A TEST OF THE BUDGET METHOD: A REFINED APPROACH TO THE MEASUREMENT OF FINE ROOT TURNOVER

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Abstract.--The measurement of fine root turnover provides important data for nutrient cycling models, carbon cycling models, and determinations of plant productivity. Previous methods - repeated sampling and ingrowth cores - have been inadequate because of conceptual and statistical difficulties and disruptions of plants and soil.

A new method - the budget method - attempts to overcome these problems, but requires considerable data: (1) repeated measures of the standing pool of live roots and (2) of dead roots, and (3) estimates of decomposition rates in units of mass/area/time. Production or mortality rate measurement may replace the latter. The method attempts to keep a continuous budget of organic material moving into the live pool (production), out of the live pool and into the dead pool (mortality), and out of the dead pool (decomposition).

This approach was tested on the upper 25 cm of soil in a mature white oak (*Quercus alba*) stand in central Missouri. Its estimates of very fine root (< 1 mm diameter) production were compared to independent estimates via (1) ingrowth cores and (2) rhizotrons. Standing pool samples were taken bimonthly for 14 months and divided into living and dead pools using techniques involving triphenyltetrazolium chloride (TTC). Decomposition rates were measured using buried root detritus bags.

Ingrowth core and budget estimates of production showed close agreement over the entire 14 month period (56 g/m^2 and 42 g/m^2, respectively). Agreement, however, was poor over the shorter, two month intervals.

The new method was judged inappropriate for short time intervals (< 6 months) but an improvement for longer intervals (1 to 2 years). Faults of all three methods were reviewed, and a modified, long-term budget approach was suggested.

Keywords: *Quercus alba*, root decomposition, root growth, root mortality

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Fine root turnover rates in forested ecosystems have received considerable research attention in the last five years (see Harris et al., 1980; Persson, 1979, 1980a, 1980b; Cox et al., 1978, Edwards and Harris, 1977; Santantonio, 1978). The major goal of most such studies has been to quantify the organic matter which is produced annually as fine roots and lost annually via decomposition. Such information is essential to models which portray the rate of incorporation of nutrients into organic matter, their temporary "immobilization" there, and the rate of their release via mineralization. Also estimates of total plant productivity frequently omit fine root production for lack of data. Further, any model of carbon cycling within the soil - and O_2 consumption and CO_2 release - would be immensely improved by adequate estimates of fine root turnover.

The chief reason for our paucity of knowledge of fine root turnover rates is the obvious methodological difficulty of studying thin, fragile strands of organic matter which are hidden from view, are extremely well-dispersed and intermixed, and which live briefly and decay rapidly. Two additional facts increase the "methodological messiness": (1) The mass of fine roots cannot be determined without killing the sample and disturbing the growth medium significantly. (2) The aliveness of each root is not immediately apparent.

The two most popular and "successful" methodological approaches to this problem have been (1) the repeated sampling method and (2) the ingrowth core method. Almost all present estimates of fine root turnover have been obtained by one of these two approaches. Both have numerous faults, however.

The repeated sampling method simply makes an estimate of the standing pool of roots (alive and dead together, usually) at different times during the year (see Edwards and Harris, 1977; Persson, 1979). Increases in the pool size are ascribed to the growth of fine roots and decreases, to the decay of dead roots. The total annual turnover rate is computed from the sum of increases and decreases. Aside from the considerable statistical difficulties, this method ignores one glaring problem - decomposition and growth occur simultaneously; only the net change in the total pool ([growth minus decomposition] or [decomposition minus growth]) is being measured in any given time period.

The ingrowth core method requires the insertion of a root-free mass of soil, enclosed in a mesh container, into a hole in the existing forest soil profile. Roots are allowed various time intervals to penetrate these cores before they are dug up and the mass of roots determined (see Persson, 1979, 1980a, 1980b). The mass of roots measured is taken as an estimate of fine root production for the volume of the core and the time interval.

We have developed and tested a new approach to measuring fine root turnover, herein called the budget method. It is basically an elaboration of the repeated sampling method. The method is based on the following two equalities:

$$S_{A_2} - S_{A_1} = G_{1-2} - M_{1-2}$$

$$S_{D_2} - S_{D_1} = M_{1-2} - D_{1-2}$$

where $^{S}A_1$ and $^{S}A_2$ are the standing pools of alive roots at time 1 and time 2, respectively. G_{1-2} is the growth during the time interval time 1 to time 2. M_{1-2} and D_{1-2} are mortality and decomposition during the same time period, respectively. $^{S}D_1$ and $^{S}D_2$ are the standing pools of dead roots.

