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1 Laboratory Analysis of Elevated Atmospheric Carbon Dioxide on Carbon Fluxes for  
2  
3 Cyanobacterial Biological Soil Crusts  
4

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59 CO<sub>2</sub>,  
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## Abstract

1  
2  
3 Metabolic activity of Biological Soil Crusts (BSCs) is principally dependent on  
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5 moisture availability, but also on temperature and light conditions. Less understood is  
6  
7 how BSCs respond to elevated atmospheric CO<sub>2</sub>. This paper reports laboratory  
8  
9 experimental results of elevated atmospheric CO<sub>2</sub> on carbon fluxes for cyanobacterial  
10  
11 BSCs. The study uses newly designed dynamic gas exchange chambers in which the  
12  
13 internal atmosphere was controlled. CO<sub>2</sub> flux was monitored during controlled  
14  
15 experiments in two phases under simulated rainfall events (2 & 5 mm plus control  
16  
17 with no wetting) each lasting 3 days with a dry period in between. Phase 1 subjected  
18  
19 crusts to 392 ppm CO<sub>2</sub> (representing ambient level) in dry air; in phase 2, the CO<sub>2</sub>  
20  
21 concentration was 801 ppm. Both phases exhibited significant efflux (respiration) of  
22  
23 CO<sub>2</sub> immediately after wetting, followed by substantial influx (sequestration) of CO<sub>2</sub>.  
24  
25 Samples subject to 2 mm wetting sequestered an order of magnitude more C under  
26  
27 elevated CO<sub>2</sub> than at ambient CO<sub>2</sub>; for samples subject to 5 mm wetting, this increase  
28  
29 was threefold. The findings highlight the role of BSCs in future carbon budgets by  
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31 enabling greater sequestration into dryland soils even under enhanced atmospheric  
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33 CO<sub>2</sub> concentrations, following both light and heavy rainfall events.  
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## 1. Introduction

Dryland soils cover approximately 41% of the Earth's terrestrial surface and support more than one-third of the global population (Reynolds et al., 2007). Recent findings suggest that sub-Saharan Africa has acted as a likely net carbon sink within the last decade (Ciais et al., 2009) with a net balance of approximately  $0.97 \text{ Pg C y}^{-1}$  uptake (Bombelli et al., 2009). However, the role of soils and their Biological Soil Crust (BSC) cover remains only sparsely assessed (see reviews by Maestre et al., 2012 a,b; Stringer et al., 2012) but with BSCs identified as a major contributor to overall  $\text{CO}_2$  efflux in drylands (e.g. Castillo-Monroy et al., 2011).

BSCs are made of cyanobacteria and other bacterial species, algae, lichens, mosses and microfungi. In general, BSCs cover up to 70 % (Belnap and Lange, 2003) of the soil surface of many drylands and in some undisturbed areas such as in Wildlife Management Areas of the Kalahari BSCs cover more than 90% of the soil surface (Dougill and Thomas, 2004). Globally, cyanobacterial biomass in dryland areas is estimated to be 56 Pg of carbon (Garcia-Pichel et al., 2003), therefore constituting a significant part of the estimated 241 Pg of soil organic carbon (SOC) contained within drylands (Lal, 2004). The estimated total global Carbon net uptake of BSCs has been approximated as  $3.9 \text{ Pg yr}^{-1}$  (corresponding to approximately 7% of global net primary production uptake) (Elbert et al., 2012) but the controls on rates of  $\text{CO}_2$  efflux remain poorly understood (Frey et al., 2013).

Substantial amounts of carbon are also being lost from BSCs via respiration (Huxman et al., 2004; Thomas et al., 2008; Thomas and Hoon, 2010; Thomas et al., 2011) particularly after rainfall events; due to activity and substrate availability increases within microbial populations (Borken and Matzner, 2009). During hours when moisture, temperature and light availability are optimum gain of carbon

1 (photosynthesis) occurs; prior to these optimum conditions (particularly in the  
2 absence of light) an initial efflux is observed (Evans and Johansen, 1999; Thomas et  
3 al., 2011; Thomas, 2012).  
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7 Free Air Carbon Enrichment (FACE) facilities have been set up in several  
8 dryland study sites to investigate the effects of increasing atmospheric CO<sub>2</sub> on  
9 vascular vegetation and soils (e.g. de Soyza et al., 2005; Steven et al., 2012). For  
10 example, ten years of FACE field studies in Nevada suggest that enhanced CO<sub>2</sub>  
11 treatments cyanobacteria will show a generalized decline with elevated CO<sub>2</sub> though  
12 the direct impacts on CO<sub>2</sub> efflux rates remains unknown (Steven et al., 2012). . A  
13 similar experiment in the Mojave desert conveys the importance of BSCs in the  
14 uptake of CO<sub>2</sub>; whereby in post rain conditions the net ecosystem CO<sub>2</sub> exchange  
15 (uptake) was unexpectedly high and was likely due to autotrophic mechanisms as  
16 vascular plant photosynthesis was low at this time (Jasoni et al., 2005).  
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32 The aim of this experimental study was to quantify the short-term temporal  
33 changes in carbon exchange (respiration and photosynthesis) of BSCs when the  
34 atmospheric concentration of CO<sub>2</sub> was doubled (~800 ppm). The study was conducted  
35 using specially designed dynamic gas exchange chambers (DGECs) and  
36 cyanobacteria-dominated BSCs sampled from Kalahari Sand soils of southern Africa,  
37 a large dryland biome which covers 2.5 million hectares (Wang et al., 2007). In  
38 addition to the doubled CO<sub>2</sub> atmospheric level in laboratory studies, two wetting  
39 treatments (2 and 5 mm) were incorporated. This also added an extra dimension and  
40 additional data on BSCs responses to key conditions of both soil moisture and  
41 atmospheric CO<sub>2</sub> concentration.  
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## 60 **2. Methods**

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## 2.1 Culturing BSCs in Controlled Conditions

Both crusts and subsoil samples were collected from a lightly grazed commercially-owned farm near Tsabong, Botswana (25°56'51"S 22°25'40"E) during a field campaign in summer 2008. The subsoil (97% fine sand, pH  $5.9 \pm 0.4$ , bulk density  $1.85 \pm 0.03 \text{ g cm}^3$  and porosity  $0.34 \pm 0.01 \text{ v/v}$ ) (Thomas and Hoon, 2010) was sieved (853  $\mu\text{m}$ ), sterilized (autoclaved at 121 °C for 15 min) and then filled (178 g each) in nine polyethylene terephthalate (PET) containers (diameter = 93 mm and depth = 25 mm). Soil depth was approximately 20 mm, a depth such that a 5 mm wetting would result in near saturation. On the soil surface, biological soil crust samples were gently broken into similarly sized pieces and distributed evenly and equally (3.32 g per container) in all PET containers. Samples were weighed again and then wetted using 10 ml of pure water (purity > 99%) applied using a calibrated water sprayer. This initial wetting consolidated the soil and the samples were left in an environmental cabinet (the conditions of which are outlined below) to settle into a circadian rhythm. The samples were allowed to establish for two months in a growth cabinet and the crust surfaces were gently moistened several times a week (1-5 mm each), a total of 53.2 mm (361 ml) of water per container. Included in several of these treatments was the addition of standard nutrient medium, BG11 (Rippka et al., 1979), which was diluted with water, to encourage growth of the cyanobacteria.

