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the dielectric constant of Layer 1, l_1 is the thickness of Layer 1, and so on. The smaller influence on the TDR reading caused by the water-filled crack in dry soil is a result of the dielectric constant being in the denominator of each term in the denominator of Eq. [1]. The water-filled crack has a much larger dielectric constant than the sand and thus contributes a smaller fraction to the equivalent total. With an air-filled crack, the low dielectric constant of the air, compared with wet sand, results in a larger effect.

Our results were not affected as much by the air- or water-filled crack as Eq. [1] would predict (Table 1) because of the spatial sensitivity of the TDR measurements. Baker and Lascano (1989) demonstrated that TDR measurements are several times more sensitive to the properties of the medium near the rods than to the medium in between them. Equation [1] does not take this spatial sensitivity into account and is therefore overpredicting the effect of the medium in the crack. Equation [1] closely predicts the observed TDR value if a weighting factor of 5 is applied to the soil as follows:

$$K_e = \frac{l_T}{(5\ l_1/K_1) + (l_2/K_2)} \qquad [2]$$

where l_1 is the thickness of soil between the rods, l_2 is the thickness of the soil crack, and $l_T = 5(l_1) + (l_2)$. The weighting factor of 5 was estimated based on the geometry of electric field lines generated between cylindrical rods, and is consistent with the general findings of Baker and Lascano (1989). Linear regression of TDR water-content measurements across the water-filled crack and the air-filled crack against predicted water content using Eq. [2] resulted in an r^2 of 0.94, an intercept of 0.006 $m^3\ m^{-3}$, and a slope of 1.02.

It is important to note that spatial sensitivity alone cannot explain the results from the soil-crack experiments. Though weighting factors can be postulated that lead to close predictions of the measurements for either the air-filled or water-filled crack, a single spatial-sensitivity correction cannot duplicate the results of both cases without considering the dielectrics as capacitors in series. In addition, the bilateral symmetry of the earlier experiments, with half wet soil and half dry soil, eliminated spatial-sensitivity issues, yet TDR readings were heavily weighted toward the lower water content medium. In all situations where spatial sensitivity of measurements is a concern (i.e., whenever there are water-content heterogeneities between the waveguide rods), the behavior of the soil as capacitors in series should be considered.

Conclusions

If water-content heterogeneities exist between the waveguide rods, TDR measurements will be influenced by varying EMW velocities and capacitor behavior. Thus, the water-content measurement will not represent a weighted average of the area but will instead be biased toward the drier material and a serious underestimation of volumetric water content is likely. Although such situations could be encountered in the field (particularly where soil cracks occur) or in some laboratory studies, care in TDR-waveguide placement can usually avoid installation of rods parallel to layers of different water content. If TDR is used to monitor infiltration of water through soil cracks, the rods should be inserted directly into the crack rather than straddling the crack. In studies using TDR to validate flow models requiring various orientations of TDR probes, closer rod spacing coupled with shorter probe lengths will lessen the probability of water-content variations between the rods. Regardless of the application, knowledge of the orientation of soil water-content heterogeneities relative to the TDR probe is necessary for correct data interpretation.

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

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Abstract

Most ^{15}N dilution techniques disturb either the soil or N-pool size. The objective of this study was to develop a method of labeling the roots of *Populus* trees with ^{15}N without physically disturbing the soil. Such a method would enable the direct measurement of the flux of ^{15}N from dead roots into the soil organic matter. 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 wk after 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.

THE APPLICATION of ^{15}N to soil either as fertilizer or plant residue has made it possible to study N 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

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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 residue) or the N-pool size.

Successful injection of ^{15}N directly into the plant allows 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 2 wk to 1 mo following application, the N was distributed throughout the plant. We report the development of a method for studying the direct flux of labeled N from trees to the soil.

Methods

Trees of a single *Populus* clone (*P.* × *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 m*M* KCl and 0.4 m*M* 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 to 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 min or filtering through a 0.2-μm Millipore filter (Millipore Corp., Bedford, MA) to avoid introducing pathogens directly into the tree.

