf litter		3.44 (0.05)	3.33 (0.76)	2.10 (0.31)	0.20 (0.02)	0.55 (0.13)
Stem		0.88 (0.05)	0.58 (0.06)	0.78 (0.07)	0.22 (0.02)	0.19 (0.02)
Roots	< 0.5 mm	0.97 (0.07)	1.43 (0.10)	0.43 (0.06)	0.20 (0.03)	0.57 (0.07)
	0.5-1 mm	1.04 (0.11)	1.58 (0.31)	0.63 (0.04)	0.25 (0.02)	0.67 (0.06)
	1-3 mm	0.88 (0.11)	1.83 (0.23)	0.64 (0.06)	0.18 (0.01)	0.51 (0.08)
	3-10 mm	1.30 (0.04)	1.76 (0.13)	0.91 (0.07)	0.18 (0.02)	0.53 (0.05)
	> 10 mm	0.97 (0.12)	0.39 (0.06)	1.14 (0.14)	0.07 (0.02)	0.25 (0.06)
Cutting		0.44 (0.06)	0.28 (0.02)	0.51 (0.15)	0.04 (0.01)	0.19 (0.09)

The ^{14}C content of the protein pools was similar to the sugar pools following the labeling periods and November sampling (Table 5.3-5.4). In July, root and leaf ^{14}C-protein accounted for 1-1.9% of the protein C and was distributed in the roots proportional to diameter. In the above-ground tissue, protein was significantly labeled in branches (2.6%) and less in the stem (0.5%). The increased ^{14}C content of root protein in September corroborated the basipetal flow of C during autumnal photosynthesis. Labeled C in the protein of roots amounted to 1.9-4.3% of the C content in September. The woody shoot protein C, including the cutting, contained approximately 1% ^{14}C and leaf protein C contained 2.1%. Large dilutions of ^{14}C occurred after each labeling and was exemplified by lower values for C derived from ^{14}C in all tissue except litter, which remained relatively unchanged. This indicates that the protein pool turns over rapidly during the growing season through dormancy.

The labeled C in the chloroform soluble fraction, though lower than any other fraction followed a distinctly different pattern than either the nonstructural carbohydrates or protein. Generally, root chloroform soluble material increased in ^{14}C content from summer to autumn. In roots (> 10 mm) and above-ground tissue, the percentage of ^{14}C declines from July and September to November. The decline was most likely due to dilution of the C component of these tissues. The increase in the percentage of ^{14}C in the chloroform soluble C in the roots (< 10 mm) was probably due to cambial activity for diameter growth, storage and winter hardening requirements. The ^{14}C needed to enrich these pools was no doubt taken from reserves which were significantly more labeled.

The disposition of ^{14}C in the residue C pool followed closely the seasonal flow of C to active sinks of growth and storage. During July, the

^{14}C content in the residue of above-ground tissue was 20 to 50% greater than the root system (Table 5.3). The above-ground preferentially utilized photosynthates to support shoot synthesis. As autumn approached, the roots became active sinks for photosynthates with residue C comprising 2-5 times more ^{14}C than residue in branch or stem tissue. The residue pools became diluted with unlabeled C from the addition of new biomass from July to November. The dilution of the residue pool during the September labeling was less due to storage of photosynthate taking precedent over the synthesis of cell wall components. The ^{14}C content of residue was lower than nonstructural carbohydrates and protein indicating that the assimilated ^{14}C had not equilibrated between structural, metabolic and storage pools two weeks following exposure in summer or autumn. This lack of equilibrium indicated that storage of reserve material in roots had already begun during mid growing season. This trend became more evident in autumn when starch and sugar pools contained 2-3 times more ^{14}C than the residue. A similar trend was evident for the above-ground components in September.

Redistribution of ^{14}C Over Time

The nonstructural carbohydrates, protein and chloroform soluble pools of roots (< 0.5 mm) contained significant amounts of ^{14}C one year latter (Tables 5.5 and 5.6). The residue C of fine roots contained a similar amount of ^{14}C to that found in November of the previous year. The biomass of fine roots increased slightly during the next growing season (Figure 5.6). The ^{14}C composition of the fine root residue may have been sustained through the utilization of labeled reserves. It was not possible to determine

Table 5.5. Concentration (mg g^{-1})of chemical fractions found in fine root components the year following ^{14}C labeling. Data is presented for subsequent sampling of trees on July 25, 1991 for the July 19, 1990 labeling and September 5, 1991 for the September 5, 1990 labeling. Standard error of the mean in parentheses.

		Sugars	Starch	Protein	Lipids	Residue
July label (7/19/1990) Sampled 7/25/1991						
Roots	< 0.5 mm	10.8 (2.2)	17.5 (2.1)	13.8 (0.8)	103.0 (6.0)	755.2 (34.9)
Sept. Label (9/5/1990) Sampled 9/5/1991						
Roots	< 0.5 mm	17.3 (1.5)	34.3 (0.1)	15.2 (1.1)	113.9 (6.2)	728.6 (4.7)

Table 5.6. Amount of labeled C (%) found in the different chemical fractions expressed as the percentage of C derived from ^{14}C one year following ^{14}C labeling. Data is presented for subsequent sampling of trees on July 25, 1991 for the July 19, 1990 labeling and September, 1991 for the September 5, 1990 labeling. Standard error of the mean in parentheses.

		Sugars	Starch	Protein	Lipids	Residue
July Label (7/19/1990) Sampled 7/25/1991						
Roots	< 0.5 mm	0.38 (0.01)	0.15 (0.04)	0.10 (0.02)	0.04 (0.00)	0.22 (0.04)
Sept. Label (9/5/1990) Sampled 9/5/1991						
Roots	< 0.5 mm	0.65 (0.06)	0.25 (0.00)	0.40 (0.03)	0.12 (0.01)	0.66 (0.01)

the metabolic state of these roots, therefore, the measurement of ^{14}C activity of this root class encompassed both live and dead roots. The residue pool of fine roots had similar amounts of ^{14}C indicating the importance of reserves in the both synthesis maintenance.

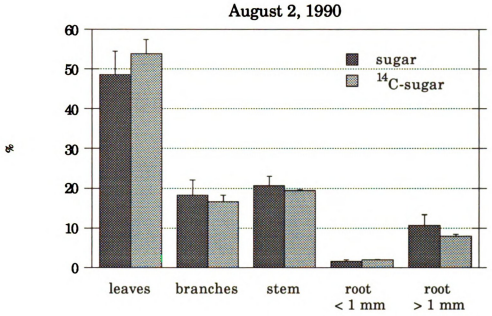
Distribution of ^{14}C in Chemical Fractions

Figures 5.2-5.4 represent the distribution of sugar, starch and residue within the tree and the ^{14}C contained within each fraction. In July, the bulk of tree sugar was located in the shoot. The ^{14}C-labeled sugar was distributed according to the distribution of sugar within the tree. This indicated that the above-ground tissue was a strong sink for labile photosynthates as well as recently fixed C during mid-growing season. The distribution of ^{14}C-sugar became diluted in roots (> 1 mm) and stem from the utilization and addition of unlabeled C (Figures 5.2a-5.2b). The September labeling showed that the predominant flow of C was basipetal. By November, some equilibration between the root system and above-ground tissue had occurred with leaf litter retaining proportionately more ^{14}C in the sugar pool.

The distribution of starch and ^{14}C-starch was always highest in the root system (Figures 5.3a-5.3b). The distribution of starch and ^{14}C starch between shoot and root was relatively evenly distributed for the July labeling and November sampling (Figure 5.3a). The location of labeled and unlabeled starch was mainly in the root system in September to November (Figure 5.3b). Again, leaves and leaf litter contained proportionately more labeled starch and was similar to leaf ^{14}C-sugar distribution.

Figure 5.2a. The distribution of total sugars and labeled ^{14}C-sugars among the shoot and root components. The July labeling with subsequent sampling August 2, 1990 and November 2, 1990 show the acropetal translocation tendency of sugars synthesized in July during mid-growing season. Standard error of the mean shown as line bars.

Figure 5.2a


August 2, 1990
%
60
50
40
30
20
10
0
sugar
14C-sugar
leaves
branches
stem
root
< 1 mm
root
> 1 mm

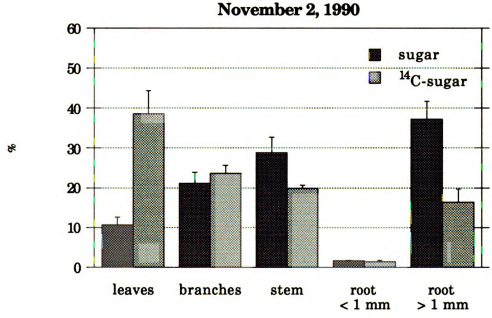
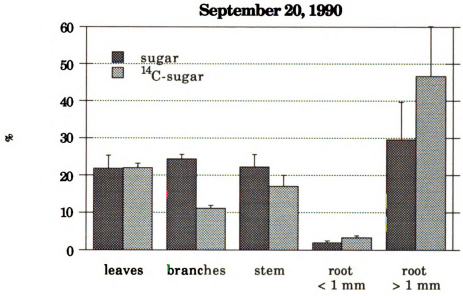

November 2, 1990
%
60
50
40
30
20
10
0
sugar
14C-sugar
leaves
branches
stem
root
< 1 mm
root
> 1 mm

Figure 5.2b. The distribution of total sugars and labeled ^{14}C- sugars among the shoot and root components. The September labeling with subsequent sampling September 20, 1990 and November 4, 1990 show the basipetal translocation of C assimilated in September during autumn for this hybrid poplar clone. Standard error of the mean shown as line bars.


September 20, 1990
%
60
50
40
30
20
10
0
sugar
14C-sugar
leaves
branches
stem
root
< 1 mm
root
> 1 mm

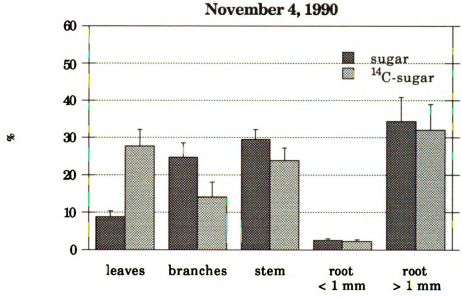
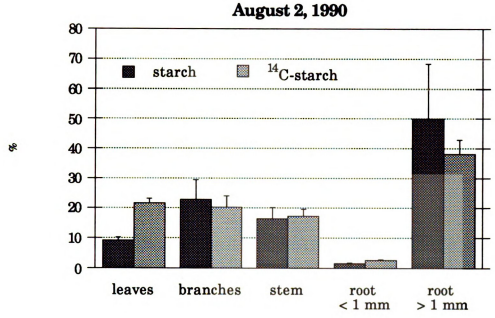

November 4, 1990
%
60
50
40
30
20
10
0
sugar
14C-sugar
leaves
branches
stem
root
< 1 mm
root
> 1 mm

Figure 5.3a. The distribution of total starch and labeled ^{14}C-starch among the shoot and root components. The July labeling and subsequent sampling August 2, 1990 and November 2, 1990 show the tendency of this clone to translocate C to the root system for storage in July during mid-growing season. The distribution of labeled starch increases in the root system as dormancy is induced. Standard error of the mean shown as line bars.

Figure 5.3a


August 2, 1990
%
starch
14C-starch
leaves
branches
stem
root < 1 mm
root > 1 mm

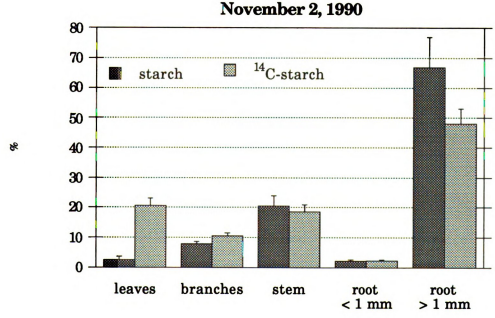
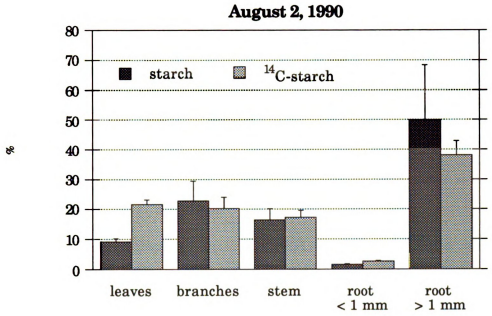

November 2, 1990
%
starch
14C-starch
leaves
branches
stem
root < 1 mm
root > 1 mm

Figure 5.3a. The distribution of total starch and labeled ^{14}C-starch among the shoot and root components. The July labeling and subsequent sampling August 2, 1990 and November 2, 1990 show the tendency of this clone to translocate C to the root system for storage in July during mid-growing season. The distribution of labeled starch increases in the root system as dormancy is induced. Standard error of the mean shown as line bars.

Figure 5.3a


August 2, 1990
%
starch
14C-starch
leaves
branches
stem
root < 1 mm
root > 1 mm

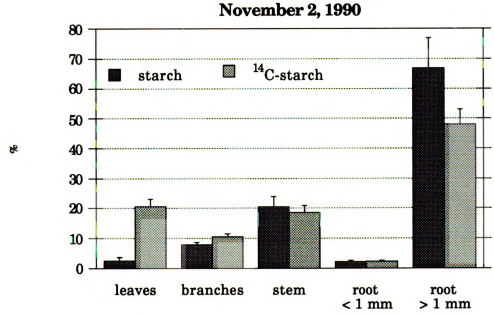
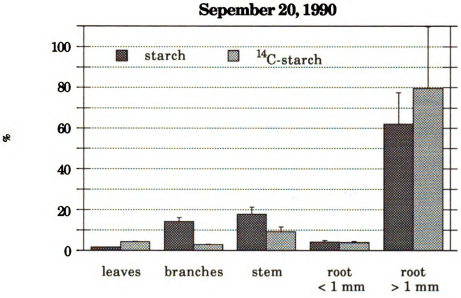
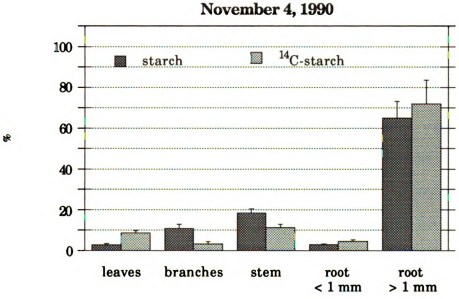

November 2, 1990
%
starch
14C-starch
leaves
branches
stem
root < 1 mm
root > 1 mm

Figure 5.3b. The distribution of total starch and labeled ^{14}C-starch among the shoot and root components for trees labeled September 5, 1990. The subsequent sampling done on September 20, 1990 and November 4, 1990 show the basipetal translocation and loading of the coarse root system with starch and labeled C assimilated during autumn. Standard error of the mean shown as line bars.


September 20, 1990
starch
14C-starch
%
100
80
60
40
20
0
leaves
branches
stem
root < 1 mm
root > 1 mm


November 4, 1990
starch
14C-starch
%
100
80
60
40
20
0
leaves
branches
stem
root < 1 mm
root > 1 mm

Approximately 60-80% of the residue was located in the woody shoot (Figure 5.4a-5.4 b). Root residue increased through the dormant season. Labeled C assimilated in July had a similar distribution to residue distribution with leaves containing proportionately more and roots less. The trend continued through November with the exception that the stem accumulates proportionately more ^{14}C and the leaf litter contained less. Carbon assimilated in July exhibits acropetal movement and stabilization in above-ground tissue. The photosynthate assimilated in September was distributed mainly to the stem and root system. The increase in ^{14}C in root residue in September and November (Figure 5.4b) clearly demonstrated active fine root growth after new leaf expansion has stopped. Branches retained proportionately less ^{14}C while leaves and litter retained an increased level. The distribution of ^{14}C within residue changes little from September to November (Figure 5.4b).

Figure 5.5a-5.5b represents the distribution of ^{14}C in the different chemical fractions expressed as a percentage of recovered from the total tree. The residue pool always contained the largest amount of ^{14}C compared to other fractions. In the trees labeled in July, the labile fractions contained no greater than 20% of the recovered ^{14}C, except in litter. The accumulation of ^{14}C in starch remained constant in roots (> 1 mm) (Figure 5.5a). In September the labile fraction of roots (> 1 mm) and branches contained up to 40% of the recovered ^{14}C (Figure 5.5b). The accumulation of ^{14}C in the labile fraction remained constant in branches and roots (> 1 mm) in November from the September labeling (Figure 5.5b). A significant amount of ^{14}C accumulated in the chloroform soluble fraction of branches and stem from the September labeled trees. The recovery of ^{14}C in root starch remained constant from September to November. The distribution

Figure 5.4a. The distribution of total residue and labeled ^{14}C-residue among the shoot and root components of trees labeled July 19, 1990. The subsequent sampling done on August 2, 1990 and November 2, 1990 show that the shoot residue comprises a significant proportion of the tree. Standard error of the mean shown as line bars.

Figure 5.4a

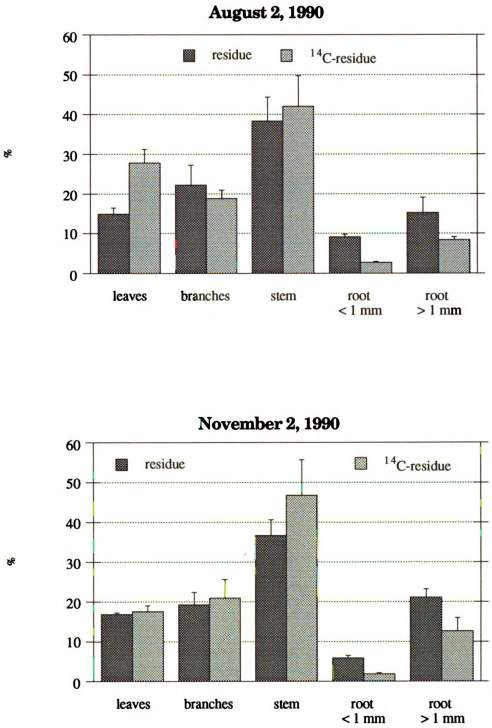
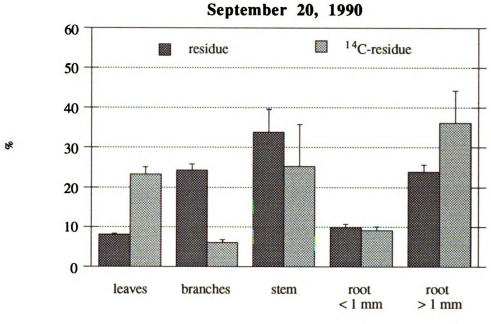

August 2, 1990
residue
14C-residue
%
60
50
40
30
20
10
0
leaves
branches
stem
root < 1 mm
root > 1 mm
November 2, 1990
residue
14C-residue
%
60
50
40
30
20
10
0
leaves
branches
stem
root < 1 mm
root > 1 mm

Figure 5.4b. The distribution of total residue and labeled ^{14}C-residue among the shoot and root components of trees labeled September 5, 1990. The subsequent sampling done on September 20, 1990 and November 4, 1990 show that the root residue is both increasing in mass and receiving more labeled C at this time during the season. Standard error of the mean shown as line bars.

Figure 5.4b


September 20, 1990
residue
14C-residue
%
60
50
40
30
20
10
0
leaves
branches
stem
root < 1 mm
root > 1 mm

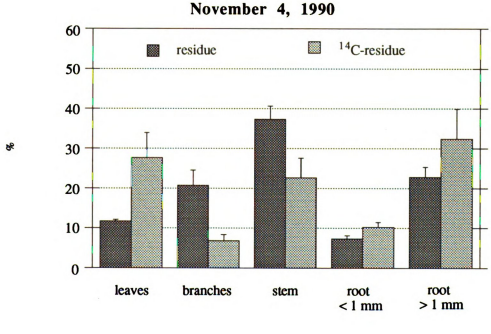
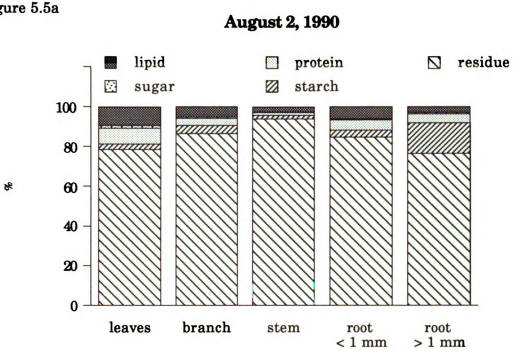

November 4, 1990
residue
14C-residue
%
60
50
40
30
20
10
0
leaves
branches
stem
root < 1 mm
root > 1 mm

Figure 5.5a. The distribution of ^{14}C within the chemical fractions of the trees labeled July 19,1990. The subsequent samplings done on August 2, 1990 and November 2, 1990 reveal that trees labeled during the growing season synthesize a significant amount of the residue fraction as a result of shoot and root expansion.

Figure 5.5a


August 2, 1990
lipid
protein
residue
sugar
starch
%
100
80
60
40
20
0
leaves
branch
stem
root
< 1 mm
root
> 1 mm

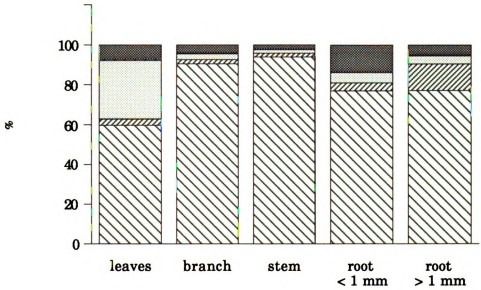
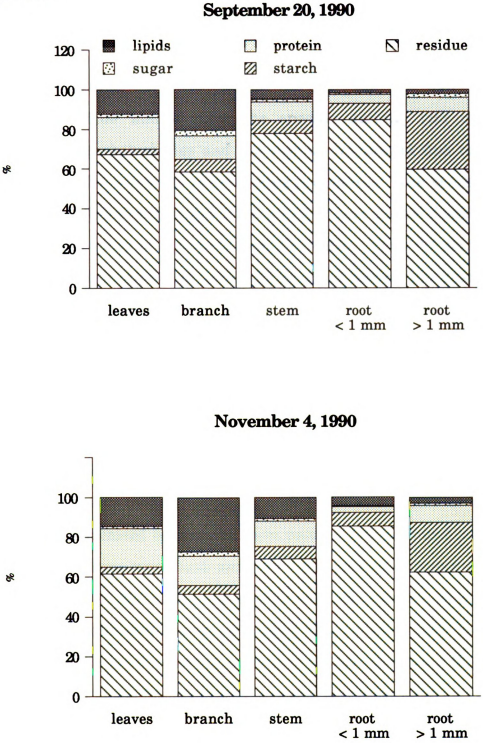

November 2, 1990
%
100
80
60
40
20
0
leaves
branch
stem
root
< 1 mm
root
> 1 mm

Figure 5.5b. The distribution of ^{14}C within the chemical fractions of the trees labeled September 5,1990. The subsequent samplings done on September 19, 1990 and November 4, 1990 show that trees labeled after budset synthesize less of the residue fraction and allocate more photosynthates to labile fractions.

Figure 5.5b


September 20, 1990
lipids
protein
residue
sugar
starch
120
100
80
60
40
20
0
%
leaves
branch
stem
root
< 1 mm
root
> 1 mm
November 4, 1990
100
80
60
40
20
0
%
leaves
branch
stem
root
< 1 mm
root
> 1 mm

of ^{14}C in the residue pool changed little regardless of date labeled or sampled (Figure 5.5a-5.b).

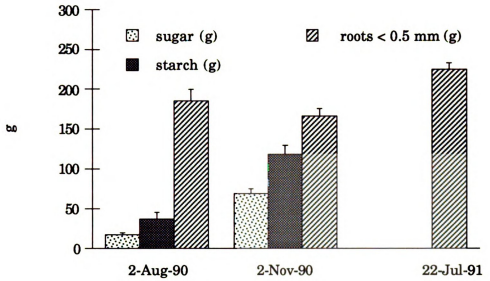
Fate of Reserves

Figure 5.6a and b show the specific activity (Bq mg^{-1} C) of nonstructural carbohydrates and the residue fraction of fine roots (< 0.5 mm) one year following the radiolabeling procedures. The specific activity of the residue fraction changed little over the year period despite a slight increase in the mass of fine roots from November. The ^{14}C activity of the nonstructural carbohydrates generally declined during the year from the utilization and accumulation of new reserves. This implied that the synthesis of fine roots was derived from labeled reserves. Since the reserves had twice the specific activity of the residue in November, it was possible that the addition of unlabeled photosynthate sustained the activity of the fine root residue at previous year levels. This implied fine root growth depended on significant stored photosynthate accumulated during the previous year. Since the fine roots themselves do not contain sufficient reserves to increase their mass, movement of reserves from roots (> 0.5 mm) and possibly the above-ground tissue must have occurred. The amount of starch reserves in the root system in November was greater than the increase in mass of fine root pool the following season indicating this scenario may be realistic. Considering the total reserves contained within the tree, the constant specific activity of the fine roots after one year seems plausible.