Restated, the equations assert that changes in the living pool ($^{S}A_2$ - $^{S}A_1$) result from gains due to growth and losses due to mortality. Similarly, changes in the dead pool result from mortality (gains) and decay (losses).

Since both equations contain the same M_{1-2} term, if all four standing pool terms ($^{S}A_2$, $^{S}A_1$, $^{S}D_2$, $^{S}D_1$) are known, only one of the three terms on the right hand side of the equations needs to be measured in order to quantify all three. For example, if M_{1-2} is known, then:

$$G_{1-2} = {}^{S}A_2 - {}^{S}A_1 + M_{1-2} \text{ and}$$

$$D_{1-2} = M_{1-2} - {}^{S}D_2 + {}^{S}D_1$$

The model does make several assumptions: (1) there are no significant translocations of materials out of the fine roots into other portions of the plant; (2) outgrowth from the lowest measured size class is zero; (3) outgrowth equals ingrowth for the other size classes. To simplify the discussion, we will accept these assumptions as true, though in reality they can be estimated and corrections made. Earlier measurements have implicitly made the same assumptions (Edwards and Harris, 1977; Persson, 1979, 1980a, 1980b).

Methodologically then, we still need methods of (1) sampling the total pool of roots, (2) determining what percentage of these are living and (3) quantifying either growth, mortality, or decomposition over the various time intervals. We chose to measure decomposition and to calculate growth and mortality from the above equations.

For comparison and testing purposes, we installed ingrowth cores to compare these measurements of growth with those obtained from the continuous budget method. As a further check on both methods we kept qualitative measurements (root length growth/time) of root growth through most of the year; these were obtained from glass-walled rhizotron measurements. If the above methods were accurate, the relative amounts of root biomass production measured in different seasons should correspond roughly with the relative amounts of root length growth observed in the rhizotrons.

METHODS

Site

The study was conducted at Ashland Wildlife Area in central Missouri, 24 kilometers southeast of Columbia, Boone County. The study plot consists of approximately 0.2 hectare with an overstory heavily dominated by white oak (*Quercus alba*) (89% of basal area), with some northern red oak (*Q. rubra*) (8%), hickory (*Carya* spp.) (2%), and sugar maple (*Acer saccharum*) (1%). Ring counts indicate that the stand is approximately 105 years old; basal area at the time of the study was 24 m^2/ha. Located on a flat ridge top, which has retained its loess cap of approximately 1 m, the soil is somewhat poorly drained with about 25 cm of silt loam A over silty clay B.

Sampling

The standing pool of live and dead roots was sampled bimonthly (except for winter) for 14 months (April 1, 1981-June 1, 1982). Only roots within the top 25 cm of soil were sampled. Thirty cores, 10.2 cm in diameter and 25 cm in depth, were taken with a cylinder equipped with a hole saw blade and mounted on a portable, gasoline-powered posthole digger. Cores were washed through a 2 mm sieve and non-root material removed by hand. Roots were sorted into four size classes (0-1, 1-2, 2-3, and 3-5 mm), then oven-dried at 105°C, weighed, ashed at 550°C, and reweighed to obtain ash-free oven-dried weights.

Living Percentage

The percentages of living and dead roots were determined from bimonthly sets of 30 separate, composited samples using 2,3,5-triphenyltetrazolium chloride (TTC) according to the methods of Parker (1953) and Jacques and Schwass (1956). The colorless TTC is converted to red stain (Formazan) via an enzymatic reduction in live tissue. All roots were stained: roots larger than 1 mm diameter were sorted into live and dead classes by individual inspection, using stain and visual appearance as criteria. Roots less than 1 mm were characterized by the spectrophotometric absorbance at 450 μm of extractable dye per 200 mg of root tissue, according to the method of Knievel (1973).

Decomposition

Decomposition rates were determined from the percent weight loss from 30 pre-weighed dead root samples buried inside nylon stockings at 5 cm for the two month intervals. The mesh size (0.5 mm, stretched) was small enough to prevent loss of roots, but large enough to allow entry of microarthropods.