The growth cabinet was set to provide 16 hours of light (intensity =  $76 \pm 1 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , measured as an average at the central position of each cabinet shelf) and a temperature of  $28 \pm 0.3 \text{ }^\circ\text{C}$ ; the remaining 8 hours with no light at a temperature of  $10 \pm 0.3 \text{ }^\circ\text{C}$ . The light intensity in the cabinet represented early morning hours during austral winter in the Kalahari where maximum microbial activity occurs (Evans and Johansen, 1999; Thomas and Hoon, 2010). Four USB loggers (USB-500

1 Measurement Computing Corporation) were placed in various positions within the  
2 cabinet, in order to record internal temperature, which ranged from 7.5-30.5°C in  
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4 phase 1 and 7.5-29.0°C in phase 2. Relative humidity was not recorded because the  
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6 sensors were located within the environmental cabinet and not in the enclosed and  
7  
8 therefore isolated (to external relative humidity) design of the DGECs.  
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## 11 **2.2 Dynamic Gas Exchange Chambers (DGECs)**

12 The design of the chambers used was developed from that proposed and used  
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14 successfully in field conditions by Hoon et al. (2009). Chambers were designed to  
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16 house the PET containers containing crust samples. Chambers were made using  
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18 transparent Perspex® material (3 mm thickness) with a height of 103 mm and 100  
19  
20 mm outer diameter, allowing 92% transmission of PAR (400 nm-700 nm),  
21  
22 transmission of thermal infrared (IR) and filtered ultraviolet radiation (UV) < 300 nm;  
23  
24 the top of the chamber was fitted with a removable cap made of Teflon® film that  
25  
26 allowed 96% transmission of incoming radiation (Figure 1). The Teflon® film was  
27  
28 able to accommodate pressure changes within the chambers by acting as a diaphragm.  
29  
30 Each chamber was made air-tight and had ports (Suba-Seals®) from which samples  
31  
32 were collected at regular intervals. Chambers were attached (via the gas inlets) to the  
33  
34 main vessel of 32 l volume, containing the CO<sub>2</sub>-air mix. Open-cell foam inserts were  
35  
36 placed into the gas inlets and outlets in order to prevent diffusion of atmospheric  
37  
38 gases into or out of the chamber, while permitting the flushing of the chamber and  
39  
40 equilibration with the external atmospheric pressure, should it become over  
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42 pressurized. A Teflon® bag was attached to the end of the flush-gas feed line in order  
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44 to confirm flow of the flush gas. Final checks were made for any leaks. After this,  
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1 inside the growth cabinet. Care was taken to minimize the disturbance to the crust  
2 samples. The final set up is given in Figure 2.  
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### 7 **2.3 Treatments and Sampling Regime**

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9 Experiments were conducted in two phases (3 days each, as during the preliminary  
10 run, mentioned below, the behavior had stabilized by the end of day three) to study  
11 the impacts of elevated CO<sub>2</sub> along with different wetting treatments. Phase 1 was  
12 carried out under ambient CO<sub>2</sub> level (392 ppm in air, with a standard deviation of 4.4  
13 ppm, blended in-laboratory from pure dry air and 1003 ppm CO<sub>2</sub>-air mix blended by  
14 Air Liquide) with three single wetting treatments (0, 2 and 5 mm) with three  
15 replicates. After phase 1, covers were removed and water added to the 0 and 2 mm  
16 treatments (5 and 3 mm water, respectively) in order to equalize the moisture content  
17 with the 5 mm wetting treatments. Covers were left off and the crusts allowed to dry  
18 out again. They reached the initial moisture content 4-5 days after this and were then  
19 moistened once a week before commencement of phase 2 (phase 2 was undertaken  
20 three months after phase 1). Phase two was undertaken using the same method as  
21 phase 1, on the same crust samples, this time with elevated CO<sub>2</sub> (801 ppm in air,  
22 blended by Air Liquide).  
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44 Approximately 12 hours prior to experiments, the CO<sub>2</sub>-air mix was used to  
45 flush the chambers. This was in order to allow diffusion through the air spaces in the  
46 soil, such that the concentration gradient between subsoil and atmosphere was  
47 reduced and to acclimatize the crust to the new environment. The first gas sample was  
48 taken (0815 GMT) the following morning, two hours before lights on (1015 GMT).  
49 Samples were then weighed and the Teflon® lids opened for wetting (simulating a  
50 single rainfall event of 2 or 5 mm) using a Jencons Powerpette for even application.  
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1 The chambers were immediately weighed and closed; then placed back into the  
2 growth cabinet and re-attached to the gas line. All chambers were again flushed with  
3 the CO<sub>2</sub>-air mix. Chambers were then left for approximately 40 min before gas  
4 samples were taken, followed by flushing; with the exception of the last flush of each  
5 day which was left overnight and the gas sample taken immediately the following  
6 morning. Each flushing was 12 min (this was determined during initial DGEC design  
7 testing in the laboratory). Chamber venting time was close to optimum (40 min),  
8 which has been determined by a sensitivity analysis of static gas chambers on soils  
9 (Ohlsson et al., 2005), in which it was concluded that it is possible to reduce the  
10 uncertainty in the CO<sub>2</sub> flux value by allowing extended CO<sub>2</sub> accumulation/reduction  
11 times. Nine gas samples (10 ml each) were collected per chamber at regular intervals  
12 during the day (0815-1715 GMT with at least two samples taken before lights on) for  
13 three days and a total of 243 samples were collected from each phase of this  
14 experiment. A preliminary run (with 410 ppm CO<sub>2</sub>-air mix) was carried out prior to  
15 the experiment reported here to check the system.  
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36 Sample weights were taken only at the beginning and end of each phase in  
37 order to quantify moisture loss. Moisture loss during the experiment was found to be  
38 negligible in the DGECs, due to the chambers being closed.  
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## 46 **2.4 Analysis of CO<sub>2</sub> Using Gas Chromatography with Helium Ionization**

