DIVISION S-10—WETLAND SOILS

A Plant–Soil–Atmosphere Microcosm for Tracing Radiocarbon from Photosynthesis through Methanogenesis

J. P. Megonigal,* S. C. Whalen, D. T. Tissue, B. D. Bovard, D. B. Albert, and A. S. Allen

ABSTRACT

We designed a CO_2-controlled cuvette and stripping system to trace a $^{14}CO_2$ pulse-label from photosynthetic assimilation by wetland plants (in this study *Orontium aquaticum* L.) to its release as $^{14}CH_4$ by microbial respiration. The system maintained cuvette CO_2 concentrations to within ±5 Pa of the set-point, and it allowed continuous recovery of $^{14}CO_2$ and $^{14}CH_4$ for 17 d without damage to the enclosed plant. The first emissions of $^{14}CH_4$ were detected <12 h after photosynthetic assimilation of the label. The $^{14}CH_4$ flux increased linearly from 0.12 Bq min^{-1} at 12 h to 3.0 Bq min^{-1} at 5 d, then plateaued at ≈2 Bq min^{-1}. We could not distinquish between $^{14}CH_4$ produced by aceticlastic methanogenesis vs. that produced by CO_2 reduction. Radiocarbon activity in the soil dissolved inorganic C pool peaked on the first day then declined slowly. We did not detect radiocarbon activity in soil solution pools of several low molecular weight organic acids (acetate, formate, lactate, and propionate), but the label was detected in the bulk dissolved organic C pool. We argue that radiocarbon will be useful for investigating the contribution of root exudates to methanogenic metabolism, but data interpretation will require separation of the relative contributions of CO_2 reduction and aceticlastic methanogenesis to overall $^{14}CH_4$ emissions. Processes such as CH_4 oxidation and acetogenesis must also be considered in quantitative estimates of photosynthetic support of methanogenesis.

Methanogenesis is the final step in a series of biochemical reactions that ultimately begins with photosynthesis. Photosynthetic assimilation of CO_2 generates organic C compounds that are released from plants either in an organic form as litter, root exudates, or leaf leachates, or in an inorganic form as CO_2 from respiration. Under anoxic conditions, plant detritus undergoes fermentation to relatively simple compounds, such as acetate and CO_2–H_2 (Stronach et al., 1986), which are the primary substrates used by methanogens. Previous studies have shown that rates of methanogenesis can be limited at each step in this process by factors such as pH (Dunfield et al., 1993), temperature (Conrad et al., 1987), redox potential (Wang et al., 1993), substrate quality (Valentine et al., 1994; Updergraff et al., 1995), or substrate competition (Achtnich et al., 1995). However, there is growing evidence that plant productivity — the first link in substrate availability — is a master variable regulating CH_4 production in wetlands. Strong correlations exist between CH_4 emissions and plant productivity across a broad sample of North American wetlands (Whiting and Chanton, 1993). Further, CH_4 production in paddy soils can be stimulated by adding plant detritus (Sass et al., 1991; Cicerone et al., 1992). Perhaps the most intriguing evidence for a tight coupling between photosynthesis and methanogenesis is that elevated CO_2 can stimulate CH_4 emissions (Dacey et al., 1994; Ziska et al., 1998), even in the absence of an increase in plant biomass (Hutchin et al., 1995; Megonigal and Schlesinger, 1997).

The most rapid pathway by which photosynthates become available to soil microbes as organic compounds is through root exudation and root turnover, collectively referred to as *rhizodeposition*. Although there have been few studies of rhizodeposition in hydric soils, it may be an important source of labile C compounds for methanogens. Rhizodeposition has been implicated as the process driving correlations between plant growth and CH_4 emissions in paddy soils (Raimbault et al., 1977; Sigren et al., 1997). Wieder and Yavitt (1994) reported that ≈10% of a $^{14}CO_2$ label applied to a sphagnum-dominated peatland was recovered from root-free soil organic matter one day after labeling, perhaps having been exported as root exudates from vascular plants. Minoda and Kimura (1996) used a ^{13}C-labeling technique to determine that recent photosynthates contributed ≈30% of the C substrates used by methanogens in rice paddy microcosms. Photosynthates are also released into soils as inorganic C (CO_2) from root respiration.