Figure 5.6a. The amount of fine roots and the total nonstructural carbohydrates (sugars and starch) in the root system for the July labeling and subsequent harvests (upper graph). The specific activities of sugar, starch and residue fraction of fine roots (< 0.5 mm) for the year following labeling (lower graph). Standard error of the mean shown as line bars.

Figure 5.6a

July



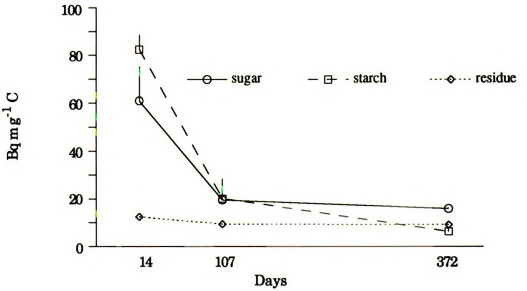
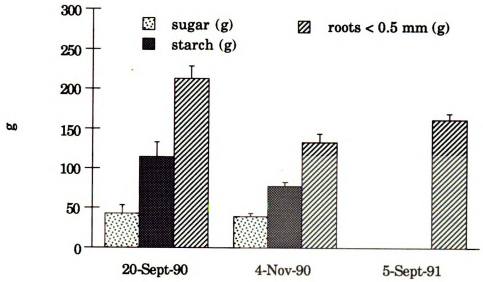
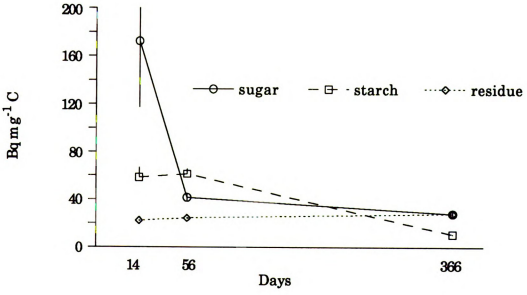


Figure 5.6b. The amount of fine roots and the total nonstructural carbohydrates (sugars and starch) in the root system for the September labeling and subsequent harvests (upper graph). The specific activities of sugar, starch and residue fraction of fine roots (< 0.5 mm) for the year following labeling (lower graph). Standard error of the mean shown as line bars.

Figure 5.6b

September





DISCUSSION

This research showed the similarity between ^{12}C and ^{14}C assimilation during normal tree growth. The amount of storage reserves, including sugar, starch and protein, increased from July to November in most tissue, especially in coarse roots. The amount of radiolabeled photosynthate in roots increased and surpassed the concentration in woody shoot tissue, verifying that the below-ground tissue had become the dominant sink following budset. In the July labeling, the amount of ^{14}C in carbohydrate and protein pools typically ranged between 1-2%. The sugar and protein pools were comprised of less ^{14}C than the starch pool showing that these pools were being diluted with unlabeled C. In contrast, the root sugar pool in September remained more than twice as enriched as the starch pool indicating that the accumulation of sugar was occurring at a rate exceeding its conversion to starch. Regardless of when the ^{14}C labeling occurred, the protein and carbohydrate pools retained labeled photosynthate into dormancy.

Few studies have followed the fate of ^{14}C-photosynthates into labile and insoluble pools in tree tissue on a seasonal basis and most do not represent field data. The results of laboratory and glasshouse studies in conifers (Schier, 1970; Glerum and Balatinecz, 1979) and hardwoods (Kandiah, 1979; McLaughlin *et al.,* 1980) indicate that the fate of assimilated C from earlier in the growing season is dynamic, never achieving equilibrium during the season of assimilation. Our data confirms that the ^{14}C is located in both labile and structural pools of fine roots in the following growing season.

The storage of carbohydrates in the root system begins before diameter growth of the coarse roots (Dickson, 1989). Diameter growth of roots follows that of the shoot growth, generally as a wave of cambial activity from developing buds (Krammer and Kozlowski, 1979). Deposits of starch in the ray and parenchyma cells of expanding roots begins in mid summer (Wargo, 1976) and is illustrated by our data (Tables 5.1-5.4). This early starch storage is not hydrolyzed to support cambial activity (Dickson, 1989). This infers that current photosynthate is used to support growth and respiratory activity of the root system. The fine root pool may also act as storage for carbohydrates (Nguyen *et al.*, 1990). The increase in carbohydrate storage, especially starch, in fine roots is evident both in increasing concentration and ^{14}C activity. The results in Figures 5.2-5.4 indicate the importance of storage by exemplifying the correlation between the distribution of ^{14}C, storage pools and structural residue C. These results are in contrast to the Marshel and Waring (1985) hypotheses which predict that starch deposition occurs during the initiation of fine (roots < 2 mm) in Douglas fir (*Pseudotsuga menziesii*). van den Dressche (1987), using Douglas fir seedlings, found that current photosynthate was important to new root growth, but also found root growth dependent on the supply of storage reserves. Clearly, the relative importance of current and storage photosynthate needs further research to adequately define the potential for and limits to growth (Glerum, 1980; Dickson, 1989).

The storage of C is also accompanied by the temporary storage of N which is necessary for the perennial growth of trees. The uptake, synthesis, distribution and recycling of nitrogen compounds is similar to the fate of photosynthates in that allocation is dependent upon competing sinks. As the growing season passes, the N requirements of the shoot

diminishes when the synthesis of photosynthetic enzymes ceases (Titus and Kang, 1982). During leaf senescence, some leaf protein is hydrolyzed to amino acids and transported back into the woody tissue of the shoot and roots. An increase in the concentration of root protein was distinguishable in November (Tables 5.1-5.2). The salvage of leaf protein nitrogen will depend on a number of factors, including the level of tree N, biotic influences such as infectious leaf diseases, and abiotic factors such as frost damage. The percentage of N retranslocated from leaves of *Populus* is generally between 60-80% (Baker and Blackmon, 1977). The present data indicate that from 6-25% of protein N was salvaged from leaves based on concentration. The low translocation of leaf N may be attributable to the nitrogen-rich site which was formerly under alfalfa cultivation and may explain the lack protein decrease in leaf litter.

Storage of N in trees can be either in the form of amino acids or proteins. The storage of amino acids increases in response to N fertilization in *Malus* (Tromp, 1983) and in *Pinus slyvestris* (Nasholm and Ericsson, 1990). Sauter *et al.* (1988) revealed the presence of protein bodies in ray cells of *Populus* during the dormant season. The occurrence of these protein storing vacuoles has subsequently been determined in other deciduous and conifer species (Sauter *et al.*, 1989). The characterization of these proteins has shown them to be high in arginine and other basic amino acids (Titus and Kang, 1982; Tromp, 1983). Our data reveals this trend showing an increase in protein content of the woody shoot and roots during dormancy. The occurrence of storage protein in trees is a subject receiving little attention and more research is needed to understand the accumulation and utilization in response to fertilization and stress (Dickson, 1989).

Trees have been classified according to the predominant form of storage during dormancy as "fat" or "starch" trees. Ziegler (1964) noted that many conifers and diffuse porous trees were "fat" trees and many ring porous trees were "starch" trees. This distinction applies mainly to the stem and not the root system, which generally stores starch regardless of species. The extraction of plant tissue with organic solvents removes considerable material besides fat. The crude extraction represents less than 10% of the tissue (Krammer and Kozlowski, 1979). Our values for methanol/chloroform extracts range from 5% in roots to 13.7% in leaf litter tissue. Chung and Barnes (1977) using a benzene/ethyl alcohol extraction of needle and axes of *Pinus taeda* found that the tissue contained 4.7% lipids and 15-20% phenolics. An extraction of *Quercus alba* tissue, including roots, determined that chloroform soluble material composed 1.3-8.7% of the dry weight (McLaughlin *et al.*, 1980). Nelson and Dickson (1981) found that stems of *Populus deltoides* contained 1-3% triglycerides (components of lipids) in dormancy induced one-year-old plants. These literature values are difficult to compare since no standard methodology exists for cross-comparative purposes.

Roots (< 10 mm) contained significantly more chloroform extractable material in September (Table 5.2). At this time the roots receive more photosynthate due to the basipetal flow of C. The transported sugars were undergoing conversion to starch for long term storage. Sauter (1988) has documented the formation of vesicular and dilated ER-cisternae during sugar-starch interconversions in *Populus* stems stored at 0°C. The formation of this vesicular material would require the accumulation of membrane lipids. During dormancy induction, sugars are accumulating and undergoing conversion into starch and may explain the increase in the

chloroform-soluble fraction in roots labeled in September. The accumulation of lipids in response to root diameter growth and for the synthesis of storage cells are additional reasons for lipid accumulation in roots.

The distribution of recovered ^{14}C in the chloroform-soluble fraction reaches a maximum of 27% in branch tissue in November from the September labeling. This agrees with Ziegler's (1964) interpretation of a diffuse porous being a fat storing tree. However, roots (> 10 mm) have an equivalent amount of chloroform soluble material. Perhaps, since the lipids in these pools were not defined, the comparison of these fractions is inappropriate. Therefore, the comparison to other research may also have no meaning. However, since this class of compounds are susceptible to nonpolar organic solvents the comparison is noteworthy. Glerum (1980) found recoveries of ^{14}C in the lipid pool of *Pinus banksina* leaves of greater than 35%, in roots 8-18% and woody shoot tissue displaying intermediate values. The distribution of ^{14}C in ether soluble material of *Pinus resinosa* trees was generally between 2.1-7.4% in the woody tissue and 16% in needles (Schier, 1970). The lipid fraction contains both significant mass and photosynthate, but is rarely studied in terms of a storage component in trees.

The residue component is a complex mixture of polysaccharides and lignin. This component is structural in nature and its synthesis is related to the initiation and expansion of shoot and root tissue. The amount of ^{14}C recovered in the residue fraction decreases as the growing season passes. In September, the relationship between the allocation of ^{12}C and ^{14}C to the mass of shoot residue does not correlate as it did in July. The increase in the amount of ^{14}C distributed to roots (> 1 mm) is probably the result of

diameter growth. The diameter growth of the root system is typical of this clone in autumn and also provides tissue for the storage of photosynthate (Pregitzer *et al.,* 1990). The retention of leaves in hybrid poplar after budset in autumn is an important factor contributing to stem and root growth as well as the formation of reserves (Nelson *et al.,* 1982; Michael *et al.,* 1988; Pregitzer *et al.,* 1990).

Glerum and Balatinecz (1979) described a similar distribution of ^{14}C in the residue component of *Pinus banksina* seedlings from July to early September. However, as the season progressed into November, less than 10% of the ^{14}C was recovered in residue, with the majority now present as sugars and starch. Dickson and Nelson (1982) also found that the amount of ^{14}C recovered from the residue declined in 1-year-old *Populus deltoides* stems as dormancy was induced under short day length regimes in growth chamber conditions. These researchers found between 20-30% of the ^{14}C in the residue fraction of budset plants 48 hours after exposing the them to $^{14}CO_2$. The lower recovery of ^{14}C in the residue fraction of *Populus deltoides* as compared to our results is probably due to sampling 48 hours after ^{14}C exposure. Our sampling was done 336 hours after ^{14}C exposure allowing more time for equilibration and incorporation of label into the residue fraction.

The incorporation of photosynthate into the residue fraction may involve both synthesis of structural and storage components. It has been suggested that hemicellulose is a form of storage photosynthate (Glerum, 1980; Bonicel *et al.,* 1987). Meyer and Splittstoesser (1971) found hemicellulose to be the primary form of storage in seedlings of *Taxus*. This could explain the high incorporation of ^{14}C into residue in September after budset, especially in leaf tissue. After budset, no initiation or expansion of

the leaf canopy occurs. Yet, a surprising amount of ^{14}C is recovered from the residue fraction of leaves (Figure 5.4). However, hemicellulose is a complex polysaccharide and has strong intermolecular bonds with lignocellulose leading physiologists to believe these carbohydrates are structural in origin (Krammer and Kozlowski, 1979). More likely, is the retention of C in leaves in response to wounding inflicted by the various leaf diseases. The higher retention of ^{14}C-photosynthate in ozone damaged leaves is an example of such a phenomenon (McLaughlin *et al.*, 1982). The increase of ^{14}C in the residue of roots may also be associated with the increase of recalcitrant polysaccharides, such as chitin, from mycorrhizal symbiosis.

The importance of storage photosynthate is exemplified in the lack of decline in the specific activity of fine roots (< 0.5 mm) one year following the exposure to $^{14}CO_2$ in both July and September of the previous year. The reserves accumulated in mid growing season (July label) and autumn (September label) played an active role in the initiation of new fine roots the following season. Other researchers have shown the importance of autumnal photosynthate storage in the growth of new shoots of apple trees (Quinlan, 1969; Kandiah, 1979). Lockwood and Sparks (1978) found that 78% of the ^{14}C in roots in November was present in roots after shoot elongation had ceased in pecan (*Carya illinoensis*). Similarly, Hansen (1967) found 69% of the ^{14}C present in apple roots in September to November of the following year. The same or increased recovery of ^{14}C was found in roots of *Pinus resinosa* labeled in July and October up to 10 months after exposure (Schier, 1970). Generally, these studies have observed C storage in relationship to shoot and fruiting dynamics, neglecting their role in the maintenance and growth of roots.

The specific activity of the reserves, sugar and starch, in the fine roots was approximately twice that of the residue pool in November regardless of when $^{14}CO_2$ exposure occurred (Figures 5.6a-b). The specific activity of these storage pools increased with increasing root diameter. Therefore, the utilization of these pools to initiate new fine root growth required that they be diluted with an equivalent amount of unlabeled C to maintain the specific activity of the fine root pool from the previous year. The source of the unlabeled C is current assimilates and indicates that the growth of fine roots is dependent on reserve and current photosynthate. The rate of reserve utilization and its subsequent dilution in the growth of fine roots was not distinguishable since the root system was composited when harvested. It is possible that depending on demography and ontogeny of fine roots the utilization of reserve material was not uniform. Any growth of roots before shoot elongation would have been entirely dependent on reserves and would produce roots of the same specific activity as the reserve material. It is evident from our data that growth of fine roots is dependent on reserves and current photosynthate. The specific activity of reserves remaining are beginning to equilibrate with structural residues one year after exposure to ^{14}C.

CONCLUSION

This research has presented evidence that the synthesis and turnover of the root system is influenced by tree activities occurring over more than one season. During the growing season, current assimilates are utilized for respiration, growth and storage of reserve material from a whole-tree perspective. In autumn, the shoot stops expanding and

assimilates are both stored and translocated at an increasing rate to the root system. The dormant period is characterized by the consumption of reserve material to maintain life and some growth of the root system before shoot expansion the following growing season. During the next growing season, reserve and current photosynthate are used in shoot and root system expansion. The persistence of reserve material and undefinable dilution of cell wall tissue in the fine root system exemplifies the importance of storage reserves in these hybrid poplars.

In conclusion, the phenological plasticity of the shoot and root system will be directly influenced by the amount and utilization of reserve material stored in current and subsequent growing seasons. The rate of accumulation of reserve material will be highly sensitive to factors such as competition, abiotic interactions and cultural practices. It is evident that the success of these hybrids in producing increased biomass at an accelerated rate will depend on the ability to sequester reserves in more than one growing season. The storage and utilization of reserves, is therefore, an extremely important process that influences the success of this and other species in managed and unmanaged ecosystems. Understanding patterns of mobilization and utilization of reserve material is necessary to fully understand the consequences of cultural practices in ecosystems designed with decreasing diversity and increasing production.

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Chapter 6

MICROBIAL BIOMASS AND DYNAMICS OF C AND N IN *POPULUS* PLANTATIONS

ABSTRACT

Soil microbial activity and organic matter dynamics are central to carbon cycles and nutrient fluxes in ecosystems. The contribution of leaf and root production and turnover to the soil microbial biomass and soil organic matter (SOM) maintenance are vaguely understood. This study was designed to determine the role of leaf and root turnover in the dynamics of carbon and nitrogen cycling through the microbial biomass and SOM. Two-year-old hybrid poplars were labeled with ^{14}C and ^{15}N in the field at different times of the growing season to characterize the flux of C and N in the tree-soil system. Labeled leaf litter was placed on unlabeled plots to determine the relative contribution of leaf and root turnover. Microbial biomass methodologies of direct microscopy, chloroform-fumigation incubation (CFI) and chloroform fumigation-extraction (CFE) were used to measure microbial biomass. The comparisons of the microbial biomass methods showed that the techniques of CFI and CFE correlated well against direct microscopy, but gave consistently higher values. The subtraction of a partial control from CFI gave values similar to direct microscopy in surface and subsurface soils. The comparison of the ^{14}C activity of microbial biomass C from CFI and CFE indicated these methods may be assaying different pools of microbial C. Long-term incubation of soil the year following labeling was used to study the

dynamics of C and N mineralization. Mathematical analysis of the mineralization curves provided decomposition rates, kinetic parameters and pool sizes of labile and more stable soil C pools. Kinetic analysis of long-term soil incubations indicated a greater contribution of C and N to soil organic fractions from leaf litter decomposition in the early stages of the rotation. The mean residence time of ^{14}C in surface and subsurface soil from leaf and root turnover was 14-64 days for labile organic soil C and 2-16 years for stabilized C.

INTRODUCTION

Soil organic matter dynamics (SOM) are intrinsically related to carbon (C) cycles and nutrient fluxes in managed and unmanaged ecosystems. These in turn are influenced by cultural-management practices, the magnitude, quality and timing of residue inputs as well as soil physical and chemical characteristics (McGill and Cole, 1981; Paul, 1984). Various components of SOM act as temporary energy and nutrient reservoirs. The determination of the pool size and the rates of release of nutrients from these pools is important in determining the nutrient cycles of ecosystems and the fertilizer requirements of managed systems.

Attempts to utilize a chemical or physical extraction technique to predict the amount of N that would be released during a crop's growth have not been very successful (Keeney, 1982; Juma and Paul, 1984). The exception can be found in dry climates where soils accumulate nitrates due to the lack of leaching over autumn, spring, or fallow periods. The SOM pool that provides nutrients required for plant growth over a reasonably short term is usually referred to as the "active fractions". The size and kinetics of active fractions can be determined quantitatively with tracer analysis (^{15}N, Jansson, 1958; ^{14}C, Coleman and Fry, 1992). These components are thought to be comprised primarily of partially decomposed plant residues, microbial biomass, and recent microbial byproducts. The size of these fractions have been determined by long-term incubation combined with mathematical analysis of the accumulated inorganic C and N (Standford and Smith, 1972; Boyle and Paul, 1989; Blet-Charaudeau *et al.*, 1990).

The measure of nitrogen (N) availability and cycling provides a tangible determination of the sustainability and production potential of ecosystems. Nitrogen availability controls the amount and substrate chemistry of plant litter (Aber and Melillo, 1982; Vitousek, 1982; McClaugherty *et al.*, 1985). Many methods of assessing N availability via incubation have been developed to determine fertilizer need and understand productivity at the stand level. These include: soil incubation at increased temperature (37°C) and periodic leaching (Standford and Smith, 1972); statically incubated soils with sampling to determine N accumulation (Vitousek *et al.*, 1982); buried polyethylene bags (Zak and Pregitzer, 1990); soil cores encased in ion exchange resin (Di Stefano and Gohlz, 1986); ion exchange resin in soil (Binkley and Matson, 1983); and anaerobic soil incubation (Powers, 1980). These methods describe differences in N availability between sites, but fare poorly when the methods are compared indicating that N availability is being qualitatively assessed (Hart and Firestone, 1989; Powers, 1990).

A number of studies have shown that the size of the microbial biomass itself appears to be well correlated with the size of the active fractions (Anderson and Domsch, 1989; Smith and Paul, 1990). In addition, the net magnitude of microbial mediated processes involved in the immobilization-mineralization reactions are reasonably well understood (Paul and Clark, 1989). The gross magnitude of nutrient flux is not well characterized. There have been a number of studies showing that the use of tracers combined with long-term incubations can be used to determine the fluxes involved in N mineralization, nitrification and immobilization of N (Robertson and Vitousek, 1981; Vitousek *et al.*, 1982).

Nutrient dynamics in forests are difficult to study due to different growth rates of the plant components and their large size, making the use and interpretation of tracer research difficult. This is complicated further by fertilization effects caused from increases in carbon dioxide and deposition of atmospheric N and other nutrients (Kauppi *et al.*, 1992). The recent interest in short-rotation forestry, such as Christmas tree farming and production of biomass for pulp and energy industries has led to a requirement for information on the N needs of these systems. Short-rotation forestry provides an amenable system to study (Horwath *et al.*, 1992). A number of reasonably sized, uniform and non-competing trees can be labeled with ^{14}C-^{15}N , (Chapter 3; Smith and Paul, 1988).

In the present study, I examined the role of root and leaf litter C and N in the production of microbial biomass and active organic matter pools. Long-term soil incubations measured the occurrence and stabilization of ^{14}C and ^{15}N in soil organic fractions derived from the labeled tree litters. The tracer content of the microbial biomass and SOM fractions was measured by analytical assays. Kinetic analysis of long-term soil incubation data provided information on pool sizes and fluxes. The uncertainty associated with microbial biomass assays has led to the development of a variety of techniques to determine microbial C, N and tracer content. To establish which assay was suitable to determine C and N content of the soil microbial biomass I compared the results of direct microscope counts, chloroform fumigation incubation (CFI) and chloroform fumigation extraction (CFE). This comparison is followed by a discussion of the results of long-term soil incubation data and kinetics of C mineralization.

MATERIAL AND METHODS

Plant ^{14}C and ^{15}N Labeling

A hybrid poplar plantation (*Populus americana* cv. Eugenei) was established in conjunction with the LTER studies at the Kellogg Biological Station, Michigan State University, Hickory Corners, Michigan. Site, plantation characteristics and ^{15}N and ^{14}C labeling procedures are described in Chapter 3. Table 6.1 describes some of the important plant and soil characteristics of this system. Two sets of eight trees labeled during the 1990 growing season were sampled the following year. July labeled trees were sampled August 2, 1990 (day 14), November 2, 1990 (day 107) and July 25, 1991 (day 372). September labeled trees were sampled September 19, 1990 (day 14), November 4, 1990 (day 60) and September 5, 1991 (day 366). Labeled leaf litter collected during autumn was placed over plots containing unlabeled trees on December 21, 1990 (Chapter 4). The litter exchange was done to compare the dynamics of ^{14}C and ^{15}N in soil and microbial biomass from leaf and root derived C and N. The leaf litter exchange plots were sampled during the following year on June 6 (day 168) and November 14, 1991 (day 328).

The soil sampling and preparation has been described previously (Chapter 3). The root labeled soil was sampled to a depth of 100 cm at intervals of 0-25 cm, 25-60 cm and 60-100 cm. The plots containing labeled leaf litter were sampled to a depth of 25 cm at 0-10 cm and 10-25 cm intervals. Soil moisture was determined gravimetrically after drying at 105°C for 24 hours. Soil ^{14}C activity was determined using a sulfuric/phosphoric acid digestion in modified culture tubes (Smith *et al.*, 1989).

Table 6.1. Characteristics of the plant-soil system studied.

Vegetation	*Populus americana* cv. Eugenei	
Spacing	2 m x 1 m	
Management	Short-rotation Intensive-culture	
Soil type	Kalamazoo fine, loamy, mixed, mesic, Typic Hapudalf	
Soil depth		
Tree plots	% C	% N
0-25 cm	1.06	0.080
25-60 cm	0.37	0.033
60-100 cm	0.27	0.025
Leaf litter plots		
0-10 cm	1.10	0.101
10-25 cm	1.01	0.092

Microbial C and N Determinations

Microbial biomass determinations were conducted three times during each long-term incubation using CFI (Horwath and Paul, 1992). Microbial C determinations by CFI were compared to direct microscopy and CFE on the first two samplings of the labeled tree plots and first sampling of the litter exchange plots. Microbial C determined by CFI was calculated without the use of a control, K_C=0.41 (Voroney and Paul 1984). The K_C for the CFE determination was 0.35 (Voroney et al., 1992). Microbial N was calculated based on the ratio of the fumigated flush of C to N, K_n (Voroney and Paul, 1984). CFI data were also recalculated using a partial control as determined by Horton, Horwath and Paul (Personal communication).