### 47 **Detection**

48 Temporal changes in CO<sub>2</sub> concentration within the DGECs reflect the activity  
49 (photosynthesis and respiration) of biological soil crusts. To measure the changes in  
50 CO<sub>2</sub> levels, 2.5 µl gas samples were taken at approximately 40 minute intervals  
51 throughout the experiment and were separated using a Porous Layer Open Tubular  
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1 (PLOT) column (PoraPLOT Q, 25 m x 0.53 mm, 20 µm i.d. Chrompack,  
2 Netherlands). A helium ionization detector (VICI, Houston, USA) was used to  
3  
4 quantify the eluate. The dimensionless integrals of the CO<sub>2</sub> peaks were converted to  
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6 ppm and then to carbon flux in mg C m<sup>-2</sup> hr<sup>-1</sup> using Equation 1;  
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$$10$$
$$11$$
$$12 \text{ C Flux Rate} = \{([\text{CO}_2]_{\text{diff}} \times n) / (A \times t)\} \times V_s \times M_C \times 3,600 \text{ mg C m}^{-2} \text{ hr}^{-1} \quad (1)$$
$$13$$
$$14$$
$$15$$

16 where [CO<sub>2</sub>]<sub>diff</sub> = difference between measured CO<sub>2</sub> concentration and flush CO<sub>2</sub>  
17  
18 concentration in ppm; n = number of moles of gas present in loop at temperature T  
19  
20 (°C);  
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22  
23 A = surface area of soil sample (6.793×10<sup>-3</sup> m<sup>2</sup>); t = time between end of last flush and  
24  
25 taking of gas sample (s); V<sub>s</sub> = volumetric scaling factor = 222,072; M<sub>C</sub> = molar mass  
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27 of carbon (12.0107 g mol<sup>-1</sup>) and 3,600 is used to represent flux over one hour.  
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31 Net carbon balances were based on the area under each carbon flux time series and  
32  
33 were calculated separately for each chamber for each day using the integral function  
34  
35 in EasyPlot™ software. In order to characterize significance of treatments (two levels  
36  
37 of CO<sub>2</sub> and three levels of wetting), analysis of variance (ANOVA) was undertaken  
38  
39 using statistical package SYSTAT 13 which determines the p-value for CO<sub>2</sub>, wetting  
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41 and also whether or not these values are statistically significant.  
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### 56 **3. Results**

#### 57 **3.1 Carbon fluxes in absence of liquid water (controls)**

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1 Temporal changes in CO<sub>2</sub> concentrations in the chambers were measured to  
2 understand the short-term responses to elevated CO<sub>2</sub> levels along with pulse wetting  
3 events. The positive values of flux in Figures 3 – 5 indicate net respiration and  
4 negative values indicate net photosynthesis. Net carbon balances for each individual  
5 chamber, on each day of both phases can be seen in Tables 1 & 2.  
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12 Figures 3a and 3b show temporal changes in the fluxes in the dry chambers  
13 (controls) for phase 1 (392 ppm CO<sub>2</sub>) and phase 2 (801 ppm CO<sub>2</sub>), respectively. The  
14 activity of the crust was very low due to there being no liquid moisture available. For  
15 phase 1, the carbon loss (net respiration) dominated over the gain of carbon (net  
16 photosynthesis) whereas for phase 2, the opposite was found. Tables 1 & 2 show that,  
17 interestingly, many of the replicates exhibited appreciable carbon fixation even when  
18 no liquid moisture was available (with the exception of air moisture). However, the  
19 net carbon budgets over three days showed that,  $0.02 \pm 0.01$  mg C was lost in phase 1  
20 and  $0.39 \pm 0.04$  mg C was sequestered for phase 2.  
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### 36 **3.2 Carbon fluxes under 2 mm wetting treatment**

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39 Figures 4a & 4b display the carbon release during the first day from the samples soon  
40 after the wetting treatment for both phases. The samples continued respiration for the  
41 majority of the first day and settled into a photosynthetic regime by the end of the day.  
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46 The second and third days show the samples to be predominantly photosynthetic in  
47 activity. This photosynthetic activity peaked toward the end of each day. The quantity  
48 of liquid moisture available for the samples for each of the three days, remained  
49 largely unchanged. Tables 1 & 2 show the net carbon balances (in mg) of all  
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65 chambers for each day of each phase. After a 2 mm wetting treatment and subject to  
the conditions of phase 1 the crust sequestered a total of  $0.14 \pm 0.14$  mg C and during

1 phase 2 this was  $1.3 \pm 0.5$  mg C. All cell group averages displayed net sequestration  
2 for both phases under 2 mm wetting, with phase 2 showing the highest quantity of  
3 carbon sequestered.  
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### 9 **3.3 Carbon fluxes under 5 mm wetting treatment**

10 When the 5 mm wetting treatment was applied the wetting front reached the bottom of  
11 each container, therefore saturating the soil. Moisture remained available for the  
12 samples for each of the three days, and the quantity remained largely unchanged.  
13  
14 Figures 5a & 5b display the carbon fluxes of the chambers subject to a 5 mm wetting  
15 treatment for each phase. It can be seen that throughout each experiment, a pulse of  
16 carbon was released at the beginning of each day, particularly during phase 1,  
17 indicating respiration. The samples exhibited very similar behavior to those subject to  
18 2 mm wetting; the main difference being a moderate carbon pulse at the beginning of  
19 each day and overall larger fluxes. For phase 2, the carbon losses were found to be  
20 similar to phase 1; however the peak influx (sequestration) was 2-3 times larger than  
21 phase 1. Tables 1 & 2 show the net carbon balances (in mg) of all chambers for each  
22 day of each phase. After a 5 mm wetting treatment and subject to the conditions of  
23 phase 1,  $0.62 \pm 0.05$  mg C was sequestered and during phase 2,  $2 \pm 0.2$  mg C was  
24 sequestered. Phase 2 showed the highest quantity of carbon sequestered.  
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46 ANOVA significance testing of daily carbon sequestration (i.e. all negative  
47 values from Tables 1 & 2) was undertaken. Tables 1 & 2 show the highly significant  
48 effects of CO<sub>2</sub> (P value = 0.005) and wetting (P value = 0.001), however the  
49 interaction between these factors was not found to be significant.  
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#### 4. Discussion

All carbon balances, with the exception of the dry samples at 392 ppm CO<sub>2</sub>, were negative. This suggests that the overall, the dominant process was photosynthesis. This shows that isolation of the autotrophs has been achieved, although it should be noted that BSCs colonies are an amalgamation of heterotrophs and autotrophs, and as such there is potential for both photosynthesis and respiration to occur simultaneously. This is further confirmed when considering the pulse CO<sub>2</sub> efflux effects observed in those samples subject to wetting treatments, although interestingly initial pulses were lower under 801 ppm CO<sub>2</sub> than under 392 ppm CO<sub>2</sub> perhaps suggesting that the CO<sub>2</sub> concentrating mechanism (CCM) (Badger and Price, 1992) activity was enhanced under the increased CO<sub>2</sub> atmosphere. Respiration was observed early each day in the samples subjected to wetting treatments due to the absence of light (and in some cases lasted for a short duration after the lights were on); again these effluxes were lower under higher ambient CO<sub>2</sub> levels.

