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Carbon dioxide dynamics of combined crops of wheat, cowpea, pinto beans in the Laboratory Biosphere closed ecological system

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Abstract

A mixed crop consisting of cowpeas, pinto beans and Apogee ultra-dwarf wheat was grown in the Laboratory Biosphere, a 40 m^3 closed life system equipped with 12,000 W of high pressure sodium lamps over planting beds with 5.37 m² of soil. Similar to earlier reported experiments, the concentration of carbon dioxide initially increased to 7860 ppm at 10 days after planting due to soil respiration plus CO₂ contributed from researchers breathing while in the chamber for brief periods before plant growth became substantial. Carbon dioxide concentrations then fell rapidly as plant growth increased up to 29 days after planting and subsequently was maintained mostly in the range of about 200–3000 ppm (with a few excursions) by CO₂ injections to feed plant growth. Numerous analyses of rate of change of CO₂ concentration. In the middle period of growth (days 31–61), fixation rates doubled for CO₂ at 450 ppm compared to 270 ppm, doubled again at 1000 ppm and increased a further 50% at 2000 ppm. High productivity from these crops and the increase of fixation rates with elevated CO₂ concentration supports the concept that enhanced CO₂ can be a useful strategy for remote life support systems. The data suggests avenues of investigation to understand the response of plant communities to increasing CO₂ concentrations in the Earth's atmosphere. Carbon balance accounting and evapotranspiration rates are included.

Keywords: Closed ecological system; Laboratory Biosphere; Carbon dioxide; Fixation; Fixation rate; Respiration; Evapotranspiration

1. Introduction

Laboratory Biosphere is a closed ecological system of some 40 m³ with two soil planting beds, each 2.68 m² by 30 cm deep. High pressure sodium lights (12×1000 W each) provided artificial light for plant growth at an intensity of about 960 µmol m⁻² s⁻¹ on a 13 h light/11 h dark regime for a total daily light exposure of 44.9 mol m⁻². A mixed crop consisting of 63 cowpea (Texas Pink-Eye) seeds on 2.6 m², 56 pinto bean (Grand Mesa) seeds on 1.6 m², and Apogee wheat on 0.8 m² were planted. The purpose

mass production with these crops and to quantify fixation and respiration of carbon dioxide as deduced from measurement of the atmospheric concentration. Net evapotranspiration was also measured. See Nelson et al. (2008a) for a detailed account of the environment and production of the crops in this experiment, which ran for 91 days from February 5 to May 7, 2005. A detailed description of the Laboratory Biosphere experimental facility is in Dempster et al. (2004).

of the present experiment was to measure edible and bio-

2. Carbon dioxide dynamics

In this paper, we investigate the interactions between the atmosphere contained in the airtight system, the plants and the soil, particularly in regard to carbon dioxide. An

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earlier paper investigating the atmospheric dynamics of two prior crops, wheat and sweet potato, has also been published (Dempster et al., 2005). There are strong similarities and also some notable differences in the observed dynamics among the different crops. That earlier paper fully describes the methodology of measurement and analysis, which will only be summarized here.

2.1. Management of carbon dioxide

Initially, the crops start from seed and there is no photosynthetic activity, but there is soil respiration and members of the study team occasionally enter the chamber using the airtight door for various reasons and contribute their respiration. Both of these are sources of CO₂ inside the chamber and result in a rise in CO_2 concentration. As the plants grow and develop, their fixation of CO₂ by photosynthesis increases until the fixation rate first matches. then overtakes the sources of CO₂ inside. The result is an initial rise in CO_2 , in this case to a high concentration of 7863 ppm on day 10, followed by a rapid descent as the growing plants fix more CO₂ than all sources within the chamber. The strong concentration of CO₂ that had accumulated is consumed by the growing plants and CO₂ falls below 500 ppm by day 29. Then injection of CO_2 from an external cylinder is initiated to provide CO₂ for plant growth. It is not the intention to maintain a steady state of CO₂ concentration because much more information is obtained by examining fixation rates at many different concentrations throughout the experiment. Therefore, CO₂ injection raises the concentration, typically to between 2000 and 3000 ppm, after which point the injection is stopped and plant growth draws CO₂ down, typically below 1000 ppm and then a new injection begins. This manifests as somewhat irregular injections. In this experiment there were 27 injections during days 29-71 after planting. The duration of injections ranged from 161 to

726 min with an average of 372 min. After day 71, plant growth rates had diminished to the point where soil respiration plus respiration from team member entries were sufficient to maintain CO_2 (see Fig. 1).

