Respiration Protocol

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Respiration protocol

Sample collection and preparation

Respiration measurements were carried out on a monthly basis at the Headley and Elgin sites.

- Approximately 150mg (f. wt) of root (extracted with 2.5cm diameter auger) or shoot was collected from coppiced poplar (Trichobel, Ghoy and Beaupre) and willow (Germany, Jorunn and Q83).
- Root samples were washed to remove soil debris.
- Each sample was weighed and sealed into a black polycarbonate tube cuvette (diameter: 12mm, length: 71mm) within 3-5 minutes of extraction.
- The cuvette was incubated in a water bath at 10ºC for 30 minutes.

Gas exchange system

The cuvette was then connected to the gas exchange system (LI-7000, Li-Cor, Nebraska, USA) as shown in Figure 1.

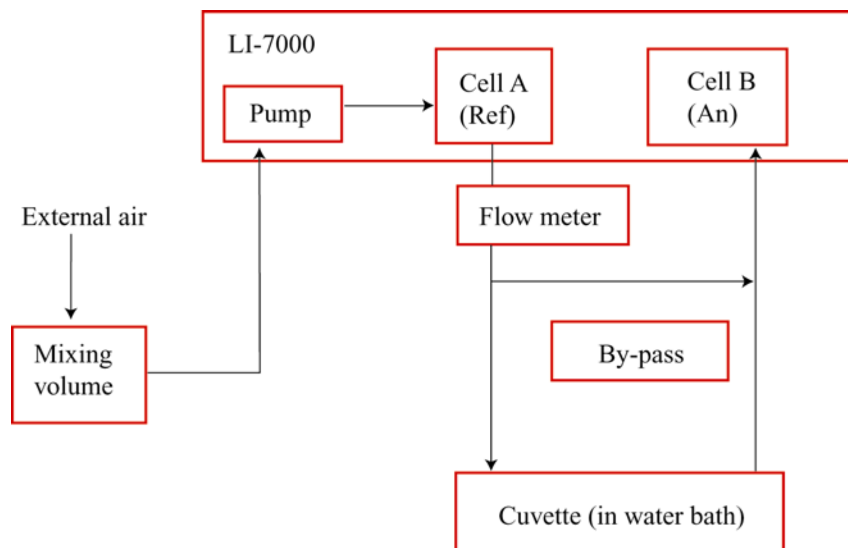

Fig. 1 Diagram of the gas exchange system

Compressed air was passed through a mixing volume (using the internal IRGA pump) maintaining a stable CO_2 concentration, and then through the IRGA reference cell (A), set to read zero. The exhaust from cell A was passed through cell B to determine the differential between the two cells. Two 3-way Solenoid valves (Monitor Labs Inc. USA) were used to divert the flow of cell A exhaust through the cuvette, over the respiring tissue and into cell B. The attenuation time (time taken for the respiration signal in cell B to stabilise or reach equilibrium) was recorded. The two 3-way solenoid valves were used to isolate the sample and cuvette, allowing the respired CO_2 to accumulate in the cuvette for exactly 1 minute. The solenoid valves were then switched to allow the exhaust from cell A to flow through the cuvette, flushing the respired CO_2 through the B (analysis) cell of the gas analyser, until the signal returned to the equilibrium value.

The flow rate was set to 170 ml/min and was recorded by a mass flow meter (AWM3100V, Honeywell). The analogue signals from the IRGA reference and analysis cells and the flow rate were recorded by a DT50 datalogger (datataker, Letchworth) every 0.25s, allowing integration of the respiration flux during the isolation period. Sample dry weight was determined after oven drying and sample nitrogen content was

analysed. Q_{10} relationships were also investigated by repeating the experiment, with the cuvette and respiring sample incubated in a water bath at 20ºC.

In each sampling period, samples were analysed randomly to prevent diurnal variation of respiration or systematic errors from masking variety differences. Measurements were made between 10:00-15:00h.

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