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Optimization of pulse labelling techniques of plant shoots with $^{14}\text{CO}_2$ or $^{13}\text{CO}_2$ to study the belowground allocation of recently assimilated C



Member of the BIORHIZ Network

Objective : to describe a procedure of plant shoots labelling with $^{14}\text{CO}_2$ or $^{13}\text{CO}_2$, with minor disturbances of the plant functioning. This is a prerequisite of studies aiming to investigate short-term assimilate partitioning in the plant and rhizodeposition. For such labelling procedure, it is particularly crucial to maintain steady the CO_2 concentration and the specific activity (or isotopic enrichment) of the atmosphere surrounding shoots.

Procedure : Prior to the labelling, the belowground compartment is hermetically separated from the labelling atmosphere with liquid silicon set between shoots and roots (Photo 1). Plants are set into a labelling chamber (Photo 2) whose climatic parameters are regulated (temperature, relative air humidity and light).

The $^{14}\text{CO}_2$ (or $^{13}\text{CO}_2$) is generated by the addition of $\text{NaH}^{14}\text{CO}_3$ (or $\text{NaH}^{13}\text{CO}_3$) in 1M lactic acid (Photo 3). CO_2 and ^{14}C specific activity of the atmosphere in the labelling chamber are regulated with a β radiations scintillation detector (Photo 4) coupled with an infra-red gas analyser (IRGA), mass flow controller for $^{12}\text{CO}_2$ and a peristaltic pump for $^{14}\text{CO}_2$ from $\text{NaH}^{14}\text{CO}_3$. For ^{13}C labelling, $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ can be regulated separately with a dedicated IRGA. The required $^{14}\text{C}/^{12}\text{C}$ or $^{13}\text{C}/^{12}\text{C}$ ratio is reached within 10 min. after the beginning of the labelling and remains constant, regulated according to the shoot demand (Fig. 2). The belowground compartment is continuously flushed with decarbonated air at the input. CO_2 evolved from this compartment at the output is either measured with an IRGA or trapped for measurement of respired ^{12}C , ^{14}C or ^{13}C (Photo 2).

At the end of labelling, air is forced to pass through a soda lime trap in order to decrease the $^{14}\text{C}/^{12}\text{C}$ or $^{13}\text{C}/^{12}\text{C}$ ratio in the chamber, while $^{12}\text{CO}_2$ is continuously injected in the chamber to compensate for trapping and shoot uptake. As a part of the ^{14}C (or ^{13}C) respired by the shoots during the chase period can be re-fixed during the chase period, the ^{14}C (or ^{13}C) is continuously fixed in a soda lime trap. Thus, the CO_2 concentration is steady during all the procedure (at 598 ± 7.5 vpm in the example shown Fig. 1).

