Contribution of Arbuscular Mycorrhizal Fungi to soil Respiration

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■ **Abstract**

Soil CO_2 release from the mycorrhizal (M) and non-mycorrhizal (NM) compartments were measured using a LICOR LI-6400 IRGA system fitted with soil respiration chamber. CO_2 fluxes were determined in the growth cabinet used to raise the maize plants, and rates of CO2 release were determined at time zero and after 30 minutes intervals. Mean values of CO_2 release of the M and NM compartments were significantly different, which rates of respiration from the central cylinders of the M chambers (M1-M4) at time zero were higher than those observed in the NM chambers (Chambers NM1-4). The magnitudes of these differences were largely the same in both M and NM systems at the second reading (0.05) indicating that equilibrium rates of respiration were being detected.

• **Keywords:** Rhizophagus, irregulars, mycorrhizal fungi, Maize

■ **Introduction**

Respirometric techniques have recently been employed to determine the activities of mycorrhizal mycelia in soil. Hogberg *et al*. (2001) showed that up to fifty percent of soil respiration was attributable to fine roots and their associated ectomycorrhizal

mycelium in boreal forest ecosystem. Unfortunately, it is not possible in nature, using approaches of the kind described by Hogberg *et al*., (2001) to discriminate between the activities of roots and associated mycelia. However, by modifying the design of the chambers described in previous studies (Franis

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and Read, 1994), it was possible to provide a root-free compartment which was both colonised by AMF and compatible with the application of an IRGA soil respiration chamber.

■ Materials and Methods

Specially designed chambers were constructed from 15cm diameter plastic drain pipe and with 40cm length. Each chamber base was sealed perforated by plastic plate to retain growth medium and permit drainage. Within each chamber, outer and inner compartments were separated by a cylinderal column of 6cm diameter, designed to accommodate the LICOR LI-6400 IRGA soil chamber. This was made up of nylon mesh with 35μm pore size, which was sealed to the base plate. The chambers were filled with course sand which was moistened with 1/5th strength Rorison's solution lacking phosphorus.

Seeds of Maize (*Zea mays* L.) were germinated in 9 cm Petri dishes on moist filter paper, and then transferred to plastic trays (37×24×6 cm). In order to produce mycorrhizal plants, inoculum of the AMF *Rhizophagus irregularis* was added to half of the trays in two thin layers each separated by a layer of sterilized sand. Trays containing sterilized sand without inoculum were used to produce non-mycorrhizal plants (controls). Both M and NM maze seedlings were maintained in a controlled environment room, where they were supplied with 1/5th strength Rorison's solution lacking phosphorus. After examination of M and NM seedlings to ensure the colonization had, or had not, occurred respectively, seedlings were transferred to the chambers .Two AMF colonised or uncolonised maze seedlings were then planted in the outer compartment of each of a series of chambers. The planted systems were returned to the controlled environment room (day temperature 25˚C, night temperature 10˚C, relative humidity from 80 to 86 % and allowed to grow for a period of 14 weeks for AM mycelium growth through the mesh cylinder of the M systems to colonise the sand of the central compartment.

At 14 weeks 4 M and 4 NM systems were selected for analysis. Soil CO_2 fluxes from the central compartments were measured using a LICOR LI-6400 IRGA system fitted with a soil respiration chamber (Fig.2). Respiratory CO_2 fluxes were determined in the growth cabinet used to raise the plants. The soil chamber was sealed onto the rim of the nylon mesh cylinder using a polystyrene

gasket (Fig.5). In each case, to obtain steady static rates of CO_2 evolution, rates were determined from a chamber at time zero and at 30 minutes intervals. After the second steady state reading (30 minutes) a knife was quickly run around the inner mesh cylinder of M and NM systems penetrating to a depth of 30 cm. This was designed to break mycelial connections with the inner cylinder in the case of the M systems and to reproduce comparable levels of disturbance in the NM systems.

Further measurements of CO_2 flux were made at 30 minutes after the cut and at 2, 4, 24, and 48 hours. Results are presented as mmoles CO_2 released $m^{-2}\,s^{-1}$.

■ **Results**

The background rates of respiration from the central cylinders of the M chambers (M1-M4) at time zero (Figs.3 and 4) were uniformly higher than those observed in the NM chambers (Chambers NM1-4) (Figs. 4 and5). The mean value of the four NM chambers (2.86 mmol CO_2 $m^{-2}\,s^{-1}$) was 31.25 % lower than those of the M systems (4.16 mmol CO_2 $m^{-2}\,s^{-1}$). The magnitudes of these differences were largely the same in both M and NM systems at the second reading (0.05) indicating that equilibrium rates of respiration were being detected.