Direct microscopic counts of acridine-orange-stained bacteria and fungi were done on the first two samplings of the July and September labeled trees. Soil (10 g) was homogenized with 200 mL of 0.2 *M* Tris (pH 8) in a Waring blender for 60 seconds. Serial dilutions of 1 mL homogenate to 9 mL Tris buffer were done to obtain 0.1 mg soil mL^{-1} for bacteria and 0.5 mg soil mL^{-1} for fungi. The final dilutions were stained with 0.5 mL acridine orange (0.1% w/v in water) for 10 minutes (Faegri *et al.*, 1977). The dilutions were preserved by adding 0.1 mL formaldehyde. The entire final dilution was drawn through a pre-wetted (1 mL 0.1% tween in water) 0.2 μm black Nucleopore membrane filter on a vacuum side arm flask. The filter was mounted on a microscope slide with a drop of immersion oil and cover slip. Graticule fields were counted randomly until at least 300 bacteria were counted per slide. Fungal hyphae length was determined with a line intersect method using an ocular grid (Hanssen *et al.*, 1974). A mean volume of 0.12 μm^3 for bacteria (Bakken and Olsen, 1983) and a 2.3 μm diameter for fungi (Dave Harris, personal communication) were used

in the calculation of microbial biomass. Carbon content of bacteria and fungi was calculated using values for specific gravity of (1.1), solids content of (0.3) and 45% C (Paul and Clark, 1989).

Additionally, microbial biomass C was determined by the CFE method (Vance *et al.*, 1987). Fumigated and unfumigated soils (25 g wet weight) were extracted with 0.5 M K_2SO_4 (5:1 v/v) for 1 hour. Soluble C was determined on extracts using a modified persulfate digestion (Horwath and Paul, 1992). The soil extract (15 mL) was digested with 1 g persulfate and 1 mL 0.05 M H_2SO_4 in a modified culture tube containing a vial with 1 mL 0.1 M NaOH. The culture tubes were sealed with Poly seal caps and digested at 120°C for 2 hours. The contents of the vial were titrated to a phenolthalien endpoint with 0.1 *M* HCl after the addition of an equivalent amount of $BaCl_2$ to determine total C. The contents of the titrated base traps were rinsed into scintillation vials, 10 mL of scintillation cocktail added and ^{14}C activity determined on a liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL).

Long-Term Soil Incubations

The soil was weighed (60 g field moist, approximately 52 g dry weight) into urine specimen cups and placed into Mason jars for long-term CO_2 mineralization studies. A vial containing 5 mL of 0.6 M NaOH was placed with the soil and the Mason jar sealed. The soil was incubated at 25°C for approximately 150 days. The vials were changed every 15-20 days with less frequent sampling as the incubations proceeded past 75 days. The contents of the vials were divided in two to determine mineralized CO_2-C and ^{14}C activity. Mineralized CO_2 was determined by titrating the NaOH with 1.5 M HCl to a phenolthalien endpoint (pH 7) after the addition of an

equivalent amount of $BaCl_2$ (Horwath and Paul, 1992). The remainder of the vial contents was combined with 10 mL of scintillation cocktail (Research Products International Corp., Mount Prospect, IL.), sufficient water to gel the mixture and the ^{14}C activity determined.

Soil (350 g, oven dry weight) for long-term N mineralization and microbial biomass determinations was placed into Mason jars with 1 mm diameter holes punched into the lids. Soil moisture was maintained gravimetrically during the incubation period. Mineralized N was determined throughout the long-term incubation at approximately 0, 10, 20, 40, 50, 60, 70, 90, 120 or 150 days. Soil (25 g) was removed from the Mason jars and extracted with 2 N KCl (5:1, v/v) for 0.5 hour. Inorganic N (NO_3^- and NH_4^+) was determined colorimetrically using a flow-injection autoanalyzer (Lachet Chemicals Co., Mequin, WI). The diffusion procedure (Brooks *et al.*, 1989) was used to determine the ^{15}N content of inorganic soil N and microbial biomass N. The ^{15}N analysis was done on a Gas chromatograph-Mass Spectrometer (Europa Scientific, Crewe, England).

Calculations

Standard deviation and error of the mean are shown to demonstrate the reproducibility of field and laboratory techniques. Potential C mineralization (C_1) was calculated using a mixed order and modified double exponential equations. The mixed order equation (1) incorporates a zero and first order component describing the cumulative C mineralization curves from the long-term soil incubations (Blet-Charaudeau *et al.* , 1990):

$$C_t = C_1*(1-\exp(-k_1*t) + k_2*t \qquad (1)$$

where C_t is the total amount of CO_2-C evolved at time t; k_1, k_2 and C_1 are constants expressing, respectively, the first order rate constant, the zero order rate constant and potentially mineralizable C. A modified double exponential equation (2) was adapted from (Bonde and Rosswall, 1987):

$$C_t=C_1*(1-\exp(-k_1*t))+((C_{soil}/2)-C_1)*(1-\exp(-k_2*t)) \qquad (2)$$

where C_{soil} is the total organic C in soil. In equation 2, the above assumes three soil C pools C_1, C_2 and C_3. The potentially mineralizable C_1 pool is described by the first order equation. C_3 was estimated from carbon dating as being 50% of the total soil C ($C_{soil}/2$) with a turnover time sufficiently long it does not affect the amount of CO_2 evolved during the long-term incubations (Martel and Paul, 1974), therefore, the more stable soil C pool is defined as $C_2=C_{soil}/2-C_1$. The parameters were developed for the cumulative C mineralization curves using a nonlinear model estimation procedure with Quasi-Newton minimization methods (Systat: Statistics, Evanston, IL). The software computes mean square errors, asymptotic standard errors and correlation by estimating the Hessian (second derivative) matrix.

RESULTS

Microbial Biomass

Direct microscopy is a traditional method to determine soil microbial biomass C and N and is used as standard to calibrate other methods (Horwath and Paul, 1992). The advantage of direct microscopy to determine

microbial biomass is the ability to differentiate fungal and bacterial populations. Fungal hyphal lengths were 109 to 146 m g^{-1} (72.9-98.1 μg C g^{-1} soil) in the surface soil (0-25 cm) (Table 6.2). They dropped by a factor of 2.9 to 3.7 (26-42 μg C g-1 soil) at the 60-100 cm depth. Bacterial numbers ranged from 1.26-1.6 x 10^9 cells g^{-1} (22.5-28.7 μg C g^{-1} soil) of surface soil. The surface to subsurface bacterial number ratio was 4.0 to 8.0, thus the subsurface soil contained more fungal C than bacterial C indicating the importance of fungi in subsurface soil. Microscopy, however, does not measure the turnover of the fungal hyphae.

Soil microbial C determined by CFE for surface soil was 112-147 μg C g^{-1} for the first two sampling of tree plots in 1990 (Table 6.3a). The CFI method gave slightly higher values of 143-219 μg C g^{-1} soil for the same sampling and an additional final sampling of the tree plots the following year (Table 6.4a). Direct counts, CFI and CFE all showed similar changes with time, therefore, the difference in the biomass for the surface soils are considered to be meaningful measurement of seasonal differences. Microbial biomass C values were lower in subsurface soil for both fumigation methods corroborating the similar trend found for direct microscopy.

The microbial biomass C values for the first sampling of the leaf litter exchange plot were approximately 350 μg C g^{-1} in the surface soil (0-10 cm), as determined by both CFI and CFE methods (Tables 6.3b and 6.4b). The high values for microbial C subsided for the second sampling of the leaf litter exchange experiment determined by CFI. The subsurface soil (10-25 cm) gave estimates of microbial C more similar to the surface soil of the labeled tree plots. The apparent increase in microbial biomass was

Table 6.2. Direct microscopic counts of bacterial numbers and hyphal lengths and biomass C conversions for the first two samplings of labeled trees. Standard error of the mean shown in parentheses.

	Bacteria		Fungi		Microbial biomass	
Soil depth (cm)	numbers x 10^9 g^{-1} soil	µg C g^{-1} soil	Hyphal lengths m g^{-1} soil	µg C g^{-1} soil	Microbial C g^{-1} soil	Fungi: bacteria
Labeled July 19, 1990				Sampled August 2,1990		
0-25	1.48 (0.14)	26.4 (2.6)	108.6 (2.5)	72.9 (2.7)	99.3 (3.7)	2.8
25-60	0.27 (0.05)	4.9 (0.9)	55.9 (13.8)	37.5 (14.5)	42.4 (14.5)	7.7
60-100	0.22 (0.03)	3.9 (0.5)	36.7 (0.6)	24.7 (0.7)	28.6 (0.9)	6.3
				Sampled November 2,1990		
0-25	1.60 (0.08)	22.5 (2.1)	116.0 (2.9)	91.8 (3.5)	114.4 (4.1)	4.1
25-60	0.53 (0.00)	8.6 (0.6)	35.9 (3.5)	42.0 (0.7)	50.5 (0.9)	4.9
60-100	0.36 (0.01)	4.4 (0.3)	26.0 (1.9)	28.2 (2.1)	32.6 (2.1)	6.5
Labeled September 5, 1990						
				Sampled September 20, 1990		
0-25	1.26 (0.12)	28.6 (1.5)	136.7 (3.3)	77.9 (3.1)	106.5 (3.4)	2.7
25-60	0.48 (0.03)	9.5 (0.1)	62.5 (0.7)	24.1 (3.7)	33.6 (3.7)	2.5
60-100	0.24 (0.02)	6.5 (0.3)	42.0 (2.0)	17.5 (2.0)	24.0 (2.0)	2.7
				Sampled November 4, 1990		
0-25	1.61 (0.11)	28.7 (2.0)	146.0 (29.3)	98.1 (30.7)	126.8 (30.8)	3.4
25-60	0.36 (0.03)	6.4 (0.6)	40.7 (20.4)	27.4 (21.4)	33.7 (21.4)	4.3
60-100	0.20 (0.01)	3.5 (0.2)	38.8 (2.2)	26.1 (2.3)	29.6 (2.3)	7.4

Table 6.3a. Microbial biomass C determined by the CFE method for the first and second samplings of trees labeled with ^{14}C and ^{15}N July 19,1990 and September 5,1990. Standard error of the mean shown in parentheses.

Soil depth (cm)	Extractable soil C $\mu g\ g^{-1}$	Uncorrected biomass C $\mu g\ C\ g^{-1}$ soil	Corrected Biomass C $\mu g\ C\ g^{-1}$ soil	Bq mg^{-1} biomass C
Labeled July 19,1990				
		Sampled August 2, 1990		
0-25	50.4 (1.0)	51.5 (4.6)	147.1 (13.2)	1.0 (0.2)
25-100	57.1 (3.9)	30.1 (8.2)	86.0 (23.4)	1.1 (0.4)
60-100	37.9 (2.5)	29.5 (4.3)	84.2 (12.4)	0.7 (0.1)
		Sampled November 2, 1990		
0-25	65.0 (3.6)	56.4 (8.0)	161.2 (23.0)	1.2 (0.1)
25-100	80.2 (4.3)	15.0 (1.1)	42.9 (3.2)	1.5 (0.2)
60-100	49.3 (4.6)	9.2 (3.1)	26.3 (8.8)	1.9 (0.4)
Labeled September 5,1990				
		Sampled September 20, 1990		
0-25	63.2 (1.1)	39.4 (4.2)	112.5 (12.1)	2.0 (0.1)
25-100	60.7 (2.7)	21.7 (4.2)	62.1 (12.1)	1.0 (0.1)
60-100	45.2 (2.5)	11.4 (1.8)	32.6 (5.3)	2.0 (0.6)
		Sampled November 4, 1990		
0-25	59.9 (2.6)	52.2 (2.4)	149.1 (6.7)	1.7 (0.2)
25-100	74.9 (4.4)	21.4 (4.4)	61.1 (12.6)	1.3 (0.9)
60-100	52.7 (0.7)	11.3 (0.3)	32.4 (0.8)	2.2 (0.3)

Table 6.3b. Microbial C determined by the CFE method for the first sampling, June 6, 1991, of the litter exchange experiment. Standard error of the mean shown in parentheses.

Soil depth (cm)	Extractable soil C $\mu g\ g^{-1}$	Uncorrected biomass C $\mu g\ C\ g^{-1}$ soil	Corrected Biomass C $\mu g\ C\ g^{-1}$ soil	Bq mg^{-1} biomass C
Leaf litter exchange, started December 21,1990				
		September litter		
0-10	29.7 (2.1)	123.4 (5.3)	352.5 (15.0)	4.1 (0.3)
10-25	28.8 (2.0)	91.6 (4.1)	261.7 (11.8)	0.5 (0.1)
		July litter		
0-10	36.6 (1.1)	122.3 (2.8)	349.3 (8.0)	1.8 (0.1)
10-25	36.0 (3.2)	80.7 (6.0)	230.6 (17.0)	0.2 (0.0)

Table 6.4a. Microbial biomass C, ^{14}C and N determined by the CFI method for all samplings of trees labeled on July 19,1990 and September 5,1990. Standard error of the mean shown in parentheses.

Soil depth (cm)	Fumigated flush $\mu g\ C\ g^{-1}$ soil	Corrected biomass C $\mu g\ g^{-1}$ soil	Bq mg^{-1} biomass C	Biomass N $\mu g\ g^{-1}$ soil	C/N
Labeled July 19, 1990					
		Sampled August 2, 1990			
0-25	58.6 (1.9)	143.0 (4.5)	1.9 (0.1)	25.7 (0.7)	5.6
25-60	29.1 (9.6)	70.9 (23.3)	1.1 (0.3)	10.7 (3.2)	6.6
60-100	20.7 (5.7)	50.6 (13.9)	0.8 (0.4)	7.5 (1.8)	6.8
		Sampled November 2, 1990			
0-25	76.3 (3.2)	186.1 (7.7)	1.1 (0.2)	32.1 (1.2)	5.8
25-60	11.1 (3.8)	27.2 (9.3)	2.0 (1.0)	4.4 (1.4)	6.2
60-100	3.1 (3.2)	7.6 (7.7)	4.2 (2.5)	1.4 (1.3)	5.5
		Sampled July 25, 1990			
0-25	87.5 (4.6)	213.3 (11.3)	0.5 (0.1)	34.9 (0.1)	6.1
25-60	26.7 (6.8)	65.0 (16.6)	0.5 (0.1)	9.7 (3.8)	6.7
60-100	10.8 (3.2)	26.4 (7.7)	0.7 (0.2)	4.0 (1.7)	6.6
Labeled September 5, 1990					
		Sampled September 20, 1990			
0-25	73.1 (0.6)	178.3 (1.4)	3.2 (0.3)	31.6 (0.4)	5.6
25-60	27.8 (1.4)	67.9 (3.4)	3.8 (0.6)	10.6 (1.0)	6.4
60-100	17.8 (1.2)	43.3 (3.0)	2.9 (0.3)	5.9 (0.5)	7.4
		Sampled November 4, 1990			
0-25	77.8 (6.6)	189.8 (16.2)	2.5 (0.2)	33.0 (4.4)	5.8
25-60	32.7 (2.6)	79.7 (6.2)	2.2 (0.2)	11.1 (0.6)	7.2
60-100	20.6 (1.6)	50.2 (3.9)	2.3 (0.2)	6.9 (0.9)	7.3
		Sampled September 5, 1990			
0-25	89.8 (2.3)	219.0 (5.7)	0.7 (0.1)	37.6 (1.6)	5.8
25-60	28.7 (0.1)	70.0 (0.4)	0.5 (0.1)	9.6 (0.6)	7.3
60-100	15.5 (1.4)	37.8 (3.5)	0.8 (0.2)	5.8 (1.1)	6.5

Table 6.4b. Microbial biomass C, ^{14}C and N determined by the CFI method for all samplings of the litter exchange experiment on June 6, 1991 and November 14, 1991. Standard error of the mean shown in parentheses.

Litter	Soil depth	Fumigated flush $\mu g\ C\ g^{-1}$ soil	Corrected biomass C $\mu g\ g^{-1}$ soil	Bq mg^{-1} biomass C	Biomass N $\mu g\ g^{-1}$ soil	C/N
Leaf litter exchange experiment started December 21, 1990						
			Sampled June 6, 1991, day 168			
Sept.	0-10	142.0 (4.0)	346.3 (9.8)	4.7 (0.5)	60.3 (1.7)	5.7
	10-25	102.0 (2.4)	248.9 (5.9)	0.6 (0.2)	42.9 (1.1)	5.8
July	0-10	145.9 (6.5)	355.9 (15.9)	2.3 (0.2)	62.6 (2.8)	5.7
	10-25	88.7 (4.2)	216.4 (10.2)	0.4 (0.1)	37.6 (1.7)	5.8
			Sampled November 14, 1991, day 328			
Sept.	0-10	89.9 (10.0)	219.3 (24.4)	4.4 (0.6)	40.7 (3.8)	5.4
	10-25	67.1 (7.6)	163.7 (18.5)	1.1 (0.5)	30.0 (3.0)	5.4
July	0-10	107.1 (16.5)	261.3 (40.3)	2.8 (0.7)	42.0 (3.2)	6.2
	10-25	60.0 (7.2)	146.3 (17.7)	0.7 (0.2)	27.2 (2.9)	5.4

perplexing, since an equivalent amount of unlabeled leaf litter was placed on the labeled tree plots.

The specific activity of the biomass C is similar at all depths for the labeled tree plots across time indicating a similar relation between roots and microorganisms and that the recently incorporated C is behaving similarly with time at all soil depths (Tables 6.3a and 6.4a). The lower ^{14}C levels in the subsurface soil is a result of less root biomass. Approximately 50% of both the fine root biomass and microbial biomass was located below 25 cm (Chapter 4). The ^{14}C activity of the microbial biomass from the leaf litter exchange experiment plots was concentrated in the surface soil (0-10 cm) (Table 6.3b and 6.4b). The higher ^{14}C concentration in the surface soil of the litter treatment was a fundamental difference between the input of C to soil from leaf litter (leaf derived C) and root turnover (root derived C).

The microbial biomass of the tree plots contained more ^{14}C activity 14 days after labeling in the September labeling than in the July labeling (Tables 6.3a, b and 6.4a, b). The higher amount of ^{14}C found in the biomass during September corresponds to the increase in the translocation of C to the root system for this *Populus* clone during autumn (Chapters 4 and 5). The biomass ^{14}C in the September labeled trees subsided to levels similar to July labeled trees one year later. The drop in ^{14}C activity in microbial C over time in the tree plots shows the turnover of the microbial biomass and the utilization of new unlabeled substrate from the soil. The specific activity of the fine roots had not dropped significantly during this time. This trend is not exhibited in the leaf litter labeled plots indicating that the labeled C input is being sustained by the continued movement from the aboveground leaf litter to the soil beneath. This demonstrates an additional fundamental difference in the role of C soil input between leaf and root derived C.

Microbial biomass N values in surface soil ranged from 26-36 μg N g^1 soil in tree plots (Tables 6.4a, b). The elevated amount of microbial N (27-62.6 μg N $g^{1)}$ found in the first sampling of the leaf litter exchange plots is similar to the microbial C results for these plots. These higher values are partly explained by dividing the surface soil in to 0-10 and 10-25 cm depth increments. However, this does not explain all of the increase which could be attributable to the spatial proximity of the leaf litter to 0-10 cm soil. Table 4a shows that there appears to be a tendency of the microbial C:N ratio to widen with depth. Since the microscopic counts showed a greater proportion of fungi with depth and since fungi have wider C:N ratios, the C:N ratio as determined by the CFI should widen. This again verifies the accuracy of the techniques employed.

The comparisons of the fumigation methods to direct microscopy for microbial biomass are shown in Figures 6.1a-c. The comparison of CFI and CFE show high correlation across time and soil depth. This indicates that the different constants and variables used to calculate microbial biomass are both meaningful and applicable, especially with soil depth. Similarly, the comparison between CFI and CFE produce a slope of 1 and R^2 of 0.98 (Figure 6.1d). The similarity in microbial C values between the to fumigation methods is remarkable since the CFE methods subtracts a control. The biomass ^{14}C activity determined by the two methods are not statistically different (Figure 6.1e), although CFE had a tendency to be somewhat lower. This indicates that the two fumigation methods maybe sampling two somewhat different C pools.

The results of all methods to determine microbial biomass were normalized to CFI values to facilitate their comparison (Table 6.5). The data include a computation that subtracts a partial control from the CFI

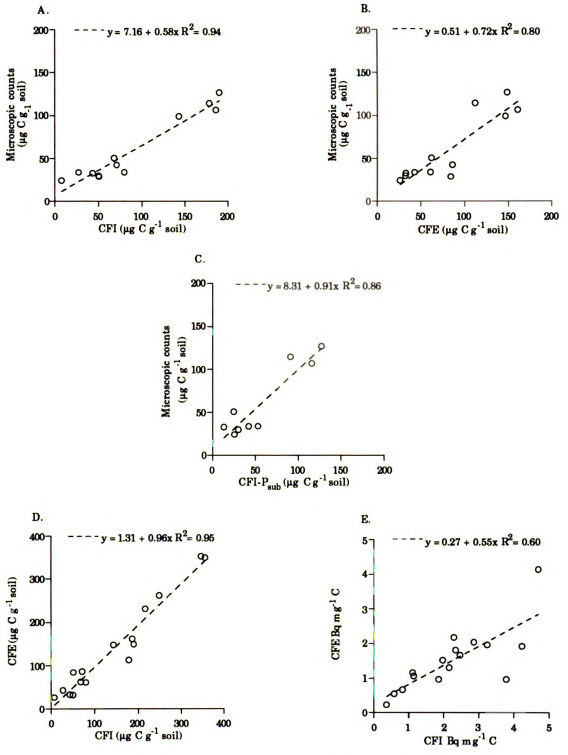


Figure 6.1. The comparison of the fumigation and direct microscopy methods of microbial biomass determination are shown in a-c. Figures d and e compare the biomass C and specific activity of biomass C from the fumigation methods.

Table 6.5. Average results from all samplings and methods of determining microbial biomass C. The data has been adjusted to 100 μg C=CFI. Standard errors of the mean shown in parentheses.

Soil depth (cm)	CFI	CFE	CFI-P_{sub}*	Direct counts
0-25	174.3 (10.7)	142.5 (10.5)	111.4 (10.7)	111.8 (5.9)
25-60	61.4 (11.7)	63.0 (8.8)	39.9 (8.2)	40.1 (4.1)
60-100	37.9 (10.3)	43.9 (13.5)	22.8 (5.1)	28.7 (1.8)
	Data normalized to CFI values for comparison purposes.			
0-25	100	82	64	64
25-60	100	103	65	65
60-100	100	116	60	76

* $=0.229+0.0882*(\text{fumigated } CO_2/\text{control } CO_2)+0.0969*(\text{fumigated } CO_2/\text{control } CO_2)^2-0.016*(\text{fumigated } CO_2/\text{control } CO_2)^3$

values. The partial control was determined by the addition of an internal standard to the fumigated and unfumigated soil. The internal standard (substrate) was uniformly labeled ^{14}C-straw. The value of the partial control was calculated by determining the difference in substrate utilization between unfumigated and fumigated samples and deriving the formula:

$$\text{Biomass } C = 0.229 + 0.0882(fum_C/con_C) + 0.0969(fum_C/con_C)^2 - 0.016(fum_C/con_C)^3$$

where, fum_C is the fumigated flush after 10 days of incubation and con_C is the amount of C mineralized in 10 days in an unfumigated soil (Horton, Horwath and Paul, personnel communication). The corrected CFI value is remarkably similar to the results of direct counting (Figure 6.1c and Table 6.5).

Long-Term Incubations

Long-term incubations were done on the soil from the second and third samplings of the labeled trees and the first two samplings of the leaf litter exchange plots. The object of the long-term soil incubations was to determine the amount of recently assimilated tracer ^{14}C and ^{15}N entering the different fractions of the SOM from root and leaf litter derived C and to determine the contribution of the microbial biomass to the mineralizable C and N pools. The data were also useful to calculate rate constants and pool sizes for C mineralization. Nitrogen mineralization was represented by a straight line, and therefore, cumulative N mineralization could not be described using first order kinetics.