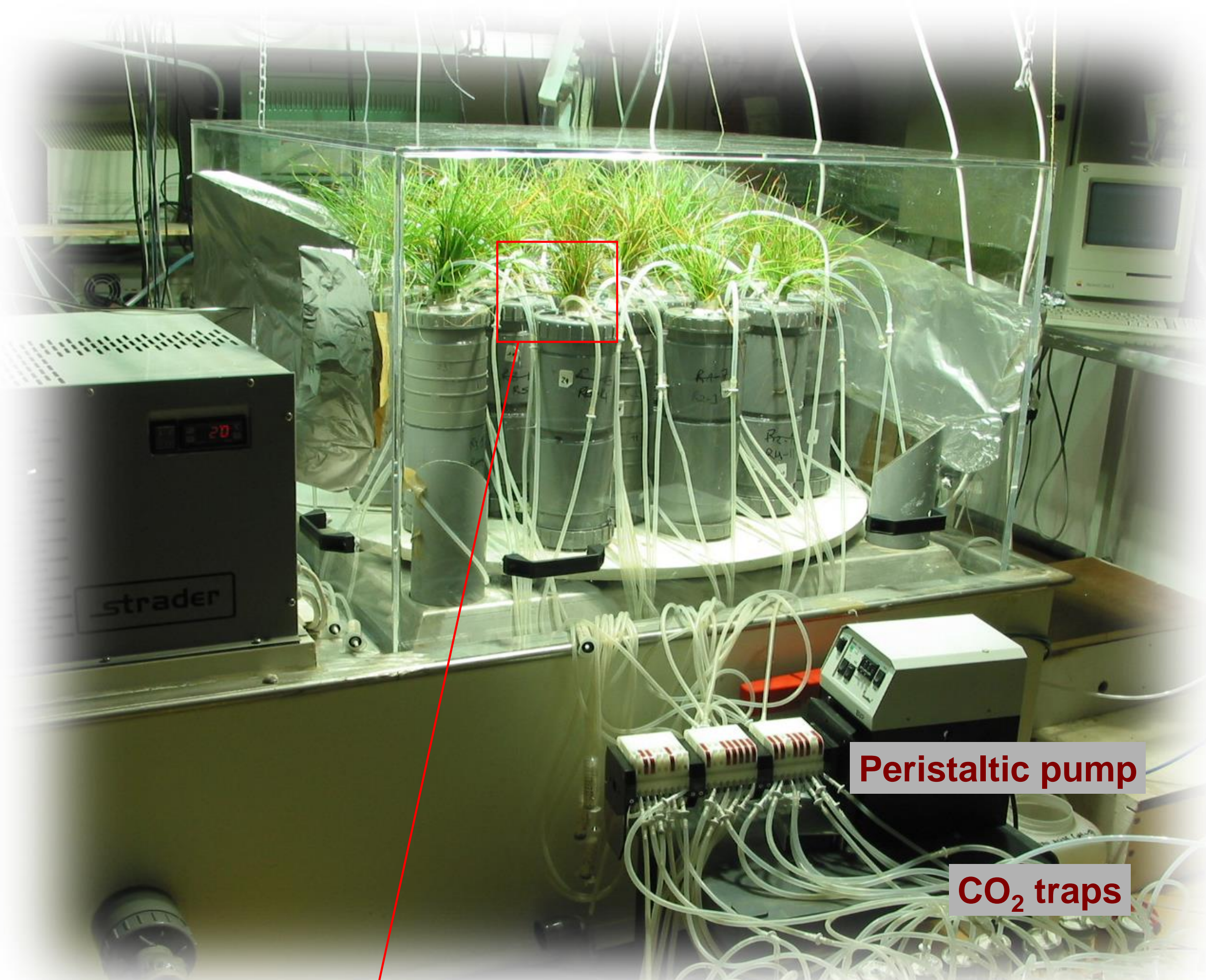


Photo 2. General view on the labelling chamber. In this example, ryegrass plants were labelled in an atmosphere enriched with CO_2 (600 vpm) at a constant specific activity.