Overall, each of the wetting treatments yielded higher influx of carbon when subject to an 801 ppm CO<sub>2</sub>-air mix than when subject to the 392 ppm CO<sub>2</sub>-air mix. Of these the most significant were the samples subjected to a 2 mm wetting treatment; carbon influx increased by an order of magnitude suggesting that photosynthetic activity in the BSCs samples responded best to 'light' wetting treatments/events. Increases in carbon influx of three fold were observed in those samples with a 5 mm wetting treatment, still representing a significant relative increase in carbon influx under increased atmospheric CO<sub>2</sub>. Samples which had no additional water added also exhibited increased carbon influx under enhanced atmospheric CO<sub>2</sub>; this is very likely due to moisture formation in the form of condensation on the soil surface during the

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‘night-time’ part of the cycle creating a source of liquid moisture for the BSCs, facilitating photosynthesis.

The finding that the relative increase in carbon influx was highest in the 2 mm wetting treatment is complementary to the field-based findings of Thomas et al. (2008) whereby not only do the autotrophs respond best to ‘light’ wetting treatments, but the light wetting also limits the depth of soil which receives moisture, hence reducing the effect that subsoil heterotrophs have on the net carbon balance. Light rainfall events are common across the Kalahari, particularly at the beginning of the wet season and have an important effect upon the annual soil carbon balance (Thomas et al., 2011). The results presented here are promising for the continuing role of BSCs as a carbon store as atmospheric CO<sub>2</sub> increases, provided rainfall patterns remain at higher frequencies of light events as currently seen (Simelton et al., 2013) rather than moving to more extreme intense rainfall events as predicted by some for dryland Africa more widely (e.g. Twomlow et al., 2008)).

Although the mechanisms of increased carbon sequestration were not investigated in this study, Badger and Price (1992) describe the CO<sub>2</sub> concentrating mechanisms of cyanobacteria, which are capable of concentrating CO<sub>2</sub> up to 1000-fold. The cyanobacterial CO<sub>2</sub> concentrating mechanism is possibly the most effective of any photosynthetic organism (Badger and Price, 1992), and therefore it would be hoped that this effect would be amplified if elevated levels of CO<sub>2</sub> were made available (up until saturation). Cyanobacterial species are among the earliest forms of life, having evolved under, and been exposed to, the high CO<sub>2</sub>/low O<sub>2</sub> atmosphere of the early (~3,000 Ma) Earth (Bowes, 1991); therefore it could be postulated that cyanobacterial photosynthetic activity is optimum when exposed to the conditions under which the organisms evolved, and hence a positive correlation between

1 photosynthetic activity and increasing CO<sub>2</sub> levels may exist. The findings of this study  
2 appear consistent with this hypothesis; however the exact mechanisms require further  
3 investigation. Further evidence is the ability of CO<sub>2</sub> concentrating mechanism to  
4 modulate activity under the influence of environmental factors; cyanobacteria (and  
5 other CO<sub>2</sub> concentrating mechanisms) can acclimate to a wide range of CO<sub>2</sub>  
6 concentrations (as also show in FACE studies of Steven et al., 2012).  
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14 This study demonstrated the carbon sequestration potential of BSCs under  
15 elevated CO<sub>2</sub> levels under two moisture regimes. The response was similar to many  
16 crops (Cure and Acock, 1986) and dryland vegetation (Naumburg et al., 2003) under  
17 elevated levels of CO<sub>2</sub> in the atmosphere. BSCs have the potential to fix carbon under  
18 limited soil moisture availability and nutrient poor soils (typical of drylands) and is  
19 especially applicable to BSCs dominated by nitrogen fixing cyanobacteria. Therefore  
20 undisturbed BSC-covered drylands could be enhanced carbon sinks, and play an  
21 increasingly significant role in global carbon budgets in years to come. With this in  
22 mind, protection of BSCs is of increasing importance, particularly considering land  
23 use pressures on these areas (Dougill and Thomas, 2004), where implementation of  
24 community-based ecosystem service schemes such as those described by Dougill et  
25 al. (2012) could be greatly beneficial to dryland areas. More long-term flux  
26 monitoring studies are required with varying environmental conditions, particularly  
27 concerning BSCS responses to increased temperatures (e.g. Frey et al., 2013) and  
28 incoming solar radiation with varying wetting treatments.  
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## FIGURE CAPTIONS

**Figure 1.** Photograph of the dynamic gas exchange chamber. A Teflon® film was attached to the top of the chamber using strong adhesive tape, and the gas inlet/gas outlet had open-cell foam inserts in order to allow pressure equilibration, but to prevent gas diffusion. Gas inlets/outlets were attached using grommets which were sealed using Instant Gasket. Gas samples were taken from the Suba-Seal® sampling port (to the rear of the chamber in this image). A hole was drilled in the bottom of the chamber in order that the samples could be inserted into the cell in a controlled manner; during experiments this hole was sealed using high strength adhesive tape.

**Figure 2.** Layout of DGECs within controlled environment cabinet. The USB data loggers (not shown) were placed at various points within the cabinet (one on top and bottom shelf, two on middle shelf). A flow rate meter (not shown) was attached to the gas feed line close to the point of exit of the main 32 l vessel.