Our first objective was to develop a system for tracing radiocarbon, applied to the plant as $^{14}CO_2$, through a plant–soil–atmosphere microcosm. The system permitted continuous recovery of the radiocarbon as $^{14}CO_2$, $^{14}CH_4$, and dissolved ^{14}C in soil pore water. Our second objective was to quantify the kinetics of C flow by sampling at intervals as short as 2 h for 14 d. We hypothesized that radiocarbon-labeled photosynthates would be cycled back to the atmosphere as $^{14}CH_4$ in a period of hours (Minoda and Kimura 1994). Our use of radiocarbon in a plant–soil–atmosphere microcosm provided unambiguous evidence that CH_4 was produced by microbial conversion of newly fixed photosynthetic C.

METHODS

Experimental System Design

Our microcosm allowed continuous sampling of plant and soil respiration gases emitted into the headspace of a cuvette

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Abbreviations: IRGA, infrared gas analyzer system; PAR, photosynthetically active radiation.

over a 17-d period (Fig. 1). The 1.2-L cuvette was made of clear acrylic round stock (7.6-cm i.d.) and a flat acrylic lid. It was modified to hold a 1.7-L s^{-1} brushless fan, a copper–constantan thermocouple, a rubber septum for manual sampling of the headspace, and Swagelok inlet–outlet ports. The cuvette attached to PVC pots (7.6-cm i.d.) with a machined PVC coupling on each end to accept an internal O-ring seal.

We used a system of manual- and computer-operated flow controllers to maintain the CO_2 concentration inside the cuvette close to a set point. Preliminary tests showed that adding 1% CO_2 to the cuvette at rates ranging from 0 to 30 mL min^{-1} would balance the CO_2 assimilation rate of our plant. A pair of model 2000 FCCM mass-flow controllers (MKS Instruments, Andover, MA) mixed CO_2-free air and 3.6% CO_2–air to a final concentration of ≈1%. The flow of this mixture into the cuvette was regulated by a PACSYS 9900 flow control system (Data Design Group, La Jolla, CA). The other flow controllers in the system were manually operated and had an upper limit of 1.0 L min^{-1} (Dwyer Instruments, Michigan City, IN). We supplemented the flow of 1% CO_2–air (0–30 mL min^{-1}) with CO_2-free air to achieve a total flow rate into the cuvette of ≈50 mL min^{-1}. This flow rate was the the maximum at which the downstream NaOH traps would operate efficiently. The CO_2-free air was produced by passing breathing air through a soda lime column to remove CO_2, and then through a charcoal filter to remove trace contaminants that can be toxic to plants during long exposures (Byrnes et al., 1995). A Licor 6200 infrared gas analyzer system (IRGA) (Licor, Lincoln, NE) pumped air through the cuvette at a rate of 1.1 L min^{-1} and transferred data on the cuvette CO_2 concentration to the PACSYS flow control module. The IRGA reference cell required a CO_2-free air flow rate of 400 mL min^{-1} for proper operation.

Several moisture traps of either Mg-perchlorate or Drierite (CaO_4S, W.A. Hammond Drierite, Xenia, OH) were located downstream of the cuvette to prevent water from accumulating in the tubing. High humidity caused us to change these traps once each day.

The cuvette atmosphere was continuously vented through a series of CO_2 traps at ≈50 mL min^{-1}. The traps were Harvey biological oxidizer traps (R.J. Harvey Instrument, Hillsdale, NJ) that were modified to accept nylon Swagelok fittings. Gas flowing to the traps was released into 10 mL of 1 *M* NaOH solution at the bottom of a glass test tube creating small bubbles; the bubbles then ascended along a spiral glass baffle that served to increase contact time with the NaOH solution. The first two traps removed nearly all of the $^{14}CO_2$, but only negligible amounts of $^{14}CH_4$ (Zehnder et al. 1979). Downstream, the $^{14}CH_4$ was oxidized to $^{14}CO_2$ in a 1.25-cm stainless steel column containing CuO and recovered as $^{14}CO_2$ in a third NaOH trap. The CuO column was heated to 900°C with a Thermolyne model 21100 tube furnace (Barnstead Thermolyne, Dubuque, IA). We determined the $^{14}CO_2$ scrubbing efficiency of the first two NaOH traps by connecting the outlet of the second trap directly to the inlet of the third trap, thus bypassing the oven (see Results and Discussion).

Soil solutions were drawn from pore water samplers inserted into the soil horizontally at depths of 2, 5, and 10 cm.

