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A ^{15}N tracer technique for assessing fine root production and mortality

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Abstract We tested a ^{15}N tracer technique to assess fine root production and mortality based on temporal measurements of the ^{15}N mass in fine root structural tissues and the ^{15}N concentration of the plant-available soil N pool. The results of a pilot study indicated that this technique is based on sound methods and reasonable assumptions. The ^{15}N tracer technique avoids most of the major limitations which hinder existing methods and may provide valuable insight into the rates and controls of fine root production and mortality in terrestrial ecosystems.

Key words Fine roots · Production · Mortality · ^{15}N · Methodology

Introduction

Fine root production and mortality are major pathways of carbon and nutrient flow in terrestrial ecosystems (Brewer 1994). It is therefore critical to gain a sound understanding of the rates and controls of these processes. However, attempts to assess fine root dynamics have been hindered by methodological limitations (Persson 1990; Mackie-Dawson and Atkinson 1991; Hendricks et al. 1993).

Current field-based assessment techniques are based on tenuous assumptions (Persson 1990; Mackie-Dawson and Atkinson 1991). Soil core methods either assume that fine root production and mortality occur asynchronously (max-min and sequential core approaches), that root biomass and necromass may be measured accurately (decision matrix approach), or that fine root decay constants may be assessed precisely (compartment flow approach) (Publicover and Vogt 1993). Rhizotron and in-growth core techniques assume that dynamics measured in experimentally disturbed rooting environments are representative of those in bulk soil (Neill 1992; Hendrick and Pregitzer 1993). The nitrogen budget method assumes that numerous ecosystem nitrogen pools and fluxes are measured accurately (Nadelhoffer et al. 1985). The carbon budget method assumes that soil carbon fluxes are in an approximate steady-state condition (Raich and Nadelhoffer 1989). Finally, while not relevant to the assumptions of the technique, the carbon isotope method is functional only at relatively small spatial scales in a limited number of terrestrial ecosystem types (Milchunas and Lauenroth 1992).

We developed a ^{15}N tracer technique to assess fine root production and mortality which may avoid many of the major limitations of the existing methods. In 1993, a pilot study using this technique was conducted in the Harvard Forest Long Term Ecological Research (LTER) Site, Petersham, Massachusetts. The objectives of this paper are to (1) describe the ^{15}N tracer technique, and (2) evaluate the results of the pilot study to assess the potential for future development and application of this technique.

Materials and methods

The ^{15}N tracer technique

The simultaneous occurrence of fine root production and mortality in most ecosystems has precluded the measurement of each process using conventional methods based on temporal changes in fine root standing biomass. The ^{15}N tracer technique overcomes this limitation by coupling isotope dilution approaches with standing biomass assessments to distinguish production and mortality dynamics

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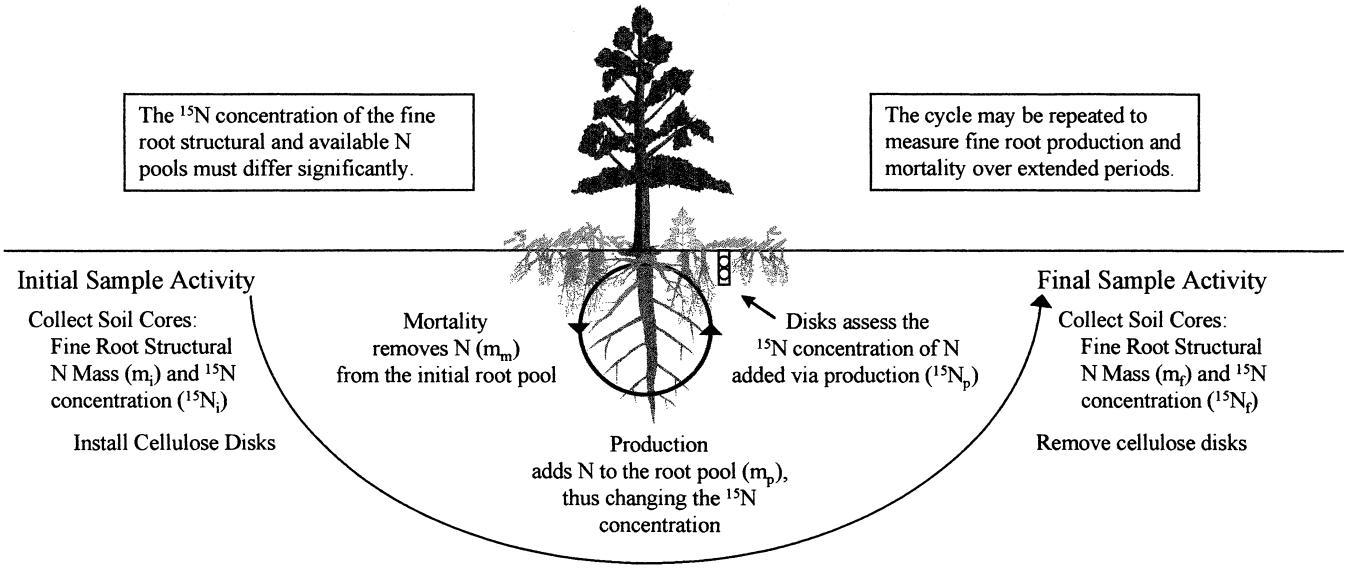