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**Figure 3a & 3b.** Gas sample data from phases 1 & 2, samples with no additional liquid water, data presented as average values of three replicates for each treatment with error bars representing standard error. Gas sample data from phases 1 (Figure 3a) & 2 (Figure 3b) (392 ppm & 801 ppm CO<sub>2</sub>, respectively, in air flush-gas), measuring carbon fluxes in chambers subject to no additional liquid water. Night-time (lights off) was 8 hours before 1 hour, between 17-25 hours and between 41-49 hours, after wetting. The left y-axis (Y1) corresponds to the solid dots / line (carbon flux of crust in chamber averaged over three chambers) and the right y-axis (Y2) corresponds to the dashed curve (internal temperature of cabinet). The Y1=0 line represents the point at which net carbon flux is zero. Y1=+ve → net respiration, Y1=-ve → net photosynthesis. The point at which water was added to the crusts is given by the line X=0.

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**Figure 4a & 4b.** Gas sample data from phases 1 & 2, samples subject to 2 mm wetting treatment, data presented as average values of three replicates for each treatment with error bars representing standard error. Gas sample data from phases 1 (Figure 4a) & 2 (Figure 4b) (392 ppm & 801 ppm CO<sub>2</sub>, respectively, in air flush-gas), measuring carbon fluxes in chambers subject to a 2 mm wetting treatment. Night-time (lights off) was 8 hours before 1 hour, between 17-25 hours and between 41-49 hours, after wetting. The left y-axis (Y1) corresponds to the solid dots / line (carbon flux of crust in chamber – averaged over three chambers) and the right y-axis (Y2) corresponds to the dashed curve (internal temperature of cabinet). The Y1=0 line represents the point at which net carbon flux is zero. Y1=+ve → net respiration, Y1=-ve → net photosynthesis. The point at which water was added to the crusts is given by the line X=0.

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9 **Figure 5a & 5b.** Gas sample data from phases 1 & 2, samples subject to 5 mm  
10 wetting treatment, data presented as average values of three replicates for each  
11 treatment with error bars representing standard error. Gas sample data from phases  
12 1 (Figure 5a) & 2 (Figure 5b) (392 ppm & 801 ppm CO<sub>2</sub>, respectively, in air flush-  
13 gas), measuring carbon fluxes in chambers subject to a 5 mm wetting treatment.  
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21 Night-time (lights off) was 8 hours before 1 hour, between 17-25 hours and between  
22 41-49 hours, after wetting. The left y-axis (Y1) corresponds to the solid dots / line  
23 (carbon flux of crust in chamber – averaged over three chambers) and the right y-axis  
24 (Y2) corresponds to the dashed curve (internal temperature of cabinet). The Y1=0 line  
25 represents the point at which net carbon flux is zero. Y1=+ve → net respiration, Y1=-  
26 ve → net photosynthesis. The point at which water was added to the crusts is given by  
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## HIGHLIGHTS

- Response to elevated atmospheric CO<sub>2</sub> of cyanobacterial samples was investigated
- Three wetting treatments were applied under fixed temperature and light conditions
- Newly designed dynamic gas exchange chambers were developed and used
- Samples subjected to 2 mm wetting sequestered 10 times more C when CO<sub>2</sub> was doubled
- Samples subjected to 5 mm wetting sequestered 3 times more C when CO<sub>2</sub> was doubled







Figure2

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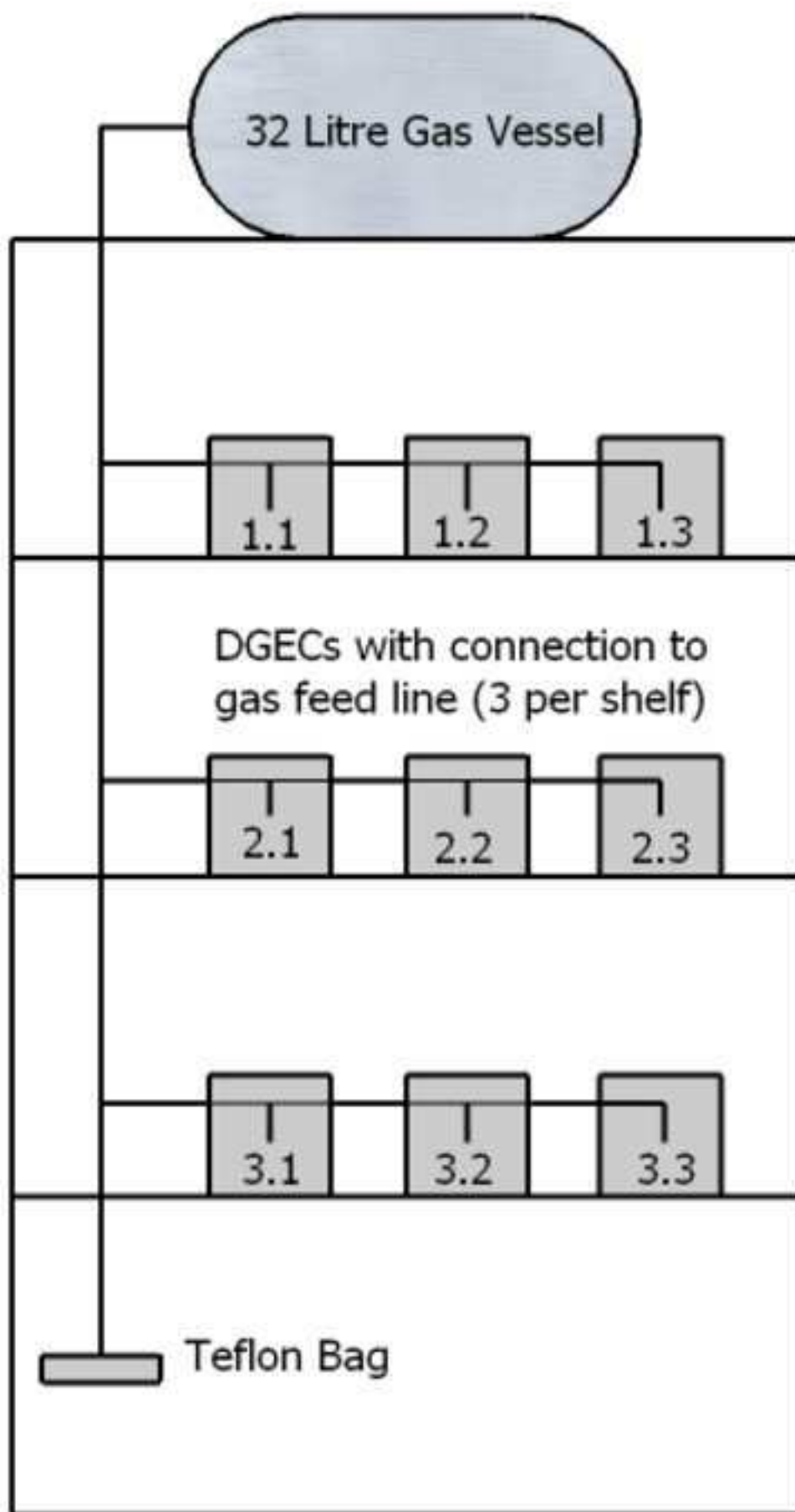


