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Techniques for Examining the Carbon Relationships of Plant-Microbial Symbioses

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I. INTRODUCTION

A. Symbioses in Which ^{14}C Is a Useful Tracer of C Flow

Symbioses between higher plant root systems and microorganisms frequently involve the exchange of energy in the form of reduced carbon compounds from photosynthesis for inorganic nutrients. Biological N fixation, particularly by legume-*Rhizobium* symbiosis, has received much attention because of its importance to agriculture and to global N cycling and its potentially high cost in terms of host plant photosynthate. Less attention has been paid to the C costs of mycorrhizal symbioses despite their extremely widespread occurrence in nature. Vesicular-arbuscular (VA) mycorrhizal fungi appear to be obligate biotrophs, deriving all their C from the host plant. While it is clear that ectomycorrhizal fungi also derive C from the host, the extent of saprophytic activity and thus the proportion of fungal C derived from the host plant is uncertain. Other looser associations of rhizosphere and rhizoplane organisms such as *Azospirillum* and various associative N-fixing organisms have no specialized structures for the exchange of materials with the host plant, but may be biochemically specialized to regulate plant growth and activity.

B. Carbon Costs to Plant

1. Theoretical Estimates

The broad limits of C costs to plants hosting microbial symbionts can be estimated by consideration of the biomass and expected molar growth yields of the various components of the symbiosis. Consider, for example, a hypothetical soybean plant that grows from a 0.1 g seed to achieve maximum biomass after 12 weeks. In the absence of symbionts a total plant biomass of 30 g (12 g C) is formed from the photosynthetic fixation of 20 g C. The molar growth yield of this plant is 0.6 and its average specific growth rate falls between the limits 0.14 and 0.07/day, representing linear and exponential growth. We now add rhizobial and VA mycorrhizal symbionts to this plant and stipulate that 50% of its total N content of 1 g is derived from N fixation. The biomass of root nodules and mycorrhizal fungus at 12 weeks are both 0.5 g (0.2 g C). If the molar growth yield of nodules is assumed to be 0.5 and the ratio of C used to N fixed by nitrogenase is 3:1 (Atkins *et al.*, 1978), the C diverted to root nodules will be 1.9 g, of which 0.4 g will be used in symbiont growth and 1.5 g by nitrogenase. The molar growth yield of VA mycorrhizal fungi appears to be low (Harris and Paul, 1987) (here we will assume a value of 0.2); thus, mycorrhizal fungus growth would require 1 g C. If plant biomass C is still to achieve 12 g, total photosynthesis must be increased to 22.9 g C, reducing plant molar growth yield to 0.52, a decrease of 13%. In the absence of increased photosynthesis, the decrease in molar growth yield would reduce the average specific growth rate by the same 13% proportion. From this we can place approximate limits on the potential effects of the C costs of these symbioses in reducing plant biomass, from 13% if growth were linear, to 45% if growth were exponential.

In real symbiotic systems many other factors must be considered. Important among these are the actual limitations to plant and symbiont growth in a particular environment and the nature of growth curves of the component partners of the symbiosis. When plant photosynthesis and C export are limited by nutrient availability or by feedback inhibition, there is potential for increased C fixation in the host which may "compensate" for the C cost of the symbiosis if an effect of the symbiosis is to relieve the limitation. This may occur through an increase in the supply of the limiting factor, for example P or N, or simply by the microbial utilization of excess carbohydrate. Symbioses may also alter the architecture of the host plant in ways that increase photosynthetic capacity through increases in the ratio of leaf area to total biomass (Harris *et al.*, 1985).

The allocation of C to microbial symbionts is not uniform throughout the growth and activity cycles of the component organisms. The mutualistic

effects of C and nutrient exchange depend therefore on the synchrony of growth cycles as well as edaphic and environmental factors. Tracer techniques can be used to measure these costs.