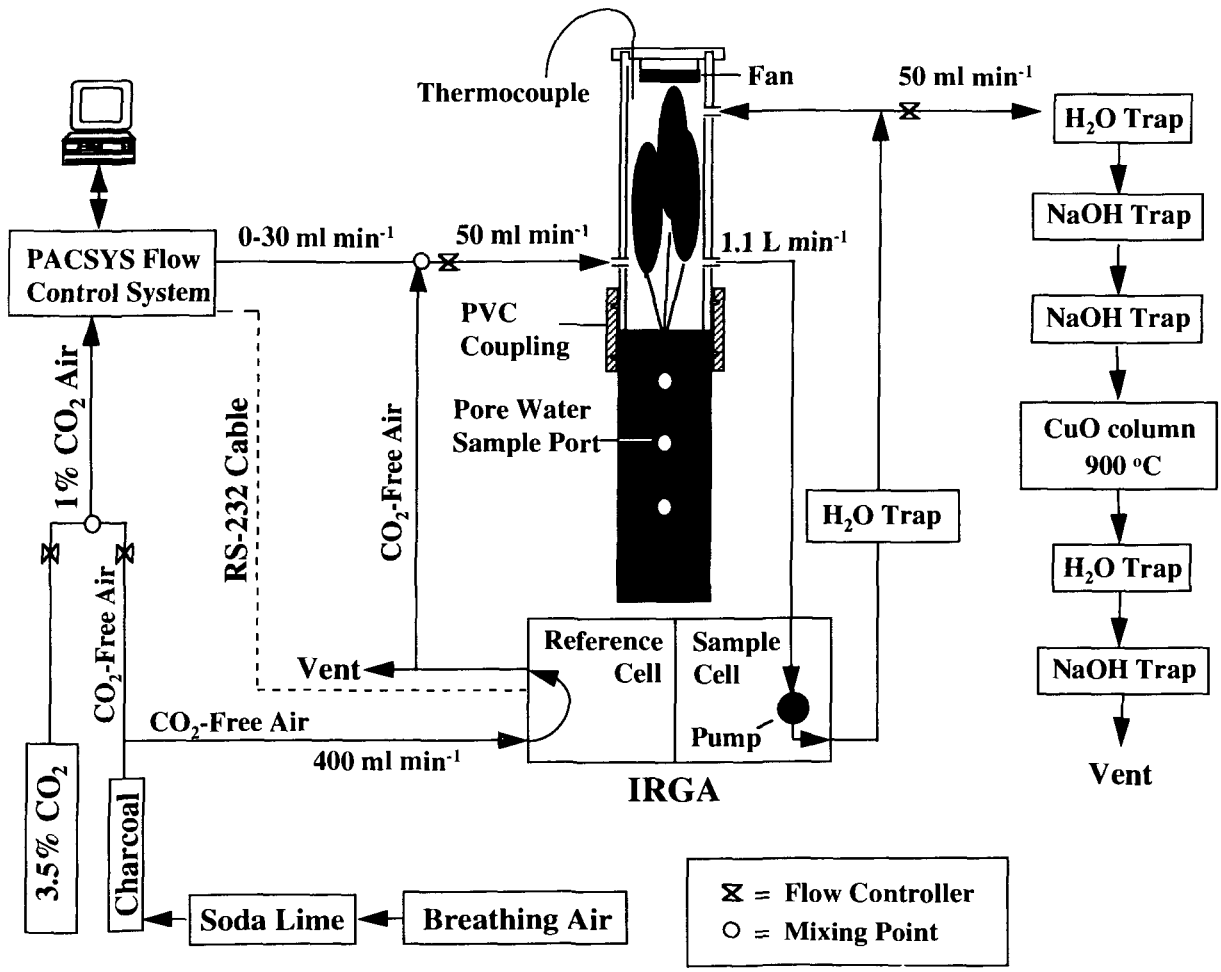

Fig. 1. Schematic of a system for continuously sampling the headspace of a plant–soil cuvette while maintaining a preset concentration of CO_2. Solid lines represent tubing and the dotted line represents a computer cable. IRGA is an infrared gas analyzer system.

The samplers were made from a cylindrical polyethylene filter element (4 cm long × 0.4-cm o.d.) with a 60 μm nominal pore size (Porex Technologies, Fairburn, GA) attached to a stainless steel tube (3 cm long × 0.3-cm o.d.) with silicon sealer. The stainless steel tube passed through a rubber septum to the outside of the PVC pot and ended in a three-way stopcock.