Fig. 1 The ^{15}N tracer technique field procedures used to measure the ^{15}N mass in fine root structural tissues and the ^{15}N concentration of the available N pool for the calculation of fine root production and mortality

(Fig. 1). To use this technique, the N bound in fine root structural tissues (such as cellulose, hemicellulose, and suberin) and the N in the plant-available soil pool (hereafter referred to as "available" N) must have different ^{15}N concentrations during the assessment period. If this prerequisite is satisfied, the ^{15}N tracer technique is based on the premise that the change in the mass of ^{15}N bound in fine root structural tissues during the assessment period is a function of (1) the incorporation of available ^{15}N into the fine root structural N pool via production, and (2) the removal of ^{15}N from the initial fine root structural N pool via mortality (Fig. 1). Mass balance equations are used to calculate fine root production and mortality based on measurements of the ^{15}N concentration of the plant-available soil N pool and the ^{15}N mass in fine root structural tissues. The ^{15}N mass of fine root structural tissues is measured to ensure that changes in root ^{15}N are due to production and mortality and not to the degradation and resynthesis of non-structural N compounds during the life span of the root.

The ^{15}N tracer technique is based on the following assumptions. First, at the time of fine root production, the ^{15}N concentration of N incorporated into new structural tissues is approximately equal to the ^{15}N concentration of the available N pool. Second, the N concentration of fine root structural tissues does not vary significantly during the assessment period. Third, there is no retranslocation of N from fine root structural tissues.

Production and mortality equations

Tracing the movement of ^{15}N into and out of the fine root structural N pool during an assessment period requires mass balance techniques (Nadelhoffer and Fry 1994). Assuming conservation of mass,

$$m_i {}^{15}N_i + m_p {}^{15}N_p - m_m {}^{15}N_m = m_f {}^{15}N_f \quad (1)$$

where

m_i = mass of N in the initial fine root structural pool
m_p = mass of available N added to the fine root structural pool via production
m_m = mass of N removed from the fine root structural pool via mortality
m_f = mass of N in the final fine root structural pool
$^{15}N_i$ = ^{15}N concentration of the N in the initial fine root structural pool
$^{15}N_p$ = ^{15}N concentration of the available N added to the fine root structural pool via production
$^{15}N_m$ = ^{15}N concentration of the N removed from the fine root structural pool via mortality
$^{15}N_f$ = ^{15}N concentration of the N in the final fine root structural pool.

Assuming that $m_i + m_p - m_m = m_f$ (conservation of mass) and $^{15}N_m = {}^{15}N_i$, the following equations may be derived to calculate the mass of N added to the fine root structural pool via production:

$$m_p = m_f\left({}^{15}N_f - {}^{15}N_i\right)/\left({}^{15}N_p - {}^{15}N_i\right) \quad (2)$$

and the mass of N removed from the initial fine root structural pool via mortality,

$$m_m = \left(m_i {}^{15}N_i + m_f {}^{15}N_p - m_i {}^{15}N_p - m_f {}^{15}N_f\right)/\left({}^{15}N_i - {}^{15}N_p\right) \quad (3)$$

during the assessment period. In turn, m_p and m_m estimates may be used with the estimate of N mass in the initial fine root structural pool, m_i, to calculate production and mortality rates.

Equation 2 does not account for the production of fine roots that complete their entire life cycle between the initial and final collection dates. Equation 3 does not account for the mortality of fine roots produced after the initial assessment.