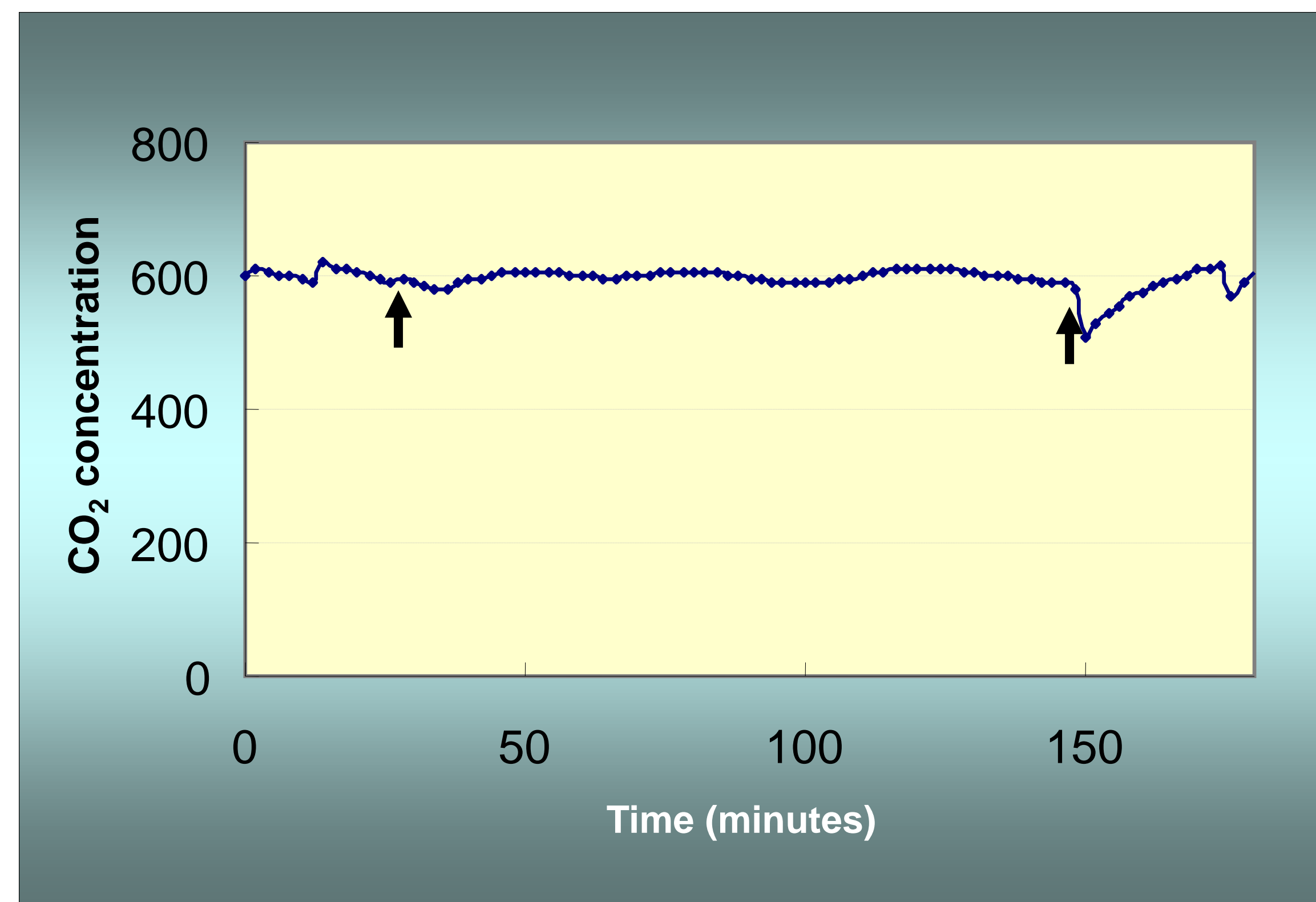


Figure 1 : Kinetics of the CO_2 concentration in the chamber. The duration of labelling was 120 min.