Radiocarbon Label Application

We tested our system on a single individual of *Orontium aquaticum*, an emergent aquatic macrophyte that occurs throughout the southeastern U.S. The 18-month-old plant was grown from seed in a PVC tube under 4 cm of continuous flooding. The life span of leaves and stems is ≈12 d, with about six shoots present at a time. Tap water was added to the soil on a daily basis, and there were occasional additions of a nutrient solution. The PVC tube (40 cm deep, 7.6-cm i.d.) was filled to within 10 cm of the top with a muck soil (Typic Medisaprists) containing 200 g kg^{-1} organic C.

We pulse-labeled the plant shoot on 3 October 1995 with 9.12 MBq of $^{14}CO_2$ applied over a 1-h period. The label was applied in a closed system consisting of a clear acrylic cuvette, a model 6200 Licor IRGA, and a reaction vessel. The reaction vessel was a 250-mL Erlenmeyer flask with a rubber stopper perforated by an inlet tube, an outlet tube, and a 10-cm-long hypodermic needle connected to a three-way stopcock. We added to the flask 10 mL of 1 *M* NaOH, 40 mL of deionized water, and 0.40 mL of $Na_2{}^{14}CO_2$. The radiocarbon was liberated as $^{14}CO_2$ by the injection of 10 mL of 5 *M* HCl. The $^{14}CO_2$ in the reaction vessel was circulated through the cuvette by the IRGA pump at 1.1 L min^{-1}. The CO_2 concentration of the cuvette was maintained between 30 and 60 Pa during most of the labeling period by the injection of small amounts of pure CO_2 gas. Three artificial lights provided 650 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) during the labeling phase, which occurred in the lab.

We took precautions to prevent $^{14}CO_2$ from coming into direct contact with the soil surface by placing a 1.3-cm thick disk of closed-cell foam on the soil surface, covering it with 4 cm of water, then adding a second foam disk of the same dimensions. The disks were slit along the radius to accommodate the plant stems. Our objective was to create a thick, undisturbed boundary layer above the soil to minimize diffusion of $^{14}CO_2$ to the soil surface during labeling.

Unfixed $^{14}CO_2$ that remained in the cuvette and the reaction vessel after the labeling period was quantified. Before dismantling the labeling system, we sampled the surface water immediately above the lowermost foam disk through a rubber septum. We then sampled the cuvette headspace, bulk surface water, and the reaction vessel solution. Solution samples were fixed with 5 *M* NaOH, and headspace gas was fixed with 1 mL of phenethylamine in a 100-mL Nalgene bottle (Farrar, 1993). Before replacing the cuvette, the foam disks and flood water were removed and the soil was flooded again with deionized water. We transferred the plant to a glasshouse at Duke University and began sampling the cuvette headspace for $^{14}CO_2$ and $^{14}CH_4$ ≈90 min after the labeling period had ended.

Carbon dioxide concentration set points were 35 Pa during the day and 50 Pa at night. Ambient sunlight in the glasshouse was supplemented with three halogen lamps for a total of 500 to 700 μmol^{-2} s^{-1} PAR on a 12 h day–night schedule.

Non-Destructive Recovery of Radiocarbon

We exchanged the NaOH solutions at 2-h intervals for the first 48 h and gradually increased the sampling interval to 12 h after two weeks. The oven was shut off for 4 h of each 12-h interval to reduce wear on the oven and CuO column. All samples were mixed with three parts scintillation cocktail (Aquasol II, New England Nuclear) to one part NaOH solution and counted on a Beckman LS 6000 scintillation counter (Beckman Instruments, Irvine, CA). Sample activities were 1 Bq for blanks, from 50 to 1 000 Bq for $^{14}CH_4$, and generally >1 000 Bq for $^{14}CO_2$.

At daily intervals, the soil solution sample ports were flushed once and sampled. Samples were passed through prerinsed 0.45-μm cellulose membrane filters and preserved in an equal volume of 1 *M* NaOH. Dissolved inorganic radiocarbon activity was calculated from the loss in activity after the samples were acidified and sparged (≈1 μL 50% HCL mL^{-1} sample). Acetate, propionate, lactate, and formate were derivitized and quantified on a Beckman model 322 gradient liquid chromatograph with an ISCO V^4 UV/VIS detector (Albert and Martens, 1997). The radiocarbon activity of the soil solution and dissolved low molecular-weight organic acids were determined by scintillation counting (Albert and Martens, 1997). Floodwater was not sampled.

