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A pulse-labelling method to generate ^{13}C- enriched plant materials

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Abstract

Plant materials labelled with ^{13}C can be used to trace litter decomposition and root carbon flow, but only if the isotope is uniformly distributed in the plant. We postulated that if $^{13}CO_2$ were applied at regular intervals, in direct proportion to the rate of photosynthesis, then the abundance of ^{13}C would be uniform among plant parts. To test this hypothesis, wheat plants were grown in the greenhouse, and exposed weekly to $^{13}CO_2$ for six hours in a closed chamber. A constant dose of $^{13}CO_2$ (about 33 atom%) was injected whenever CO_2 concentration fell below a prescribed limit, so that $^{13}CO_2$ was added in proportion to photosynthetic rate. Wheat exposed for 13 weeks (starting 11 days after seeding) had reasonably consistent ^{13}C abundance among plant parts: grain = 3.41, chaff = 3.41, stem = 3.65, and root = 3.50 atom%. The 'leaf' fraction had slightly higher abundance (3.99 atom%), perhaps because recently-fixed ^{13}C was not translocated from senescing tissue. Exposing plants only during early stages of the growing season increased differences among plant parts. The approach offers a practical way to label plants with ^{13}C.

Introduction

Storing more carbon in soils (carbon sequestration) has been proposed as one way of mitigating atmospheric CO_2 increases. To develop methods of increasing and retaining soil carbon, we need to understand better the fate of plant litter, the source of new soil carbon (C).

Isotopic techniques have been widely used to study C cycling in soil-plant systems (Meharg, 1994; Warembourg and Kummerow, 1991). Until recently, such studies usually used ^{14}C because of availability and sensitivity of analysis. Recently, advances in the analysis of stable isotopes have prompted interest in the use of ^{13}C as a tracer, and methods have been developed for labelling plants with $^{13}CO_2$ (Berg et al., 1991; Gaillard et al., 1999; Schmidt and Scrimgeour, 2001; Svejcar et al., 1990; Thompson, 1996). The main advantage of ^{13}C over ^{14}C is that it is not radioactive, so that labelling chambers do not need extensive precautions against leaks and analysis can be conducted routinely and safely.

Plant residues must be uniformly labelled to obtain quantitative measurements of C dynamics during decomposition. Homogeneous labelling of plants with C isotopes has been accomplished through continuous exposure of plants to an atmosphere with relatively constant CO_2 concentrations and ^{14}C activity or ^{13}C abundance (Kouchi and Yoneyama, 1984; Martin et al., 1992). But these systems require complex control systems to maintain constant environmental conditions (CO_2 concentration, ^{14}C activity or ^{13}C abundance, temperature and soil moisture). As a result, continuous labelling systems are expensive and not always accessible.

Repeated pulse-labelling systems, in which plants are exposed periodically to labelled CO_2 for short periods of time, circumvent many of the logistical constraints of continuous labelling systems. Sophisticated automated control of atmospheric conditions and soil moisture is not required because the chambers are sealed for a short duration (e.g. several hours).

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With pulse-labelling systems, however, it may be more difficult to achieve homogeneous labelling of plant components (e.g. Berg et al., 1991), because the ratio of $^{13}C/^{12}C$ (or $^{14}C/^{12}C$) in assimilated C may vary with time.

We postulate that the uniformity of ^{13}C enrichment can be improved by adjusting the amount of label applied for the change in photosynthetic rate over the growing season. If the amount of label applied is proportional to the rate of photosynthesis at each labelling period, and the pulses occur at regular intervals over the entire growing season, then the label should be uniformly distributed in the plant. A pulse-labelling system, therefore, which monitors rate of net photosynthesis and applies labelled CO_2 accordingly should allow reasonably uniform labelling of the plant without the complexity of continuous labelling techniques.

Our objective was to test this hypothesis by evaluating the distribution of ^{13}C in wheat plants regularly exposed to $^{13}CO_2$, in amounts proportional to the rate of photosynthesis.