Soil C

The accumulation of mineralized C (μg CO_2-C g^{-1}) declined as the long-term incubations progressed for both tree and leaf litter plots (Figure 6.2a and 6.3a, Appendix B.1-B.10). Carbon mineralization values for surface soil from the tree plots were 315-708 μg C g^{-1} soil. Carbon mineralization values for subsurface soils dropped to one sixth of this value. The decline in the accumulation rate of C is associated with the mineralization of a labile C soil pool and the subsidence of a possible disturbance effects associated with sampling. The C mineralization curves for the labeled tree plots remained essentially similar over time, especially in the surface soil (Figure 6.2a). The C mineralization from the leaf litter exchange plots were higher than the tree plots and decreased from the first to second sampling (Figure 6.3a). This was similar to the results of the microbial biomass determination for tree plots. The level subsides to values resembling the tree labeled plots for the second sampling and long-term incubation and was similar to the results of the microbial biomass determinations.

The mineralization of ^{14}C followed patterns similar to that of the native C for both tree and leaf litter plots (Figures 6.2b and 6.3b). The amount of ^{14}C mineralized declined in the second long-term incubations (soils sampled at greater than 300 days in the field) for all plots. The decline in specific activity of the mineralized C from both the leaf litter and tree plots is less pronounced for the long-term incubations from soils sampled between 328-372 days (Figures 6.4a-d) showing that field microbial activity had removed much of the available ^{14}C. This pattern of decline is influenced by the date of ^{14}C labeling. The ^{14}C data for the September labeled trees showed greater translocation below-ground.

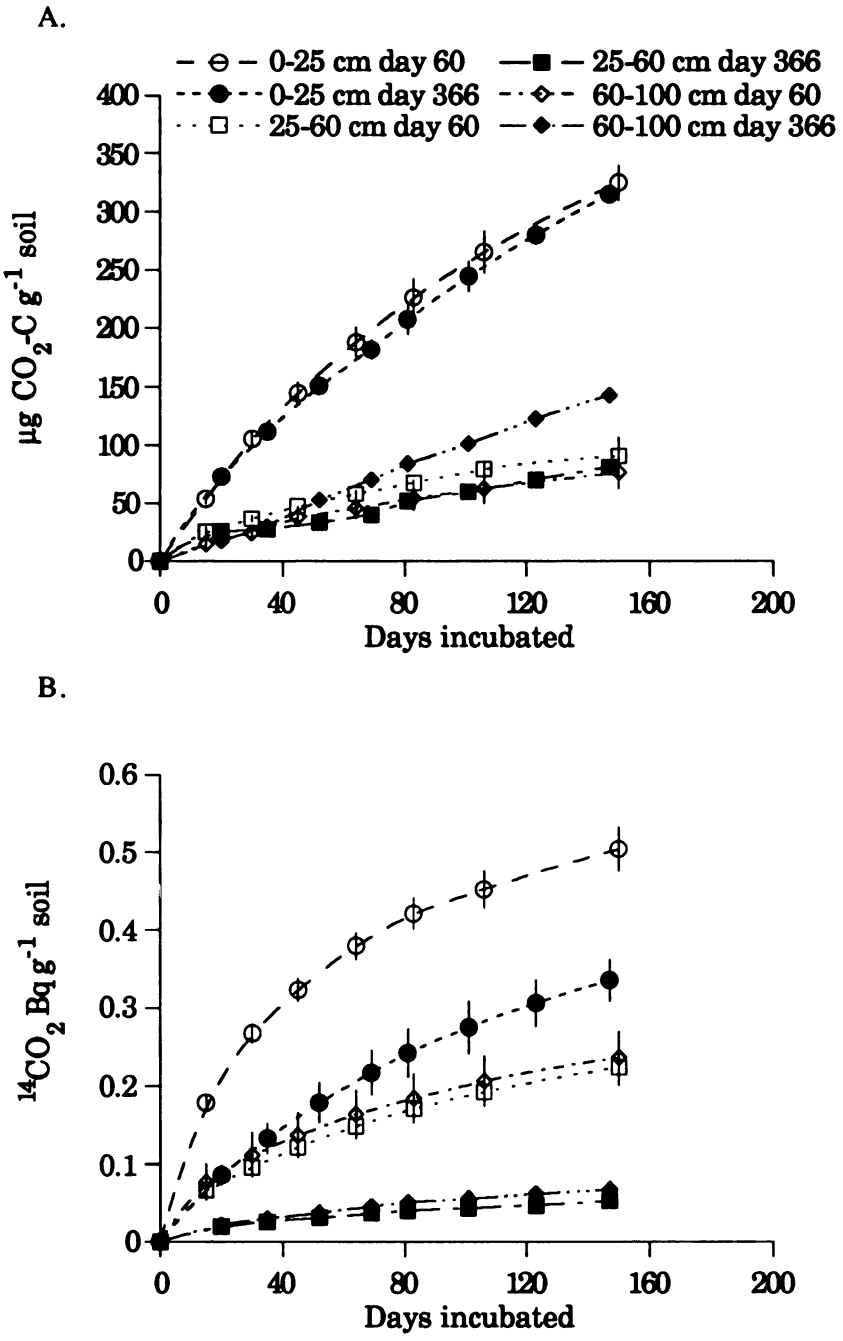


Figure 6.2. The C (Fig. 6.2a) and ^{14}C (Fig. 6.2b) mineralization data for the first (60 days) and second (366 days) long-term incubation of September labeled trees. The graph lines are simulated values calculated using equation 2. Standard error of the mean shown as line bars.

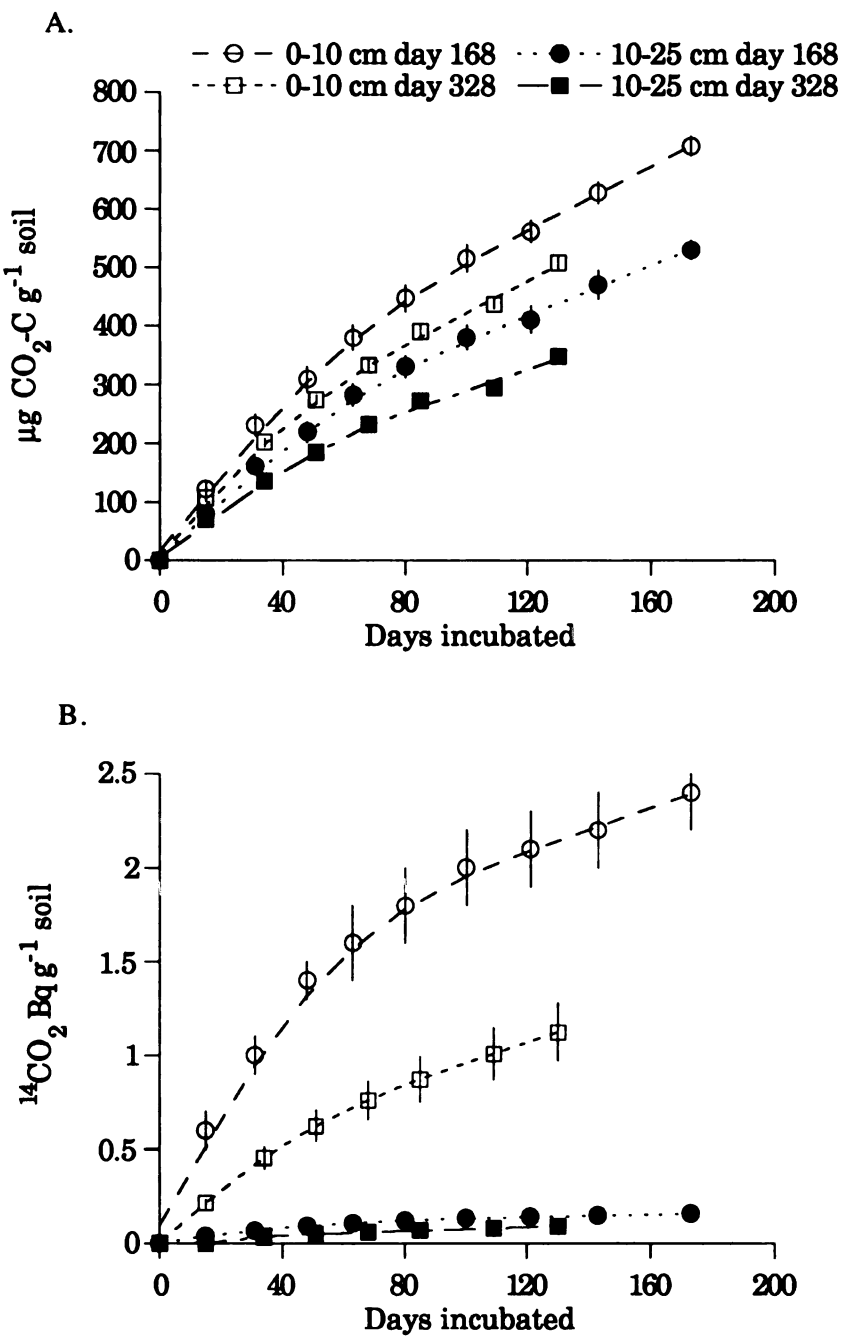


Figure 6.3. The C (Fig. 6.3a)and 14 C (Fig. 6.3b) mineralization data for the first (168 days) and second (328 days) long-term incubation of September litter exchange. The graph lines are simulated values calculated using equation 2. Standard error of the mean shown as line bars.

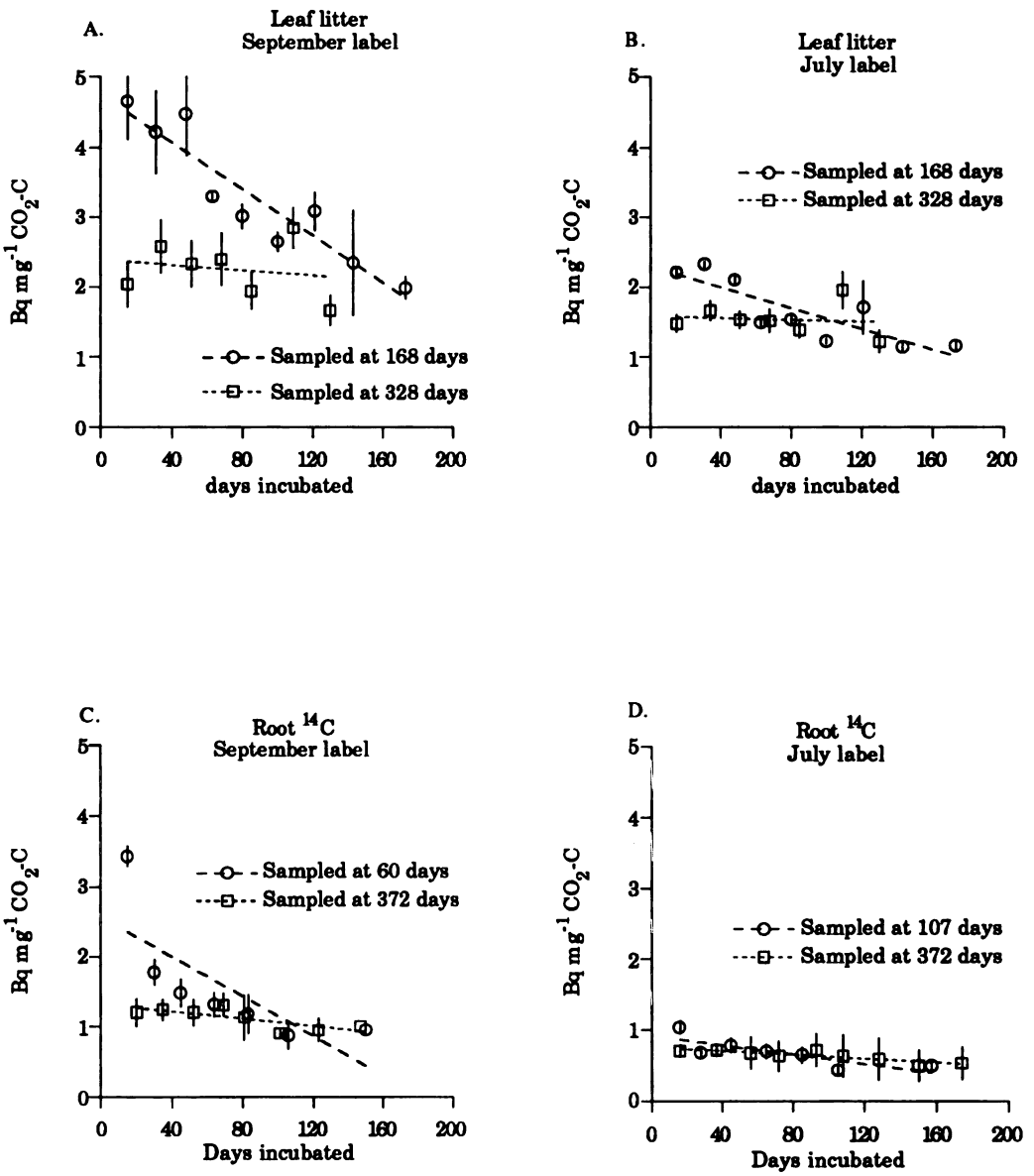


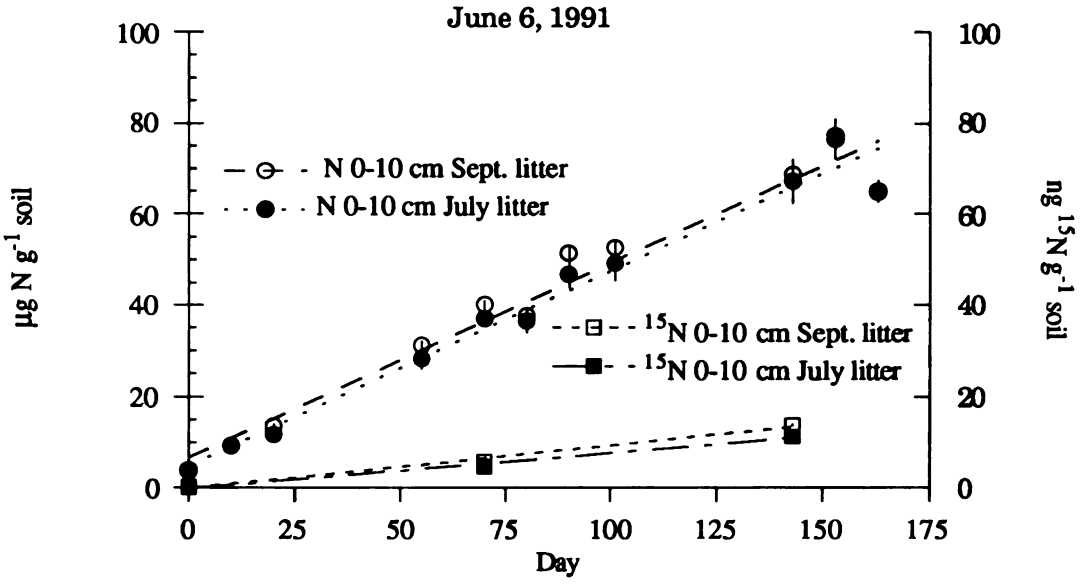
Figure 6.4. The decline in specific activity of mineralized C during the long-term incubation of soils labeled through leaf and root derived C. Figure 6.4a and 6.4b are from leaf litter plots and 6.4c and 6.4d are from plots from the whole tree labeling. Standard error of the mean shown as line bars.

The differences in the specific activity of root and leaf derived C indicate that the disposition of assimilated ^{14}C is depended on growing season and phenology as discussed in Chapter 5. The consistency in slope of specific activity in soil incubated for 300 days from the field shows that this material had become equilibrated with constituents of SOM fractions.

N Dynamics

N mineralization occurred at a constant rate for all the long-term soil incubations (Figure 6.5,6.6; Appendix B.11-B.15). The N was mineralized at a constant rate of 0.426 $\mu g\ N\ g^{-1}\ d^{-1}$ soil for surface soil of all plots even though C mineralization data had shown differences between root and leaf litter. Subsurface soils released 0.08 $\mu g\ N\ g^{-1}\ d^{-1}$. The lack of first order kinetics suggests a large potentially mineralizable N pool. The recently incorporated ^{15}N from leaf litter decomposition also displayed a linear rate of mineralization during the long-term soil incubations (Figure 6.5). The similar response of native N and recently incorporated N suggests that the potentially mineralizable N pool is being utilized similarly. However, the mineralization of unlabeled and labeled material display different slopes or rates of accumulation. These rate differences may be attributable to the spatial differentiation of more recently incorporated N. The turnover of ^{15}N in from root labeled soil was not detectable in the incubation. The N content of the roots, especially fine roots, was not large enough to enrich soil N pools through turnover (Chapter 4). Similarly, labeled N was not detected below 10 cm in leaf litter labeled soil. The lack of N movement past 10 cm soil depth is probably due to efficient microbial immobilization and tree uptake. Since some of the ^{14}C moved to 10-25 cm and ^{15}N did not, one

A.



B.

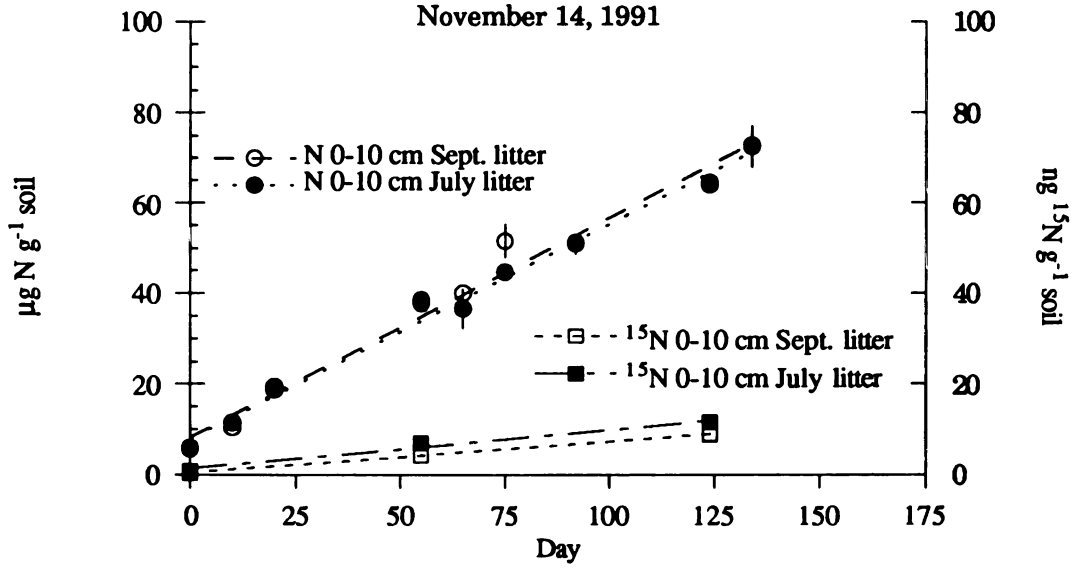
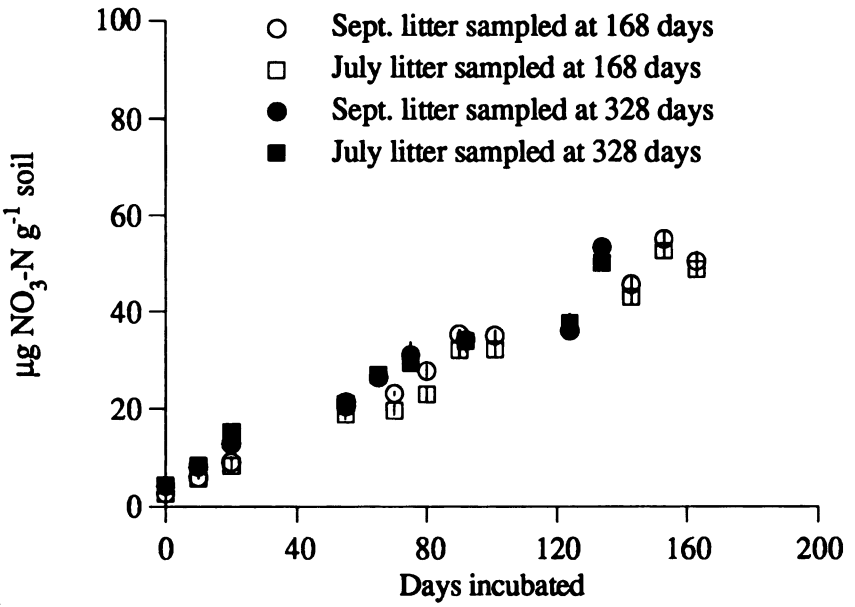


Figure 6.5. Cumulative N and ^{15}N mineralization curves from long-term soil incubations of surface soil (0-10 cm) from the litter exchange experiment. Figure 6.5a represents the first sampling on June 6, 1991 and figure 6.5b the sampling done on November 14, 1991 of both September and July litter. Standard error of the mean shown as line bars.

A.



B.

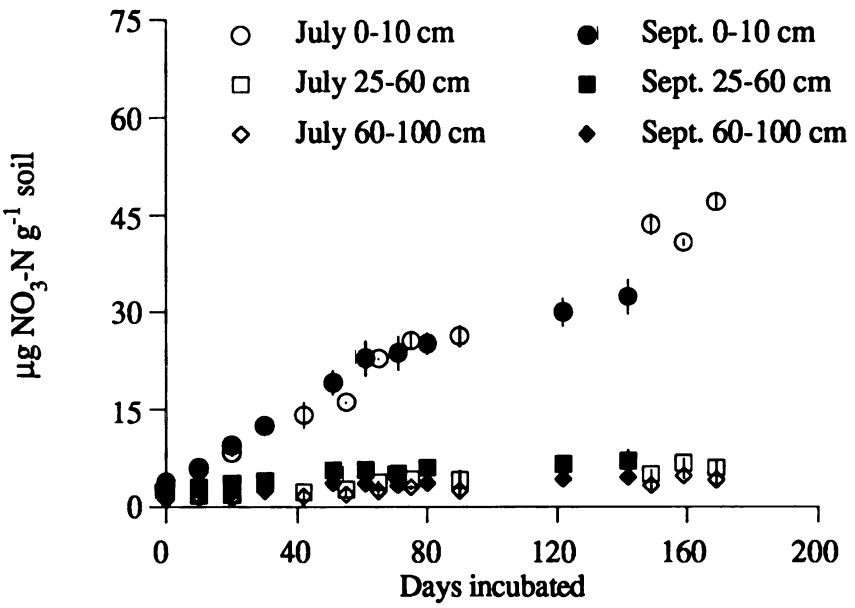


Figure 6.6. Nitrogen mineralization data for all the subsurface (10-25 cm) samplings of the leaf litter exchange (Fig 6.6a) and all soil depths of the first long-term incubation of July and September labeled tree plots (Fig. 6.6b). Soil depth and dates are indicated. Standard error of the mean shown as line bars.

would speculate that the ^{14}C movement was by leaching of high C material. Soil fauna movements should have moved equal concentrations of both ^{14}C and ^{15}N through the soil profile.

Microbial Biomass C and N

The microbial biomass C measurements at day zero of the long-term incubations corroborate the field measurements discussed earlier. On incubation, the biomass C dropped in the surface soil samples (Tables 6.6-6.9) by approximately one third. Subsurface samples of the July label did not show a similar drop, but those from the September label did (Table 6.7). The leaf litter experiments again showed the increase in biomass C (Table 6.8) and the incubations of the later samplings showed that the biomass was at similar levels to the tree plots.

Biomass N again showed somewhat similar results to that of biomass C (Tables 6.9-6.11). The microbial biomass N from subsurface soils of the July labeled trees increased upon incubation in the lab. Conversely, the subsurface soil from the September labeled trees dropped in biomass N during lab incubations. The microbial biomass N from the leaf litter plots behaved similarly to the surface soils of both July and September labeled trees.

The specific activity of the microbial biomass N (Tables 6.11a, b) was similar to that of biomass C, dropping during the long-term incubation (Tables 6.6-6.8). The drop in biomass C is an artifact of the extended incubations and is attributable to the lack of soil C input. The drop in specific activity of the biomass C shows turnover and increased dependence on nonlabeled soil C pools.

Table 6.6. Microbial biomass C, specific activity and percent of applied ^{14}C from long term soil incubations of tree plots labeled July 19, 1990. Standard error of the mean shown in parentheses.