Field procedures

The prerequisite difference in ^{15}N concentrations between the fine root structural N and the available N pools may be achieved without altering fine root dynamics by applying small quantities of ^{15}N depleted or enriched fertilizer ($^{15}NH_4$ and/or $^{15}NO_3$ labeled sources) to the soil (Nadelhoffer and Fry 1994). Technically, the ^{15}N fertilizer may be applied any time prior to the initiation of the assessment. However, allowing time for the ^{15}N label to become integrated into the N cycle of the soil system may yield more reliable results.

The ^{15}N mass of fine root structural tissues must be measured at the beginning and end of the assessment period by collecting soil cores, isolating live fine root tissues, extracting non-structural components, and measuring the ^{15}N content of the residual structural tissues. The resulting estimate is sensitive to the fine root standing biomass measurement so the soil core collection intensity should be sufficient to reduce the standard error to within at least 20% of the mean (see Vogt et al. 1986). Also, since Eq. 2 does not

account for the production of fine roots that complete their entire life cycle between the initial and final collection dates, fine root biomass should be quantified on sample intervals shorter than the average fine root life span, but sampling should not be conducted so frequently as to introduce error due to random variation in biomass estimates (see Singh et al. 1984).

There is no standard technique for measuring the ^{15}N concentration of the available N pool. We tested a new approach which consisted of incubating cellulose disks in the soil to serve as a carbon source for microbes resulting in the immobilization of N from the available pool. Disk installation and removal were conducted during the initial and final soil core collections, respectively to provide an integrated estimate of the available ^{15}N concentration during the assessment period.

Pilot study

The pilot study was conducted in the Harvard Forest red pine (*Pinus resinosa* Ait.) control and low chronic N addition plots (Aber et al. 1993; Magill et al. 1996). Since 1988, the control and low plots have received 0 and 50 kg N · ha^{-1} $year^{-1}$, respectively, as dissolved ammonium nitrate. In conjunction with the regular applications, nitrate labeled fertilizer ($NH_4{}^{15}NO_3$; 0.6451 atom % ^{15}N or $\delta\ ^{15}N = 766‰$) was applied in six equal applications per growing season in 1991 and 1992 (the total N additions remained constant, except for control plots which received approximately 0.1614 kg N · ha^{-1} $year^{-1}$ as $^{15}NO_3$). Non ^{15}N-enriched fertilizer additions were continued in 1993.

Fine root samples were collected on 26 April and 25 October 1993. On each date, 25 cores (5.5 cm diameter) of the organic and mineral (0–10 cm) soil horizons were removed and randomly composited to five samples per horizon for each treatment. Fine roots (live tissues generally ≤1 mm in diameter) in each sample were hand sorted and washed using a #18 mesh (1.08 mm openings) sieve. In conjunction with core collections, nine cellulose disk sets (4 Whatman #5, 5.5 cm diameter disks per set placed inside undyed, fine net stockings to reduce soil contamination) were incubated in the organic and mineral soil horizons of each plot from 14 June to 16 November 1993. After collection, the nine disk sets were randomly composited to three samples per horizon for each treatment.

Fine root and cellulose disk samples were dried (70°C to a constant weight), ground, and subsampled for ash determination (500°C for 5 h). Fine root structural components were isolated by boiling subsamples in water for three hours and filtering the residual tissues (TAPPI 1975). Structural fine root tissues and cellulose disk samples were analyzed for total N and ^{15}N using an Europa Scientific Roboprep CN Analyzer coupled with a Tracer Mass Isotope Ratio Mass Spectrometer.

Results and discussion

The ^{15}N concentration of the fine root structural and available N pools differed in the organic and mineral soil horizons of each treatment plot during the assessment period, thereby satisfying the fundamental prerequisite for using the ^{15}N tracer technique (Fig. 2). Equations 2 and 3 were used with fine root structural and available ^{15}N concentration data (Fig. 2) and fine root structural N mass data (the product of structural biomass and N concentrations in Table 1) to provide estimates of fine root production and mortality rates during the assessment period (Fig. 3).

Fine root production estimates were relatively low for the control and fertilized treatments (Fig. 3). The production of fine root structural biomass increased with fertilization in the organic and mineral horizons. The mortality rates for the initial root pool were generally high and increased with N fertilization (Fig. 3). The finding that root mortality increased with N fertilization and fine root N concentration is corroborated by the results of other studies (see Hendricks et al. 1993).