Materials and methods

Plant culture

Wheat (*Triticum aestivum* L. cv. 'Katepwa') was seeded in pots (20-cm tall × 10-cm diameter) containing 1.8 kg (oven-dry basis) of sieved (< 2 mm) soil obtained from the A horizon of a loamy sand soil (84% sand, 3% clay) with C content of 8 g C kg^{-1}. Soils were moistened and fertilized with N, P, K, S, and selected micronutrients prior to seeding. After establishment, wheat was thinned to three plants per pot, and pots were placed in a greenhouse and watered regularly to prevent moisture stress. Pots were fitted with lids containing holes for watering and aeration, and lids were sealed around wheat stems with medical grade silicone rubber (Dow Corning, Midland, MI, USA).

The plants were fertilized with ^{15}N-enriched urea (10.1 atom%) to generate residues labelled with both ^{13}C and ^{15}N. The enriched urea was applied prior to seeding (100 mg N kg^{-1} soil), and at 15 (100 mg N kg^{-1} soil), 44 (50 mg N kg^{-1} soil), 51 (50 mg N kg^{-1} soil), and 62 (25 mg N kg^{-1} soil) days after seeding.

Experimental treatments

The wheat plants were exposed to $^{13}CO_2$ according to one of 3 treatments – 'full' exposure: Weekly pulses for 13 weeks starting 11 days after seeding; 'early' exposure: Weekly pulses for the first 5 weeks of the growing season; and 'late' exposure: Weekly pulses for the last 8 weeks. Each treatment was replicated four times so that eight pots were treated every week (4 'full' and 4 'early' or 4 'late'). In addition, there were five control pots not exposed to $^{13}CO_2$; two were grown among the exposed plants in the greenhouse, three were grown on another bench to avoid possible contamination with ^{13}C.

^{13}C labelling

Once a week, the designated wheat plants were placed inside a closed acrylic chamber (60-cm deep × 120-cm wide × 104-cm high) in a controlled climate growth cabinet, with temperature set at 16 °C. Closed plastic containers with ice were also placed inside the chamber to prevent heating and to condense excess humidity. During labelling, holes in pot lids were closed to avoid release of respired soil C into the chamber air.

The CO_2 concentration in the sealed chamber was monitored continuously using an infrared gas analyzer (EGM-1; Environmental Gas Monitor, PP Systems, Hitchin, UK) and average values were recorded every minute. Because of the shift in absorption spectrum of $^{13}CO_2$, relative to that of $^{12}CO_2$, infrared gas analyzers detect only a small proportion of the $^{13}CO_2$ in air (Mordacq et al., 1986). Consequently, our measurements of CO_2 concentration underestimated the true concentration when CO_2 was highly enriched with ^{13}C; in our study (∼33 atom% $^{13}CO_2$), we estimated that the analyzer reading was about 72% of the actual concentration. As long as the ^{13}C abundance of the CO_2 is consistent, the apparent CO_2 reading is proportional to the actual concentration, though the latter can be only roughly estimated.

^{13}C-labelled CO_2 was generated by injecting $Na_2{}^{13}CO_3$ solution through a septum into a flask containing 1 M H_2SO_4. The CO_2 evolved was swept into the sealed chamber through a closed loop of tubing with a small pump, and a fan circulated the CO_2 inside the chamber.

The intended enrichment of CO_2 in the chamber was 33 atom% ^{13}C. To compensate for the diluting effect of unlabelled CO_2 initially in the air, the first injection every week was with $^{13}CO_2$ enriched to about 97%. For this first injection, CO_2 concentration was allowed to fall to 327 ppmv (by photosynthesis or, where photosynthesis was too slow, by suspending a