Figure3a

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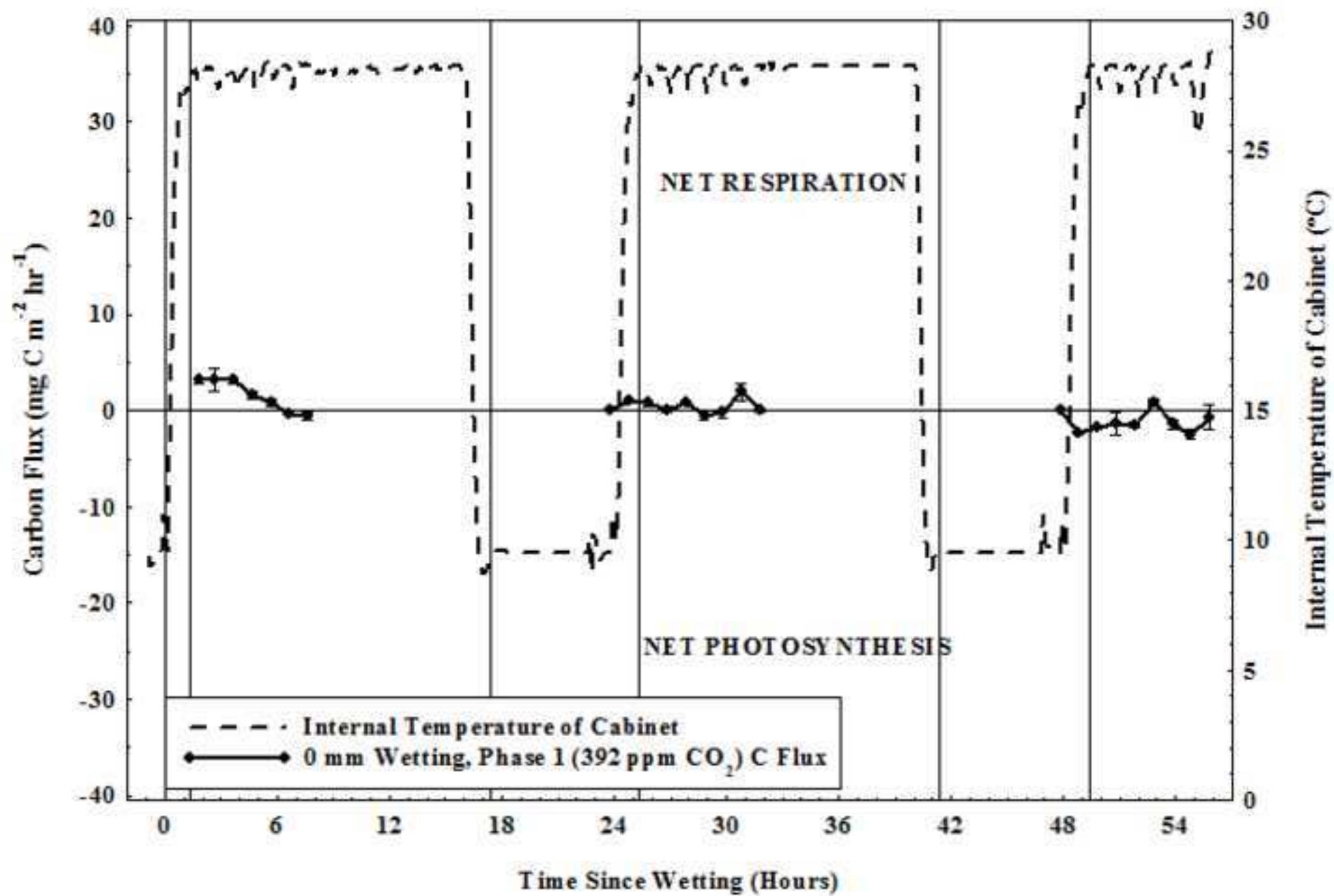


Figure3b  
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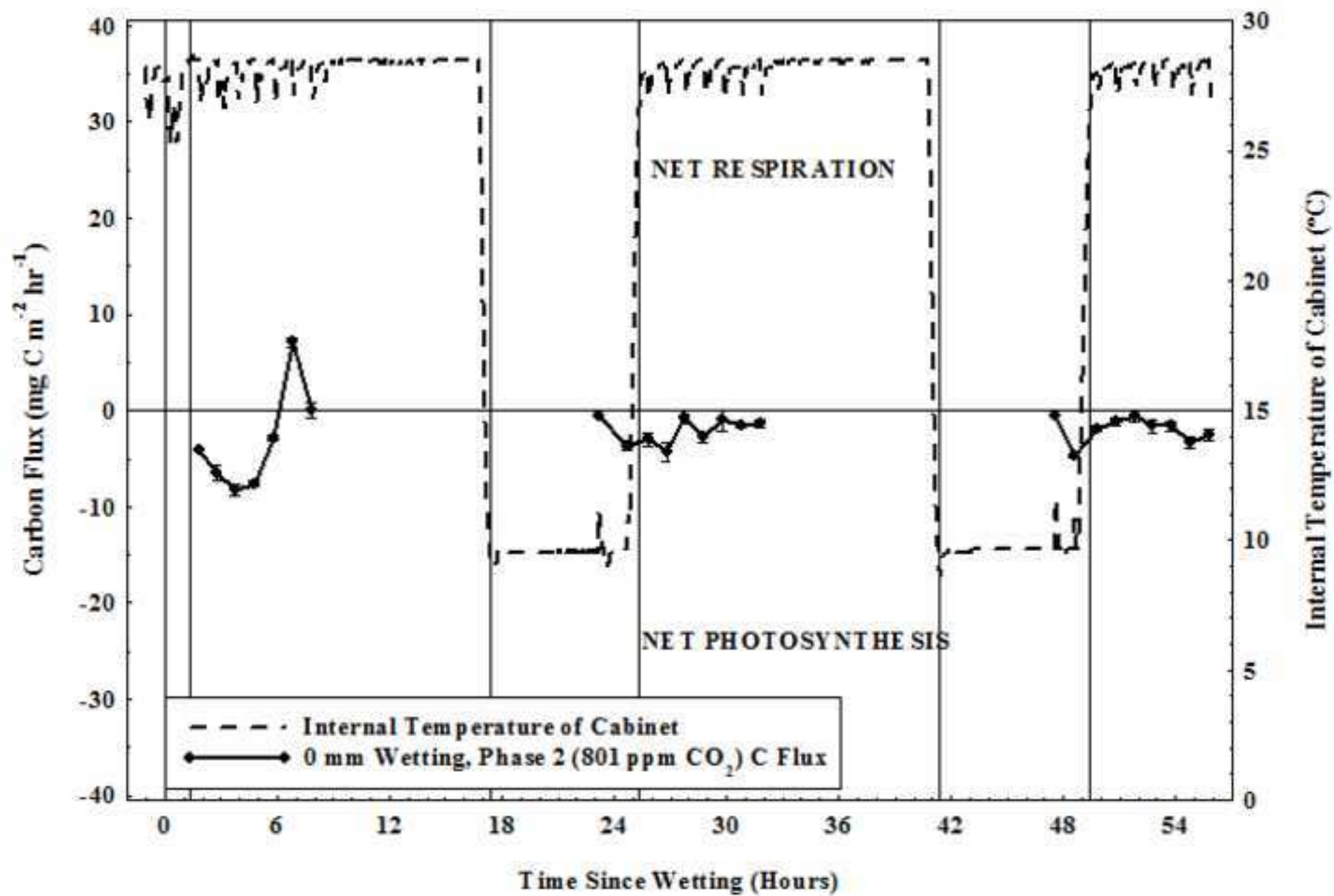