Destructive Recovery of Radiocarbon

After 17 d we completely harvested the shoots and coarse roots. A large, but unknown, portion of the fine roots were recovered by gently washing the soil through a 2-mm sieve. Before washing, soil subsamples were recovered from 1-, 11-, and 23-cm depth and picked nearly free of fine roots. These root-free subsamples were analyzed for microbial biomass and microbial-^{14}C as described by Horwath and Paul (1994), and for soil organic matter ^{14}C content. Plant and soil material were dried to constant mass at 70°C for 5 d and ground to powder in a Wiley mill. We digested 6 to 11 mg of sample in 2 mL of Protosol for 2 d at 45°C, added 8 mL of Aquasol II and counted with a Beckman LS 6000 scintillation counter.

Methane Emissions and Carbon Dioxide Assimilation

We measured CH_4 emissions from the plant–soil system 1-h before applying the radiocarbon label by capping the pot with an opaque static chamber and sampling the headspace at 5-min intervals for 20 min (Megonigal and Schlesinger, 1997). Methane was separated at 50°C using a Porapak Q 80–100 mesh column (Alltech Assoc., Deerfield, IL) and a helium carrier gas, and quantified with a flame ionization detector on a Varian 3700 gas chromatograph (Varian, Palo Alto, CA). We used a Licor 6200 photosynthesis system to measure CO_2 assimilation on a subsample of leaves before and after the experiment. These measurements were made in the glasshouse under ambient light conditions.

RESULTS AND DISCUSSION

System Performance

Our system normally maintained the cuvette CO_2 concentration within ±5 Pa of the set-point and it required adjustment two to three times per day, primarily at dawn and dusk. Carbon dioxide levels varied by ±30 Pa when the system was shut down for periods of 10 to 15 min for sampling or maintenance. Cuvette temperature normally reached 30 to 35°C during the day and 15 to 20°C at night. Condensation inside the cuvette indicated that relative humidity was always 100%. Failure to change the moisture traps on the third day caused the system to malfunction for 5 h because of water in the IRGA tubing.

During the 17-d experiment, the *Orontium aquaticum*

Table 1. Gas exchange characteristics of an *Orontium aquaticum* L. plant before and after 17 d inside a flow-through cuvette. The measurement conditions are for the Licor 6200 photosynthesis system (Licor, Lincoln, NE) cuvette, not the microcosm cuvette.

	Experimental treatment	
	Before	After
Photosynthesis, μmol m^{-2} s^{-1}	14.7 (0.7)†	11.8 (2.4)
PAR,‡ μmol m^{-2} s^{-1}	641 (57)	531 (24)
Ambient [CO_2], Pa	38.2 (1.5)	34.1 (1.6)
Relative humidity, %	46 (15)	67 (5)
Number of leaves measured	3	5

† Standard deviations are in pareutheses.
‡ PAR is photosynthetically active radiation.

plant lost one of eight leaves due to natural senescence, but otherwise remained healthy in appearance. Photosynthetic assimilation was lower at the end of the experiment (14.7 vs. 11.8 μmol m^{-2} s^{-1}, Table 1), most likely because of lower ambient light levels at the time of the second measurement.

The first two CO_2 traps were 99.7% efficient at removing $^{14}CO_2$, resulting in an overestimate of $^{14}CH_4$ emissions of <4%. Radiocarbon activity in the second trap was 2% of the first trap on average (range 0.21% to 11%). From previous research, we estimated the oven was 98% efficient at oxidizing $^{14}CH_4$ under the experimental conditions.

Byrnes et al. (1995) described a wetland plant microcosm similar to ours that featured temperature control of the plant chamber, automated sampling of CH_4 concentrations in the headspace, and separation of CH_4 emitted from soils vs. plants. It was not intended to recover a radiocarbon label. The primary advantage of our system is that it allows large volumes of headspace gases to be collected continuously and quantitatively. This feature maximizes the ability to detect radiocarbon activity because none of the label is lost to the atmosphere, and the label can be concentrated in a NaOH solution over long time periods when efflux rates are low. Both systems are expensive to build, which limits the amount of replication that is practical. If continuous recovery of the label is not desired, the cuvette could be used on replicate plants for intervals of ≤1 h. A less expensive alternative to our design would collect $^{14}CH_4$ under darkness, thus obviating the CO_2 control system. However, allowances should be made for the possibility that CH_4 emissions may be different in light and dark conditions (Fig. 2).