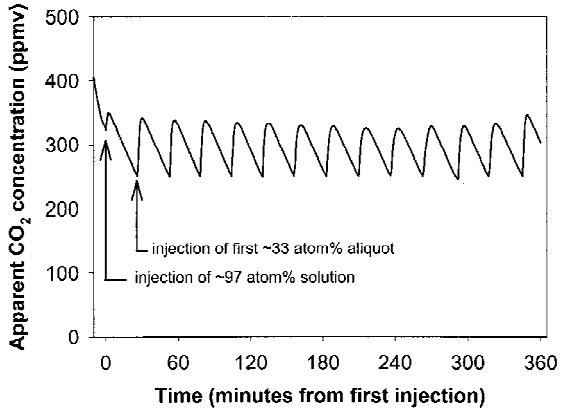

Figure 1. Apparent CO_2 concentration during one labeling period (fourth pulse) showing the initial decline upon closing of the chamber, the initial injection of highly-enriched $^{13}CO_2$ (~97 atom%), and subsequent injections of ~33 atom% $^{13}CO_2$. After the initial injection, the apparent CO_2 concentration underestimates the actual concentration because of incomplete detection of $^{13}CO_2$.

soda lime trap into the chamber). Once concentration had fallen to 327 ppmv, 4.8 mmoles of highly-enriched Na_2CO_3 (~97 atom%) was injected into the generation flask, yielding an estimated ^{13}C abundance in chamber air CO_2 close to the intended 33 atom%. All injections thereafter were made using $Na_2{}^{13}CO_3$ with an abundance of about 33%, prepared by diluting various highly enriched sources (close to 100%) with reagent grade Na_2CO_3. (Had we used 33 atom% $^{13}CO_2$ for the first injection, the ^{13}C abundance would have been much lower initially, then gradually increased toward 33 atom% with successive injections.)

After the first injection, 5 mL of Na_2CO_3 solution (33 atom%, 4.2 mmol C) was added whenever the *apparent* CO_2 concentration fell to about 250 ppmv (corresponding to an actual CO_2 concentration of about 350 ppmv) (Figure 1). In this way, the frequency of injections increased proportionally with the rate of CO_2 removal and, by definition, $^{13}CO_2$ addition was therefore always proportional to photosynthesis, regardless of plant growth stage (assuming all CO_2 removal was by photosynthesis). In later growth stages, plant height exceeded the chamber height so that plants touched the upper surface during labelling. The chamber was opened after 6 h and wheat plants were returned to the greenhouse.

During the eighth pulse, a power outage briefly interrupted the labelling. The chamber was opened to allow escape of $^{13}CO_2$, then re-sealed, and the labelling was continued after an initial injection of highly-enriched $^{13}CO_2$ (about 97 atom%).

Harvest and analyses

During the experiment, leaves were removed and dried as they senesced. At maturity (111 d after seeding), wheat plants were harvested and separated into grain, chaff, stem, leaf (including the leaf sheath) and root fractions. A sub-sample of soil was also collected for analysis. Plant parts were oven-dried (70C), ground and analyzed for total C, N, atom% ^{13}C and atom% ^{15}N using a Carlo-Erba C and N analyser (Milan, Italy) coupled with an Optima mass spectrometer (Micromass, Manchester, UK). Soil samples were air-dried and similarly analyzed.

Aliquots of injected $Na_2{}^{13}CO_3$ solutions were also analyzed for ^{13}C abundance, after dilution of the ^{13}C with reagent grade Na_2CO_3, to ensure they were close to the target enrichments. All atom% values for the solutions and resulting atmospheric CO_2 (e.g. 33 atom%) are only approximate.

'Root' samples are often contaminated with adhering soil residues. Because the density of soil is so much higher than that of dry plant tissue, even contamination that appears slight can affect dry matter yield, elemental concentration, and atomic abundance data. We corrected for this contamination as follows:

$$f = M_r/M_t = (C_t - C_s)/(C_r - C_s)$$

Where f is the fraction of sample mass from root tissue; M_r, M_t is the mass of root tissue and total 'root' sample, respectively (g); C_s, C_t, C_r is the C concentration in soil, total 'root' sample, and pure root tissue, respectively. C_r (458 mg C g^{-1}) was measured on a few carefully-cleaned root sub-samples and was assumed to be constant for all treatments. Then:

$$M_r = f^* M_t$$

To correct ^{13}C abundance values:

$$A_r = \frac{12A_tC_t/(12 + A_t) - 12A_sC_s(1 - f)/(12 + A_s)}{C_rf + A_sC_s(1 - f)/(12 + A_s) - A_tC_t/(12 + A_t)}$$

Where A_r, A_t, A_s is the abundance of ^{13}C in root, total 'root' sample, and soil, respectively (atom%/100) (If ^{13}C enrichment is small, then the atomic mass of C can be assumed to be constant and the equation is much simpler.)