2. Measurements

The theory of photosynthetic pulse-chase labeling as a method of examining the C economy of systems and the general methodology of exposing plants to pulses of $^{14}\text{CO}_2$ have been described by Warembourg and Kummerow, in Chapter 2, this volume. We will deal with methodologies important to the examination of symbioses and point out features of experiment and equipment design that we have found to be important in some of the symbiotic relationships examined over the last 20 years.

II. MATERIALS AND PROCEDURES

A. Chamber Design

Much of the carbon flux through the symbiotic root system will be released as respired CO_2 ; it is therefore important to design the exposure system to separate and contain both the aboveground and belowground atmospheres. Separation of the belowground atmosphere can be achieved by growing the plants in cylinders that can be closed at the top and bottom. This requires a gas-tight seal around the plant stem. Terostat or some brands of modeling clay (Permaplast, American Art Clay Co., Indianapolis, Indiana) work very well as stem sealants for woody or herbaceous plants with distinct primary stems; grasses pose severe problems because the seal must usually be made above the first node and gas leaks between the inner leaves are frequent. Polyvinyl chloride (PVC) drain pipe is inexpensive, locally available in many diameters, easily machined, and can be glued readily to itself. It is thus a good material for the construction of belowground containers. Cylinders may be split longitudinally and clamped or bolted together to facilitate dissection of the soil-plant-symbiont system at the destructive harvest of the experiment. A typical apparatus for exposing plants and measuring or collecting respired CO_2 is shown in Fig. 1.

Cylinder size must be a compromise between sufficient rooting volume and the difficulty of harvesting root and other biological components from large volumes of soil or other media. In cylinders of any reasonable size roots will inevitably be concentrated at the cylinder walls. *In situ* experiments have been conducted in undisturbed soil blocks (1 m³) made with a narrow ditching machine ("Ditch Witch"). Plywood dividers were inserted into a square array of slots in the field soil and the vertical faces of the monoliths wrapped in 0.18 mm vinyl sheet before back-filling the slots.

Large volumes of soil can reduce the sensitivity and dynamic accuracy of measurements of the respiration of the plant-symbiont system by (at least) two mechanisms. First, by buffering the exchange of respired $^{14}\text{CO}_2$ with the collection system and second, by diluting the plant-symbiont-derived CO_2 with CO_2 from mineralization of organic matter in the soil. Most experiments on symbiotic systems will involve comparisons between plants of