Radiocarbon Budget

After 17 d plant roots contained 50 to 55% of the radiocarbon label and plant shoots contained 12% (Table 2). We recovered 5% of the label in bulk soils, but some unknown fraction of this was associated with fine roots that we were unable to separate from soil organic matter in this histosol. Emissions of $^{14}CO_2$ and $^{14}CH_4$ integrated over the course of the study totaled 0.54 and 0.043 MBq, respectively; this amounted to 5.5 and 0.5% of the radiocarbon fixed by the plant. Microbial biomass ^{14}C could not be distinguished from soil ^{14}C because the radiocarbon activity of the microbial pool was low and

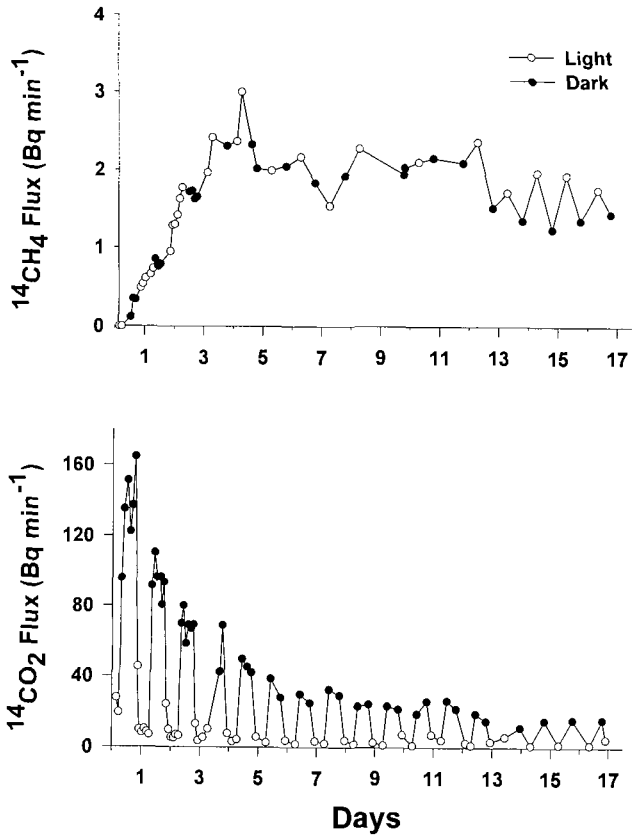

Fig. 2. Emissions of $^{14}CH_4$ and $^{14}CO_2$ from a flooded soil following a radiocarbon pulse-label of the leaf photosynthate pool in a single individual of *Orontium aquaticum* L. The label was accumulated for periods of 2 to 8 h. Samples with open circles represent daytime fluxes and closed circles are nighttime fluxes.

variability among the three soil subsamples was large. We could not account for 28% of the added label and suspect that it was lost during washing as fine root biomass — the largest single radiocarbon pool and the most difficult to recover. Fine roots were removed from soil subsamples used to estimate the activity of the bulk soil reservoir (Table 2).

Net CH_4 emission immediately before the label was applied was 41 n*M* min^{-1} (12.2 mmol m^{-2} d^{-1}). Assuming the same rate for Day 3 of the experiment when $^{14}CH_4$ emissions peaked at 3 Bq min^{-1}, less than 0.1% of CH_4 emissions were derived from newly fixed photosynthate. The contribution of recently assimilated photosynthates to methanogenesis would be small even if 90% of the $^{14}CH_4$ was oxidized to $^{14}CO_2$ by methanotrophs (Megonigal, 1996). By contrast, Minoda and Kimura (1996) reported that recent photosynthates contributed ≈30% of the C substrates used by methanogens in rice paddy microcosms.

Radiocarbon Transfer Kinetics

We detected $^{14}CH_4$ within 12 h of exposing the plant to the label (Fig. 2). The flux of $^{14}CH_4$ increased linearly from 0.12 Bq min^{-1} at 12 h to 3.0 Bq min^{-1} at 5 d, then plateaued at ≈2 Bq min^{-1} for the duration of the study. Our results suggest that photosynthates were translocated to roots, released to the soil as exudates, root

Table 2. Recovery of initial $^{14}CO_2$ added to cuvette (9.14 MBq).