Usually one or more times per "day" (during lighted hours), CO_2 in the chamber is transitioning from a high concentration (e.g. above 2000 ppm) to a lower concentration (e.g. a few hundred ppm) due to photosynthetic fixation and its concentration is recorded every 15 min by the data acquisition system. This gives the opportunity to determine the net fixation rate of the whole system at many different concentrations and also at virtually every stage of growth. Similarly, CO_2 rises by respiration at "night" (during dark hours). Of course, there are a few data analysis problems to be dealt with:

- (1) We must select undisturbed time segments for analysis. Disturbances that must be avoided are periods of CO_2 injection, entry into the chamber by a team member, changes between "day" and "night" when the lights are switched on or off.
- (2) Data is always accompanied by some noise level. These irregularities are smoothed out by fitting a cubic curve to a set of data points. The first derivative of the cubic fit determines the fixation or respiration rate.
- (3) A long enough undisturbed time period must be used to provide enough data points to obtain a meaningful curve fit. Eight data points, and a time period of two hours, is considered to be the minimum disturbancefree data set.

Altogether, 152 runs of declining CO_2 during fixation (over 19 data points average) and 91 runs of rising CO_2 during respiration (over 40 data points average) that satisfied these criteria were obtained. More detail of the procedure is described in Dempster et al. (2005).



Fig. 1. CO_2 concentration in the Laboratory Biosphere with combined crops of wheat, cowpea and pinto bean grown in soil. Initially, while plants have not yet sprouted or are very small, CO_2 rises due to soil respiration (to day 10). As they grow and develop, increasing fixation rapidly draws CO_2 down (to day 29). It becomes necessary to inject CO_2 to supply feedstock (27 times from days 29 to 71). Occasionally humans enter the chamber and respire CO_2 . Two prominent occasions are indicated.

The pattern of fixation and respiration rates vs. day after planting is shown in Fig. 2. We arbitrarily assign positive rate numbers to respiration and negative rate numbers to fixation. It is to be understood that we show here the net rates of the whole system, combining soil and plants, since there is no way in this system to separate the atmospheric exchange of plants from the atmospheric exchange of soils. It is likely that during lighted hours, soil respiration is simultaneously proceeding which has some net cancellation effect against the plant fixation rates. If it were possible to measure the fixation rate of the plants isolated from the soil, we would expect to observe somewhat higher rates than the net fixation rates plotted in Fig. 2. Conversely, plant respiration at night and soil respiration combine additively to give the net respiration rates shown. The data shown in Fig. 2 are qualitatively similar to observations of other investigators (Wheeler et al., 1993, 2003).

We take particular interest in determining the response of the plants to varying CO₂ concentrations. From the widely varying fixation rates seen in Fig. 2 it is obvious that it would be meaningless to simply compare all rates observed at, say, 1000 ppm CO₂ with all rates observed at, for example, 700 ppm CO_2 without matching the day that each observation is taken. However, because we have observations of fixation rate on nearly every day across a wide range of CO_2 concentrations, we can compare the rate at 1000 ppm CO_2 with the rate at 700 ppm CO_2 on the same day for many different days. This gives a set of several ratios, (rate at 700 ppm CO₂)/(rate at 1000 ppm CO₂), each ratio taken from different days. This set of ratios has an average and standard deviation. We arbitrarily pick 1000 ppm CO_2 as the reference concentration to which the rates at other concentrations are compared. Here we only use 700 ppm CO_2 as an example to describe the



Fig. 2. Net fixation and respiration rates with a mixed crop at 1200 ppm (squares) and at 2000 ppm (dots) CO₂. Note the general shift of fixation rates toward higher rates for the higher concentration of CO₂. Fixation is represented as negative, respiration as a positive number.



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1

0.5

0

0

0

500

1000

Fig. 3. Fixation rates (line) at various CO₂ concentrations relative to the arbitrarily chosen rate at 1000 ppm. The dots represent ± 1 standard deviation. Circles represent the number of ratios sampled (reference right axis). Data taken from the full duration of this experiment.

1500

PPM CO₂

2000

procedure, and have calculated the ratios for every multiple of 100 ppm from 200 to 2900 ppm. The results of this analysis are plotted in Fig. 3. The solid line represents the average of all rate ratios for a given CO₂ concentration to the rate at 1000 ppm CO₂. The dots represent the average ± 1 standard deviation. (We choose not to use error bars to avoid clutter on the graph.) The small circles represent the number of ratios from which the average and standard deviations are derived (reference the right axis).