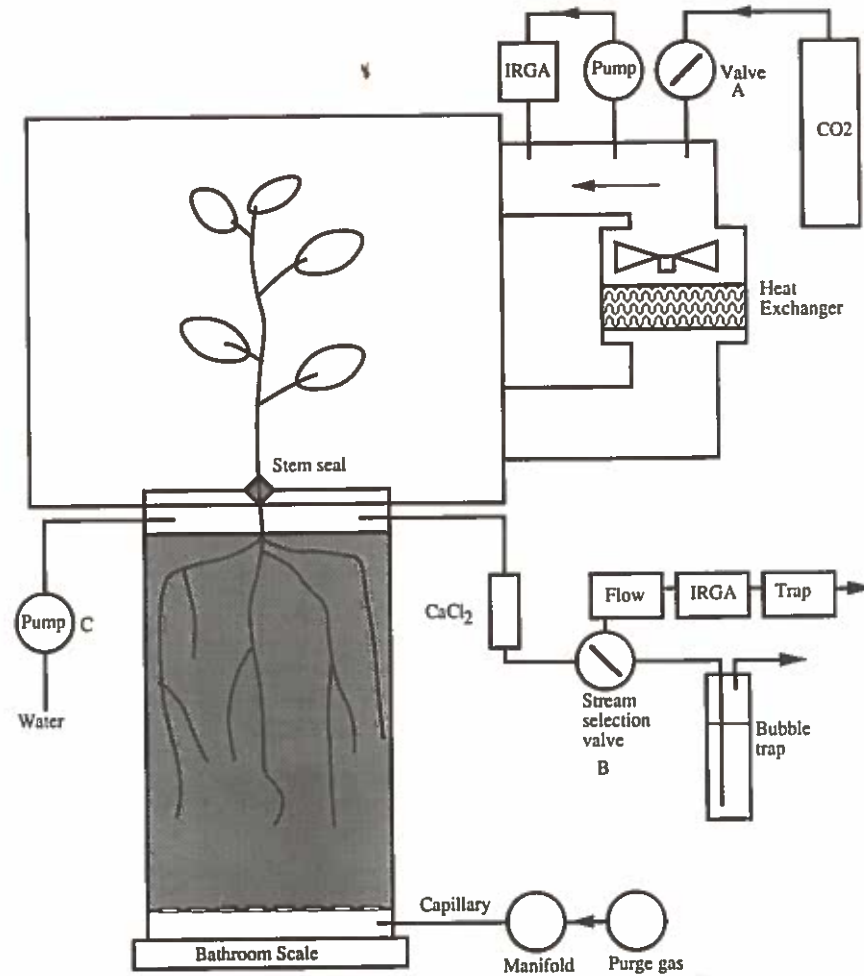


Fig. 1 Diagram of $^{14}\text{CO}_2$ labeling and respiration chamber. Analog signals for aboveground and belowground CO_2 concentrations, belowground purge flow rate, canopy air temperature, and cylinder weight are digitized and read by a microcomputer. The program controls canopy CO_2 concentration by admitting $^{14}\text{CO}_2$ through valve A and valve B sequentially selects each of several purge flows. Water is added to cylinders by pump C.

different symbiotic states so that several plants will be needed for each experiment. It is expensive to duplicate the control apparatus for the aboveground atmosphere of each plant. We use an aboveground chamber common to all plants. This requires only a single set of environmental control devices and ensures that all plants are exposed to the same aboveground atmosphere and environment. Disadvantages are that CO_2 consumption by photosynthesis and production by shoot dark respiration cannot be attributed to individual plants.

We fabricate aboveground canopies from a variety of transparent plastic films depending on the required size and application. Small, self-supporting canopies for indoor use are made from Propafilm C (Imperial Chemical Industries, Wilmington, North Carolina), which is relatively impervious to gaseous diffusion in very thin films, is transparent in the infrared, and easily heat sealed. This material is too fragile for very large canopies or in the field where stress from wind can be expected; here we use acrylic sheet glazing material (4 mm). This material can be glued and, when supported by a frame, forms large, durable canopies.

B. Environmental Control

Control of the aboveground atmosphere and temperature within the canopy is essentially similar to the methods described elsewhere. $^{14}\text{CO}_2$ may be generated as required by the addition of acid to $\text{Na}_2^{14}\text{CO}_3$ within the labeling apparatus. Alternatively, it may be advantageous to prepare $^{14}\text{CO}_2$ at the required specific activity in advance and trap the gas cryogenically in a small cylinder. The apparatus comprises a manifold with connections to: (1) an empty 2-lb CO_2 cylinder; (2) a Buchner flask containing $\text{Na}_2^{14}\text{CO}_3$ solution and fitted with separatory funnel containing H_2SO_4 (1 M); and (3) a vacuum pump and gauge. The manifold has an inline CaCl_2 trap for water. The system is evacuated, the vacuum pump is then isolated, and the CO_2 cylinder cooled by partial immersion in liquid N_2 . $^{14}\text{CO}_2$ is generated by addition of acid such that the pressure within the manifold remains somewhat less than atmospheric. The $^{14}\text{CO}_2$ is trapped as a solid within the cylinder. Once the $^{14}\text{CO}_2$ collection is complete the cylinder valve is closed and the temperature allowed to return to ambient.