Ingrowth Cores

One hundred and eighty ingrowth cores were buried at the beginning of the experiment (April, 1981). These cores had been extracted from an adjacent field by the identical procedure described under "Sampling" and placed in plastic mesh bags (mesh size = 5 mm). These cores were then transferred to identically-sized, fresh auger holes on the site and good soil contact assured by using a silt slurry. Ten to fifteen of these ingrowth cores were dug up at two month intervals and the total ash-free oven-dry weight of root ingrowth determined.

Rhizotron

New root extension growth was measured (biweekly, except in winter) in three, nearby, glass-walled rhizotrons established three years previously. Only the upper 25 cm of soil were observed.

RESULTS

Over the entire 14 months, the two estimates of fine root production (growth) are very close. The ingrowth cores show a production rate of 56 g/m^2 for the 14 month period (see Table 1); the budget approach, using decay-

ing root detritus bags, indicates a production of 42 g/m^2 (roots < 1 mm diameter, top 25 cm for the same time period) (see Tables 1 and 3).

Table 1.--A comparison of estimates of fine root (< 1 mm) production by the budget method, ingrowth core measurements, and rhizotron observations. Upper 25 cm of a *Quercus alba* stand. Rhizotron percentages are percentages of the total length of extension growth observed during the 14 months.

Time Period	Budget	Ingrowth Core	Rhizotron
	g/m^2	g/m^2	% of total
April 1 - June 1 (1981)	34 (81%)	4[a] (7%)	(22%)[d]
June 1 - August 1	-4 (-10%)	24[b] (43%)	(39%)
August 1 - October 1	-14 (-33%)	12[c] (21%)	(12%)
October 1 - April 1, 1982	-20 (-48%)	5[c] (9%)	(4%)
April 1 - June 1 (1982)	46 (110%)	12[c] (21%)	(22%)
Total, 14 months	42 (100%)	56 (100%)	(100%)

[a] n = 11
[b] n = 10
[c] n = 15
[d] Rhizotron data for April 1 - June 1, 1981 is based on data collected from April 1 - June 1, 1982.

When we examine these production measures over shorter time intervals, however, there is quite a discrepancy between the budget method and the ingrowth core method. The budget method repeatedly shows almost all growth occurring in the spring, with "negative growth" occurring in late summer and winter. The ingrowth cores, on the other hand, indicate a summer peak in growth and relatively little occurring in the spring. The rhizotron data agree closely with the ingrowth core data; both are in agreement with previous rhizotron observations on the seasonality of white oak root growth in the same setting (Dougherty, 1977).

Tables 2 and 3 summarize some additional information the budget method is capable of providing, on the size of the standing pools of living roots and of dead roots (Table 2) and on decomposition and mortality rates (Table 3). In Table 2, the total pool of root material remained fairly constant, while pools of living and dead roots fluctuated by as much as 70-80 g/m^2. The live pool remained steady until late summer when it declined, apparently during a dry period in September; it had failed to return to its initial level by the end of the following May. Table 3 indicates that decay and growth were in equilibrium over the 14 month period, but that high mortality over the 14 months had, at least temporarily, enlarged the dead pool and reduced the live pool.

Table 2.--Seasonal distribution of fine root (< 1 mm) biomass in the top 25 cm of a *Quercus alba* stand. Each total standing biomass figure (1) and percent living figure (2) represents 30 samples. Live (3) and dead (4) pools were calculated from the product of (1) and (2).

Point in Time	(1) (S_T) Total Standing Biomass[a]	(2) Percent Living Roots	(3) (S_A) Live Standing Biomass	(4) (S_D) Dead Standing Biomass
	g/m^2	%	g/m^2	g/m^2
April 1, 1981	272 ± 31	92	251	21
June 1	300 ± 14	80	241	59
August 1	290 ± 28	91	263	27
October 1	267 ± 23	70	186	81
April 1, 1982	236 ± 28	79	185	51
June 1	275 ± 26	67	185	90

[a]95% confidence intervals.

Table 3.--Quantifications of fine root (< 1 mm) decomposition, production, and mortality by the budget method. Upper 25 cm of the same *Quercus alba* stand as in Tables 2 and 3. Decomposition rates (%) were measured directly and converted to g/m^2 using standing dead pool data. Production and mortality rates were computed from decomposition data and standing pool data. Numbers in parentheses are 95% confidence intervals.