The substantial variation in fixation rates begs the further question if the rate ratios will be different at different stages of growth. To that we also add the question of differences associated with different plant species. To answer both of these questions, we re-visit data taken in Laboratory Biosphere for past experiments with wheat and sweet potato crops (Dempster et al., 2005) plus data from this experiment. We also subdivide the days of each experiment into thirds of the 90 days (wheat), of the 125 days (sweet potato) and of the 91 days (this experiment, mixed crops). This produces analyses of nine cases, three experiments with different crops \times three periods of growth within each experiment. The analysis for each of the nine cases is shown in Fig. 4, which follows the same format as Fig. 3.

Qualitative similarity is evident among all nine plots. Regardless of species and of the stage of growth, we see a strong dependence of fixation rate on CO₂ concentration. The data set for the mixed crops for the middle third period was the most complete. It shows a doubling of CO₂ fixation rate as the concentration increases from about 270 ppm to about 450 ppm, another doubling to 1000 ppm and a further 50% increase at the concentration of about 2000 ppm. These results suggest applications for understanding the response of growing plants to increasing concentration of atmospheric CO₂ associated with climate changes that the Earth is presently undergoing, but we do not suggest that these results be considered definitive

NUMBER OF DATA POINTS (circles)

20

0

3000

° °°°÷

0 000

2500



Fig. 4. Comparison of fixation rates at different CO_2 concentrations for three different crop experiments at three different stages of growth of each crop. From left to right, the crops are wheat, sweet potato and the mixed crops described in this paper. From top to bottom the growth stages are the first third, the middle third and the last third of the duration of each experiment. Comparison (by ratio) of fixation rates at every 100-multiple of CO_2 concentration are made to the fixation rate at 1000 ppm CO_2 . The average ratio to the 1000 ppm rate, taken on the same day, is plotted as the solid line. The dots represent ± 1 standard deviation from the average and the circles represent the number of ratios sampled (reference right axis).

irrespective of the circumstances and conditions found in nature or in agricultural fields. We also note that these results reflect instantaneous fixation rates in the context of rapidly changing CO_2 concentrations and may not represent final biomass produced (edible or total) for plants exposed to a given CO_2 level for all or most of their growth. Indeed, other work suggests that continuous high level CO_2 exposure may reduce plant productivity (Grotenhuis et al., 1997).

2.3. Soil and plant respiration

We turn now to dark period data as indicative of soil and plant respiration. Fig. 5 shows the average respiration rate (rising CO₂ concentration) for each dark period. There is considerable scatter in the plotted data, but an overall pattern is clear: dark respiration increases to a maximum at around 65 days after planting, then decreases rapidly to the end of the experiment. We have some clues about distinction of soil from plant respiration. As is evident in Fig. 5, initial respiration appears to be on the order of $\sim 3 \text{ mmol h}^{-1} \text{ m}^{-2}$. This is at a time before the plants had sprouted and could be considered to be due to soil respiration. We also kept the system closed and dark for a 7-day period (168 h) after the end of the experiment when all



Fig. 5. Respiration rate during dark periods. Plant respiration evidently peaked at about day 65. Soil respiration is likely nearly constant at \sim 3 mmol h⁻¹ m² throughout.

above ground biomass had been removed for weighing. CO_2 rose in a nearly uniform straight line during this period. A linear fit by regression analysis showed a rise from 2521 to 5145 ppm CO₂, $r^2 = .9942$, slope of 374.83 ppm

2.4. Effect of leakage

We must consider if undetected leakage could be distorting the CO₂ measurements and affecting the analyses presented above. We have previously assessed leakage of the Laboratory Biosphere system to be approximately 1% per day of air exchange between outside and inside (Dempster et al., 2004). We also note that rigorously closed systems subjected to typical barometric pressure fluctuations of Earth combined with their own internal temperature and humidity variations must either be equipped with an expansion/contraction chamber or be built to routinely withstand pressure differentials between inside and outside on the order of 5000 pascal (about 100 lbs/square foot) (Dempster, 1994, 2008). In practice, "closed" systems that have no expansion/contraction chamber and are not rigorously sealed will approach a leak rate of 10% per day due to air driven in and out through small holes as pressure differences between inside and outside fluctuate. (Dempster, 2008). The Laboratory Biosphere is equipped with an expansion/contraction chamber called the "lung" (Dempster et al., 2004). Here, we examine what effect a probable leak rate of 1% per day would have on the fixation rate ratios we have presented in Figs. 3 and 4.