Cutting of the connections between outer and inner compartments immediately after the second reading (0.5h), led to a reduction of respiration rates in the 4 M chambers (see 0.5h vs 1h release in the Fig.5). The mean reduction of M respiration rates between the readings taken before and after cutting was 23%. The mean values of CO_2 release of the M and NM systems were significantly different at $p<0.05$. In contrast to the M systems, the NM chambers did not yield a significant reduction of respiration after cutting in any chamber (Fig.4). After 2h (1.5 after cutting), the M chambers showed further significant reduction in respiratory CO_2 release from the central compartment by 18.82% (Fig.3). In all cases this brought respiratory output from the M systems to levels close to these seen in the NM systems (Fig.5). At the 4h measurement stage all the M systems showed a very small and not statistically significant rise in respiration rates relative to the levels seen at 2h. This very small rise was repeated at the 24 & 48h readings in the M systems (Fig.3). At 2, 4, 24 and 48h the output from the NM systems were not significantly different from those of the M systems (Fig.5).

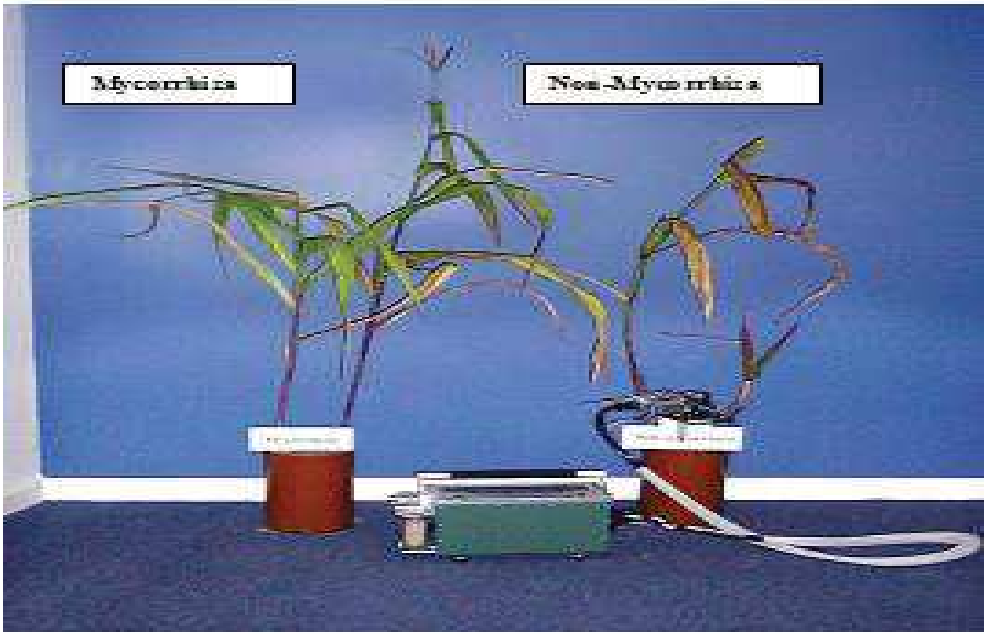

Figure 1 .View of experimental chambers and LICOR LI-6400 IRGA analyser used. Mycorrhizal chamber (left) and Non-mycorrhizal chamber (right) where *Zay mays* plants were grown for 14 weeks.