A valve is used to add the gas directly to the canopy atmosphere to maintain the required concentration and specific activity throughout the tracer pulse. The valve is computer controlled using digitized signals from an infrared gas analyser (IRGA) or rate meter equipped with a thin-window Geiger-Müller tube (see Chapter 2, this volume). Each demand for CO_2 results in the addition of a preset volume of gas from the storage cylinder followed by a latent period in which no addition is allowed; this damps oscillation in the control system. The amount and rate of $^{14}\text{CO}_2$ addition is

derived from a record of the operation of the addition valve. This simplifies the handling of the $^{14}\text{CO}_2$ during the experiment and may provide better control of $^{14}\text{CO}_2$ addition than direct generation from carbonate within the apparatus.

It is important to obtain good control of soil moisture in cylinder experiments because of its effects on plant C partitioning and symbiont or soil microbial activities. Plant transpiration transfers water from the belowground to the aboveground canopy, where it condenses on the cooling coils. It may be necessary to include a drainage system to remove this water. Return of the condensate to the soil should be avoided because of its dissolved $^{14}\text{CO}_2$ content. Soil water loss is usually replaced by watering to weight at frequent intervals. In experiments with small cylinders the primary problem is the frequency at which water content must be adjusted. Large cylinders are difficult to move onto a suitable scale. In both cases such manipulations disrupt the apparatus used to contain and monitor the aboveground and belowground atmospheres. It is also difficult to maintain synchrony in wetting and drying cycles in different cylinders. Electronic bathroom scales that detect weight using strain gauges can be modified to be read by a computer. Cylinders of up to 150 kg can be permanently mounted on the scales. It then becomes possible to continuously monitor water content and either to maintain soil water within narrow limits or to control wetting and drying cycles by additions of water under programmed control. Tensiometers or conductivity measurements using Bouyoucos blocks can also be used. We prefer gravimetric methods; they are more reliable and are not restricted in working range.

C. $^{14}\text{CO}_2$ Pulse

The specific activity of the applied $^{14}\text{CO}_2$ and the length of the tracer pulse can be varied to obtain sufficient label in the pools of interest. A simple model of the anticipated ^{14}C distribution is useful in predicting tracer dilution and turnover times in various pools so that experiments can be designed to be sensitive to changes in the most dilute and slow pools of interest and approximate detection limits calculated. In general, investigations of C allocation to root systems and microbial symbionts require relatively large amounts of label to be assimilated because the tracer must pass through several intermediate compartments before reaching the symbiotic components. The "shape" of the ^{14}C pulse becomes broader and shallower in each successive C pool and may be difficult to detect, either in pools remote from the origin or in those with slow turnover, if insufficient tracer is incorporated. Short pulses at very high specific activity should yield the highest resolution of tracer dynamics because the shape of the peak of tracer concentration (specific activity) in dilute pools will be more easily

discerned than with a broader pulse at lower specific activity. However, there are several other factors that do not favor the use of short pulses:

1. The possibility of radiation damage to plant leaf tissues, components of which may initially achieve specific activities close to that of the applied tracer. Sauerbeck and Führ (1963) found effects on growth in plants continuously exposed to $^{14}\text{CO}_2$ at a specific activity of 3.7 MBq/g C but not at 0.37 MBq/g C. This limit can be exceeded in pulse label experiments because of the transient nature of the radiation dose. We have used specific activities of 3.7–37 MBq/g C for short (1–2-hr) pulses without obvious effects on plant growth.
2. The transitions between $^{12}\text{CO}_2$ and $^{14}\text{CO}_2$ in the aboveground chamber cannot be instantaneous. In short pulses (<2 hr), the periods during which input tracer concentration is changing constitute an important part of the overall pulse and may greatly complicate subsequent interpretation.
3. The rebuilding of leaf photosynthate pools early in the photoperiod after nighttime depletion is likely to alter the source-sink balance such that belowground C allocation is reduced early in the photoperiod. A useful strategy for integrating diurnal variation in C allocation is to apply the label for one full photoperiod.