The low fine root production rates and the large disparity between production and mortality estimates were unexpected for this system (McClaugherty et al. 1982). Although there is no standard method for assessing the accuracy of these production and mortality

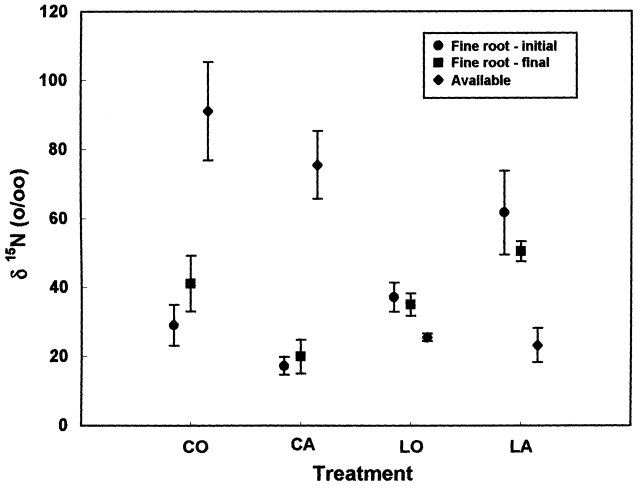

Fig. 2 The ^{15}N concentration of the fine root structural (initial and final assessments) and available N pools. *Error bars* represent the standard error of the mean. See Table 1 for the treatment abbreviation key

Table 1 Fine root structural biomass (g m^{-2}) and structural nitrogen (%) for the initial and final assessments. Values in each cell represent the means with standard errors in parentheses. For each column set, different letters within a row represent significant differences ($P < 0.05$, Student's *t*-test) between the initial and final assessments

Treatment	Biomass		Nitrogen	
	Initial	Final	Initial	Final
Control				
Organic (CO)	31.05 (10.16)[a]	25.84 (12.54)[a]	1.81 (0.14)[a]	1.70 (0.04)[a]
Mineral (CM)	62.97 (14.91)[a]	19.59 (5.24)[b]	1.34 (0.04)[a]	1.31 (0.03)[a]
Low				
Organic (LO)	101.58 (25.85)[a]	31.26 (10.10)[b]	2.30 (0.14)[a]	2.20 (0.07)[a]
Mineral (LM)	95.5 (16.04)[a]	20.49 (2.32)[b]	1.84 (0.04)[a]	1.78 (0.07)[a]

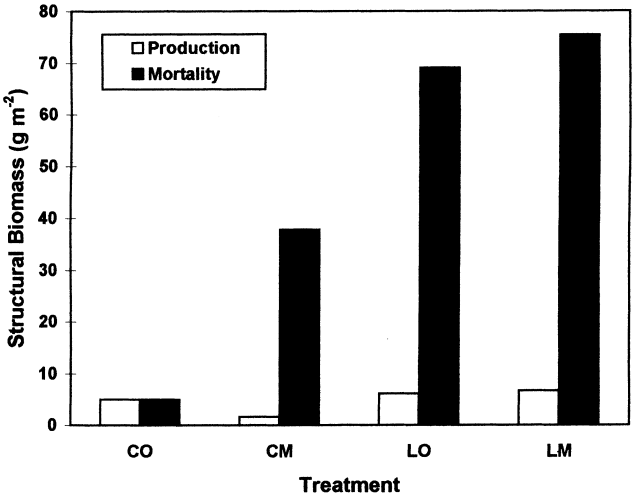

Fig. 3 Fine root production and mortality estimates obtained using the ^{15}N tracer technique. See Table 1 for the treatment abbreviation key

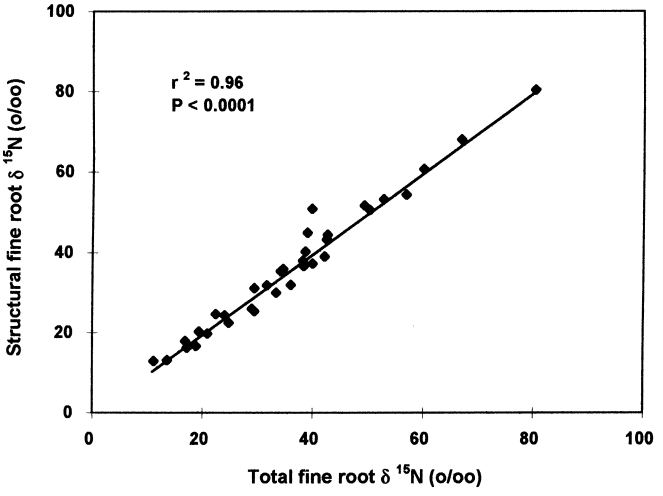

Fig. 4 The relationship between total and structural fine root ^{15}N concentrations

estimates, an evaluation of the assumptions and methods of the ^{15}N tracer technique may provide insight into potential sources of error.