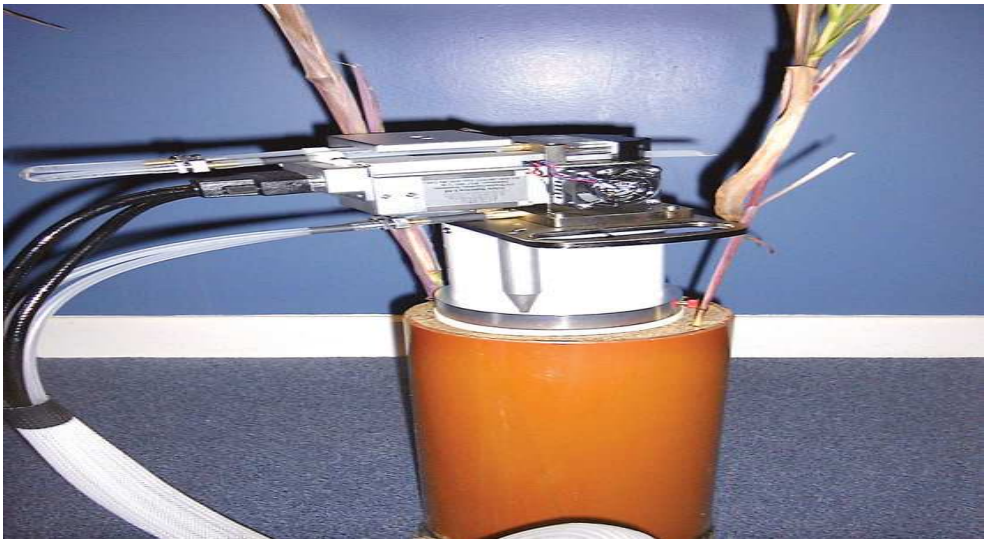

Figure 2. View of polystyrene gasket of LICOR LI-6400 IRGA fitted with the ram of the nylon mesh cylinder for measuring soil CO_2 release.

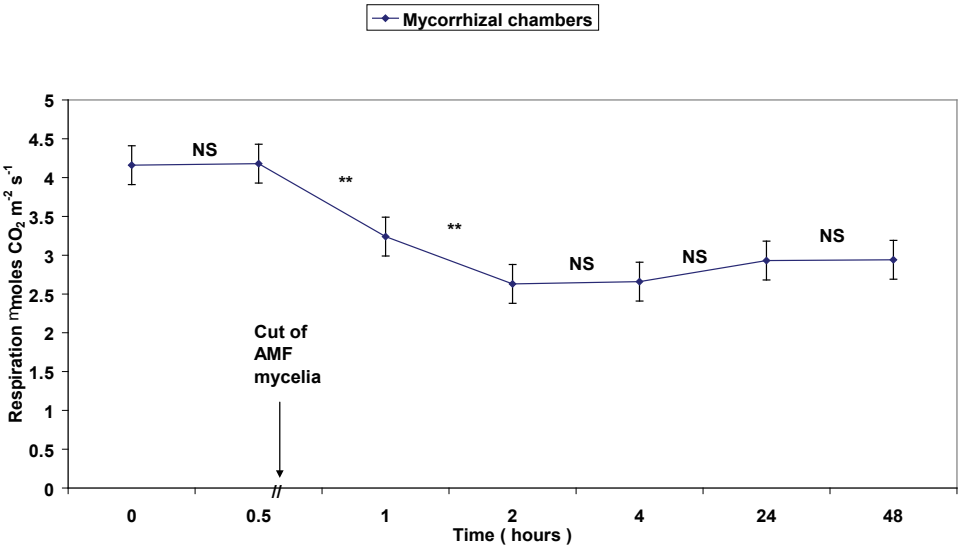

Figure 3. CO_2 release in mycorrhizal chambers. Each value represents a mean of 4 measurements. The vertical bars represent standard errors of the mean. (Asterisks indicate significant differences between current and previous reading at $p < 0.05$; NS indicate no significant differences). CO_2 measurements were taken at time zero and 30minutes prior to breakage of mycelium and then further 30 minutes (1hour from first reading), 2h, 4h, 24h and 48h.

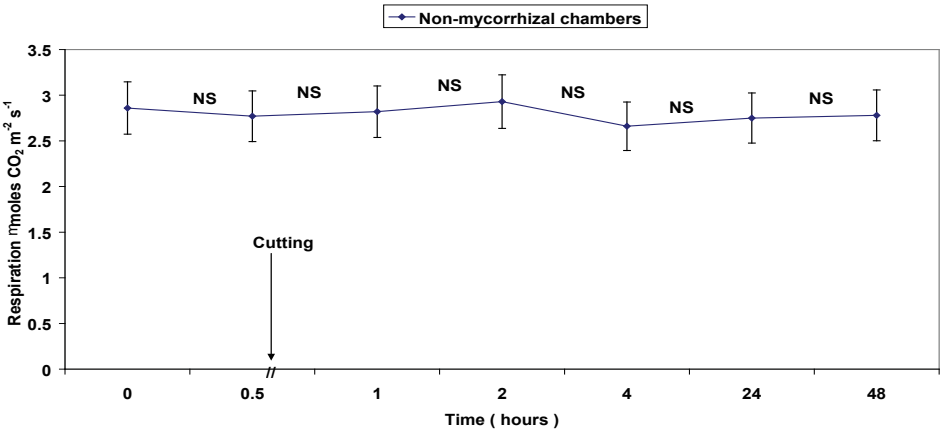

Figure 4. CO_2 release in non-mycorrhizal chambers. (Asterisks indicate significant differences between M and NM readings at $p < 0.05$; NS – differences were not significant at $p > 0.05$).