Time Period	(D) Decomposition	(G)[b] Production	(M)[c] Mortality
	g/m^2	g/m^2	g/m^2
April 1 - June 1, 1981	6 (±3)	34 (±48)	44 (±27)
June 1 - August 1	6 (±3)	-4 (±45)	-26[a] (±30)
August 1 - October 1	9 (±3)	-14 (±54)	64 (±28)
October 1 - April 1, 1982	12 (±4)	-20 (±55)	-19[a] (±31)
April 1 - June 1, 1982	7 (±3)	46 (±57)	46 (±35)
Total, 14 months	40 (±16)	42 (±73)	109 (±29)

[a] Negative mortality is impossible. These figures indicate some form of error. Negative growth is quite possible; see text.

[b] $G_{1-2} = S_{T_2} - S_{T_1} + D_{1+2}$.

[c] $M_{1-2} = S_{D_2} - S_{D_1} + D_{1+2}$.

DISCUSSION

Why should the ingrowth core method and the budget method agree over the long term and disagree so completely over the short-term? At first glance, the ingrowth core method would appear to be an easier and more accurate method of measuring root production than the budget method. The former agrees more closely with rhizotron data, shows no anomalous "negative growth", and has

smaller confidence intervals. Upon closer examination, however, the first two of these reasons lose significance, and may even cast doubts on the validity of the ingrowth core method.

The seasonal discrepancies between the two methods, so apparent in Table 1, can be partly attributed to the fact that the two methods are really measuring different aspects of root production. The budget method looks at changes in the total weight of both new and old roots (in the 0-1 mm size class, here); the ingrowth core method looks only at recent extension growth. In fact, the "older" (i.e., existing prior to ingrowth installation) fine roots might be expected to show fluctuations in weight through the seasons as carbohydrate reserves fluctuate (Ford and Deans, 1977). "Negative growth" is hardly anomalous, but to be expected as high respiration rates in summer and the lack of new photosynthate in winter deplete carbohydrate reserves. Likewise the outgrowth of "older" roots (0-1 mm) into the next size class can contribute to "negative growth." The ingrowth cores, failing to reflect these changes in "older" fine roots, would be expected to agree more closely with data from rhizotron records which measure the length of recent extension growth. But the mass changes of the "older" fine roots, which make up about 80% of the fine roots at the end of the growing season, are an important part of fine root production.

The wide confidence intervals around the budget method estimates are another possible reason for the lack of agreement seasonally between the budget method and the ingrowth core method. The large variability within the sample population of fine roots makes a good estimate of its true mean size difficult. When dealing with small changes in pool size due to small amounts of growth, mortality, and decay, errors in estimates of the true mean can easily obscure these changes. Hence, the budget method is of little use for small, short-term measurements. The large confidence intervals are apparently the major problem with the budget method; any effort to reduce them is especially beneficial, because the confidence intervals are additive in the budget model.

Are the ingrowth core method and/or the budget method valid? The budget method appears logically sound, though the assumptions made should not be ignored. The 14-month agreement between budget method estimates and ingrowth core estimates of production make both appear reasonable. Further, the agreement between decay estimates and production estimates enhance the value of each, as one would expect a mature forest to be near equilibrium in this respect. Finally, agreement between spring, 1981 and spring, 1982 estimates of decomposition, production, and mortality (see Table 3) is further evidence of the budget method's validity.

The ingrowth core method, however easy and accurate it may appear, has several features which cast doubt on its validity. The act of disturbing both the soil and the root system may distort growth patterns. The method seems to be subject to an initial lag period, during which the severed roots are apparently adjusting to this shock. This lag has been observed here as well as elsewhere (Persson, 1979). Certainly the removal of live roots from the core alters somewhat the moisture and nutrient conditions within. Ingrowth core measurements are also influenced by the core's geometry. The surface to volume ratio of the core itself certainly affects the rate of reoccupation. The diameter of the core similarly determines the total amount of

time required to reoccupy the total core column. Finally, the ingrowth core method is limited with respect to time span. With time, mortality and decay become significant (but unmeasurable) within the core.