Reservoir	Activity	Initial label
	MBq	%
Cuvette gas	0.001	<0.1
Erlenmeyer flask	0.003	<0.1
Floodwater	0.01	0.1
Shoots	1.1	12.1
Root	4.5	49.2
Total $^{14}CH_4$	0.04	0.4
Total $^{14}CO_2$	0.5	5.5
Bulk soil	0.4	4.4
Total recovered	6.55	71.7

biomass, or root-respired $^{14}CO_2$, and then microbially reduced to $^{14}CH_4$ within 12 h of applying the label. Using rice microcosms, Minoda and Kimura (1994, 1996) recovered the first traces of a ^{13}C label as $^{13}CH_4$ in 3 to 19 h. Wieder and Yavitt (1994) found higher levels of radiocarbon activities in soils than in roots 1 d after applying a $^{14}CO_2$ label to a peatland. Collectively, these studies argue for a strong temporal coupling between plant and microbe C biochemistry.

Pathways for movement of radiocarbon into the soil other than photosynthesis appeared to have been effectively eliminated. The radiocarbon activity of floodwater that lay just above the lowermost foam barrier was 1.4 Bq mL^{-1}, while the bulk floodwater (after mixing) was 26 Bq mL^{-1}. We expect that the radiocarbon activity below the second barrier was considerably lower than 1.4 Bq mL^{-1}. In a worst case scenario, if the entire pore volume of the soil core contained 1.4 Bq $^{14}CO_2$ mL^{-1} that was quantitatively converted to $^{14}CH_4$ by CO_2 reduction, this pathway would account for 3.5% of the $^{14}CH_4$ recovered during the study. Although $^{14}CO_2$ may have diffused into the vascular tissue through plant stems, we believe the importance of this pathway was minimal. Minoda and Kimura (1996) labeled rice plants with ^{13}C in the dark for 1 h but could not detect ^{13}C in soil pore water dissolved CH_4 or inorganic C pools. Furthermore, because the stems of *Orontium aquaticum* are photosynthetic, $^{14}CO_2$ diffusing through stomata would have been largely assimilated.

We observed a trend for higher emissions of $^{14}CH_4$ during daylight that was particularly evident in the last 5 d of the experiment when the flux was integrated for two 8-h periods that coincided with night and day (Fig. 2). During this period, $^{14}CH_4$ emissions were 41% higher during day than at night. Nighttime depression of CH_4 fluxes have been previously observed in rice (Byrnes et al., 1995) and *Peltandra virginica* (L.) Schott & Endl. (Chanton et al., 1992; Frye et al., 1994), a plant that is morphologically similar to *Orontium aquaticum*. The pattern could have been caused by diurnal variations in a number of processes, including stomatal conductance, pressurized ventilation, photosynthate supply to methanogens, transpiration rate, or rhizosphere CH_4 oxidation.

Nighttime fluxes of $^{14}CO_2$ from soil and plant respiration peaked within 12 h of labeling then declined exponentially, dropping from 160 Bq to 60 Bq min^{-1} in 4 d, and to 20 Bq min^{-1} in 13 d (Fig. 2). During daylight, $^{14}CO_2$ fluxes were <30 Bq min^{-1} throughout the experiment because of photosynthetic reassimilation of the label.

Issues Associated with Data Interpretation

Our results from the trial run of this system raised several issues associated with data interpretation. The implications of a tight coupling between plant and microbe C cycling for methanogenesis depend strongly on whether C is released from the plant in a reduced form as organic C from rhizodeposition or in an oxidized form as CO_2 from root respiration. Acetate is produced during anaerobic decomposition of organic C compounds and is considered to be the dominant precursor of CH_4 in freshwater soils (Oremland 1988; Zinder 1993):

$$CH_3COOH \rightarrow CH_4 + CO_2$$

Microbial reduction of CO_2 accounts for most of the remaining CH_4 production, but it may be the dominant CH_4 source in some peatland ecosystems (Lansdown et al., 1992):

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

Because aceticlastic methanogenesis can be limited by the availability of organic C compounds (Yavitt and Lang, 1990), the appearance of $^{14}CH_4$ produced from acetate (or other organic compounds) is evidence that photosynthates have directly stimulated methanogenesis. However, CO_2 reduction is not limited by CO_2 availability, but by H_2 from organic C fermentation (Conrad, 1989; Bridgham and Richardson, 1992). Concentrations of H_2 are typically at nanomolar levels while ΣCO_2 in soil pore water is at millimolar levels (Kelley et al., 1990; Zinder, 1993; Conrad, 1996). Thus, the appearance of $^{14}CH_4$ produced by the CO_2 reduction pathway does not necessarily indicate that C from photosynthesis has directly stimulated the production of CH_4.