A centered 6-mm-diam. hole was drilled 75% of the way through a 5.0-cm-diam. 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- to 5-cm-diameter trees with success. The injection method relies on an active transpiration stream and, therefore, irrigation may be necessary when soil matric potential is low. With species other than *Populus*, the ideal protocol may have to be obtained through experimentation.

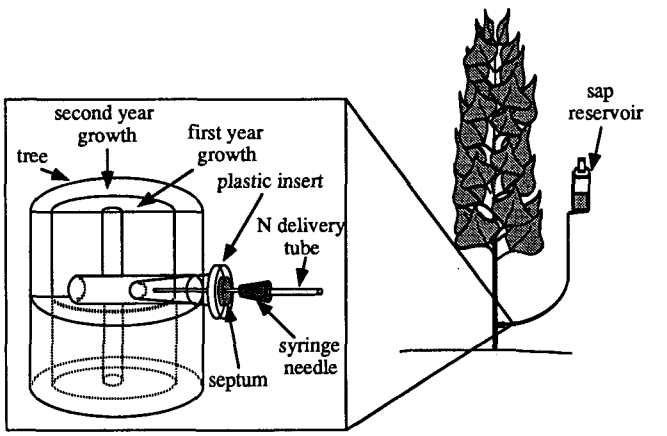

Fig. 1. Diagram of the technique used to inject ^{15}N into trees.

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 (Fig. 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 (Fig. 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 s.

Each replicate tree was centered in a 1-m^2 soil monolith (Owosso sandy loam, a fine-loamy, mixed, mesic Typic Hapludalf) by trenching to a depth of 45 cm and wrapping the soil column with two layers of plastic sheeting 2 mo prior to ^{15}N injection. Tree Set 1 contained four replicate trees injected on 13 July 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 contained four replicate trees, and they were injected on 12 Sept. 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 2 and 8 wk after injection. Four cores were taken randomly from each of the soil monoliths 2 wk after injection. Five additional cores were taken from each monolith 8 wk after injection. In order to avoid dead roots created by extracting the first set of cores, the cores extracted at 8 wk 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.

Leaf and root samples were dried in a convection oven

Table 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 d after injection of Tree Set 1 (standard errors given in parentheses).

Crown aspect	N content	Atom % ^{15}N excess†
	g kg^{-1}	
N	36.4a (1.8)	0.93a (0.17)
NE	39.1a (2.1)	1.22a (0.24)
E	41.1a (2.2)	0.78a (0.18)
SE	39.3a (1.5)	0.85a (0.23)
S	42.8a (3.3)	1.05a (0.22)
SW	42.0a (1.5)	0.80a (0.24)
W	34.9a (1.4)	0.66a (0.17)
NW	34.1a (2.0)	0.89a (0.22)

† Atom % ^{15}N excess is defined as the percentage of ^{15}N above background (0.3663%).

at 65°C for 48 h. 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, Europe Scientific, Crewe, England) 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, Europe Scientific) and thermal-conductivity detector for CO_2 (Carle, Fullerton, CA). Urea–^{15}N (0.60571 atom % ^{15}N) and 44% C (as cellulose) Whatman no. 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 1 shows no significant aspect differences of the injected N or ^{15}N 72 h after injection, using Tukey's procedure for comparison of means at $\partial = 0.05$. Following leaf fall, the leaves from Tree Sets 1 and 2 contained only 17 and 18% of the applied ^{15}N, respectively (Table 2). 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 to 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 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

Table 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 errors given in parentheses).

	N content	C content	Atom % ^{15}N excess†	^{15}N Recovered
	g kg^{-1}	g kg^{-1}		%
Tree Set 1	13.8 (1.3)	367.0 (2.5)	0.39 (0.05)	17.2
Tree Set 2	17.0 (0.5)	377.0 (3.2)	1.07 (0.11)	18.2

† Atom % ^{15}N excess is defined as the percentage of ^{15}N above background (0.3663%).

Table 3. Mean labeled N and N concentrations of different classes of roots collected with time form Tree Sets 1 and 2 (standard errors given in parentheses).