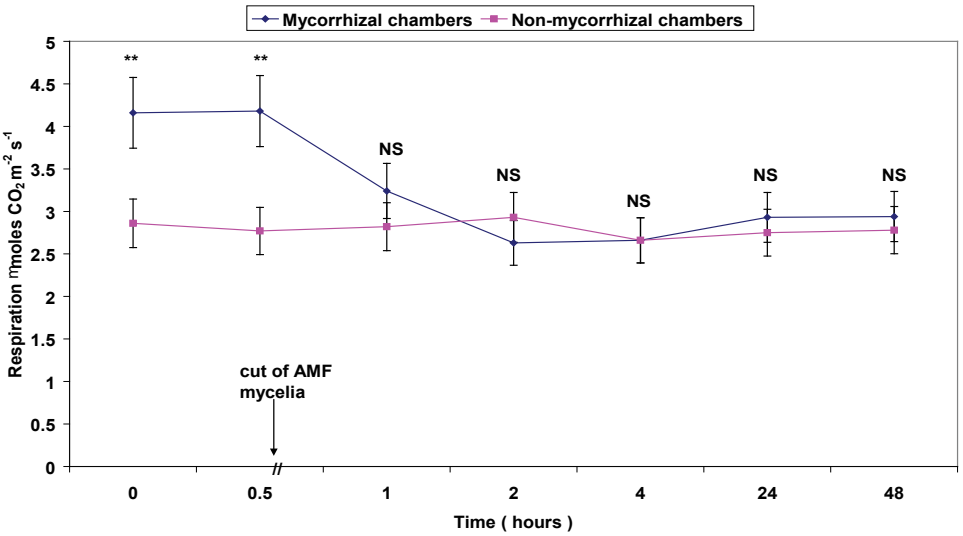

Figure 5. Comparison of measurements of CO_2 release in mycorrhizal and non-mycorrhizal chambers. (** indicate significant differences at $p< 0.05$; NS – differences were not significant, $p> 0.05$).

■ Discussion

Interpretation of the results of the respiration study can be assisted by microscopic analysis in so far as the latter suggests that AMF mycelium are the major heterotrophic components in the root free compartments of the mycorrhizal chambers. However, it should be stated that the chambers lacking AMF also had a measurable respiration rate at (2.79μmoles CO_2 m^2 s^{-1}) and it is necessary to consider the possible sources of CO_2. Clearly some of it may have been attributable to saprophytes, (bacteria and non-AMF fungi) but as pointed out above, the low organic content would mean that there was little substrate to maintain such activity. The most likely explanation is that CO_2 released from the root-containing outer compartments is diffusing into the central compartments and being detected by the IRGA.

Cutting of the sand columns would presumably not break the diffusion channels which were enabling this gas transfer to take place.

If this 'leakage' is contributing to respiration in the non-mycorrhizal chambers, it must be assumed to be doing so also in the mycorrhizal system. However, if the cutting process does not alter the physical pathway in the

non-mycorrhizal system then it is unlikely to do so in those supporting the mycorrhizal plants so both the higher initial respiration rates and the large drop in the rate after cutting to appear largely to be attributable to the AMF fungal network. It is of interest to compare the rates of respiration obtained in the AMF $^{+}$ and AMF $^{-}$ systems with those reported in the literature for corn and grassland systems.

In a comparative analysis of a large number of studies of soil respiration Raich and Tufekcioglu (2001) converted disparate unites of measurements to single measure of (grams of Carbon, m2, day). Two values for soil supporting corn are reported (Lessard *et al*., 1994; Tufekcioglu *et al*., 1999) these being 1.00 and 2.4 g C m^2 d respectively. Conversion of the mean pre –cutting rate (4.16 μmoles m^2 s^{-1}) rates obtained in the mycorrhizal chambers form μmole/ sec. to g d gives value of 4.33 g C m^2 d. If the drop in respiration following cutting of AMF mycelial connections (From 4.18 to 3.24 μmoles is considered to provide an indicator of AMF mycelial respiration, this is equivalent to 3.35g C m^2 day. They confirm that, as in the case of EMF, the AMF mycelium has the potential to contribute very significantly to total soil respiration.

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