Figure4a

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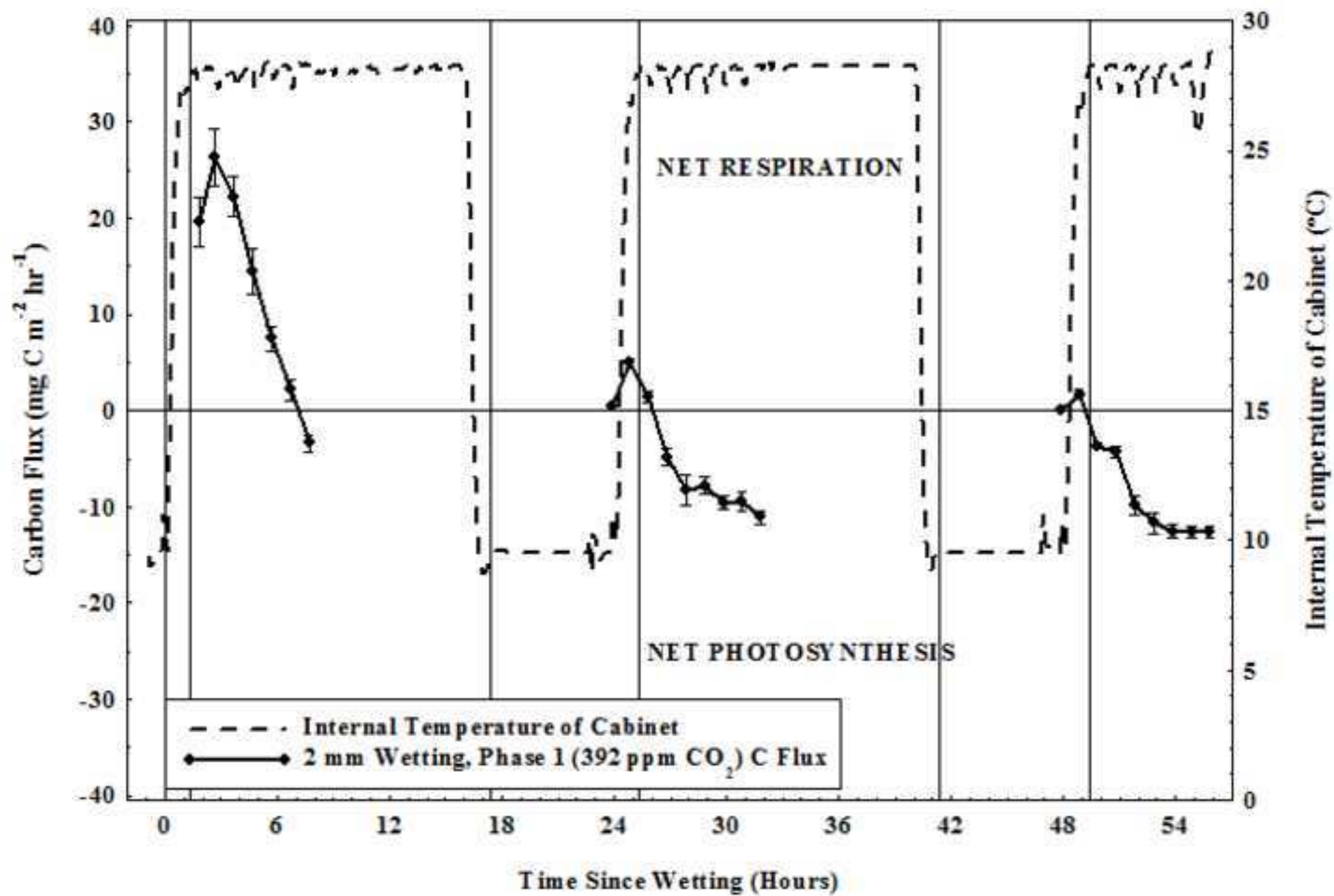