Root class	Root size	Atom % ^{15}N excess†		N content	
		2 wk‡	8 wk	2 wk	8 wk
	mm			g kg^{-1}	
		Tree Set 1 (Injected 13 July 1988)			
New white	<0.5	0.21 (0.03)	0.08 (0.01)	17.8 (0.5)	28.3 (1.6)
	0.5–1.0	0.38 (0.10)	0.18 (0.04)	19.7 (3.3)	14.6 (1.4)
Older brown	<0.5	0.19 (0.03)	0.12 (0.02)	16.7 (0.4)	27.5 (1.4)
	0.5–1.0	0.29 (0.03)	0.19 (0.02)	9.9 (0.5)	17.1 (1.5)
	1.0–3.0	0.30 (0.04)	0.21 (0.02)	9.8 (0.7)	13.6 (0.7)
	>3.0	0.28 (0.02)	0.23 (0.3)	7.5 (0.8)	10.3 (2.5)
Dead	<0.5	— —	0.07 (0.01)	— —	23.0 (1.3)
	0.5–1.0	— —	0.07 (0.02)	— —	13.1 (1.3)
	1.0–3.0	— —	0.18 (0.08)	— —	16.2 (0.5)
		Tree Set 2 (Injected 12 Sept. 1988)			
New white	<0.5	0.19 (0.09)	0.17 (0.06)	20.3 (0.6)	16.4 (1.1)
	0.5–1.0	0.55 (0.13)	0.30 (0.10)	15.5 (2.3)	14.5 (1.1)
Older brown	<0.5	0.15 (0.04)	0.14 (0.04)	16.2 (0.8)	16.1 (0.8)
	0.5–1.0	0.54 (0.11)	0.21 (0.05)	11.1 (0.6)	14.4 (0.9)
	1.0–3.0	0.93 (0.21)	0.44 (0.22)	12.6 (1.1)	18.1 (2.0)
	>3.0	0.70 (0.20)	0.55 (0.19)	10.3 (1.5)	12.7 (1.4)
Dead	<0.5	0.04 (0.02)	0.21 (0.09)	16.2 (0.7)	17.7 (1.0)
	0.5–1.0	0.01 (0.01)	0.51 (0.17)	13.4 (1.0)	16.5 (1.0)
	1.0–3.0	— —	0.17 (0.09)	— —	13.7 (2.4)

† Atom % ^{15}N excess is defined as the percentage of ^{15}N above background (0.3663%).
‡Time since injection of ^{15}N.

the enrichment of the root biomass occurred within 5 d (data not shown) and was stabilized about 2 wk after injection, regardless of the time of addition (Table 3). The increased enrichment of the dead roots in Tree Set 2 between 2 and 8 wk 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 3 for the dead-root pool and current field studies on root production (Horwath, 1990, unpublished data), the potential atom % ^{15}N excess of soil microbial-biomass N is 0.022%. The standard deviation of analysis of our mass spectrometer is 0.004 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 percentage of N in the plant biomass derived from ^{15}N with time (Table 4). This calculation is independent

Table 4. Percentage of N derived from ^{15}N in various root classes 2 and 8 wk after injection.†

Root class	Time since injection: 2 wk, Tree Set 1	2 wk, Tree Set 2	8 wk, Tree Set 1	8 wk, Tree Set 2	Change‡
New white roots					
<0.5 mm	0.54	0.19	0.21	0.17	−0.18
0.5–1	0.97	0.56	0.46	0.31	−0.38
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	−0.30
1.0–3.0	0.77	0.95	0.54	0.45	−0.37
>3.0	0.72	0.71	0.59	0.56	−0.14
Dead roots					
<0.5	—	0.04	0.18	0.21	+0.16
0.5–1	—	0.01	0.18	0.52	+0.34
1.0–3.0	—	—	0.46	0.17	+0.32

†Calculated according to Rennie and Rennie (1983).
‡Change calculated from the average of Tree Sets 1 and 2 at 2 and 8 wk minus the average of Tree Sets 1 and 2 at 2 wk after injection.

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 from time zero is due to litter turnover, and that all ^{15}N 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 wk after injection, with the exception that more label was found in the new-white-root pool of Tree Set 1 (Table 4). The dead-root pool for both tree sets at 8 wk contained similar amounts of N derived from the injected ^{15}N. Based on the July and September injections, the data indicate that 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 stem-injected ^{15}N 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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