The fixation rates observed (Fig. 2) are mostly within the range 20–80 mmol h^{-1} m⁻² in a system with planted area of 5.37 m². The absolute fixation rate is therefore 0.107- 0.428 mol h^{-1} . The volume of the chamber is about 40 m³. At the site barometric pressure of about 810 mbar and typical temperatures of about 25 °C, the chamber holds about 1300 mol of atmosphere. Exchange of 1 mol with outside air at a concentration difference of 1500 ppm inside -380 ppm outside =1120 ppm CO₂ difference and results in a loss of 0.00112 mol CO₂ per mole of air exchanged. At a leak rate of 1% per day (13 mol per day), or 0.54 mol h^{-1} of air, loss of inside CO₂ is $0.54 \times 0.00112 = 0.0006 \text{ mol } h^{-1}$. This is ~180-720 times smaller than the observed fixation rates and is insufficient to significantly affect the results shown in the graphs of Figs. 3 and 4. Even a gross underestimate of the leak rate would not result in leakage faster than 10% per day based on barometric/temperature/humidity variations as explained above. Even then, leakage would have negligible effect on these observations.

2.5. Carbon accounting

Measurement of injected CO_2 and estimates of leakage and accumulated human respiration enable a net carbon accounting. The 27 injections of CO_2 were individually measured by flow rate using a Rotameter and duration of the injection. The Rotameter measurements are not highly accurate, but the CO_2 cylinder source was accurately weighed before and after the experiment which determined that 5.625 kg or 127.8 mol of CO_2 were injected altogether. The estimates of all individual injections are then scaled to make their total equal the total amount of CO_2 injected. Cumulatively, the injection durations totaled 10,038 min.

Human respiration is estimated at 1000 g (22.72 mol) per person per day, or $0.0158 \text{ mol min}^{-1}$. Approximate confirmation of this rate has also been tested in the same chamber by measuring CO₂ rise with a person inside prior to the introduction of soil or plant materials. Handwritten records were kept of each occasion and the duration of personnel entries inside during this experiment. There were 243 entries (sometimes with more than one person) for a total of 2036 person-minutes of occupancy, which released an estimated 32.1 mol of CO₂ inside. The entry purposes were only for light work and would not have incurred exceptional respiration rates.

Previous leak rate estimates ("general leakage") had been made by measuring progressive dilution of helium inside the chamber and the estimate is about 1% of the chamber's volume exchanged with outside air per day (Dempster et al., 2004). There is a small vestibule, called the "airlock", with a volume of 2.64 m³ immediately outside the airtight door. It is estimated that about 1/8 of this air volume exchanges with the inside air each time the airtight door is opened/closed for a person to pass between inside and outside (assuming some reasonable degree of caution to not leave the door open longer than necessary). This results in an exchange of about 1% of the chamber's air with the outside per door usage.

Using the above parameters, we account for moles of atmospheric CO_2 in the system by computer simulation. At each hour of the experiment, the CO_2 concentration is known which determines how much CO_2 is exchanged along with a given air exchange due to leakage. Each entry/exit and associated human respiration is known and also the injections and their amounts. We plot the simulation results in Fig. 6. The net CO_2 exchange is an addition of 147.4 mol.

Above ground biomass produced amounted to 120 mol dry weight (Nelson et al., 2008a) using the generic approximation that plant biomass is represented chemically by CH₂O. We note that leaves a shortfall of 27.4 mol compared to the net atmospheric additions. There remain some uncertainties in the overall carbon accounting: (1) root biomass has not been determined, (2) soil respiration is not included here, and (3) change in soil organic matter has not been determined. Soil respiration has been discussed above and would cumulatively amount to about 37 mol at the conjectured rate if it were uniform for both light and dark periods of the entire experiment. But, for the soil to be a net source of carbon would mean the soil becomes correspondingly carbon depleted. There are about 2000 kg of soil in the system. An uncertainty of 0.1% by weight organic matter would amount to 66 mol of carbon. Determination of soil organic matter by method of small samples



Fig. 6. Carbon accounting between inside and outside. Leakage in general and via door usage result in carbon loss. Injected CO_2 and human respiration are external additions.

and soil analysis will not yield reliable results to this degree of accuracy. Nevertheless, carbon accounting as far as it is measurable is useful for understanding the dynamics of closed systems.