Soil depth (cm)	Days in field	Days incubated	Biomass C μg C/0.41 g^{-1} soil	Bq mg^{-1} biomass C g^{-1} soil	% of applied ^{14}C*
0-25		0	186.1 (7.7)	1.1 (0.22)	1.62 (0.19)
	107	51	152.5 (13.5)	0.5 (0.11)	0.55 (0.15)
		124	137.3 (9.4)	0.3 (0.04)	0.37 (0.05)
		0	213.3 (19.5)	0.5 (0.11)	0.84 (0.02)
	372	55	195.8 (3.5)	0.4 (0.00)	0.67 (0.11)
		149	139.7 (2.2)	0.4 (0.09)	0.48 (0.03)
25-60		0	27.2 (9.3)	2.0 (0.96)	0.62 (0.11)
	107	51	29.0 (11.3)	0.7 (0.42)	0.24 (0.08)
		124	36.9 (6.7)	0.4 (0.17)	0.19 (0.06)
		0	65.0 (28.7)	0.5 (0.23)	0.34 (0.05)
	372	55	44.5 (16.6)	0.4 (0.19)	0.23 (0.00)
		149	52.6 (2.9)	0.3 (0.17)	0.19 (0.10)
60-100		0	7.6 (7.7)	4.2 (2.46)	0.31 (0.11)
	107	51	14.1 (8.6)	0.7 (0.20)	0.11 (0.07)
		124	18.0 (8.0)	0.4 (0.08)	0.11 (0.06)
		0	23.3 (9.1)	0.8 (0.32)	0.21 (0.04)
	372	55	22.6 (5.6)	1.2 (0.86)	0.13 (0.03)
		149	25.4 (2.1)	0.3 (0.03)	0.08 (0.01)

* Based on the amount of ^{14}C found in the root system and soil from the first sampling.

Table 6.7. Microbial biomass C, specific activity and percent of applied ^{14}C from long term soil incubations of tree plots labeled September 5, 1990. Standard error of the mean shown in parentheses.

Soil depth (cm)	Days in field	Days incubated	Biomass C μg C/0.41 g^{-1} soil	Bq mg^{-1} biomass C g^{-1} soil	% of applied ^{14}C*
0-25		0	189.8 (28.1)	2.5 (0.38)	1.16 (0.12)
	60	51	118.8 (8.6)	1.4 (0.14)	0.41 (0.05)
		124	110.0 (10.1)	1.1 (0.19)	0.31 (0.05)
		0	219.0 (9.9)	0.7 (0.12)	0.57 (0.28)
	366	61	162.5 (29.5)	0.6 (0.00)	0.26 (0.06)
		151	104.8 (1.3)	0.6 (0.13)	0.19 (0.05)
25-60		0	79.7 (10.8)	2.2 (0.29)	0.66 (0.07)
	60	51	43.2 (6.1)	2.5 (0.42)	0.37 (0.06)
		124	47.1 (15.8)	1.9 (0.89)	0.31 (0.03)
		0	70.0 (0.6)	0.5 (0.10)	0.17 (0.03)
	366	61	62.2 (9.4)	0.4 (0.10)	0.12 (0.01)
		151	36.0 (3.9)	0.6 (0.08)	0.09 (0.01)
60-100		0	50.2 (6.8)	2.3 (0.38)	0.49 (0.02)
	60	51	16.8 (1.1)	5.4 (1.27)	0.30 (0.02)
		124	20.7 (4.6)	3.3 (0.69)	0.27 (0.02)
		0	37.8 (6.0)	0.8 (0.27)	0.13 (0.01)
	366	61	35.1 (3.5)	0.5 (0.07)	0.08 (0.03)
		151	12.0 (4.7)	1.8 (0.63)	0.07 (0.00)

* Based on the amount of ^{14}C found in the root system and soil from the first sampling.

Table 6.8. Microbial biomass C, specific activity and percent of applied ^{14}C from long term soil incubations of the litter exchange plots. Standard error of the mean shown in parentheses.

Soil depth (cm)	Days in field	Days incubated	Biomass C µg C/0.41 g^{-1} soil	Bq mg^{-1} biomass C g^{-1} soil	% of applied ^{14}C*
September litter exchange					
		0	346.3 (17.0)	4.70 (0.78)	3.68 (0.66)
0-10	168	70	186.1 (19.8)	4.98 (0.52)	2.08 (0.42)
		143	195.4 (6.1)	3.04 (0.13)	1.34 (0.18)
		0	219.3 (42.2)	4.42 (0.98)	2.02 (0.22)
	328	55	186.5 (15.2)	3.61 (0.18)	1.47 (0.29)
		124	199.1 (7.0)	2.44 (0.29)	1.10 (0.25)
		0	248.9 (10.3)	0.59 (0.32)	0.41 (0.15)
10-25	168	70	149.8 (62.5)	0.52 (0.19)	0.18 (0.08)
		143	160.7 (12.1)	0.38 (0.21)	0.17 (0.07)
		0	163.7 (32.1)	1.06 (0.82)	0.33 (0.10)
	328	55	146.9 (22.8)	0.55 (0.19)	0.22 (0.05)
		124	154.5 (39.0)	0.46 (0.18)	0.22 (0.12)
July litter exchange					
		0	355.9 (31.8)	2.34 (0.37)	1.82 (0.47)
0-10	168	70	211.4 (42.3)	2.43 (0.77)	1.03 (0.11)
		143	204.7 (37.1)	1.32 (0.06)	0.62 (0.10)
		0	261.3 (80.6)	2.80 (1.46)	1.40 (0.14)
	328	55	189.5 (18.4)	2.33 (0.55)	1.00 (0.13)
		124	219.5 (17.9)	1.32 (0.18)	0.71 (0.10)
		0	216.4 (20.3)	0.37 (0.21)	0.24 (0.16)
10-25	168	70	148.8 (18.3)	0.16 (0.09)	0.06 (0.03)
		143	156.4 (11.2)	0.16 (0.05)	0.08 (0.02)
		0	146.3 (35.3)	0.68 (0.44)	0.20 (0.06)
	328	55	125.9 (34.4)	0.47 (0.15)	0.16 (0.01)
		124	165.2 (16.7)	0.28 (0.04)	0.13 (0.02)

* Based on the amount of ^{14}C found in the leaf litter.

Table 6.9. Microbial biomass N from the long-term incubations of soil from the tree plots labeled July 19, 1990. Standard error of the mean shown in parentheses.

Soil depth (cm)	Days in field	Days incubated	Biomass N $\mu g\ N/k_n$* g^{-1} soil
0-25		0	32.1 (1.2)
	107	51	26.6 (2.0)
		124	24.2 (1.5)
		0	34.9 (0.1)
	372	55	34.7 (1.1)
		149	24.9 (0.0)
25-60		0	4.4 (1.4)
	107	51	5.3 (0.2)
		124	6.2 (1.0)
		0	9.7 (3.8)
	372	55	7.9 (2.7)
		149	8.7 (0.7)
60-100		0	1.4 (1.3)
	107	51	2.3 (1.6)
		124	2.9 (1.1)
		0	3.6 (1.1)
	372	55	3.9 (0.9)
		149	4.2 (0.1)

* K_n=0.148*(fumigated CO_2 flush/fumigated NH_4^+ flush)+0.39

Table 6.10. Microbial biomass N from the long-term incubations of soil from the tree plots labeled September 5, 1990. Standard error of the mean shown in parentheses.

Soil depth (cm)	Days in field	Days incubated	Biomass N $\mu g\ N/k_n$* g^{-1} soil
0-25		0	33.0 (4.4)
	60	51	21.2 (1.9)
		124	19.7 (2.0)
		0	37.6 (1.6)
	366	55	29.1 (4.5)
		149	19.0 (0.4)
25-60		0	11.1 (0.6)
	60	51	7.0 (1.4)
		124	8.1 (2.6)
		0	9.6 (0.6)
	366	55	10.3 (1.4)
		149	5.6 (0.8)
60-100		0	6.9 (0.9)
	60	51	2.9 (0.3)
		124	3.5 (0.6)
		0	5.8 (1.1)
	366	55	5.8 (0.7)
		149	2.0 (0.8)

* K_n=0.148*(fumigated CO_2 flush/fumigated NH_4^+ flush)+0.39

Table 6.11a. Microbial biomass N, ^{15}N and percent of applied ^{15}N from soil incubations of September litter plots. Standard error of the mean in parentheses.

Soil depth (cm)	Days in field	Days incubated	Biomass N μg N/k_n* g^{-1} soil	Biomass ^{15}N ng $^{15}NH_4^+$-N g^{-1} soil	% of applied ^{15}N§
September litter exchange					
0-10		0	60.3 (3.0)	9.1 (1.9)	8.2 (1.2)
	168	70	34.4 (3.1)	4.2 (1.2)	3.7 (0.8)
		143	33.9 (0.9)	2.8 (0.4)	2.2 (0.4)
		0	40.7 (6.6)	4.8 (2.1)	4.3 (1.8)
	328	55	34.0 (2.7)	3.6 (1.5)	3.3 (1.6)
		124	34.5 (0.8)	3.3 (1.5)	2.9 (1.4)
10-25		0	42.9 (1.9)		
	168	70	27.4 (10.3)		
		143	28.3 (1.9)		
		0	30.0 (5.2)		
	328	55	26.7 (3.7)		
		124	27.4 (6.5)		

* K_n=0.148*(fumigated CO_2 flush/fumigated NH_4^+ flush)+0.39

§ Based on the amount of ^{15}N in the litter.

Table 6.11b. Microbial biomass N, ^{15}N and percent of applied ^{15}N from soil incubations of July litter plots. Standard error of the mean in parentheses.

Soil depth (cm)	Days in field	Days incubated	Biomass N μg N/k_n* g^{-1} soil	Biomass ^{15}N ng $^{15}NH_4^+$-N g^{-1} soil	% of applied ^{15}N§
July litter exchange					
0-10		0	62.6 (5.5)	8.5 (0.5)	4.4 (0.3)
	168	70	39.0 (6.9)	4.4 (1.6)	2.3 (0.8)
		143	35.8 (5.8)	2.9 (1.0)	1.5 (0.5)
		0	42.0 (6.5)	6.9 (2.2)	4.0 (1.2)
	328	55	35.1 (3.0)	3.7 (2.3)	2.1 (1.3)
		124	38.2 (3.1)	4.1 (2.6)	2.1 (1.3)
10-25		0	37.6 (3.5)		
	168	70	27.4 (3.0)		
		143	27.4 (2.0)		
		0	27.2 (5.7)		
	328	55	23.3 (5.7)		
		124	28.6 (2.7)		

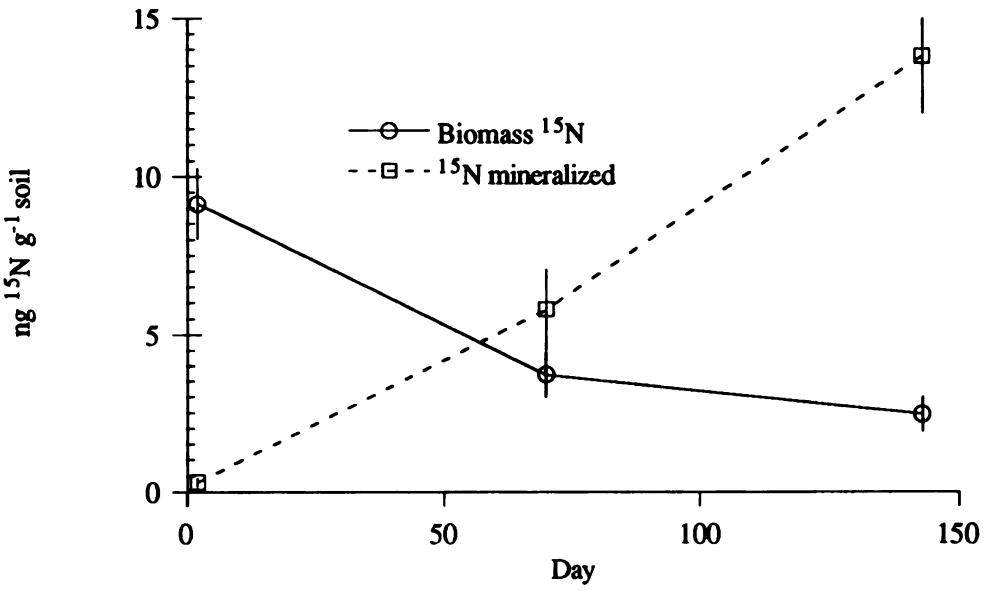
* K_n=0.148*(fumigated CO_2 flush/fumigated NH_4^+ flush)+0.39

§ Based on the amount of ^{15}N in the litter.

The changes in the leaf litter experiment (Tables 6.11a, b) showed a four fold drop in biomass ^{15}N for the long-term incubations of soil sampled at 168 days. The soil sampled at 328 days showed lower amounts of ^{15}N in the biomass, but only a 30% drop during the lab incubation showing that similar results are found during field and laboratory exposure. The microbial biomass accounted for 8.2% of the ^{15}N at 168 days and only 4.3% of the biomass N at 328 days in the September litter. The July litter showed a similar drop in ^{15}N (ng g^{-1} soil), but did not show a similar drop when expressed as a percent of applied. The lack in the decrease in the amount of ^{15}N applied in the July litter is in most part attributable to the larger amount of labeled N in the leaf litter lain on the plots. The larger amount of ^{15}N found in the July leaf litter was a result of the increased sink strength for N of leaves during mid-growing season (Chapter 3 and 4).

The increase in mineralized ^{15}N corresponds to a decrease in microbial ^{15}N in the leaf litter plots (Figure 6.7). A similar decline in microbial biomass ^{14}C is associated with the accumulation of mineralized ^{14}C (Figure 6.8). This demonstrates the importance of the microbial biomass as a component of the labile C and N fraction of the SOM. The microbial biomass N pool may also represent the portion of the labile N fraction that mineralizes at a different rate. The loss of N from the microbial biomass amounts to approximately 50% of the total N mineralized in the 0-25 cm soil during the long-term soil incubations. About 25% of the C mineralized can be accounted for from the loss of microbial biomass C during the long-term incubations.

A.



B.

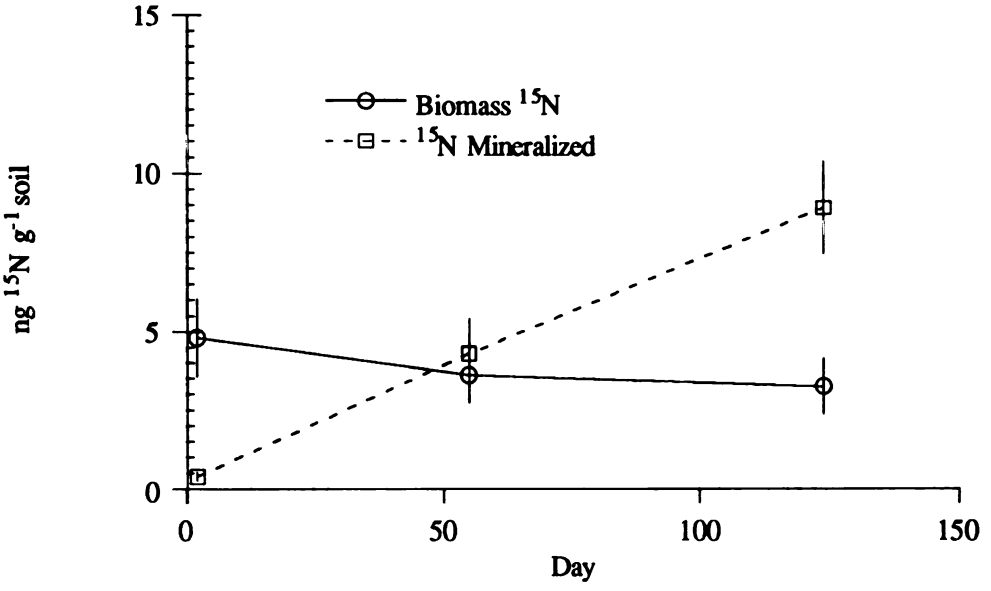
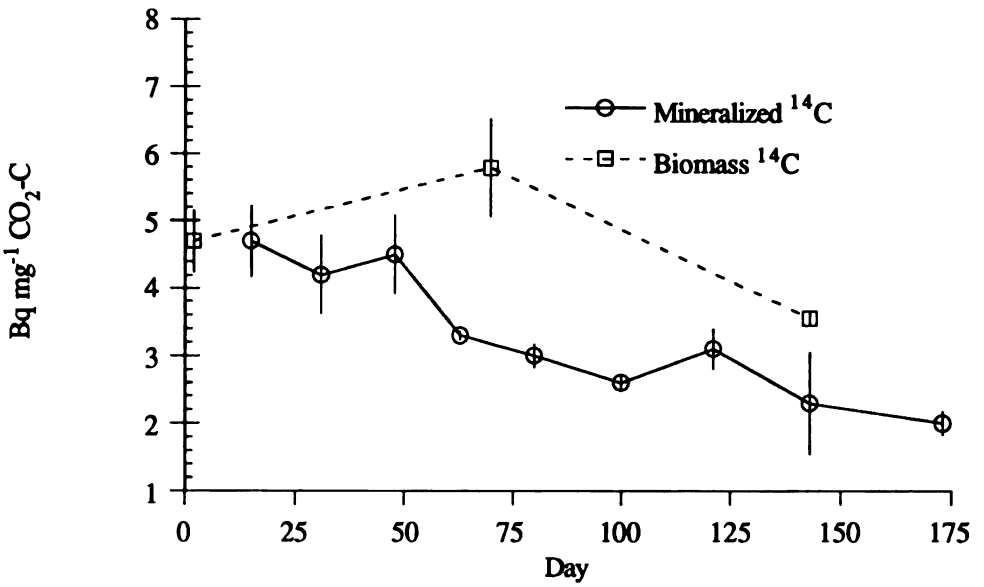


Figure 6.7. The amount of mineralized ^{15}N and microbial biomass ^{15}N for the surface soil of the September labeled litter. Figure A represents the first sampling on June 6, 1991 and figure B the sampling done on November 14, 1991. Standard error of the mean shown as line bars.

A.



B.

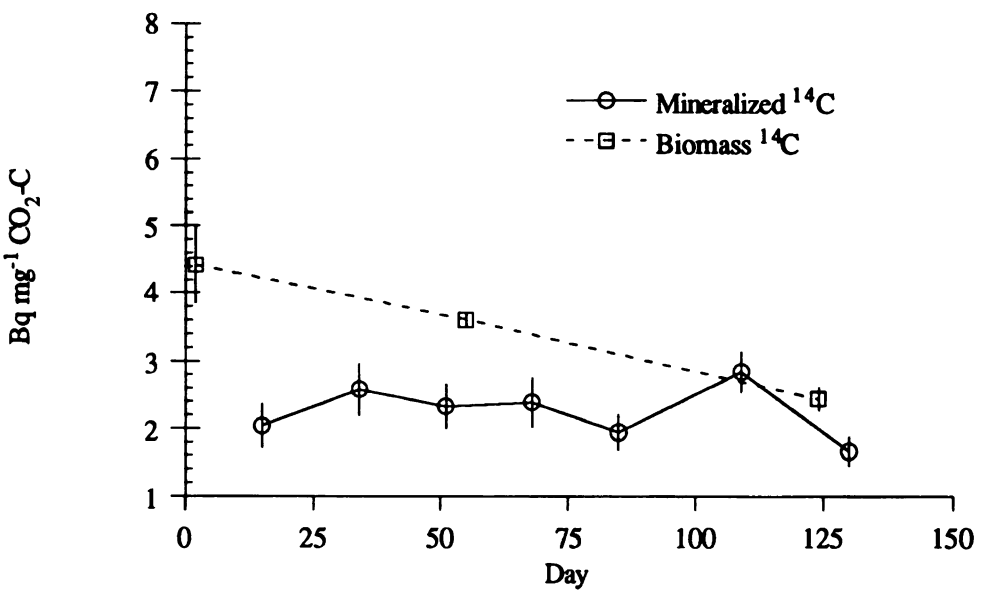


Figure 6.8. Specific activity of mineralized C and microbial biomass C for the surface soil of the September labeled litter. Figure A represents the first sampling on June 6, 1991 and figure B the November 14, 1991 sampling. Standard error of the mean shown as line bars.

Potentially Mineralizable C

The models to describe C mineralization produced good fits (Figures 2b and 3b), consequently, no difference between equations 1 and 2 were observed. Equation 2 was used to develop all of the rate parameters and pool sizes of SOM fractions. The C mineralization during the first 50 days of the long-term soil incubations followed first order kinetics. The amount of C mineralized during this period was approximately 50% of the total C mineralized. Zero and first order kinetics described the C mineralization during the latter part of the long-term incubations equally well. The mineralization of C at soil depths below 25 cm was substantially lower than the surface soil. This indicates that SOM pools are smaller in subsurface soils.

First order rate constants for labile unlabeled C (C_1) lie between 0.014-0.036 d^{-1} for surface soil above 25 cm for all plots using equation 2 (Table 6.12-6.14a). The first order rate constants for the C_1 pool below 25 cm are variable and ranged from 0.01-0.09 d^{-1}. The variability is associated with the low amount of C mineralized in subsurface soil and the possible greater effects of disturbance from sampling. Rate parameters for the more stable C pool (C_2) in surface soil ranged from 0.0001-0.0005 d^{-1}. The mean residence time (MRT) for the C_1 pool was 24-74 days for surface soil and 11-54 days for subsurface soils for all plots. The C_1 pool amounted to 1-3% of the SOM. The MRT of the C_2 pool was variable and lies between 6 to 23 years for all soil depths. The C_2 pool was 47-49% of total C in SOM as estimated by subtracting out the C_1 and considering the remaining soil C (50%) to be recalcitrant and not contributing to mineralized C during the incubations.

Table 6.12. Parameters developed from the modified exponential equation applied to the CO_2 mineralization data from long-term incubations of soil from the sampling of labeled tree plots. MRT is mean residence time.

Soil depth (cm)	Days in field	K_1 d^{-1}	K_2 d^{-1}	C_1 MRT d^{-1}	C_2 MRT y^{-1}	C_1 µg C g-1 soil	C_2 µg C g^{-1} soil
July labeled trees							
0-25	107	0.04	0.0004	26.9	7.6	112.3	5187.7
	372	0.04	0.0003	23.7	8.8	105.8	5194.2
25-60	107	0.09	0.0002	11.2	13.7	25.9	1824.1
	372	-	-	-	-	-	-
60-100	107	0.09	0.0003	11.2	9.8	29.1	1320.9
	372	-	-	-	-	-	-
September labeled trees							
0-25	60	0.01	0.0002	73.7	17.1	237.7	5062.3
	366	0.03	0.0003	33.2	8.8	85.0	5215.0
25-60	60	0.04	0.0002	26.5	14.4	39.7	1810.3
	366	-	-	-	-	-	-
60-100	60	0.02	0.0001	54.3	22.8	45.5	1304.5
	366	-	-	-	-	-	-

Table 6.13. Amount of C found in the soil microbial biomass of the trees labeled in July and September 1990 and C mineralized at 150 (C_{150}). C_{soil} is total soil C. Biomass C is C in the soil biomass.

Soil depth (cm)	Day	C_{150} $\mu g\ C\ g^{-1}$ soil	C_1/C_{soil} %	Biomass C/ C_{150} %	Biomass C/ C_{soil} %
July labeled trees					
0-25	107	384.61	1.06	48.4	1.8
	372	341.63	1.00	62.4	2.0
25-60	107	79.80	0.70	34.1	0.7
	372	111.96	-	58.1	1.8
60-100	107	80.23	1.08	9.5	0.3
	372	94.84	-	24.6	0.9
September labeled trees					
0-25	60	326.68	2.24	58.1	1.8
	366	321.02	0.80	68.2	2.1
25-60	60	90.47	1.07	88.1	2.2
	366	84.10	-	83.2	1.9
60-100	60	65.92	1.69	76.2	1.9
	366	148.97	-	25.4	1.4

Table 6.14. Parameters developed from the modified exponential equation applied to the CO_2 mineralization data from long-term incubations of soil from the sampling of leaf litter exchange plots. MRT is mean residence time. C_{soil} is total soil C.

Soil depth (cm)	Days in field	K_1 d^{-1}	K_2 d^{-1}	C_1 MRT d^{-1}	C_2 MRT y^{-1}	C_1 $\mu g\ C\ g^{-1}$ soil	C_2* $\mu g\ C\ g^{-1}$ soil
September litter exchange							
0-10	168	0.02	0.0005	46.6	5.7	308.0	5192.0
	328	0.03	0.0005	33.2	5.7	185.7	5314.3
10-25	168	0.02	0.0003	64.4	8.3	288.4	4761.6
	328	0.02	0.0003	49.1	10.1	186.0	4864.0
July litter exchange							
0-10	168	0.01	0.0003	68.4	8.3	421.6	5078.4
	328	0.02	0.0005	41.1	6.0	213.3	5286.7
10-25	168	0.02	0.0004	42.9	6.4	123.0	4927.0
	328	0.02	0.0003	59.3	8.8	177.2	4872.8

Table 6.14a Represents the amount of C found in the soil microbial biomass of the leaf litter exchange experiment and C mineralized at 150 (C_{150}) days. C_{soil} is total soil C. Biomass C is C in the soil biomass.