Is the budget method an improvement over other methods? In addition to its apparently higher validity, the budget method provides a more complete set of data than either the ingrowth core or the traditional repeated sampling approach. Since most newly-extending roots are less than 1 mm in diameter, what is happening to larger roots is virtually ignored by the ingrowth core. With the budget method data has also been collected for 1-2, 2-3, and 3-5 mm size classes. The ingrowth core method obviously also provides no data on the standing pool sizes, the mortality rate, or the decomposition rate.

The repeated sampling method not only does not allow simultaneous measurement of mortality, decay, and growth rates, but confuses the three by using net growth as a measure of gross growth and net loss of fine root material as a measure of decomposition. Finally the budget method does not require the complex statistical approach that the repeated sampling method requires.

CONCLUSIONS

A new "budget method" was applied to the measurement of fine root production, decomposition, and mortality in a white oak stand and found to be reasonably accurate over a 14-month period, but inappropriate for shorter time intervals. It seems to us that a modification of this budget approach and a combining of it with other techniques would be the simplest, most complete, and most accurate approach to measuring fine root turnover. The budget approach, if used over a long time period (e.g., one year), would require only two good standing pool measurements. This reduction in the number of sampling times, would, from a labor standpoint, allow larger sample sizes and therefore more accurate estimates of the true mean. To avoid fluctuations in mass due to changes in carbohydrate reserves, samples should probably be taken at the same time of year. With live-dead percentage data and decay rate information, annual amounts of decomposition, growth, and mortality could be deduced accurately. For a more precise division of these events seasonally, decaying litter bag data and/or rhizotron observations and/or ingrowth cores and/or even temperature and moisture calculations might be used to subdivide the annual rates. The major information lost by this modification of the method is an accurate seasonal pinpointing of mortality. Careful monitoring of the size of the dead root pool for large fluctuations could be useful here.

LITERATURE CITED

Cox, T. L., W. F. Harris, B. S. Ausmus, and N. T. Edwards. 1978. The role of roots in biogeochemical cycles in an eastern deciduous forest. Pedobiologica 18:264-271.

Dougherty, P. M. 1977. Net carbon exchange characteristics of a dominant White oak tree (Quercus alba L.). Unpublished Ph.D. Diss., Univ. of Missouri, Columbia. 222 p. Diss. A. Int. B. 38:3487.

Edwards, N. T. and W. F. Harris. 1977. Carbon cycling in a mixed deciduous forest floor. Ecology 58:431-437.

Ford, E. D. and J. D. Deans. 1977. Growth of a Sitka spruce plantation: spatial distribution and seasonal fluctuations of lengths, weights and carbohydrate concentrations of fine roots. Plant and Soil 47:463-485.

Harris, W. F., D. Santantonio, and D. McGinty. 1980. The dynamic below-ground ecosystem. In: Forests: Fresh Perspectives from Ecosystem Analysis, Proceedings of the 40th Annual Biology Colloquium (R. H. Waring, ed.), p. 119-129. Oregon State Univ., Corvallis.

Jacques, W. A. and R. H. Schwass. 1956. Root development in some common New Zealand pasture plants. VII. Seasonal root replacement in perennial ryegrass (Lolium perenne), Italian ryegrass (L. multiflorum) and tall fescue (Festuca arundinacea). N.Z.J. of Science and Technology 37:569-583.

Knievel, D. P. 1973. Procedure for estimating ratio of living to dead root dry matter in root core samples. Crop Science 13:124-126.

Parker, J. 1953. Some applications and limitations of tetrazolium chloride. Science 118:77-79.

Persson, H. 1979. Fine root production, mortality, and decomposition in a young Scots pine stand in central Sweden. Vegetatio 41:101-109.

Persson, H. 1980a. Spatial distribution of fine root growth, mortality, and decomposition in a young Scots pine stand in central Sweden. Oikos 34: 77-87.

Persson, H. 1980b. Death and replacement of fine roots in a mature Scots pine stand. Ecol. Bull. (Stockholm, Sweden) 32:251-260.

Santantonio, D. 1978. Seasonal dynamics of fine roots in mature stands of Douglas-fir of different water regimes. A preliminary report. In: Symposium: Root Physiology and Symbiosis, Nance, 11-15 September, 1978 (A. Riedecker and J. Gagnaire-Michard, eds.). p. 190-203. CNRF Champenoux-54280 Seichamps, France.