D. Belowground Respiration

The measurement of belowground respiration is important for several reasons. Respired CO_2 comprises an important component in the calculation of the C balance of all plants. This is particularly true of plants supporting microbial symbionts where up to 40% of the total C flux through the plant can be released as CO_2 belowground. Additionally, the respired $^{14}\text{CO}_2$ provides a valuable insight into the dynamics of tracer movement in the plant-symbiont system, which could otherwise be obtained only by sequential destructive harvest of many replicate plants or by the use of relatively expensive and inaccessible ^{11}C tracer techniques.

1. Methods of Measurement

The belowground evolution of CO_2 and $^{14}\text{CO}_2$ has been measured by two methods that may be classed as point sampling and total collection approaches. Point sampling has been applied particularly to plants labeled *in situ* (Warembourg and Paul, 1977). The method relies on the collection of small gas samples from tubes or sampling ports embedded at various positions in the rooting volume of the experimental plants. Samples withdrawn periodically for analysis are assumed to be in equilibrium with the soil atmosphere at the collection point. Such samples can give excellent informa-

tion on the dynamics of the tracer pulse in the belowground atmosphere. However, derivation of quantitative estimates of total $^{14}\text{CO}_2$ from these data for C balance calculations requires an accurate model of diffusion in the soil atmosphere. It also involves uncertainty both in the volume of soil atmosphere sampled and in the distribution of the samples in relation to points of biological activity.

Where C balance data are required and the root-symbiont system can be at least enclosed in an open-bottomed container, it is preferable to collect or measure all CO_2 released in the belowground system. This has the advantage that the recovery of $^{14}\text{CO}_2$ will be essentially complete and avoids some of the uncertainties of the point collection approach. In the case of an open-bottomed, *in situ* cylinder, CO_2 is continuously purged from a headspace to be replaced by CO_2 diffusion from depth. Gas exchange will also occur through the embedded cylinder base; this must be estimated from a model of diffusion. The dynamics of the $^{14}\text{CO}_2$ release (and loss through the cylinder base) will be confounded by the lengths of diffusion paths from sources at different depths in the soil column to the surface headspace. An alternative may be to estimate ^{14}C respired belowground by mass balance calculation:

$$\begin{aligned} \text{net } ^{14}\text{C belowground respiration} &= \text{net } ^{14}\text{C assimilation} \\ &- [^{14}\text{C biomass} + ^{14}\text{C shoot respiration} + ^{14}\text{C soil}]. \end{aligned}$$

In a closed cylinder the soil atmosphere can be purged by continuous passage of a flushing gas through the soil volume. The temporal accuracy of the measurements of respiration depend on the absorptive capacity of the soil and soil solution for CO_2 and on the efficiency of flushing. Figure 1 shows a closed cylinder design with airspaces above and below the soil column, which is supported on a perforated PVC plate. The flushing gas (air or CO_2 -free air) is introduced into the tailspace of the cylinder, passes through the soil volume, and exits via the headspace. The flushing gas will flow primarily along preferential pathways and there may be pockets of soil atmosphere not in equilibrium with the flushing gas. The purge efficiency can be tested by introducing $^{14}\text{CO}_2$ into tubes prepositioned through the soil column base and observing washout. Purge flow rates depend on the respiratory activity of the system, but rates of about 2 ml/min/kg soil will yield exit concentrations of 1–2% CO_2 in typical systems.

Several plant-symbiont systems will normally be exposed simultaneously; it is therefore necessary to implement parallel soil atmosphere purge systems for each cylinder. We have used flow systems based either on suction or

pressure as driver which, while very similar in design and using identical components, differ in their responses to leaks. This difference can be important, particularly in extended experiments where leaks at some point seem inevitable.