Soil depth (cm)	Day	C_{150} $\mu g\ C\ g^{-1}$ soil	C_1/C_{soil} %	Biomass C/ C_{150} %	Biomass C/ C_{soil} %
September litter exchange					
0-10	168	656.36	2.80	52.8	3.1
	328	552.88	1.69	39.7	2.0
10-25 10/25/1992	168	490.32	2.86	50.8	2.5
	328	370.29	1.84	44.2	1.6
July litter exchange					
0-10	168	619.74	3.83	57.4	3.2
	328	560.25	1.94	48.4	2.5
10-25	168	427.01	1.22	50.7	2.1
	328	384.45	1.75	38.1	1.4

The amount of C mineralized at 150 days (C_{150}) of incubation decreases 3 fold with soil depth. Microbial biomass C decreases 6 fold with depth (Tables 6.6 and 6.7). The microbial biomass C to C_{150} ranges from 0.1-0.88 from subsurface to surface soil, suggesting that the microorganisms in subsurface soil appear to account for proportionately more of the C mineralized during the long-term incubation. Both equations 1 and 2 did not describe C mineralization data from subsurface soil from the third sampling, one year latter, for the labeled tree plots. Cumulative C mineralization curves for these subsurface soils never approach asymptotic values (Figure 6.2a). Soil handling procedures and storage may influence subsurface soil to a greater extent because of their small potentially mineralizable C pools. The potentially mineralizable C is generally less than 3% of total soil C. This labile C pool is equivalent to the amount of C contained in the microbial biomass (Table 6.13-6.14b). Microbial biomass C accounts for approximately 50% of the C mineralized during 150 days of incubation, especially in surface soil, showing the potential cycling of C through the microbial biomass.

The first order rate constants for labeled C are similar to the rate constants for native C (Table 6.15 and 6.17a). However, the pool size of the recently incorporated C dwarfs that of the native C. As a result, a greater proportion of the small pool of recently incorporated C is mineralized during the long-term soil incubations, $^{14}C_o/^{14}C_{soil}$, where $^{14}C_o$ is the mineralizable ^{14}C and $^{14}C_{soil}$ is total ^{14}C in soil (Tables 6.17 and 6.17a). The potentially mineralizable ^{14}C and mineralization rate of more stable soil ^{14}C (C_2-^{14}C) was higher in leaf labeled soil. The labile ^{14}C pool (C_1-^{14}C) accounted for 1.8-12.6% of total soil ^{14}C in the labeled tree plots and 5.4-36% in leaf litter plots. The MRT of C_1-^{14}C is between 14-64 days for all soil

Table 6.15. Parameters developed from the modified exponential equation applied to the $^{14}CO_2$ mineralization data from long-term incubations of soil from the sampling of labeled tree plots. MRT is mean residence time.

Soil depth (cm)	Days in field	K_1 d^{-1}	K_2 d^{-1}	C_1-^{14}C MRT d^{-1}	C_2-^{14}C MRT y^{-1}	C_1-^{14}C Bq g^{-1} soil	C_2-^{14}C* Bq g^{-1} soil
July labeled trees							
0-25	107	0.04	0.0006	26.8	4.3	0.14	1.3
	372	0.03	0.0007	31.0	3.8	0.10	1.1
25-60	107	0.07	0.0002	13.9	12.5	0.03	0.6
	372	0.07	0.0004	14.6	6.8	0.03	0.7
60-100	107	0.05	0.0002	20.6	14.4	0.02	0.5
	372	0.07	0.0003	14.3	8.3	0.01	0.4
September labeled trees							
0-25	60	0.04	0.0006	22.7	4.4	0.31	2.2
	366	0.02	0.0003	54.8	9.4	0.21	3.3
25-60	60	0.05	0.0011	21.7	2.6	0.10	0.9
	366	0.05	0.0002	18.3	16.1	0.02	1.2
60-100	60	0.05	0.0010	19.6	2.8	0.11	0.9
	366	0.03	0.0005	29.9	5.7	0.03	0.5

* C_2-^{14}C calculated assuming no recalcitrant ^{14}C has formed during the duration of the experiment.

Table 6.16. Represents the amount of radiolabeled C found in the soil microbial biomass and ^{14}C mineralized at 150 days ($^{14}C_{150}$). $^{14}C_{soil}$ is total soil ^{14}C. Biomass ^{14}C is ^{14}C in the soil biomass.

Soil depth (cm)	Day	$^{14}C_{150}$ Bq ^{14}C g^{-1} soil	C_1-^{14}C/ $^{14}C_{soil}$ %	Biomass ^{14}C/ $^{14}C_{150}$ %	Biomass ^{14}C/ $^{14}C_{soil}$ %
July labeled trees					
0-25	107	0.26	9.45	82.2	14.5
	372	0.21	8.36	51.7	9.2
25-60	107	0.06	5.08	89.8	7.3
	372	0.07	4.01	42.7	4.1
60-100	107	0.03	3.56	57.7	3.6
	372	0.03	2.58	71.6	5.2
September labeled trees					
0-25	60	0.51	12.64	90.9	18.5
	366	0.34	6.13	55.9	5.4
25-60	60	0.23	9.88	75.4	17.5
	366	0.05	1.84	75.9	3.3
60-100	60	0.24	10.71	46.3	10.5
	366	0.07	5.64	44.2	5.4

Table 6.17. Parameters developed from the modified exponential equation applied to the $^{14}CO_2$ mineralization data from long-term incubations of soil from the sampling of the litter exchange plots. MRT is mean residence time.

Soil depth (cm)	Days in field	K_1 d^{-1}	K_2 d^{-1}	C_1-^{14}C MRT d^{-1}	C_2-^{14}C MRT y^{-1}	C_1-^{14}C Bq g^{-1} soil	C_2-^{14}C* Bq g^{-1} soil
September litter exchange							
0-10	168	0.02	0.0008	44.7	3.3	1.77	4.7
	328	0.02	0.0005	63.8	5.4	0.85	6.0
10-25	168	0.02	0.0006	44.2	4.4	0.12	0.4
	328	0.03	0.0001	34.5	22.8	0.03	0.5
July litter exchange							
0-10	168	0.02	0.0011	46.1	2.4	0.83	1.8
	328	0.02	0.0009	53.4	2.9	0.44	3.3
10-25	168	0.03	0.0017	31.8	1.6	0.08	0.1
	328	0.02	0.0013	44.0	2.2	0.04	0.4

* C_2-^{14}C calculated assuming no recalcitrant ^{14}C has formed during the duration of the experiment.

Table 6.17a. Amount of radiolabeled C found in the soil microbial biomass of the litter exchange experiment and ^{14}C mineralized at 150 ($^{14}C_{150}$). $^{14}C_{soil}$ is total soil ^{14}C. Biomass ^{14}C is ^{14}C in the soil biomass.

Soil depth (cm)	Day	$^{14}C_{150}$ Bq ^{14}C g^{-1} soil	C_1-^{14}C/ $^{14}C_{soil}$ %	Biomass ^{14}C/ $^{14}C_{150}$ %	Biomass ^{14}C/ $^{14}C_{soil}$ %
September litter exchange					
0-10	168	2.25	27.52	71.9	25.2
	328	1.21	12.44	73.8	13.1
10-25 10/25/1992	168	0.15	24.64	93.1	28.4
	328	0.04	5.36	284.7	21.5
July litter exchange					
0-10	168	1.09	30.92	76.5	31.0
	328	0.84	11.87	67.9	15.4
10-25	168	0.11	35.96	72.6	36.5
	328	0.10	9.52	76.6	18.9

depths and plots. The C_2-^{14}C pool accounted for 37.4-48.2% of total soil ^{14}C for both tree and leaf litter plots. The MRT for the C_2-^{14}C fraction was between 2-16 years.

The microbial biomass ^{14}C content was 44-91% in tree plots and 68-93% in leaf litter plots of the ^{14}C mineralized during 150 days of incubation. Again this shows the important aspect of cycling C through the microbial biomass. The litter labeled soil contains more labeled C in both microbial biomass and potentially mineralizable fractions than does the root labeled soil. This suggests that leaf litter incorporates more C into both labile and more stable SOM pools during the first year following the incorporation of labeled substrates to soil. However, as mentioned earlier, the incorporation of C into soil from leaf litter is spatially limited to the surface soil. Movement of labeled C through the soil profile is becoming evident in leaf labeled soil and can be seen in both biomass and fractions of SOM (Table 6.17b). The duration of the current experiment was not long enough to predict the magnitude of the contribution of leaf litter C to subsurface soils as compared to root derived C.

Table 6.18, shows the consistency of N mineralization over time and soil depth. The microbial biomass N and N mineralized at 150 days (N_{150}) accounted for 4-8% of total soil N in the surface soil of all plots. The microbial biomass N to N mineralized during 150 days of incubation was from 0.5-1.6, indicating the importance of microbial N as a component of labile N fraction and contribution to the potentially mineralizable N pool. The ratio of C_{150} to N_{150} generally declines with soil depth indicating that preferentially more C than N was mineralized deeper in the soil profile. The differences in C and N mineralization occurred at a relatively constant

Table 6.18. N mineralized was calculated by linear regression. N_{150} is the N mineralized 150 days. N_{soil} is total soil N. Biomass N/N_{150} is a ratio.

Soil depth (cm)	Days in field	N mineralized at 150 days $\mu g\ g^{-1}$ soil	N_{150}/N_{soil}. %	Biomass N/ N_{soil} %	Biomass N: N_{150}
July labeled trees					
0-25	107	35.85	4.48	4.01	0.90
	372	41.35	5.17	4.36	0.84
25-60	107	3.28	0.99	1.33	1.34
	372	6.17	1.87	2.39	1.28
60-100	107	2.23	0.89	0.56	0.63
	372	4.13	1.65	1.44	0.87
September labeled trees					
0-25	60	37.81	4.73	4.13	0.87
	366	40.80	5.10	4.70	0.92
25-60	60	7.70	2.33	3.36	1.44
	366	6.24	1.89	2.91	1.54
60-100	60	5.01	2.00	2.76	1.38
	366	3.61	1.44	2.32	1.61
Litter exchange experiment					
Soil depth (cm)	Days in field	N mineralized at 150 days $\mu g\ g^{-1}$ soil	N_{150}/N_{soil}. %	Biomass N/ N_{soil} %	Biomass N: N_{150}
September litter exchange					
0-10	168	68.87	6.82	5.97	0.88
	328	76.99	7.62	4.03	0.53
10-25	168	49.78	5.41	4.66	0.86
	328	51.61	5.61	3.26	0.58
July litter exchange					
0-10	168	67.18	6.65	6.20	0.93
	328	79.66	7.89	4.91	0.62
10-25	168	48.52	5.27	4.09	0.77
	328	52.67	5.73	2.96	0.52

C:N in the microbial biomass. This indicates that N may have been limiting in subsurface soil.

DISCUSSION

This research was established to measure the contribution of tree root and leaf litter to microbial biomass and SOM dynamics. The pools of C and N were examined using long-term laboratory incubations of a field labeled tree-soil system. Additionally, I were interested in comparing and estimating the amount of C and N in microbial biomass. There are a large number of CFI and CFE studies, but these are rarely standardized against direct microscopy, against each other or conducted on subsurface soil. The intent of these comparisons was to select and verify a consistent assay for both the determination of microbial biomass size and content of tracer ^{14}C and ^{15}N.

Microbial Biomass Determinations

Fungal biomass values of 153-215 μg C g^{-1} soil and bacterial biomass of 30-57.8 μg C g^{-1} soil in the temperate grasslands of Canada (data recalculated from Paul et al., 1979) are similar to our results. Babiuk and Paul (1970) found bacterial numbers of 2.2-4.6 10^9 g^{-1} in 0-30 cm grassland surface soil and 1.2 x 10^9 g^{-1} soil at 75-90 cm. My bacterial numbers are similar for surface soils , but much lower for subsurface soils. Variations in total hyphal lengths, bacterial numbers and diameters will be influenced by soil physiochemical characteristics and nature of the plant community. Hyphal diameters have been shown to decrease with decreasing amounts of organic matter present in the soil (Schnürer *et al.*, 1985). These

researchers found that hyphal diameters ranged from 1.6 to 2.7 μm in arable soils including a fallow treatment. The hyphal diameters averaged 2.3 μm for surface soils (0-25 cm) across a range of cultural treatments on the same soil type (Harris, private communication). No hyphal diameter estimates were done on subsurface soils in this study. Hyphal diameters may have been influenced by the decreasing amount of organic matter in the subsurface soil.

The degree of hyphal vacuolization can also influence fungal C estimates. I did not separate active from inactive hyphae. The viability of fungi can be determined with flourecien diacetate which is cleaved to flourecien by cytoplasmic esterases in living hyphae. This technique has shown that between 2-10 % of hyphae contain cytoplasm (Schnürer *et al.*, 1985; Paul and Clark, 1989). If the hyphal cytoplasmic content changed with soil depth and cytoplasm represents such a low percentage of the fungi we must ask why all of our microbial biomass measurements give such well correlated results.

Long-Term Soil Incubations

The mineralization rate of C and N will be affected by the quality and amount of organic C and N. The labeling of trees in the field made it possible to examine the steady state flux of C and N from tree litters into two of the active fractions (C_1 and C_2) of the SOM. The size of the microbial biomass declines in all of the long-term soil incubations. A constant C/N indicates that the representatives of the microbial population are diminishing at a constant rate. The decline in biomass C and N is typical of long-term soil incubations (Carter and Rennie, 1982; Bonde *et al.*, 1988; Robertson *et al.*, 1988) and is thought to contribute to the C and N

mineralization (Paul 1984; Juma and Paul, 1984; Bonde and Rosswall, 1987).

In this study, a substantial amount of the N mineralized (35-69 μg N g^{-1} soil) could be accounted for by the decrease in microbial biomass N over the long-term incubation in surface soil of all plots. In subsurface soil, the amount of N mineralized during 150 days of incubation was equal to biomass N. The decline in biomass C accounted for approximately half the C mineralized during the incubations. Under field conditions the biomass is regenerated by the addition of new substrate. However, the size of the microbial biomass is sufficiently large to act as a temporary pool of C and N. This reservoir of C and N plays an important role in supplying nutrients for plant uptake.

The rate of N mineralization remained unchanged during the long-term soil incubations. As a consequence, pools of active and stable N were mathematically undefinable. The mineralization of recently incorporated ^{15}N from leaf litter decomposition behaved similarly. However, the calculated slopes from the mineralization of labeled and unlabeled N indicated that the rates of mineralization differed. The difference in mineralization rates of labeled and unlabeled N suggest that the recently incorporated ^{15}N resides in a separate pool of organic N such as microbial biomass (Juma and Paul, 1984; He *et al.*, 1988). My data indicated that a decline in microbial biomass ^{15}N corresponded to an increase in mineralized ^{15}N. The exchange of ^{15}N between these pools exemplifies the importance of the microbial biomass as an important labile reservoir of N.

The trees exhibited no change in total N from the second to third growing season (Chapter 4). The loss of N through leaf fall amounted to approximately 60% or 12.8 g N of total tree N. The microbial biomass

contained 19.4 g N m^{-2} or one and one half times one years litter fall. The amount of N mineralized during 150 days of laboratory incubation was equivalent to the amount of N found in the microbial biomass in the surface soil. The comparison of 150 days of laboratory incubation to one year of field conditions indicates that sufficient N mineralization would be available to replace the loss of N through leaf fall.

Potentially Mineralizable Pools of C

First order rate kinetics have been used in simulation models to describe the decomposition of organic matter (Jenkinson and Rayner, 1977; Paul and van Veen, 1978). The use of first order models implies that the ability of the microbial biomass to decompose plant residues is not rate limiting (Paul and Clark, 1989). These attributes of the microbial biomass include large numbers and rapid growth rate (Voroney *et al.*, 1989). Models used to describe potential C mineralization included both mixed (first and zero order) and modified double exponential components. The mixed order and double exponential equations both described C and ^{14}C mineralization well. The unlabeled and labeled C mineralization curves were fit well using both models. Bonde et al. (1988) comparing both mixed and double exponential models found that the mixed order model gave the best fit to N mineralization data except in straw amended soil. The mixed model described the linear characteristics of the N mineralization in their soil. The constant rate of N mineralization was attributed to a constant rate of fungal hyphae growth without an equivalent increase in cytoplasm (Bonde and Rosswall, 1987).

N mineralization failed to approach first order kinetics during our long-term soil incubations indicating that C and N mineralization kinetics

are different and that the N pool was very large. Nitrogen and C mineralization also proceeded at different rates within the soil profile. Proportionately less N was mineralized in subsurface soil. Most studies on N availability have concentrated on surface soils. This study indicates that subsurface soil N mineralization behaves differently to surface soil. A refined approach to potential N mineralization potential should include N mineralization in subsurface soil and C and N relationship of the microbial biomass to accurately define potential N mineralization.

The labeled and unlabeled C had similar first order decay rates describing C_1 fraction in the surface soil. In contrast, decay rates were higher for recently incorporated C into the more stable C_2 SOM fraction. The higher decay rate for the recently added C in the C_2 SOM pool indicates that this C does not have the same characteristics as the entire resistant fraction. This observation was seen for the incubation of labeled residues (Jenkinson 1965) and microbial products in soil (Shields *et al.*, 1974; McGill *et al.*, 1975; van Veen *et al.*, 1987). These results can be explained by the fact that the equations describe the C mineralization curves well, but may oversimplify the processes occurring (Voroney *et al.*, 1989). The equations do not take into account the formation and decay of the microbial biomass, production of stable organic matter and or protection and priming of substances in the presence of others. Additionally, the individual reactions influencing the rate of decomposition and stabilization of C into SOM fractions may not follow first order kinetics, rather sum into a first order description of the entire process.

It is difficult to exclude microbial biomass C and N from the measure of the potentially mineralizable pools. Indeed, the microbial biomass maybe an excellent indicator of the potentially mineralizable pools (Paul, 1984;

Bonde *et al.*, 1988). The reproducibility of the biomass and mineralization rates make their combination an excellent parameter in determining SOM dynamics. These attributes were very useful in detecting the difference between root and leaf litter labeled soil.

The root labeled soil from the September labeled trees contained more potentially mineralizable labeled C than the July root labeled soils and is similar to leaf labeled soil. The increased ^{14}C in this soil was evident shortly after the September labeled trees had assimilated ^{14}C (Table 6.4). The increased enrichment of microbial biomass with ^{14}C is probably a result of the increased export of C to the root system during autumn. The rapid incorporation of ^{14}C by the microbial biomass may have been a result of the assimilated C leaking into the rhizosphere. An increase in soluble sugars occurs in the root system at this time of year and may explain the above scenario (Chapter 5). The mineralization rate of ^{14}C and microbial ^{14}C subside during the following season and are similar to the July root labeled soil.

CONCLUSIONS

The comparison of microbial biomass determination methods showed good agreement. No significant differences and high correlation values indicated that the different constants and variables used were applicable across the soil depths analyzed. The use of a partial control for the CFI method produced uncannily similar values to direct microscopy results. The comparison of CFI and CFE methods were also very similar despite the subtraction of a control in the CFE method.

Long-term soil incubations of the root and leaf litter labeled soil was a useful method to characterize the pools of SOM. In combination with labeled C and N, this method is able to distinguish the fate of recently incorporated soil C and N. The comparison of root versus leaf litter labeled soil indicates that leaf derived C and N has enriched SOM pools to a greater extent than root labeled soil. Both the kinetic analysis of pool size and mineralization of recently incorporated C and N confirm that in the year following the dual labeling of trees with ^{15}N and ^{14}C, leaf litter has conserved more organic matter and contributed a greater quantity of C and N to potential nutrient cycling processes.

The amount of N mineralized during the long-term incubations was sufficient to supply the N uptake of trees during the next season of growth. The substrate to drive potential N mineralization in the initial years of plantation establishment was determined to be dominantly leaf litter. This conclusion was reached from results on C and N allocation patterns determined for these trees. However, the root labeled soil has enriched the SOM to greater soil depths. The spatial difference in the quantity of C and N recycled by root and leaf litter suggests these litters may have different roles or strategies that conserve SOM in the soil solum. Movement of recently incorporated C from leaf litter past 25 cm soil will probably occur as time progresses.

It is evident from the duration of this experiment that the final contribution of C and N from root and leaf litter to SOM conservation was not determined. Future research in this area will require extended periods of study beyond one year to fully assess the contribution of root and leaf litter to the conservation of SOM and nutrient cycling processes.

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Chapter 7

CONCLUSION

The interpretation of C, N and other associated nutrient cycling processes has become a necessity in systems managed for increased productivity. The managed ecosystem often contains limited plant species and is subject to an increase in cultural inputs. The practical management of ecosystems designed to increase production ideally predicates embodying the concept of sustainable nutrient cycles. Intensively managed ecosystems may require a compromise between sustainability and reliance on cultural inputs which may have environmental pollution control potential.

The study of litter turnover, nutrient cycling and SOM dynamics has been a theme of countless studies endeavoring to elucidate the functions and processes underlying the nutrient cycles of ecosystems. Unfortunately, the lack of suitable techniques and limited examination of long-term field processes has led to an incomplete understanding of sustainable nutrient cycles. Nutrient cycles are strongly influenced by the production and turnover of necromass from primary producers. The simultaneous growth and decomposition of nutrient-rich plant tissue is a difficult process to resolve using periodic sampling methods to examine the change or loss of standing biomass. These problems are exemplified in the study of below-ground systems characterized by spatially complex root systems undergoing continual production and turnover.

Field dual labeling with ^{14}C and ^{15}N was used to examine below-ground C flow and the allocation and turnover of N in the tree. The advantage of isotope methods over samplings methods that estimate growth

and loss was that production and turnover processes could be simultaneously isolated by following long-term tracer flux and dilution. The use of isotope methods provided a direct estimate of production and turnover of the root system by following tracer dilution in standing biomass and flux to soil microbial biomass and organic matter. Though tracer techniques have been used extensively, their application under field conditions, such as in the current study, to probe long-term nutrient cycling processes has been done infrequently.

The application of tracers (^{14}C and ^{15}N) to field grown two-year-old trees with heights of greater than three meters to study nutrient cycles of the above- and below-ground system was unique. The complexity of field engineering used was necessary to ensure that the flux of photoassimilates was characteristic of the field environment. This complex system was chosen over other ^{14}C labeling techniques, such as short-duration pulse-chase and open-top chambers, because of the degree of environmental control afforded by closed systems.

The ^{15}N was injected into the stem to preferentially label tree components. This unusual approach was done to follow both internal N retranslocation and flux of N through plant biomass turnover. The dual labeling approach provided the opportunity to determine the flux of both tracers in the plant biomass, especially the below-ground system. The application of ^{14}C and ^{15}N and traditional methods of plant-soil sampling under field conditions represented a infrequently used quantitative approach to studying C and N dynamics in tree-soil systems.

The standing biomass of leaf litter contained significantly more C and N than fine root biomass. The standing biomass values in themselves indicated than the fine roots would need to turnover frequently to attain the

nutrient cycling potential of leaf litter. The standing biomass values did not correlate to the amount of ^{14}C found in soil from leaf and root derived C sources. The discrepancy in the amount of ^{14}C found in soil was further complicated by the slow rate of ^{14}C dilution in the cell wall fraction and stable mass of fine roots during the following year after labeling. However, a dilution of ^{14}C in nonstructural root components occurred between the periods of labeling and the beginning of dormancy for the July labeling and into the next growing season for the September labeling. During these periods, the highest level of ^{14}C was found in the soil containing labeled roots.

The slow dilution of the root cell wall fraction could have resulted from the synthesis of new roots from labeled reserves. Reserve material in the root system was sufficient to reconstruct the fine root system 1.3 times. Above-ground reserves may have contributed additional substrate for the growth of roots. It is plausible to maintain the ^{14}C content of the fine root cell wall fraction with no significant changes in mass during the year following labeling. In combination with the synthesis of new roots, additional loss of ^{14}C may have occurred from sloughing, exudation and flux to mycorrhizal symbionts.

The ^{15}N from leaf litter was detected in soil and microbial biomass within 168 days in plots containing labeled leaf litter. In root labeled plots, no ^{15}N was detected in the soil. This result was apparent from the small amount of N contained within the standing biomass of roots as compared to leaves. The detection limit of ^{15}N may have become compromised due to the small levels of N associated with the growth and turnover of roots. Though similar amounts of C are involved in both leaf and root turnover, leaf litter

turnover appears to dominant N cycling processes in the early stages of the rotation.