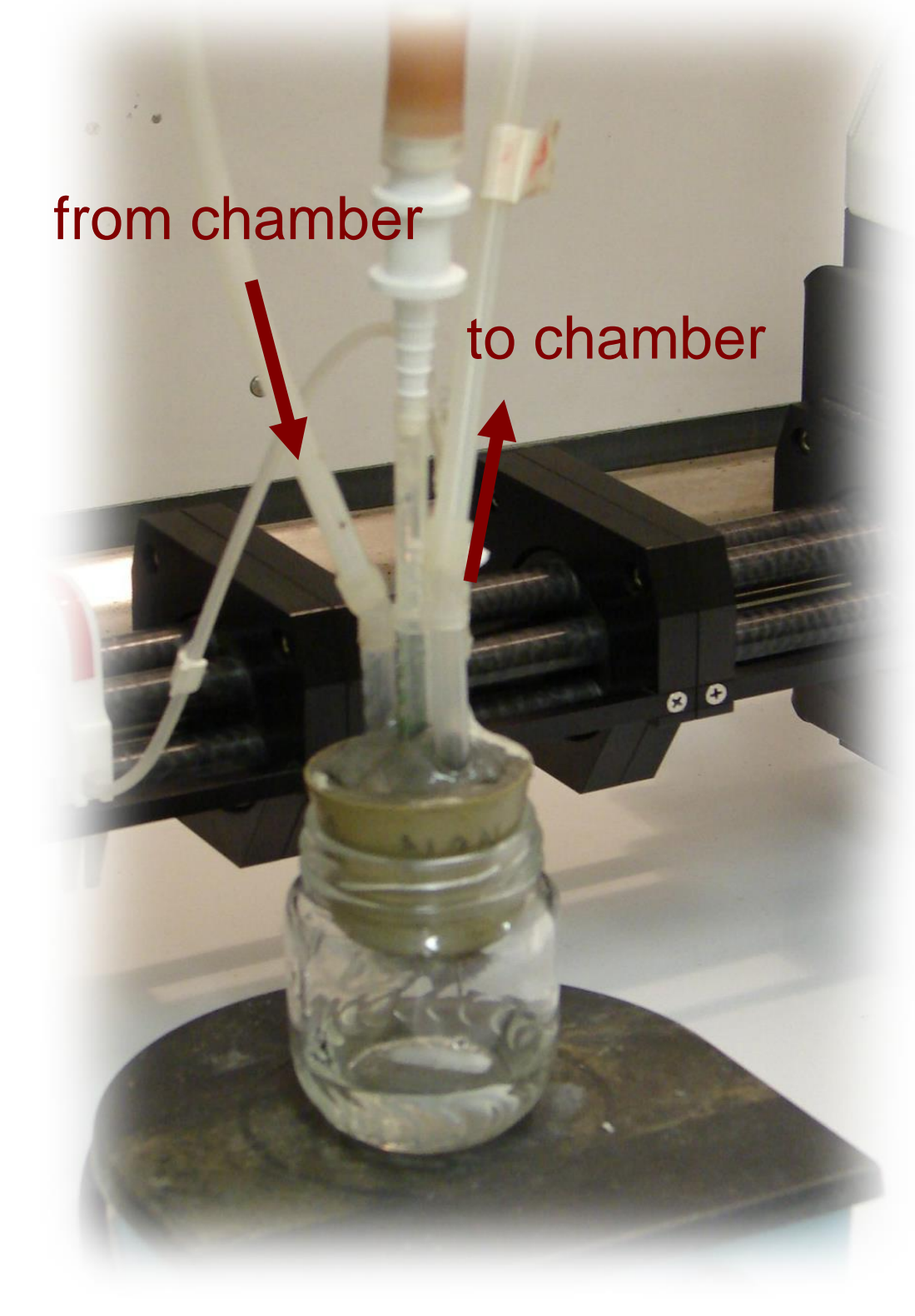


Photo 3. Generation of $^{14}\text{CO}_2$ (or $^{13}\text{CO}_2$)