2.6. Evapotranspiration

In a closed system, as well as in nature, moisture evapotranspiring from soil and plants transits through the atmosphere. In Laboratory Biosphere the temperature and humidity control system consists of two air handlers that recirculate the air within the chamber. The artificial lights impose a large heat load in the space. The air handlers remove the heat by passing the airstream across cold coils that are charged with cold refrigerant circulating inside sealed piping from refrigeration compressors outside. When the moist air contacts the cold coils, water condenses and is collected and measured. The water is subsequently pumped back into the irrigation system to deliver water to the planting beds.

Fig. 7 shows the amounts of water collected by this system on a daily basis. Cooling and water collection only occur during lighted hours when it is necessary for the air handlers to remove the heat produced by the lights. In this experiment the lights were on 13 h per day. As shown in Fig. 8, about 50 l were typically collected during days 10–60 which converts to $0.72 \, l \, h^{-1} \, m^{-2}$ of planted area.

It is relevant what are the conditions of temperature and humidity during this process. Fig. 8 shows the daily range of both temperature and humidity during lighted hours. The bounding curves for daily minimum and maximum are plotted. The humidity spike on day 29 was due to a control system error and lasted about $\frac{1}{2}$ h. At night, relative humidity was allowed to equilibrate without active control and was typically in the range 73–83%.



Fig. 7. Evapotranspiration collected daily during lighted hours. None collected at night.



Fig. 8. Daily ranges of temperature and humidity, lighted hours.

3. Conclusions

The atmospheric dynamics of a closed system with various crops have been observed. We contemplate future large scale closed systems to support humans in locations remote from Earth (Alling et al., 2005; Nelson et al., 2008b) and that such systems could maintain a breathable atmosphere for the occupants while producing their food supply. For such purposes, crop demands for CO_2 and the reciprocal production of oxygen by photosynthesis must be understood in considerable detail. The experiments in Laboratory Biosphere show distinctive patterns for the fixation rates of crops as a function both of stage of growth and of CO_2 concentration. Continued experiments along similar lines will be necessary to build up an extensive database to enable assembling complete life support systems that will behave within reasonably predictable limits.

References

- Alling, A., Van Thillo, M., Dempster, W., Nelson, M., Silverstone, S., Allen, J. Lessons learned from Biosphere 2 and Laboratory Biosphere closed systems experiments for the Mars on Earth project. Biol. Sci. Space 19 (4), 250–260, 2005.
- Dempster, W.F. Methods for measurement and control of leakage in CELSS and their application and performance in the Biosphere 2 facility. Adv. Space Res. 14 (11), 331–335, 1994.
- Dempster, W.F., Alling, A., Van Thillo, M., Allen, J.P., Silverstone, S., Nelson, M. Technical review of the Laboratory Biosphere closed ecological system facility. Adv. Space Res. 34, 1477–1482, 2004.
- Dempster, W.F., Allen, J., Alling, A., Nelson, M., Silverstone, S., Van Thillo, M. Atmospheric dynamics in the "Laboratory Biosphere" with wheat and sweet potato crops. Adv. Space Res. 35, 1552–1556, 2005.

- Dempster, W.F. Tightly closed ecological systems reveal atmospheric subtleties – experience from Biosphere 2. Adv. Space Res. 42, 1951– 1956, 2008.
- Grotenhuis, T., Reuveni, J., Bugbee, B. Super-optimal CO2 reduces wheat yield in growth chamber and greenhouse environments. Adv. Space Res. 20 (10), 1901–1904, 1997.
- Nelson, M., Dempster, W.F., Silverstone, S., Alling, A., Allen, J.P., Van Thillo, M. Cowpeas and pinto beans: yields and light efficiency of candidate space crops in the laboratory biosphere closed ecological system. Adv. Space Res. 41, 748–753, 2008a.
- Nelson, M., Allen, J., Alling, A., Dempster, W.F., Silverstone, S., Van Thillo, M. Integration of lessons from recent research for "Earth to Mars" life support systems. Adv. Space Res. 41, 675–683, 2008b.
- Wheeler, R.M., Sagar, J.C., Prince, R.P., Knott, W.M. Gas exchange characteristics of wheat stands grown in a closed, controlled environment. Crop Sci. 33, 161–168, 1993.
- Wheeler, R.M., Corey, K.A., Sagar, J.C., Knott, W.M. Crop production for advanced life support systems – observations from the Kennedy Space Center Breadboard Project. NASA/TM-2003-211184, 2003.