Results and discussion

Dry matter yield

Wheat growth was only slightly affected by ^{13}C treat-

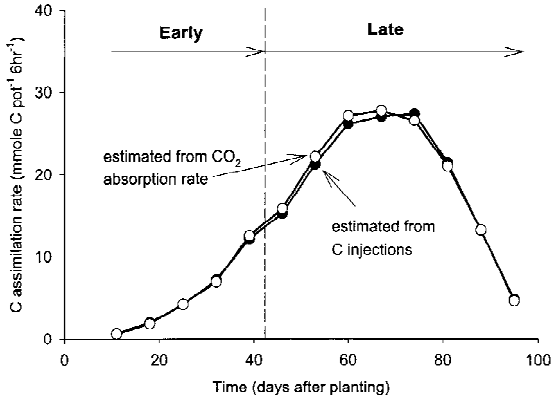

Figure 2. Rate of CO_2 assimilation over time as estimated in two ways: (a) from the amount of C added to maintain CO_2 during the six hour exposure period, and (b) from the average rate of CO_2 decline as determined by the infrared analysis of CO_2 between injections. The latter was corrected for partial detection of $^{13}CO_2$ by the analyzer. Estimates from both methods rely on simplifying assumptions and are only approximate.

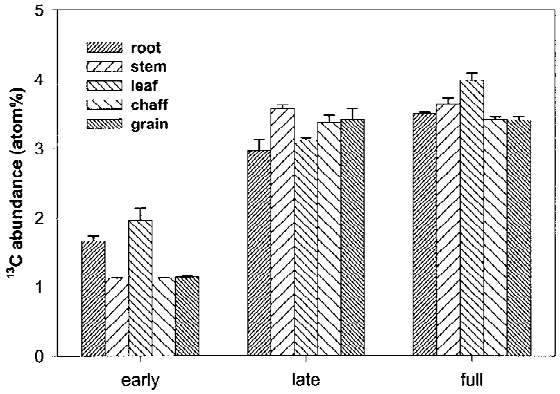

Figure 3. ^{13}C enrichment of wheat heads, leaves, stems and roots receiving early, late or full exposure to $^{13}CO_2$ labelling. Error bars represent one standard deviation (n=4) above the mean. Mean ^{13}C abundance in the control plants grown apart from the enriched plants (n=3) was as follows: roots 1.0865 atom%, stem 1.0792 atom%, leaf 1.0792 atom%, chaff 1.0820 atom%, and grain 1.0815 atom%.

ment. Mean dry matter yields (excluding roots) ranged from 32.8 to 33.9 g pot^{-1} for the three treatments exposed to $^{13}CO_2$ compared to 34.5 g pot^{-1} for controls grown among the treated plants and 34.3 g pot^{-1} for plants grown on another bench (data not shown). Differences among the three exposed treatments were not significant (P=0.05) for total above-ground yield or for yield of any plant fractions, except for leaves which had highest yield in the 'late' treatment (9.3 g pot^{-1}) and lowest in the 'full' treatment (8.5 g pot^{-1}).