In the pressure-driven system (Fig. 1) a pressurized manifold (approximately 500 Pa) supplies gas to the tailspace of each cylinder via a Teflon capillary (1 m \times 0.1 mm). The capillaries form almost all the resistance to flow through the system and thus regulate the flow rates. The gas leaving the cylinder is dried, passed to a stream selection valve and either directly to an individual CO_2 collection tube or, via an electronic flow transducer and IRGA, to a common CO_2 trap. The stream selection valve, flow transducer, and IRGA are under computer control using analog-to-digital (A-to-D) converters for signal measurement and relays to convert logic signals for valve and instrument control. Each gas stream is selected and measured sequentially. The headspace in the pressure-driven system is at slightly greater than atmospheric pressure because of the resistance to flow through the subsequent measuring devices and thus tends to leak out. This is immediately obvious because the measured flow rate decreases and the leak can be located and repaired. The concentrations of CO_2 and $^{14}\text{CO}_2$ are unaffected because the real purge flow rate of the cylinder is unchanged by the leak. The suction-driven system has the capillary and manifold moved to the effluent side. The headspace pressure is less than atmospheric and thus tends to leak inwards. While this arrangement is slightly less susceptible to leaks because the pressure differential across the headspace seals is smaller than that of the pressure-driven system, a leak is not signaled by any obvious change in system behavior. The resulting contamination of the effluent stream by aboveground or canopy atmosphere may lead to undetected errors in the data.

2. Specific Activity of Belowground Respiration

In a pulse-chase experiment the specific activity of the CO_2 respired by the root-symbiont-rhizosphere biota will show a general form similar to that of the curves in Fig. 2. Specific activity reaches a maximum, then decays to an asymptote after several days. Curves may be complex, with subsidiary local maxima, when source pools diurnally alternate between sucrose and stored starch reserves. At the asymptote it is assumed that the labeled C has achieved a stable distribution within the plant-symbiont biomass and that further release results from maintenance and turnover rather than growth. Destructive harvest and analysis of the plant, microbial, and soil components for ^{14}C at this point can be used to determine the C allocation pattern of the

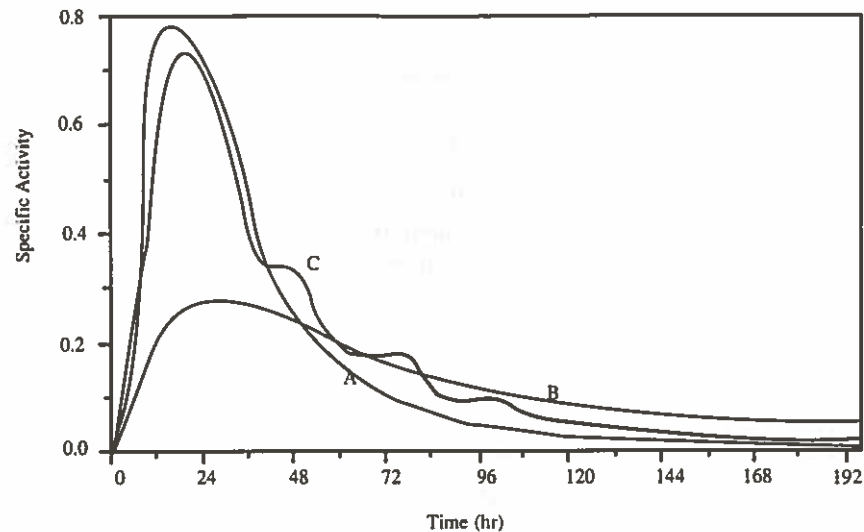


Fig. 2 Simulated curves showing the specific activity of belowground $^{14}\text{CO}_2$ after a $^{14}\text{CO}_2$ pulse from 0 to 16 hr in soybean plants: (A) with VA mycorrhizal and rhizobial symbioses; (B) no symbionts; and (C) with symbionts when starch reserves are remobilized at night.