The analysis of microbial biomass and associated soil organic fractions was important in determining the contribution of C and N from leaf and root turnover. The examination of different microbial biomass techniques compared biomass estimates and tracer contents of soil microorganisms. In general, direct microscopic counts and chloroform fumigation methods (chloroform fumigation incubation, CFI and chloroform fumigation extraction, CFE) were well correlated across time and soil depth. The subtraction of a partial control from the CFI estimates resulted in remarkably similar biomass values to that obtained by microscopic counting. The CFI and CFE methods gave similar biomass C values, but differed in ^{14}C content, indicating that the techniques were sampling different components of the soil microbial biomass.

The stabilization of ^{14}C in soil is a measure of the amount of C entering organic matter from litter turnover. Similar amounts of C entered the soil from both leaf and root turnover. The difference between leaf and root turnover lies in the spatial deposition of the stabilized C. The C entering the soil from leaf litter decomposition was localized in the surface 0-10 cm layer. An increase in the amount of ^{14}C below 10 cm indicated that eventually more C from leaf litter turnover would leach or move by faunal action to deeper soil depths, especially during successive rotations.

Kinetic analysis of C mineralization during long-term soil incubations showed that the labile C pools of both leaf and root labeled soil had similar turnover rates. The intermediate resistant fraction also was not different for the two treatments. The turnover of labile soil ^{14}C occurred in 14-64 days. The ^{14}C stabilized into pools of intermediate resistance had a turnover time

of 2-16 years. In contrast, the unlabeled C fractions had turnover times of 11-74 days for labile C and 6-23 years for fractions of intermediate resistance. The intermediate resistant C pool turnover time increased with soil depth for both litters. This showed that C stabilization increased with soil depth and was probably associated with the change in amount, activity and makeup of subsurface microbial populations. The discrepancy between the turnover times of intermediate resistant C of recently incorporated ^{14}C and native C indicated that this pool should be further divided. Separating the stabilized C into meaning fractions will enhance the understanding of the processes involved in stabilization and mineralization of soil organic matter under field conditions.

The characterization of the tree-soil system with ^{14}C and ^{15}N represented a complete tracer and total C and N budget. The combination of traditional component analysis combined with tracers proved to be a powerful approach in determining the role of the different litters in nutrient cycling processes. This approach should be broaden to include a diversity of species, cultural practices and natural systems to fully comprehend the processes associated with nutrient cycling in ecosystems.

APPENDICES

APPENDIX A

Chapter 4

Table A.1. The carbon concentration of tree components sampled one year after the dual labeling procedure on July 19, 1990. Standard error of the mean shown in parentheses.

	Sampling date		
	2-Aug-90	2-Nov-90	25-Jul-91
stem	43.0 (0.6)	38.8 (1.2)	39.3 (0.1)
branches	42.5 (0.5)	39.4 (2.1)	38.9 (0.4)
leaves	40.4 (0.3)	38.0 (0.9)	38.6 (0.5)
root 0-25 cm			
< 0.5 mm	27.1 (2.6)	25.8 (2.0)	20.9 (6.6)
.5-1 mm	36.9 (2.0)	29.8 (1.9)	34.2 (2.2)
1-3 mm	38.2 (1.7)	34.4 (1.5)	32.8 (3.8)
3-10 mm	40.3 (0.7)	36.2 (0.0)	34.4 (2.2)
> 10 mm	40.8 (0.1)	35.9 (2.0)	37.4 (0.2)
cutting	41.2 (1.2)	41.1 (0.9)	39.2 NA
root 25-60 cm			
< 0.5 mm	26.6 (3.6)	20.4 (1.1)	28.2 (4.4)
.5-1 mm	35.3 (2.1)	28.4 (0.7)	27.8 (2.9)
1-3 mm	37.7 (1.8)	31.5 (0.4)	33.4 (2.6)
3-10 mm	37.5 (1.1)	34.7 (0.2)	34.7 (0.1)
> 10 mm	40.4 (0.3)	35.4 (0.8)	34.0 NA
root 60-100 cm			
< 0.5 mm	31.8 (1.2)	21.2 (2.0)	30.7 (0.7)
.5-1 mm	36.4 (2.1)	28.5 (1.9)	34.7 (0.4)
1-3 mm	38.8 (1.6)	33.8 (0.4)	32.3 (4.2)
3-10 mm	39.5 (1.1)	36.0 (0.8)	36.6 (0.3)
> 10 mm	43.4 NA	36.8 (1.1)	39.0 NA

Table A.2. Carbon concentration of tree components sampled one year after the dual labeling procedure on September 5, 1990. Standard error of the mean shown in parentheses.

	Sampling date		
	20-Sep-90	4-Nov-90	5-Sep-91
stem	40.0 (0.8)	39.7 (0.6)	40.2 (0.1)
branches	42.0 (2.0)	40.1 (0.1)	39.0 (0.4)
litter	39.5 (2.2)	38.9 (0.7)	40.3 (0.1)
root 0-25 cm			
< 0.5 mm	30.2 (3.0)	27.2 (1.4)	28.4 (3.1)
.5-1 mm	32.1 (3.2)	31.5 (0.5)	33.6 (2.7)
1-3 mm	33.0 (0.4)	33.2 (1.0)	35.7 (0.1)
3-10 mm	36.5 (1.3)	34.0 (1.3)	34.5 (1.4)
> 10 mm	38.2 (1.6)	36.2 (1.3)	35.1 NA
cutting	43.0 (0.6)	40.3 (1.4)	39.4 (0.1)
root 25-60 cm			
< 0.5 mm	26.7 (2.9)	24.4 (3.8)	33.7 (0.4)
.5-1 mm	31.9 (1.4)	31.5 (0.2)	35.6 (1.2)
1-3 mm	34.7 (1.4)	34.1 (1.1)	36.8 (0.4)
3-10 mm	35.6 (0.7)	37.1 (0.9)	38.0 (1.1)
> 10 mm	36.0 (0.5)	37.3 (0.3)	38.1 (0.5)
root 60-100 cm			
< 0.5 mm	25.8 (0.1)	25.6 (4.9)	34.8 (0.6)
.5-1 mm	32.3 (0.8)	30.2 (1.1)	37.1 (0.4)
1-3 mm	37.4 (0.7)	33.4 (1.5)	38.0 (0.0)
3-10 mm	37.6 (0.9)	35.3 (0.7)	37.1 (1.2)
> 10 mm	35.2 NA	35.2 (3.3)	38.6 NA

Table A.3. Nitrogen concentration of tree components sampled one year after the dual labeling procedure on July 19, 1990. Standard error of the mean shown in parentheses.

	Sampling date		
	2-Aug-90	2-Nov-90	25-Jul-91
stem	0.43 (0.03)	0.56 (0.02)	0.25 (0.01)
branches	0.59 (0.04)	0.86 (0.05)	0.41 (0.01)
leaves	2.41 (0.09)	1.58 (0.06)	1.74 (0.04)
roots 0-25 cm			
< 0.5 mm	0.75 (0.07)	0.61 (0.06)	0.40 (0.07)
.5-1 mm	0.75 (0.06)	0.69 (0.05)	0.48 (0.00)
1-3 mm	0.74 (0.06)	0.88 (0.04)	0.46 (0.04)
3-10 mm	0.78 (0.03)	1.13 (0.11)	0.57 (0.02)
> 10 mm	0.58 (0.07)	0.99 (0.02)	0.40 (0.13)
cutting	0.47 (0.02)	0.62 (0.03)	0.28 NA
roots 25-60 cm			
< 0.5 mm	0.64 (0.07)	0.42 (0.01)	0.57 (0.11)
.5-1 mm	0.72 (0.07)	0.55 (0.01)	0.50 (0.08)
1-3 mm	0.73 (0.11)	0.68 (0.02)	0.47 (0.03)
3-10 mm	0.77 (0.09)	0.82 (0.01)	0.51 (0.04)
> 10 mm	0.68 (0.12)	0.82 (0.02)	0.55 NA
roots 60-100 cm			
< 0.5 mm	0.77 (0.06)	0.47 (0.06)	0.57 (0.03)
.5-1 mm	0.83 (0.09)	0.54 (0.03)	0.53 (0.06)
1-3 mm	0.74 (0.06)	0.66 (0.04)	0.53 (0.08)
3-10 mm	0.78 (0.03)	0.90 (0.13)	0.71 (0.03)
> 10 mm	1.07 NA	1.04 (0.07)	0.58 NA

Table A.4. Nitrogen concentration of tree components sampled one year after the dual labeling procedure on September 5, 1990. Standard error of the mean shown in parentheses.

	Sampling date		
	20-Sep-90	4-Nov-90	5-Sep-91
stem	0.48 (0.01)	0.59 (0.02)	0.42 (0.06)
branches	0.87 (0.04)	0.96 (0.05)	0.72 (0.15)
leaves	2.44 (0.03)	2.05 (0.03)	1.97 (0.39)
roots 0-25 cm			
< 0.5 mm	0.74 (0.02)	0.75 (0.03)	0.64 (0.17)
.5-1 mm	0.81 (0.08)	0.92 (0.03)	0.82 (0.12)
1-3 mm	0.96 (0.03)	1.16 (0.03)	0.88 (0.22)
3-10 mm	1.07 (0.02)	1.42 (0.06)	0.97 (0.25)
> 10 mm	0.88 (0.01)	1.29 (0.09)	1.25 NA
cutting	0.63 (0.05)	0.77 (0.02)	0.49 (0.15)
roots 25-60 cm			
< 0.5 mm	0.71 (0.07)	0.57 (0.06)	0.77 (0.15)
.5-1 mm	0.84 (0.05)	0.80 (0.04)	0.86 (0.21)
1-3 mm	0.95 (0.03)	1.03 (0.07)	0.91 (0.20)
3-10 mm	1.09 (0.06)	1.22 (0.07)	1.18 (0.32)
> 10 mm	0.95 (0.03)	1.17 (0.11)	1.01 (0.29)
roots 60-100 cm			
< 0.5 mm	0.64 (0.02)	0.52 (0.11)	1.02 (0.12)
.5-1 mm	0.86 (0.03)	0.73 (0.07)	0.86 (0.13)
1-3 mm	0.90 (0.05)	0.90 (0.07)	1.01 (0.28)
3-10 mm	1.06 (0.11)	1.20 (0.06)	1.34 (0.18)
> 10 mm	0.84 (0.03)	1.15 (0.17)	0.87 NA

APPENDIX A

Chapter 6

Table B.1. Results of C and ^{14}C mineralization from long-term laboratory soil incubations done on the last two samplings of trees labeled July 19, 1990. The soil depth is 0 to 25 cm. Standard error of the mean shown in parentheses.

Sampled (days)	days incubated	µg CO_2-C g^{-1} soil d^{-1}	µg CO_2-C g^{-1} soil cumulative	Bq mg^{-1} CO_2-C	Bq g^{-1} soil cumulative	% of applied ^{14}C*	% of original ^{14}C§
	0	- -	- -	- -	- -	- -	- -
	16	4.6 (0.2)	73.4 (3.1)	1.04 (0.09)	0.07 (0.01)	0.59 (0.06)	0.07 (0.01)
	28	4.9 (0.5)	132.6 (6.3)	0.68 (0.06)	0.11 (0.01)	0.90 (0.09)	0.10 (0.01)
	45	2.3 (0.1)	172.2 (6.4)	0.78 (0.10)	0.14 (0.02)	1.13 (0.12)	0.12 (0.01)
107	65	2.3 (0.0)	218.1 (7.1)	0.70 (0.10)	0.18 (0.02)	1.38 (0.15)	0.15 (0.02)
	85	2.0 (0.0)	258.4 (7.9)	0.65 (0.11)	0.20 (0.03)	1.58 (0.17)	0.18 (0.02)
	105	2.0 (0.1)	299.3 (9.0)	0.44 (0.07)	0.22 (0.03)	1.72 (0.19)	0.19 (0.02)
	157	1.6 (0.1)	380.6 (9.7)	0.50 (0.07)	0.26 (0.04)	2.03 (0.22)	0.23 (0.02)
	0	- -	- -	- -	- -	- -	- -
	16	5.1 (0.3)	82.0 (5.5)	0.71 (0.08)	0.06 (0.00)	0.45 (0.04)	0.05 (0.00)
	37	2.7 (0.1)	139.7 (7.4)	0.72 (0.08)	0.10 (0.00)	0.77 (0.06)	0.09 (0.01)
	56	2.2 (0.3)	182.4 (12.3)	0.68 (0.22)	0.12 (0.01)	0.98 (0.04)	0.11 (0.00)
372	72	2.4 (0.2)	220.3 (16.0)	0.64 (0.21)	0.15 (0.01)	1.15 (0.03)	0.13 (0.00)
	93	1.5 (0.2)	252.2 (19.8)	0.72 (0.23)	0.17 (0.02)	1.31 (0.01)	0.15 (0.00)
	108	1.6 (0.4)	276.5 (25.8)	0.64 (0.29)	0.18 (0.02)	1.42 (0.00)	0.16 (0.00)
	128	1.7 (0.5)	311.3 (35.3)	0.59 (0.29)	0.20 (0.02)	1.54 (0.01)	0.17 (0.00)
	150	1.5 (0.3)	345.1 (41.0)	0.50 (0.22)	0.21 (0.03)	1.66 (0.03)	0.18 (0.00)
	174	1.4 (0.3)	378.0 (47.4)	0.54 (0.23)	0.23 (0.03)	1.78 (0.06)	0.20 (0.01)

* Calculated from the amount in the root system and soil two weeks after labeling.

§ Calculated form the ^{14}C applied during labeling.

Table B.2. Results of C and ^{14}C mineralization from long-term laboratory soil incubations done on the last two samplings of trees labeled July 19, 1990. The soil depth is 25-60 cm. Standard error of the mean shown in parentheses.

Sampled (days)	days incubated	μg CO_2-C g^{-1} soil d^{-1}	μg CO_2-C g^{-1} soil cumulative	Bq mg^{-1} CO_2-C	Bq g^{-1} soil cumulative	% of applied ^{14}C*	% of original ^{14}C§
	0	- -	- -	- -	- -	- -	- -
	16	1.5 (0.3)	24.7 (4.2)	1.06 (0.15)	0.03 (0.01)	0.35 (0.08)	0.04 (0.01)
	28	0.9 (0.1)	35.0 (4.4)	0.73 (0.08)	0.03 (0.01)	0.44 (0.08)	0.05 (0.01)
	45	0.5 (0.2)	42.9 (2.1)	1.42 (0.57)	0.04 (0.01)	0.50 (0.09)	0.06 (0.01)
107	65	0.2 (0.0)	47.6 (1.2)	3.83 (2.55)	0.05 (0.01)	0.59 (0.11)	0.07 (0.01)
	85	0.4 (0.1)	55.2 (1.5)	0.34 (0.05)	0.05 (0.01)	0.63 (0.11)	0.07 (0.01)
	105	0.5 (0.1)	66.0 (4.1)	0.25 (0.03)	0.05 (0.01)	0.65 (0.12)	0.07 (0.01)
	157	0.3 (0.1)	82.0 (7.1)	0.53 (0.10)	0.06 (0.01)	0.75 (0.13)	0.08 (0.01)
	0	- -	- -	- -	- -	- -	- -
	16	1.6 -	25.0 -	0.86 -	0.02 (0.00)	0.33 (0.05)	0.04 (0.01)
	37	0.7 -	39.8 -	0.82 -	0.04 (0.00)	0.48 (0.06)	0.05 (0.01)
	56	0.5 -	49.7 -	0.62 -	0.04 (0.00)	0.57 (0.07)	0.06 (0.01)
372	72	0.8 -	62.9 -	1.02 -	0.05 (0.00)	0.66 (0.06)	0.07 (0.01)
	93	0.5 -	73.3 -	0.87 -	0.06 (0.00)	0.73 (0.04)	0.08 (0.00)
	108	0.6 -	81.9 -	0.84 -	0.06 (0.00)	0.78 (0.04)	0.09 (0.00)
	128	0.7 -	95.3 -	0.86 -	0.06 (0.00)	0.84 (0.00)	0.09 (0.00)
	150	0.6 -	108.3 -	0.35 -	0.07 (0.00)	0.90 (0.00)	0.10 (0.00)
	174	0.9 -	128.9 -	0.57 -	0.08 (0.00)	1.01 (0.02)	0.11 (0.00)

* Calculated from the amount in the root system and soil two weeks after labeling.

§ Calculated form the ^{14}C applied during labeling.

Table B.3. Results of C and ^{14}C mineralization from long-term laboratory soil incubations done on the last two samplings of trees labeled July 19, 1990. The soil depth is 60-100 cm. Standard error of the mean shown in parentheses.

Sampled (days)	days incubated	µg CO_2-C g^{-1} soil d^{-1}	µg CO_2-C g^{-1} soil cumulative	Bq mg^{-1} CO_2-C	Bq g^{-1} soil cumulative	% of applied ^{14}C*	% of original ^{14}C§
	0	- -	- -	- -	- -	- -	- -
	16	1.4 (0.3)	22.8 (4.6)	0.64 (0.22)	0.01 (0.00)	0.18 (0.06)	0.02 (0.01)
	28	0.8 (0.3)	31.8 (1.0)	3.90 (3.43)	0.02 (0.00)	0.25 (0.08)	0.03 (0.01)
	45	0.5 (0.2)	38.4 (5.7)	1.07 (0.48)	0.02 (0.01)	0.30 (0.10)	0.03 (0.01)
107	65	0.2 (0.1)	39.5 (7.6)	3.52 (0.72)	0.03 (0.01)	0.37 (0.11)	0.04 (0.01)
	85	0.2 (0.0)	44.3 (8.2)	0.43 (0.15)	0.03 (0.01)	0.40 (0.12)	0.04 (0.01)
	105	0.2 (0.0)	48.6 (8.5)	1.57 (0.44)	0.03 (0.01)	0.42 (0.13)	0.05 (0.01)
	157	0.2 (0.1)	58.7 (8.3)	0.99 (0.58)	0.03 (0.01)	0.50 (0.15)	0.06 (0.02)
	0	- -	- -	- -	- -	- -	- -
	16	0.9 -	13.9 -	1.87 -	0.01 (0.00)	0.11 (0.04)	0.01 (0.00)
	37	0.9 -	20.6 -	0.26 -	0.01 (0.00)	0.17 (0.05)	0.02 (0.01)
	56	0.8 -	36.5 -	0.10 -	0.02 (0.00)	0.20 (0.06)	0.02 (0.01)
372	72	0.6 -	46.1 -	0.24 -	0.02 (0.00)	0.24 (0.07)	0.03 (0.01)
	93	0.3 -	52.5 -	0.30 -	0.02 (0.00)	0.26 (0.07)	0.03 (0.01)
	108	0.8 -	64.9 -	0.18 -	0.02 (0.00)	0.28 (0.07)	0.03 (0.01)
	128	1.2 -	89.5 -	0.16 -	0.03 (0.00)	0.32 (0.07)	0.04 (0.01)
	150	0.3 -	96.3 -	3.93 -	0.03 (0.00)	0.34 (0.07)	0.04 (0.01)
	174	0.4 -	106.6 -	0.37 -	0.03 (0.00)	0.39 (0.07)	0.04 (0.01)

* Calculated from the amount in the root system and soil two weeks after labeling.

§ Calculated form the ^{14}C applied during labeling.

Table B.4. Results of C and ^{14}C mineralization from long-term laboratory soil incubations done on the last two samplings of trees labeled September 5, 1990. The soil depth is 0-25 cm. Standard error of the mean shown in parentheses.

Sampled (days)	days incubated	μg CO_2-C g^{-1} soil d^{-1}	μg CO_2-C g^{-1} soil cumulative	Bq mg^{-1} CO_2-C	Bq g^{-1} soil cumulative	% of applied* ^{14}C	% of original ^{14}C§
	0	- -	- -	- -	- -	- -	- -
	15	3.6 (0.2)	53.6 (3.5)	3.43 (0.15)	0.18 (0.01)	0.45 (0.03)	0.15 (0.01)
	30	3.4 (0.3)	105.0 (5.5)	1.77 (0.18)	0.27 (0.01)	0.67 (0.05)	0.22 (0.02)
	45	2.6 (0.3)	144.6 (9.4)	1.48 (0.20)	0.32 (0.02)	0.81 (0.06)	0.27 (0.02)
60	64	2.3 (0.2)	187.8 (12.4)	1.31 (0.17)	0.38 (0.02)	0.95 (0.08)	0.31 (0.03)
	83	2.1 (0.2)	226.2 (15.9)	1.18 (0.28)	0.42 (0.02)	1.06 (0.09)	0.35 (0.03)
	106	1.7 (0.1)	265.0 (18.0)	0.87 (0.19)	0.45 (0.02)	1.14 (0.11)	0.38 (0.04)
	150	1.3 (0.1)	324.5 (14.8)	0.95 (0.04)	0.50 (0.03)	1.27 (0.12)	0.42 (0.04)
	0	- -	- -	- -	- -	- -	- -
	20	3.6 (0.1)	72.4 (2.9)	1.20 (0.20)	0.09 (0.01)	0.26 (0.03)	0.09 (0.01)
	35	2.6 (0.0)	111.3 (3.4)	1.24 (0.15)	0.13 (0.02)	0.40 (0.05)	0.13 (0.02)
	52	2.3 (0.1)	150.6 (5.5)	1.20 (0.19)	0.18 (0.02)	0.54 (0.07)	0.18 (0.02)
366	69	1.8 (0.2)	181.5 (8.2)	1.31 (0.16)	0.22 (0.03)	0.65 (0.08)	0.21 (0.03)
	81	2.2 (0.3)	207.3 (12.4)	1.13 (0.32)	0.24 (0.03)	0.73 (0.09)	0.24 (0.03)
	101	1.8 (0.0)	244.3 (12.8)	0.90 (0.05)	0.28 (0.03)	0.82 (0.09)	0.27 (0.03)
	123	1.6 (0.2)	280.0 (7.6)	0.95 (0.16)	0.31 (0.03)	0.92 (0.08)	0.30 (0.03)
	147	1.4 (0.0)	314.7 (6.7)	1.00 (0.01)	0.34 (0.03)	1.00 (0.07)	0.33 (0.02)

* Calculated from the amount in the root system and soil two weeks after labeling.

§ Calculated form the ^{14}C applied during labeling.

Table B.5. Results of C and ^{14}C mineralization from long-term laboratory soil incubations done on the last two samplings of trees labeled September 5, 1990. The soil depth is 25-60 cm. Standard error of the mean shown in parentheses.

Sampled (days)	days incubated	µg CO_2-C g^{-1} soil d^{-1}	µg CO_2-C g^{-1} soil cumulative	Bq mg^{-1} CO_2-C	Bq g^{-1} soil cumulative	% of applied ^{14}C*	% of original ^{14}C§
	0	- -	- -	- -	- -	- -	- -
	15	1.7 (0.3)	25.2 (3.9)	2.92 (0.09)	0.07 (0.01)	0.27 (0.04)	0.09 (0.01)
	30	0.7 (0.1)	36.3 (2.3)	2.87 (0.61)	0.10 (0.01)	0.38 (0.04)	0.13 (0.01)
	45	0.7 (0.1)	46.8 (0.5)	3.43 (0.95)	0.12 (0.01)	0.49 (0.05)	0.16 (0.02)
60	64	0.6 (0.2)	57.6 (2.7)	3.04 (0.91)	0.15 (0.00)	0.60 (0.05)	0.20 (0.02)
	83	0.5 (0.0)	67.2 (2.2)	2.52 (0.10)	0.17 (0.00)	0.68 (0.05)	0.23 (0.02)
	106	0.5 (0.1)	79.0 (4.2)	2.00 (0.25)	0.19 (0.00)	0.77 (0.06)	0.25 (0.02)
	150	0.3 (0.2)	90.4 (15.6)	3.83 (1.74)	0.22 (0.00)	0.89 (0.07)	0.29 (0.02)
	0	- -	- -	- -	- -	- -	- -
	20	1.3 -	25.7 -	0.78 -	0.02 -	0.31 (0.22)	0.10 (0.07)
	35	0.1 -	27.6 -	22.11 -	0.03 -	0.47 (0.35)	0.15 (0.11)
	52	0.3 -	32.9 -	2.22 -	0.03 -	0.61 (0.47)	0.20 (0.15)
366	69	0.4 -	39.7 -	2.53 -	0.04 -	0.74 (0.57)	0.24 (0.19)
	81	1.0 -	51.9 -	0.31 -	0.04 -	0.82 (0.63)	0.27 (0.21)
	101	0.4 -	59.2 -	0.62 -	0.04 -	0.91 (0.71)	0.30 (0.23)
	123	0.1 -	70.2 -	2.70 -	0.05 -	1.00 (0.78)	0.33 (0.26)
	147	0.4 -	80.8 -	1.00 -	0.05 -	1.09 (0.84)	0.36 (0.28)

* Calculated from the amount in the root system and soil two weeks after labeling.