Pattern of ^{13}C assimilation

The rate of CO_2 assimilation was estimated in two ways: (a) from the average rate at which CO_2 concentration declined between injections (corrected for partial detection of $^{13}CO_2$ by the analyzer), and (b) from the amount of Na_2CO_3 injected during the exposure (corrected for incomplete absorption of the last injection). Both calculations showed similar patterns: minimal absorption during the first week, increasing to a maximum in the ninth or tenth week, then falling abruptly (Figure 2). The two sets of estimates were very close, though this agreement may be partly fortuitous because both methods relied on rough approximations (e.g. volume of chamber, assumption of Ideal Gas law, estimate of chamber temperature and pressure, recovery of $^{13}CO_2$ by the infrared analyzer).

^{13}C abundance in plant parts

Wheat receiving the 'full' treatment had similar ^{13}C abundance in the root, stem, chaff and grain tissues, with atom% values ranging from 3.41 to 3.65 (Figure 3). Enrichment in the 'leaf' fraction was somewhat higher, perhaps because senescing leaves absorbed but did not effectively translocate ^{13}C.

In plants exposed to ^{13}C only during early growth stages, the roots and stems had appreciably higher enrichment than other fractions, reflecting the disproportionate assimilation of C into vegetative growth (Gregory et al., 1997; Swinnen et al., 1994). The chaff, grain, and stem tissues had ^{13}C abundance not much higher than that in the control plants, indicating limited translocation into these plant parts of C absorbed early.

Distribution of ^{13}C in plants exposed only during 'late' growing stages was the inverse of that in the 'early' treatment. Highest enrichments were observed in the stem, chaff and grain fractions; lowest in the roots and leaves. Because much of the ^{13}C assimilation occurred during the exposure of the 'late' treatment (Figure 2), the ^{13}C abundance in this treatment was much higher than that in the 'early' treatment.

Other labelling trials in our laboratory, based on the approach outlined here, have furnished similar results, though the higher enrichment of the 'leaf' fraction was not always observed.

Applications of the labelling technique

The pulse-labelling technique furnished plant material sufficiently enriched with ^{13}C (and ^{15}N) for decomposition studies. For example, if 2 g of residue (3.5 atom% ^{13}C) were applied to 1 kg of soil with a C concentration of 20 g C kg^{-1}, then the average enrichment of the soil C pool after 90% of the residue had decomposed would still be about 0.011 atom% above background, a difference easily detected by mass spectrometry. Because the leaves had slightly elevated enrichment, relative to other fractions, some caution in the use of this fraction may be advisable.

The labelling method yielded roots with ^{13}C abundance similar to that in above-ground plant parts, making it possible to estimate rhizodeposition of C. In the 'full' treatment of our study, the soil had an average ^{13}C abundance of 1.2358 atom%, compared to that in the control soil of 1.0886 atom% (mean of three pots grown on separate greenhouse bench). If the ^{13}C abundance of the deposited C was the same as that of the root (3.5009 atom%), then about 6% of the C in the soil was derived from the roots. This amounts to about 0.8 g C, equivalent to about 5% of the total plant C at harvest (including plant-derived C in soil). This calculation is approximate, but it illustrates the potential use of the pulse-labelling technique for measuring C deposition.

The approach has a number of advantages over some other labelling methods. The design is simple, and the equipment required is less costly than equipment used for continuous labelling chambers. There is no radiation risk associated with labelling plants with ^{13}C, so pulse-labelling can be conducted routinely in conventional laboratories without producing hazardous waste. The method could easily be made more efficient and less labor-intensive by using an automated injection system controlled by the gas analyser. Increasing the volume of the chamber per plant would reduce frequency of injection required (the rate of CO_2 drawdown would be slowed) and would accommodate taller plants. If required, the N in the residue can also be easily labelled by fertilizing with ^{15}N, as in our experiment.

The pulse-labelling technique presented here may have useful application in C cycling studies. Until it has been more widely evaluated, however, some analyses may be required for each set of residues produced to ensure sufficient homogeneity of the plant material for its specific application. Our analysis demonstrates reasonable homogeneity of ^{13}C among plant parts, but does not yet confirm homogeneity among different C fractions within plant parts (e.g. C in cellulose vs. soluble C). Where such homogeneity is essential, it could be established by ^{13}C analysis after fractionation.

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