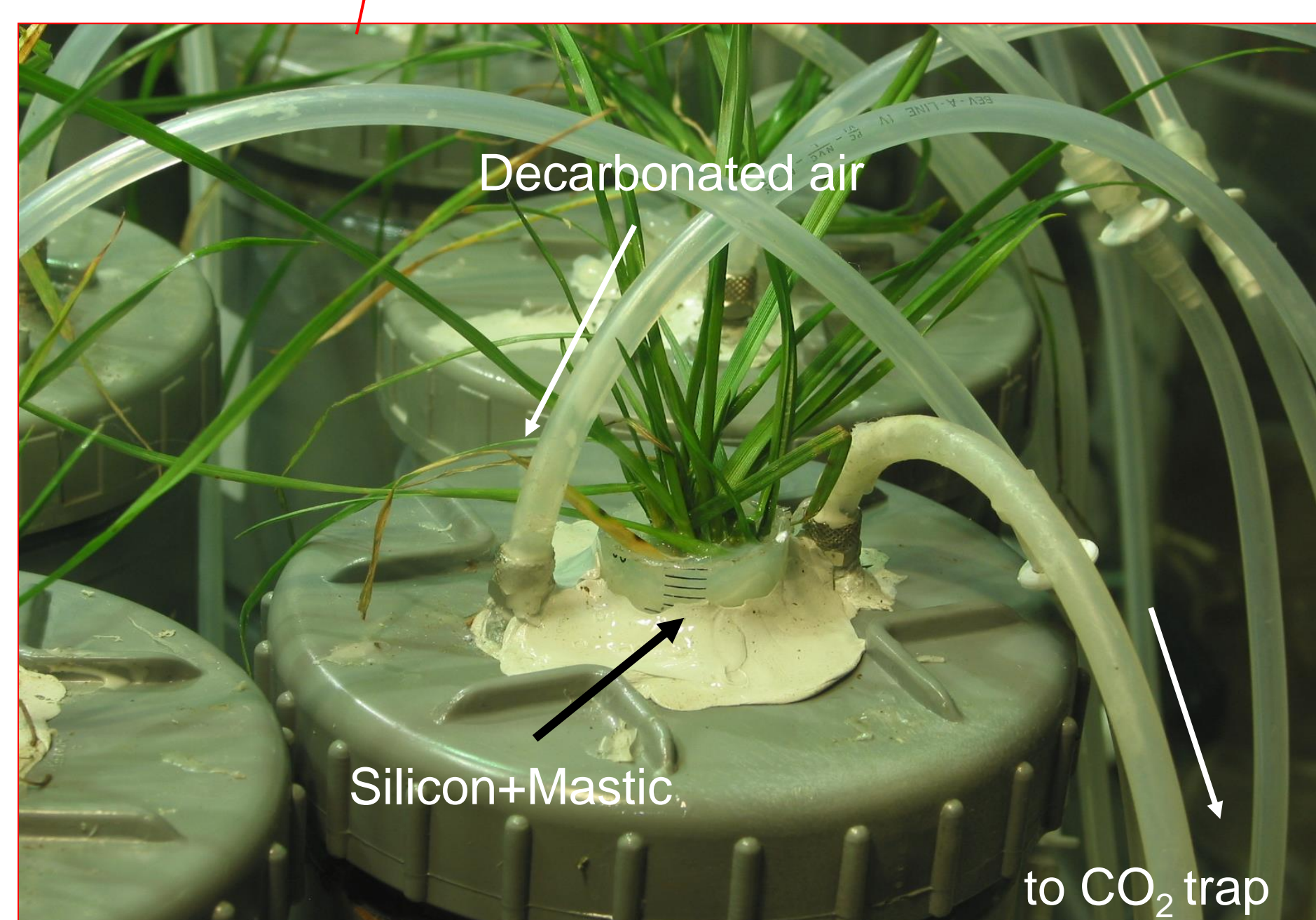


Photo 1. Airtight separation of shoot and roots. The belowground compartment is flushed with decarbonated air at a flow $50\text{-}120 \text{ ml min}^{-1}$