system. Summation of ^{14}C released as $^{14}\text{CO}_2$ throughout the experiment completes the C balance:

$$\text{net photosynthesis} = ^{14}\text{C in biomass (plant + symbiont + soil microbes)} + ^{14}\text{C respiration.}$$

It is often possible to detect two or three components with different rate constants within the decay curve of belowground $^{14}\text{CO}_2$ respiration by curve peeling using nonlinear regression. In principle, these rate constants describe measurable pools (e.g., root nodule sucrose) and could thus be used to differentiate respiratory activity in different biological components of symbiotic root systems. In practice, any realistic compartmental model of root-symbiont C flow is much too complex to be susceptible to analysis by respiratory data alone. Measurements of the specific activities of many intermediate and precursor pools, which are currently not well understood for many systems even qualitatively, would be required.

Respiration of individual symbiotic partners can be estimated by between-treatment comparisons of total belowground $^{14}\text{CO}_2$. Calculations are based on specific rates of respiration ($^{14}\text{CO}_2/\text{g}$ per unit tracer pulse length)

to enable root systems of differing masses to be compared:

$$\text{symbiotic respiration} = \text{total respiration} - \left\{ \left[\frac{\text{nonsym. root resp.}}{\text{nonsym. root dw}} \right] \times [\text{sym. root} - \text{symbiont dw}] \right\}$$

This calculation involves a number of simplifying assumptions:

1. Respiration in root-symbiont associations is strictly additive, that is, the presence of a microbial symbiont does not affect the respiration of the host root or of the native soil organisms.
2. All $^{14}\text{CO}_2$ released in a cylinder containing a root system without symbiotic associates is from plant root respiration plus nonsymbiotic native soil organisms.
3. $^{14}\text{CO}_2$ once released is not recycled by carboxylase enzymes to a significant extent.

While it is true that because of the above simplifications this calculation cannot accurately determine microbial symbiont respiration per se, it does represent the overall cost of the symbiosis in terms of additional belowground respiration; this information is equally useful.

E. Measurement of ^{14}C in Plant and Microbial Biomass

Labeled C assimilated during the applied pulse of $^{14}\text{CO}_2$ will eventually achieve a relatively stable distribution in structural and long-term storage components of plants and microbes and reallocation and metabolism of labeled C within the plant-symbiont system will be slow. This is revealed by the asymptote of the release of $^{14}\text{CO}_2$ in belowground respiration (Fig. 2). Analysis of the ^{14}C content of components such as roots, nodules, and VA mycorrhizal fungi at this point will reveal the C-allocation pattern of the system. Physical and chemical fractionation of plant, soil, and microbes can be performed to the required level of detail. We are here most concerned with allocation within the root-microbe symbiosis, but allocation between plant organs and measurements of C uptake rates by leaves are also of interest.

At harvest it is desirable to stop biological activity as rapidly as possible. Plant shoot organs should be excised, any measurements such as leaf area, requiring fresh tissue, should be made immediately, and the tissues transferred to a forced air oven for rapid drying. For measurements of labile biochemical components, subsamples frozen in liquid nitrogen and freeze dried may be preferable. It is much more difficult to rapidly harvest the belowground system. Activity can be slowed by cooling the cylinders to 4°C

Table 1
¹⁴C Allocation in a Soybean-Rhizobium-Glomus symbiosis

Week	6		9	
	C(mg)	%	C(mg)	%
Shoot	67.2	50.7	127.2	61.2
Shoot respiration	8.4	6.3	8.2	3.9
Roots	10.4	7.8	16.9	8.1
Nodules	2.6	2.0	3.6	1.7
Mycorrhizal fungus	3.6	2.7	5.8	2.8
Root washings (+) soil ¹	2.4	1.8	2.7	1.3
<i>Belowground respiration</i>				
Roots + soil	6.9	5.2	13.5	6.5
Nodules ²	12.4	9.3	20.4	9.8
Mycorrhiza ²	18.2	13.7	9.7	4.7
Total	132.5		207.8	

Source: Adapted from Harris *et al.* (1985).