§ Calculated form the ^{14}C applied during labeling.

Table B.6. Results of C and ^{14}C mineralization from long-term laboratory soil incubations done on the last two samplings of trees labeled September 5, 1990. The soil depth is 60-100 cm. Standard error of the mean shown in parentheses.

Sampled (days)	days incubated	μg CO_2-C g^{-1} soil d^{-1}	μg CO_2-C g^{-1} soil cumulative	Bq mg^{-1} CO_2-C	Bq g^{-1} soil cumulative	% of applied ^{14}C*	% of original ^{14}C§
	0	- -	- -	- -	- -	- -	- -
	15	1.0 (0.1)	14.5 (2.2)	5.40 (0.67)	0.08 (0.02)	0.34 (0.11)	0.11 (0.03)
	30	0.6 (0.1)	24.2 (3.1)	4.26 (0.32)	0.11 (0.03)	0.49 (0.13)	0.16 (0.04)
	45	0.9 (0.3)	37.7 (7.0)	2.66 (0.58)	0.14 (0.03)	0.60 (0.14)	0.20 (0.04)
60	64	0.4 (0.1)	45.5 (8.8)	10.17 (5.38)	0.16 (0.03)	0.72 (0.15)	0.24 (0.05)
	83	0.4 (0.1)	54.0 (10.6)	4.16 (1.23)	0.18 (0.03)	0.81 (0.15)	0.27 (0.05)
	106	0.4 (0.1)	62.2 (12.4)	3.89 (0.92)	0.21 (0.03)	0.91 (0.15)	0.30 (0.05)
	150	0.3 (0.1)	76.0 (13.2)	2.55 (0.39)	0.24 (0.03)	1.03 (0.16)	0.34 (0.05)
	0	- -	- -	- -	- -	- -	- -
	20	0.9 -	17.4 -	2.43 -	0.02 (0.00)	0.10 (0.02)	0.03 (0.01)
	35	0.8 -	29.6 -	0.77 -	0.03 (0.00)	0.14 (0.02)	0.05 (0.01)
	52	0.9 -	52.6 -	1.21 -	0.04 (0.00)	0.17 (0.03)	0.06 (0.01)
366	69	1.0 -	69.9 -	4.11 -	0.05 (0.01)	0.21 (0.04)	0.07 (0.01)
	81	1.2 -	83.8 -	0.72 -	0.05 (0.01)	0.23 (0.04)	0.08 (0.01)
	101	0.9 -	100.9 -	1.27 -	0.06 (0.01)	0.26 (0.05)	0.08 (0.02)
	123	1.0 -	122.6 -	0.55 -	0.06 (0.01)	0.28 (0.06)	0.09 (0.02)
	147	0.8 -	142.8 -	0.60 -	0.07 (0.01)	0.31 (0.07)	0.10 (0.02)

* Calculated from the amount in the root system and soil two weeks after labeling.

§ Calculated form the ^{14}C applied during labeling.

Table B.7. Results from long-term laboratory soil incubations done on the first two samplings of the litter exchange experiment started December 21, 1990. Litter is from the September labeled trees. The soil depth is 0 to 10 cm. Standard error of the mean shown in parentheses.

Sampled (days)	days incubated	μg CO_2-C g^{-1} soil d^{-1}	μg CO_2-C g^{-1} soil cumulative	Bq mg^{-1} CO_2-C	Bq g^{-1} soil cumulative	% of applied ^{14}C*	% of original ^{14}C§
	0	- -	- -	- -	- -	- -	- -
	15	4.35 (0.37)	121.7 (10.2)	4.65 (0.55)	0.55 (0.05)	1.26 (0.17)	0.20 (0.03)
	31	3.40 (0.26)	230.6 (18.4)	4.22 (0.58)	1.00 (0.10)	2.29 (0.30)	0.37 (0.05)
168	48	2.30 (0.09)	308.9 (21.4)	4.48 (0.59)	1.35 (0.14)	3.07 (0.40)	0.49 (0.06)
	63	2.35 (0.18)	379.4 (21.5)	3.30 (0.07)	1.58 (0.16)	3.59 (0.45)	0.58 (0.07)
	80	1.98 (0.05)	446.8 (22.9)	3.01 (0.18)	1.78 (0.17)	4.05 (0.49)	0.65 (0.08)
	100	1.70 (0.14)	514.8 (23.1)	2.64 (0.13)	1.96 (0.18)	4.45 (0.51)	0.72 (0.08)
	121	1.11 (0.11)	561.3 (19.1)	3.09 (0.28)	2.09 (0.18)	4.75 (0.53)	0.76 (0.08)
	143	1.51 (0.08)	627.9 (17.9)	2.35 (0.75)	2.21 (0.19)	5.02 (0.54)	0.81 (0.09)
	173	1.33 (0.03)	707.9 (16.3)	1.99 (0.16)	2.37 (0.20)	5.38 (0.58)	0.87 (0.09)
	0	- -	- -	- -	- -	- -	- -
	15	3.57 (0.20)	107.1 (6.0)	2.04 (0.32)	0.21 (0.02)	0.48 (0.07)	0.08 (0.01)
	34	2.48 (0.07)	201.2 (7.5)	2.58 (0.38)	0.45 (0.06)	1.04 (0.18)	0.17 (0.03)
328	51	2.15 (0.06)	274.3 (7.8)	2.33 (0.33)	0.62 (0.08)	1.42 (0.25)	0.23 (0.04)
	68	1.71 (0.07)	332.3 (10.1)	2.39 (0.37)	0.76 (0.10)	1.74 (0.31)	0.28 (0.05)
	85	1.70 (0.08)	390.0 (10.1)	1.94 (0.26)	0.87 (0.12)	1.99 (0.36)	0.32 (0.06)
	109	0.98 (0.06)	437.0 (8.2)	2.84 (0.29)	1.01 (0.14)	2.30 (0.41)	0.37 (0.07)
	130	1.67 (0.07)	507.1 (10.3)	1.66 (0.21)	1.12 (0.15)	2.56 (0.45)	0.41 (0.07)

* Calculated from the amount in leaf litter.

§ Calculated form the ^{14}C applied during labeling.

Table B.8. Results from long-term laboratory soil incubations done on the first two samplings of the litter exchange experiment started December 21, 1990. Litter is from the September labeled trees. The soil depth is 10 to 25 cm. Standard error of the mean shown in parentheses.

Sampled (days)	days incubated	μg CO_2-C g^{-1} soil d^{-1}	μg CO_2-C g^{-1} soil cumulative	Bq mg^{-1} CO_2-C	Bq g^{-1} soil cumulative	% of applied ^{14}C*	% of original ^{14}C§
	0	- -	- -	- -	- -	- -	- -
	15	2.83 (0.24)	79.3 (6.6)	0.52 (0.09)	0.04 (0.01)	0.10 (0.02)	0.02 (0.00)
	31	2.46 (0.11)	161.2 (11.8)	0.38 (0.10)	0.07 (0.02)	0.20 (0.03)	0.03 (0.00)
168	48	1.69 (0.17)	218.7 (17.2)	0.40 (0.11)	0.09 (0.02)	0.26 (0.04)	0.04 (0.01)
	63	2.12 (0.04)	282.1 (18.2)	0.25 (0.08)	0.11 (0.03)	0.30 (0.05)	0.05 (0.01)
	80	1.42 (0.13)	330.5 (18.0)	0.37 (0.18)	0.12 (0.03)	0.34 (0.06)	0.06 (0.01)
	100	1.25 (0.08)	380.3 (20.8)	0.26 (0.09)	0.13 (0.04)	0.38 (0.06)	0.06 (0.01)
	121	0.73 (0.11)	410.8 (22.5)	0.24 (0.07)	0.14 (0.04)	0.40 (0.07)	0.06 (0.01)
	143	1.34 (0.12)	469.6 (24.0)	0.18 (0.08)	0.15 (0.04)	0.42 (0.07)	0.07 (0.01)
	173	1.00 (0.14)	529.7 (16.2)	0.19 (0.05)	0.16 (0.04)	0.45 (0.08)	0.07 (0.01)
	0	- -	- -	- -	- -	- -	- -
	15	2.33 (0.20)	69.8 (6.1)	0.26 (0.04)	0.02 (0.00)	0.05 (0.01)	0.01 (0.00)
	34	1.73 (0.15)	135.5 (0.9)	0.29 (0.06)	0.04 (0.01)	0.10 (0.02)	0.02 (0.00)
328	51	1.44 (0.17)	184.5 (5.1)	0.27 (0.05)	0.05 (0.01)	0.14 (0.02)	0.02 (0.00)
	68	1.39 (0.19)	231.7 (11.7)	0.24 (0.04)	0.06 (0.01)	0.17 (0.03)	0.03 (0.00)
	85	1.21 (0.02)	272.8 (10.8)	0.24 (0.04)	0.07 (0.01)	0.20 (0.03)	0.03 (0.00)
	109	0.45 (0.08)	294.2 (8.3)	0.65 (0.26)	0.08 (0.01)	0.23 (0.04)	0.04 (0.01)
	130	1.27 (0.01)	347.6 (8.6)	0.23 (0.02)	0.09 (0.01)	0.26 (0.04)	0.04 (0.01)

* Calculated from the amount in leaf litter.

§ Calculated form the ^{14}C applied during labeling.

Table B.9. Results from long-term laboratory soil incubations done on the first two samplings of the litter exchange experiment started December 21, 1990. Litter is from the July labeled trees. The soil depth is 0 to 10 cm. Standard error of the mean shown in parentheses.

Sampled (days)	days incubated	μg CO_2-C g^{-1} soil d^{-1}	μg CO_2-C g^{-1} soil cumulative	Bq mg^{-1} CO_2-C	Bq g^{-1} soil cumulative	% of applied ^{14}C*	% of original ^{14}C§
	0	- -	- -	- -	- -	- -	- -
	15	4.13 (0.36)	115.5 (9.9)	2.21 (0.08)	0.25 (0.01)	0.56 (0.04)	0.09 (0.01)
	31	2.91 (0.15)	203.8 (10.2)	2.32 (0.03)	0.47 (0.03)	1.03 (0.07)	0.16 (0.01)
168	48	2.39 (0.11)	285.0 (13.6)	2.10 (0.06)	0.64 (0.03)	1.40 (0.11)	0.22 (0.02)
	63	2.53 (0.18)	360.8 (18.9)	1.50 (0.04)	0.75 (0.04)	1.64 (0.13)	0.26 (0.02)
	80	1.91 (0.18)	425.7 (24.1)	1.54 (0.09)	0.85 (0.05)	1.86 (0.14)	0.30 (0.02)
	100	1.76 (0.11)	496.0 (28.2)	1.23 (0.03)	0.93 (0.05)	2.05 (0.16)	0.33 (0.03)
	121	1.18 (0.11)	545.7 (30.1)	1.71 (0.38)	1.00 (0.05)	2.19 (0.16)	0.35 (0.03)
	143	1.30 (0.13)	602.7 (35.5)	1.15 (0.08)	1.06 (0.06)	2.33 (0.17)	0.37 (0.03)
	173	1.17 (0.08)	673.0 (37.9)	1.16 (0.07)	1.14 (0.06)	2.51 (0.18)	0.40 (0.03)
	0	- -	- -	- -	- -	- -	- -
	15	3.37 (0.13)	101.1 (3.8)	1.48 (0.12)	0.15 (0.01)	0.36 (0.03)	0.06 (0.00)
	34	2.57 (0.17)	198.7 (9.8)	1.67 (0.14)	0.31 (0.02)	0.76 (0.05)	0.12 (0.01)
328	51	2.21 (0.08)	273.7 (12.2)	1.54 (0.12)	0.42 (0.03)	1.04 (0.07)	0.17 (0.01)
	68	1.84 (0.03)	336.3 (11.3)	1.52 (0.16)	0.52 (0.04)	1.27 (0.10)	0.20 (0.02)
	85	1.68 (0.09)	393.6 (14.4)	1.39 (0.11)	0.60 (0.05)	1.47 (0.11)	0.24 (0.02)
	109	1.00 (0.04)	441.4 (14.8)	1.96 (0.27)	0.69 (0.06)	1.69 (0.14)	0.27 (0.02)
	130	1.74 (0.10)	514.6 (17.3)	1.23 (0.16)	0.78 (0.06)	1.91 (0.15)	0.31 (0.02)

* Calculated from the amount in leaf litter.

§ Calculated form the ^{14}C applied during labeling.

Table B.10. Results from long-term laboratory soil incubations done on the first two samplings of the litter exchange experiment started December 21, 1990. Litter is from the July labeled trees. The soil depth is 10 to 25 cm. Standard error of the mean shown in parentheses.

Sampled (days)	days incubated	μg CO_2-C g^{-1} soil d^{-1}	μg CO_2-C g^{-1} soil cumulative	Bq mg^{-1} CO_2-C	Bq g^{-1} soil cumulative	% of applied ^{14}C*	% of original ^{14}C§
	0	- -	- -	- -	- -	- -	- -
	15	2.50 (0.17)	69.9 (4.6)	0.45 (0.15)	0.03 (0.01)	0.09 (0.03)	0.01 (0.00)
	31	1.91 (0.18)	131.1 (9.9)	0.40 (0.18)	0.06 (0.02)	0.16 (0.06)	0.03 (0.01)
168	48	1.35 (0.14)	176.9 (14.6)	0.32 (0.08)	0.07 (0.03)	0.20 (0.07)	0.03 (0.01)
	63	1.56 (0.09)	223.5 (15.7)	0.24 (0.08)	0.08 (0.03)	0.24 (0.07)	0.04 (0.01)
	80	1.19 (0.17)	264.1 (21.0)	0.19 (0.04)	0.09 (0.03)	0.26 (0.08)	0.04 (0.01)
	100	1.29 (0.06)	315.7 (22.5)	0.12 (0.03)	0.10 (0.03)	0.28 (0.08)	0.05 (0.01)
	121	0.89 (0.10)	353.0 (24.9)	0.16 (0.05)	0.10 (0.04)	0.30 (0.09)	0.05 (0.01)
	143	1.12 (0.06)	402.4 (26.8)	0.14 (0.02)	0.11 (0.04)	0.31 (0.09)	0.05 (0.01)
	173	1.04 (0.05)	465.0 (28.4)	0.11 (0.03)	0.12 (0.04)	0.33 (0.10)	0.05 (0.02)
	0	- -	- -	- -	- -	- -	- -
	15	2.02 (0.30)	60.7 (8.9)	0.31 (0.02)	0.02 (0.00)	0.05 (0.01)	0.01 (0.00)
	34	1.76 (0.13)	127.5 (11.5)	0.32 (0.03)	0.04 (0.00)	0.11 (0.01)	0.02 (0.00)
	51	1.55 (0.08)	180.3 (14.1)	0.25 (0.01)	0.05 (0.00)	0.15 (0.01)	0.02 (0.00)
328	68	1.27 (0.07)	223.3 (16.0)	0.25 (0.00)	0.06 (0.00)	0.18 (0.01)	0.03 (0.00)
	85	1.33 (0.11)	268.4 (19.0)	0.27 (0.01)	0.07 (0.00)	0.21 (0.02)	0.03 (0.00)
	109	0.70 (0.06)	301.8 (20.3)	0.40 (0.05)	0.09 (0.00)	0.25 (0.02)	0.04 (0.00)
	130	1.27 (0.14)	355.3 (22.9)	0.20 (0.02)	0.10 (0.00)	0.28 (0.02)	0.04 (0.00)

* Calculated from the amount in leaf litter.

§ Calculated form the ^{14}C applied during labeling.

Table B.11. N mineralization results are shown for the last two samplings of tree labeled July 19, 1990. All soil depths are indicated. Standard error of the mean shown in parentheses.

		Soil depth		
		0-25 cm	25-60 cm	60-100 cm.
Sampled	Days incubated	Cumulative μg N g^{-1} soil	Cumulative μg N g^{-1} soil	Cumulative μg N g^{-1} soil
107 days	0	2.3 (0.1)	1.7 (0.2)	0.7 (0.1)
	10	4.0 (0.4)	1.4 (0.2)	0.6 (0.3)
	20	8.5 (0.5)	1.6 (0.6)	0.9 (0.2)
	30	10.9 (2.3)	3.0 (0.9)	1.1 (0.4)
	51	16.9 (4.0)	3.7 (0.3)	2.2 (0.4)
	61	17.7 (2.9)	2.4 (0.2)	1.3 (0.4)
	71	19.8 (2.8)	2.3 (0.1)	1.6 (0.4)
	80	26.7 (1.8)	7.3 (0.9)	4.5 (0.7)
	122	30.1 (0.8)	3.1 (0.1)	2.2 (0.5)
	132	31.1 (1.3)	3.7 (0.2)	2.4 (0.3)
	142	31.0 (0.6)	3.6 (0.1)	2.4 (0.5)
372 days	0	3.5 (0.0)	2.4 (0.2)	1.2 (0.3)
	10	5.8 (0.2)	1.8 (0.4)	1.6 (0.5)
	20	8.3 (0.5)	1.8 (0.4)	1.3 (0.4)
	42	14.1 (2.1)	2.3 (0.3)	1.6 (0.4)
	55	16.2 (0.1)	2.7 (0.2)	1.9 (0.3)
	65	22.8 (0.0)	3.8 (0.1)	2.4 (0.5)
	75	25.6 (1.0)	4.2 (0.0)	3.0 (0.3)
	90	26.3 (1.6)	4.1 (1.4)	2.5 (0.5)
	149	43.5 (1.6)	5.1 (0.5)	3.3 (0.9)
	159	40.8 (0.4)	6.8 (0.6)	4.8 (0.4)
	169	47.0 (1.3)	6.0 (0.9)	4.1 (0.9)

Table B.12. N mineralization results are shown for the last two samplings of tree labeled September 5, 1990. All soil depths are indicated. Standard error of the mean shown in parentheses.

		Soil depth		
		0-25 cm	25-60 cm	60-100 cm.
Sampled	Days incubated	Cumulative μg N g^{-1} soil	Cumulative μg N g^{-1} soil	Cumulative μg N g^{-1} soil
60 days	0	3.8 (0.7)	3.1 (0.5)	1.4 (0.4)
	10	6.0 (0.9)	2.9 (0.5)	2.5 (1.2)
	20	9.5 (0.4)	3.6 (0.3)	2.2 (0.2)
	30	12.5 (1.0)	3.9 (0.3)	2.4 (0.4)
	51	19.2 (1.9)	5.6 (0.8)	3.7 (0.5)
	61	22.9 (2.6)	5.7 (0.8)	3.5 (0.4)
	71	23.7 (2.5)	5.1 (0.7)	3.3 (0.4)
	80	25.1 (1.6)	6.0 (0.4)	3.6 (0.3)
	122	30.0 (2.1)	6.6 (1.2)	4.3 (0.5)
	142	32.3 (2.7)	7.1 (1.7)	4.6 (0.8)
366 days	0	3.1 (0.3)	2.3 (0.5)	0.8 (0.0)
	42	17.4 (0.1)	5.5 (0.1)	3.7 (0.0)
	61	20.4 (0.4)	3.3 (0.2)	1.4 (0.0)
	71	20.2 (0.6)	5.2 (0.2)	2.8 (0.0)
	81	28.4 (0.1)	5.0 (0.5)	2.3 (0.1)
	96	27.5 (2.2)	4.9 (0.9)	2.5 (0.6)
	151	42.1 (0.0)	7.0 (1.2)	3.8 (0.3)

Table B.13. Results from N and ^{15}N mineralization during long-term soil incubations. Soil collected from the 0-10 cm depth under the September labeled litter. Standard error of the mean shown in parentheses.

Sampled (Days)	Days incubated	Cumulative μg N g^{-1} soil	Cumulative ng ^{15}N g^{-1} soil	Atom % ^{15}N excess		% of ^{15}N applied*	% of original injected ^{15}N
	0	3.5 (0.1)	0.24 (0.09)	0.007	(0.002)	0.2 (0.1)	0.03 (0.01)
	10	9.3 (0.4)					
	20	13.5 (0.4)					
	55	31.1 (0.9)					
168 days	70	33.3 (0.7)	4.82 (1.06)	0.014	(0.003)	4.3 (0.8)	0.63 (0.12)
	80	37.6 (0.2)					
	90	51.4 (0.9)					
	101	52.6 (1.5)					
	143	66.0 (1.3)	13.25 (1.73)	0.020	(0.002)	11.9 (1.3)	1.75 (0.19)
	153	77.2 (1.2)					
	163	64.7 (1.1)					
	0	4.7 (0.3)	0.33 (0.10)	0.007	(0.002)	0.3 (0.1)	0.05 (0.02)
	10	10.3 (0.4)					
	20	18.6 (0.5)					
	55	32.3 (0.2)	3.58 (0.92)	0.011	(0.003)	3.3 (0.9)	0.48 (0.13)
328 days	65	40.0 (0.8)					
	75	51.5 (3.6)					
	92	51.2 (1.0)					
	124	54.0 (0.6)	7.54 (1.22)	0.014	(0.002)	6.8 (1.3)	1.00 (0.19)
	134	72.8 (1.9)					

* Calculated from the amount in leaf litter.

Table B.14. Results from N and ^{15}N mineralization during long-term soil incubations. Soil collected from the 0-10 cm depth under the July labeled litter. Standard error of the mean shown in parentheses.

Sampled (Days)	Days incubated	Cumulative μg N g^{-1} soil	Cumulative ng ^{15}N g^{-1} soil	Atom % ^{15}N excess		% of ^{15}N applied*	% of original injected ^{15}N
	0	3.2 (0.3)	0.18 (0.05)	0.005	(0.001)	0.1 (0.0)	0.02 (0.01)
	10	9.3 (0.5)					
	20	11.7 (0.8)					
	55	28.2 (2.1)					
168 days	70	30.8 (0.8)	3.91 (0.24)	0.013	(0.001)	2.0 (0.1)	0.51 (0.02)
	80	36.5 (2.5)					
	90	46.8 (2.8)					
	101	49.2 (3.8)					
	143	64.6 (4.6)	10.83 (0.71)	0.017	(0.001)	5.6 (0.3)	1.40 (0.07)
	153	76.5 (4.4)					
	163	65.0 (2.3)					
	0	5.0 (0.2)	0.74 (0.11)	0.015	(0.002)	0.4 (0.1)	0.12 (0.02)
	10	11.4 (0.6)					
	20	19.4 (0.9)					
	55	31.7 (1.2)	5.76 (0.14)	0.018	(0.000)	3.3 (0.1)	0.87 (0.02)
328 days	65	36.6 (4.3)					
	75	44.6 (1.4)					
	92	50.8 (2.2)					
	124	54.5 (1.5)	9.74 (0.47)	0.018	(0.001)	5.7 (0.3)	1.49 (0.07)
	134	72.5 (4.5)					

* Calculated from the amount in leaf litter.

Table B.15. N mineralization results are shown from both samplings of September and July litter exchange experiment. Soil was collected from the 10-25 cm depth. Standard error of the mean shown in parentheses.

	September labeled litter plot		July labeled litter plot
	Day 168		Day 168
Days incubated	Cumulative μg N g^{-1} soil	Days incubated	Cumulative μg N g^{-1} soil
0	2.7 (0.2)	0	2.7 (0.0)
10	6.0 (0.1)	10	5.7 (0.1)
20	9.1 (1.0)	20	8.3 (0.3)
55	21.4 (0.8)	55	18.8 (0.8)
70	23.2 (0.4)	70	19.7 (1.0)
80	27.8 (1.2)	80	23.0 (1.6)
90	35.4 (0.8)	90	32.2 (1.2)
101	35.1 (1.0)	101	32.4 (1.2)
143	45.6 (1.7)	143	43.0 (1.3)
153	55.1 (1.7)	153	52.5 (1.3)
163	50.4 (1.9)	163	48.8 (1.5)
	Day 328		Day 328
0	4.2 (0.2)	0	4.3 (0.1)
10	8.1 (0.9)	10	8.4 (0.4)
20	12.8 (0.3)	20	15.2 (1.1)
55	20.5 (0.5)	55	21.1 (0.9)
65	26.5 (0.5)	65	27.0 (1.4)
75	31.0 (2.8)	75	29.5 (1.1)
92	34.2 (0.9)	92	34.0 (2.1)
124	36.1 (0.9)	124	37.6 (1.8)
134	53.3 (1.5)	134	50.1 (1.5)