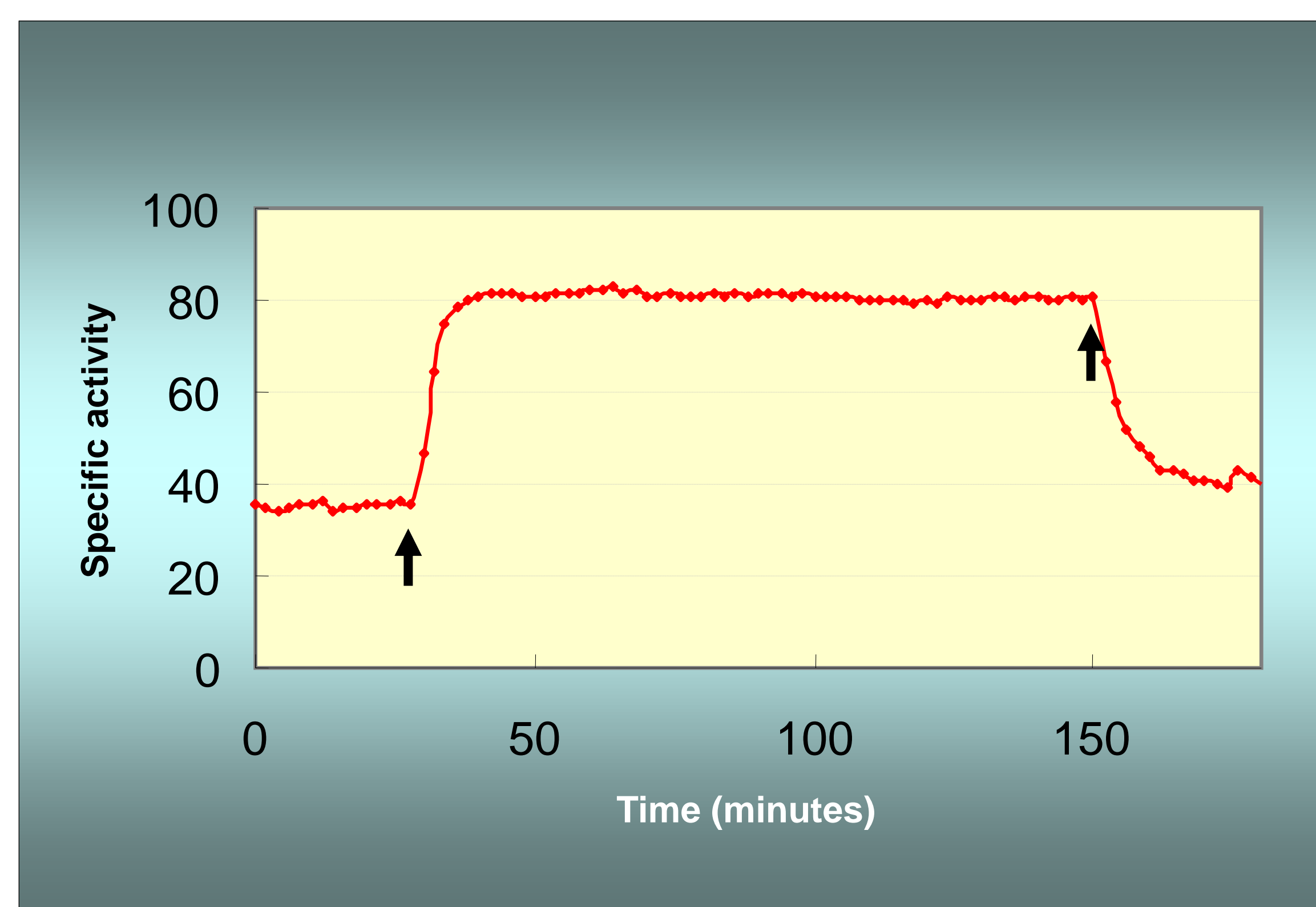


Figure 2 : Specific activity ($^{14}\text{C}/^{12}\text{C}$, in raw counts in the graph) of the atmosphere fixed at $50 \text{ KBq mg}^{-1} \text{ C}$ during labelling. Arrows represent the beginning and the end of the labelling period. ^{14}C is detected with a scintillation detector Radiomatic A500 TR Packard Instruments (one acquisition every two min.; photo 4) and CO_2 is measured with an IRGA.



Photo 4. Counting of $^{14}\text{CO}_2$ β radiations within a 15 ml plastic scintillant cell

Atmospheric CO_2 can be regulated from very low concentrations up to 1000 vpm. Pulse labelling or continuous labelling is feasible, either with a single C isotope or for dual ^{13}C and ^{14}C labelling. See poster number by Koller et al. showing an example of ^{13}C pulse labelling.

Related references :

- Introduction of the Chapter 3.2 "Element cycling and organic matter turnover". In "Handbook of Methods in rhizosphere research". Action COST 631 « Understanding and Modelling the plant-soil interactions in the rhizosphere environment ». 2007. Jörg Luster and Roger Finlay (Eds), pp 62-68. ISBN 3-905621-35-5.
Allard V., Robin C., Soussana J.F., Liefering M., Newton P.C.D., 2006. Short and long-term effects of elevated CO_2 on *Lolium perenne* rhizodeposition and its consequences on soil organic matter turnover and plant N yield. *Soil Biology and Biochemistry* 38: 1178-1187.
Bazot S., Ulf L., Blum H., Nguyen C., Robin C., 2006. Effects of elevated CO_2 concentration on rhizodeposition from *Lolium perenne* grown on soil exposed to 9 years of CO_2 enrichment. *Soil Biology and Biochemistry* 38: 729-736.