Evaluation of assumptions

Fine root structural ^{15}N concentrations tracked the available ^{15}N concentrations in the organic and mineral soil horizons of each treatment plot during the assessment, suggesting that the newly incorporated structural ^{15}N was derived from the local available N pool without isotopic fractionation (assumption 1) (Fig. 2). Previous studies also have indicated that the structural N of individual fine roots is derived primarily from the local rooting zone (Friend et al. 1990; Gebauer and Schulze 1991). In addition, N isotopic discrimination during root uptake and assimilation is insignificant relative to the ^{15}N concentrations used in this study (Nadelhoffer and Fry 1994), as supported by the significant linear relationship between total and structural fine root ^{15}N concentrations observed in this study (Fig. 4).

While fine root structural N concentrations in the organic and mineral horizons of the fertilized plots were higher by approximately 0.5% N than corresponding control plot values, root structural N concentrations did not differ significantly between collection dates in either soil horizon of the two treatment plots (assumption 2) (Table 1). This finding is consistent with the results of other studies which indicated that fine roots less than 1 mm in diameter do not exhibit significant temporal variation in N concentrations (Nambiar 1987; Goldfarb et al. 1990).

While not measured directly in this study, it is probable that ^{15}N exited the fine root structural pool via mortality rather than retranslocation (assumption 3). Although Goldfarb et al. (1990) indicated that N compounds may be retranslocated from root tissues during development, it is likely that this N is derived from non-structural rather than structural constituents (Ting 1982). Nitrogen retranslocation during fine root senescence is generally considered to be insignificant (Nambiar 1987).

Evaluation of methods

The finding that fine root structural ^{15}N concentrations tracked the ^{15}N concentrations in incubated cellulose disks on each plot suggests that the cellulose disk method may provide reliable integrated estimates of the available ^{15}N concentration during the assessment period (Fig. 2). However, the cellulose disk method is a new approach for measuring the ^{15}N concentration of the available N pool and future applications of the ^{15}N tracer technique should employ a combination of methods to corroborate results. In addition to the cellulose disk method, diffusion of available N from in situ buried bag incubation and ion exchange resin bag extracts may prove useful in this effort (Binkley et al. 1986; Brooks et al. 1989).

Fine root structural biomass estimates used to calculate the mass of N in the initial and final fine root structural pools (m_i and m_f, respectively) were conservative and relatively variable due, in part, to the heterogeneous presence of hardwood and herbaceous roots in the core samples which were not quantified; this may reduce the accuracy of production and mortality estimates (Table 1). Also, the long interval between the initial and final sample dates probably permitted fine roots to complete their life cycle unaccounted for, thereby contributing to the underestimation of production. Furthermore, the single sample period used in this study may not have adequately encompassed the peak periods of fine root production which generally occur in the spring and fall in this site (McClaugherty et al. 1982;

Aber et al. 1985). As a result, the large disparity between fine root production and mortality estimates may be primarily attributed to underestimation of fine root production due to shortcomings in the experimental design of the pilot study rather than the actual methods of the ^{15}N tracer technique.

Conclusions

The ^{15}N tracer technique may provide valuable insight into the rates and controls of fine root production and mortality in terrestrial ecosystems. This technique avoids most of the major limitations that hinder existing methods in that it may be used on sites with synchronous phases of fine root production and mortality; it does not require the quantification of fine root necromass or necromass decomposition rates; it does not disturb the soil prior to the assessment of root dynamics; it is based on the assessment of only two ecosystem N pools; and it is applicable at relatively large scales in virtually all terrestrial ecosystem types. In addition, this technique is considered to be practical based on the relatively low cost of ^{15}N enriched fertilizers and sample analyses, and sample processing times that are comparable to or less than those of the other methods.

This evaluation indicated that the ^{15}N tracer technique is based on sound methods and realistic assumptions. However, this technique also has limitations; most notably it does not account for the production and mortality of roots that complete their entire life cycle between sample events and it only measures the mortality of roots present in the initial pool. Future applications of this technique should be conducted in conjunction with other methods that may be used to compensate for these shortcomings and possibly corroborate results. It is important to continue to test and, if necessary, refine this technique to ensure the reliability of fine root production and mortality estimates. In turn, the ^{15}N tracer technique may be used to gain an improved understanding of the role of fine roots in the structure and function of terrestrial ecosystems.

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