¹ Includes exudates and microbial biomass.

² Calculated by difference between symbiotic and nonsymbiotic plants.

but a complete dissection of the soil-root system may take several hours. It is seldom practical to completely separate roots from the whole soil column and some form of subsampling must be used. The sampling scheme should take account of the distribution of roots within the soil column.

Root nodules can easily be collected by excision. Intraradicle mycorrhizal fungal biomass can be estimated by morphometric methods (Toth and Toth, 1982) or by chemical estimation of chitin. The chemical method (Pacovsky and Bethlenfalvay, 1982) is preferred because it is quicker and because the hydrolysis products of chitin (*n*-acetylglucosamines) are isolated; these can then be assayed for ¹⁴C. The biomass of extraradicle mycorrhizal fungus is difficult to measure in natural soils. Reliable measurements await the development of a suitable method for recognizing or separating mycorrhizal hyphae from the general soil fungal population. In soil-free systems the ratio of intraradicle to extraradicle fungal tissue is approximately unity in young soybean plants but increases with age. In natural soils it is probable that the extraradicle component will be reduced by grazing and is therefore overestimated by comparison to soil-free cultures. The ¹⁴C content of extraradicle hyphae and chlamydospores can be measured directly after picking material from root surfaces using fine forceps and a dissecting microscope. The ¹⁴C distribution in a tripartite symbiosis is shown in Table 1 as an example C budget for a symbiotic system.

III. COMMENTS

Calculations of C allocation and respiration in plant-symbiont systems based on comparisons between symbiont-infected and uninfected control plants assume that the control plant is physiologically similar to the infected plant except for the additional activity of the microbial symbiont. It is possible to avoid "big plant versus little plant" comparisons by adding appropriate nutrients to the uninfected plants such that all are of similar biomass at the time of the pulse label experiment. However, tissue nutrient concentrations, instantaneous growth rates, and other physiological parameters will vary between treatments, confounding measurements of the activity of the symbionts. This problem is inherently unavoidable in such comparisons whenever the presence of the symbiont has physiological effects.

One possible approach that avoids some of this difficulty is to arrange for physical compartmentalization of the symbiosis within a single host. This can be achieved, for example, in split-root systems where the symbiont(s) are confined to a portion of the root system but both infected and uninfected root portions are supplied by the same shoot. Pulse-chase label experiments are "snapshots" of C uptake and allocation at the time of the pulse. Single experiments cannot therefore illustrate the development and decay of symbiont activities, and several sequential experiments are needed. An alternative approach is to combine fewer pulse-chase experiments with conventional growth analysis and continuous measurements of belowground respiration.

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4

Photosynthesis/Translocation: Aquatic

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I. INTRODUCTION

Some of the earliest scientific use of ^{14}C was in the study and measurement of photosynthesis and plant production (e.g., Steeman Nielsen, 1952). The high sensitivity with which ^{14}C can be localized and quantified matches the rapidity with which plant cells assimilate and transform carbon. Thus, use of this isotope pervades the study of plant physiology and production. The chief theoretical drawback to use of ^{14}C for the study of production is the difficulty or ambiguity of measuring cell respiration relative to carbon assimilation; that is, ^{14}C assimilation probably measures something in between net production and gross production.

Partly owing to this difficulty, but more frequently owing to the greater cost and precautions for using isotope, aquatic production studies often use a variety of alternative methods. The most widespread of these is the oxygen change, or light-bottle dark-bottle method, which determines net photosynthesis in transparent bottles and respiration in darkened bottles. The change in oxygen with time may be followed as endpoint differences from Winkler titrations of dissolved oxygen content or continuously by recording oxygen electrodes. The technique is simple, inexpensive, and reproducible. However, while one can measure net photosynthesis and respiration, and thus gross photosynthesis, it must be remembered that the changes are due to activities of the entire plankton community rather than just those of auto-