We could not distinguish between $^{14}CH_4$ produced by aceticlastic methanogenesis and CO_2 reduction, although both processes were probably occurring. The residual radiocarbon activity remaining after acid stripping is evidence of substantial ^{14}C in unidentified nonvolatile soil organic C pools, such as glucose and ethanol, particularly after 1 d (Table 3). Ethanol is produced in wetland plants under anoxic conditions (Mendelssohn et al., 1981). However, we did not detect radiocarbon activity in pools of acetate, formate, lactate, or propionate despite a relatively large dose of $^{14}CO_2$ to the plant. Only one acetate sample had a radiocarbon activity substantially larger than the blanks (2.5 vs. 0.8 Bq mL^{-1}). Large organic acid pools suggested that rapid consumption did not interfere with our ability to detect the label (Table 3).

From Day 2 forward, 81 to 91% of the radiocarbon activity in the soil solution was dissolved inorganic C that could have been consumed by CO_2 reduction (Table 3). It is likely that roots rather than microbes were the dominant source of the dissolved $^{14}CO_2$. Translocation of photosynthates to roots and subsequent oxidation in respiration is rapid. The evolution of $^{14}CO_2$ from roots

Table 3. Dissolved organic C concentrations and ^{14}C activity in soil solutions during the course of a $^{14}CO_2$ pulse-chase experiment. Data on lactate and propionate concentrations and organic acid radioactivity were not reported because they were generally below our detection limits.

Days†	Radiocarbon activity		Concentration	
	Dissolved organic C‡	Dissolved inorganic C§	Acetate	Formate
	Bq mL^{-1}¶		μL	
1.2	449.4	63.0	3.7	2.9
1.4	–	–	18.5	10.4
2.1	32.0	330.3	21.7	11.5
3.9	51.7	231.5	>20	9.5
5.0	–	–	>20	10.9
7.0	28.1	283.1	19.4	12.1
11.5	–	–	17.7	9.5
13.9	32.3	236.2	16.3	9.1

† Consecutive days from the application of the $^{14}CO_2$ label.
‡ The residual activity after acidification and sparging.
§ Difference between bulk soil activity and residual activity.
¶ Activity is the unweighted average of samples collected at depths of 2, 5, and 10 cm. Values are corrected for the radiocarbon activity of the blank (0.8 Bq mL^{-1}).

in upland soils occurs within 30 min of labeling the shoots (Cheng et al., 1993; Rattray et al., 1995). Root respiration generates upwards of 75% of the soil CO_2 pool in terrestrial ecosystems (Bowden et al., 1993; Pulliam, 1993; Robinson and Scrimgeour, 1995). It is likely that root respiration is a major source of CO_2 for CO_2 reduction in wetlands. We recommend determining the absolute importance of CO_2 reduction and aceticlastic methanogenesis as CH_4 precursors in a parallel experiment in which soils from similar, but unlabeled, plants are incubated with either $H^{14}CO_3^-$ or [2-^{14}C]-acetate, and then $^{14}CH_4$ is recovered (Rothfuss and Conrad, 1993). These data can be combined with measurements of $^{14}CO_2$ and ^{14}C-acetate in the soil pore water of labeled plants to yield an estimate of $^{14}CH_4$ production via the two pathways.

CONCLUSIONS

Our system successfully traced radiocarbon from photosynthetic assimilation to consumption by methanogenic bacteria, and then to its emission into the atmosphere. Although tracer studies will be useful for defining the conditions under which methanogenic bacteria are dependent on rhizodeposition for an organic C source, care must be taken to separate the $^{14}CH_4$ derived by CO_2 reduction of root-respired $^{14}CO_2$ from that derived from fermentation of root exudates. Other biogeochemical processes to consider during experimental design and data interpretation are microbial conversions between $^{14}CH_4$ and $^{14}CO_2$ by oxidation (King, 1996; Lombardi et al., 1997; Bosse and Frenzel, 1997) and acetogenesis (Alperin et al., 1992). The potential benefit of refining this technique is a deeper understanding of the C-mediated coupling between free-living microbes and wetland plants.

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