Figure4b  
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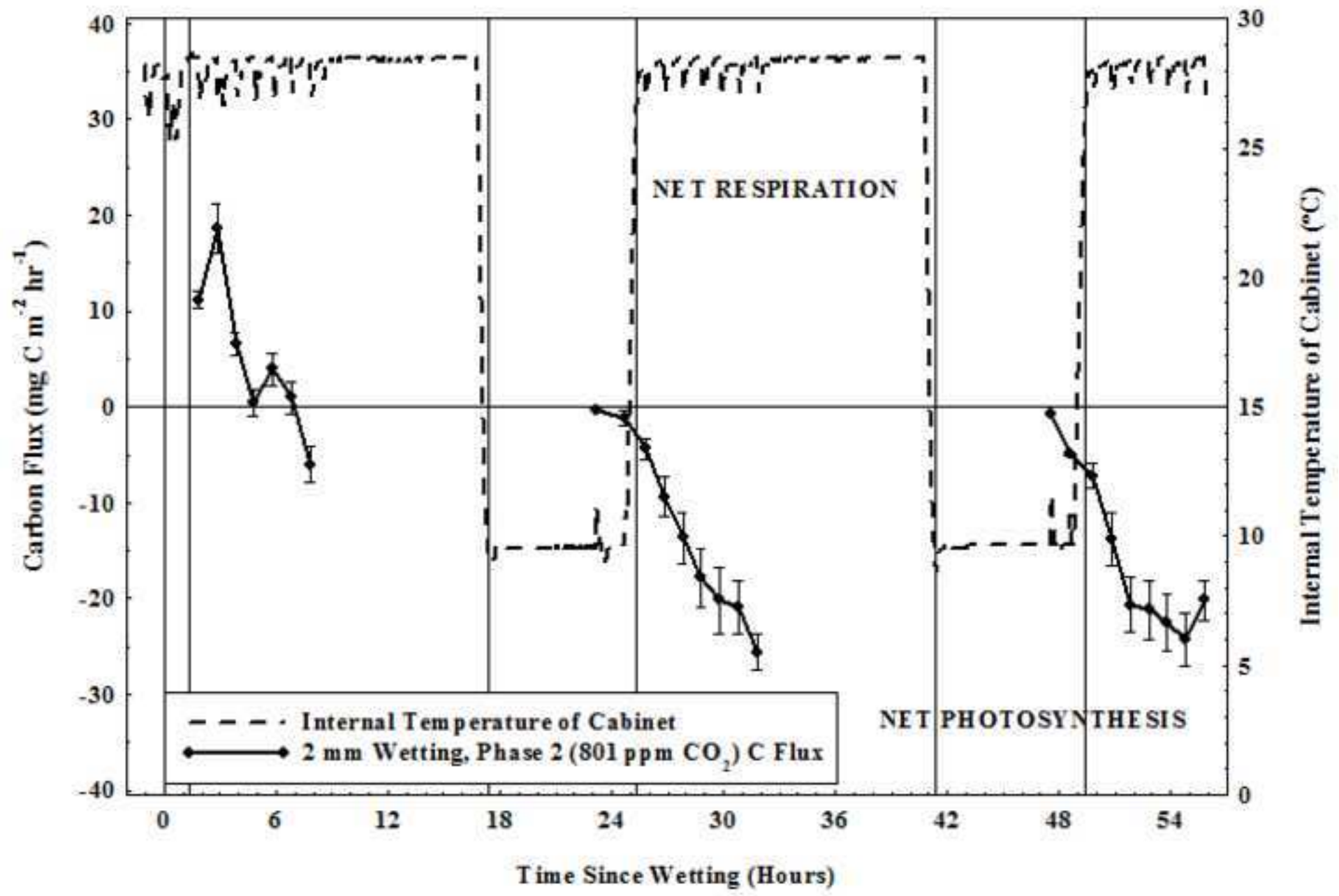


Figure5a

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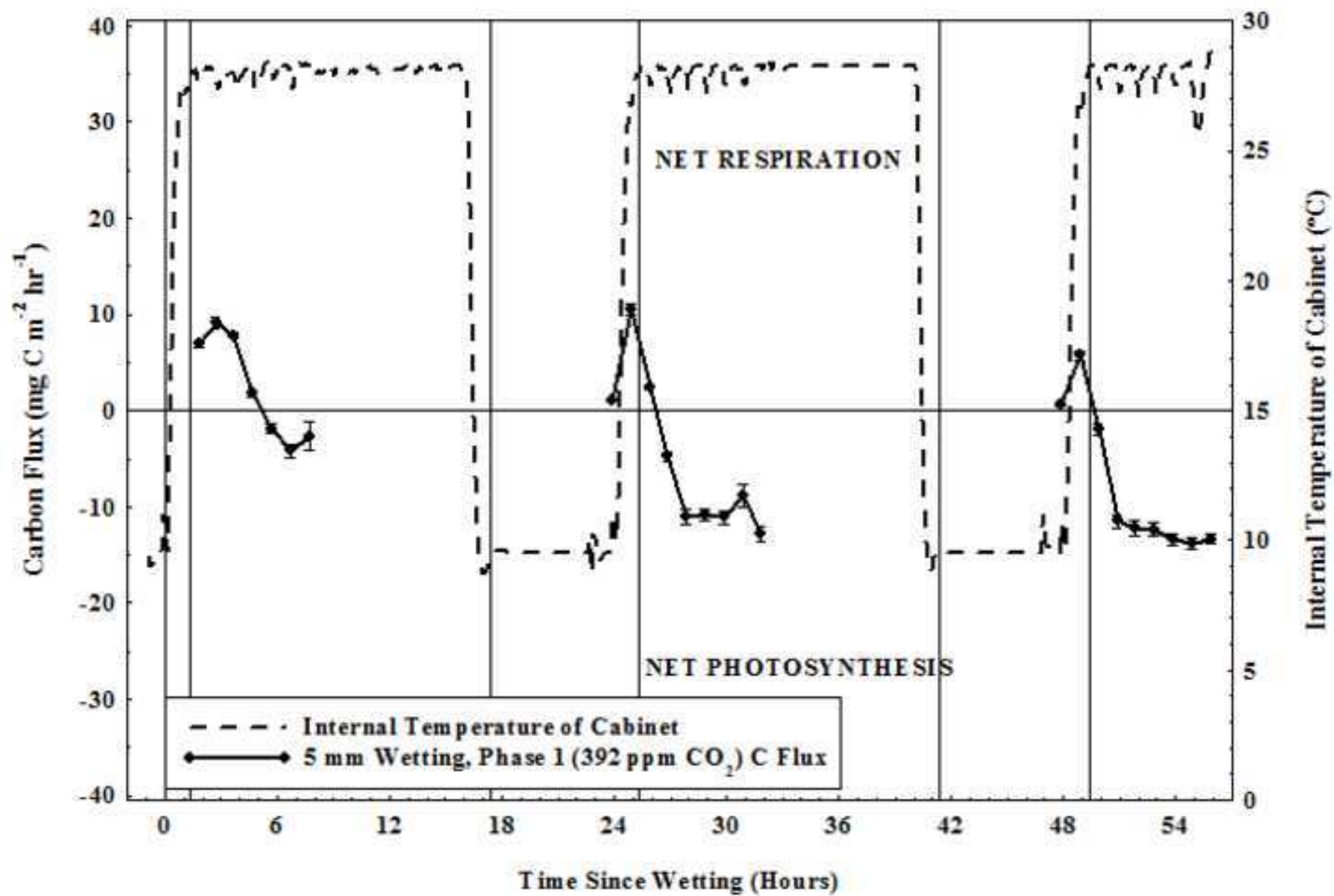
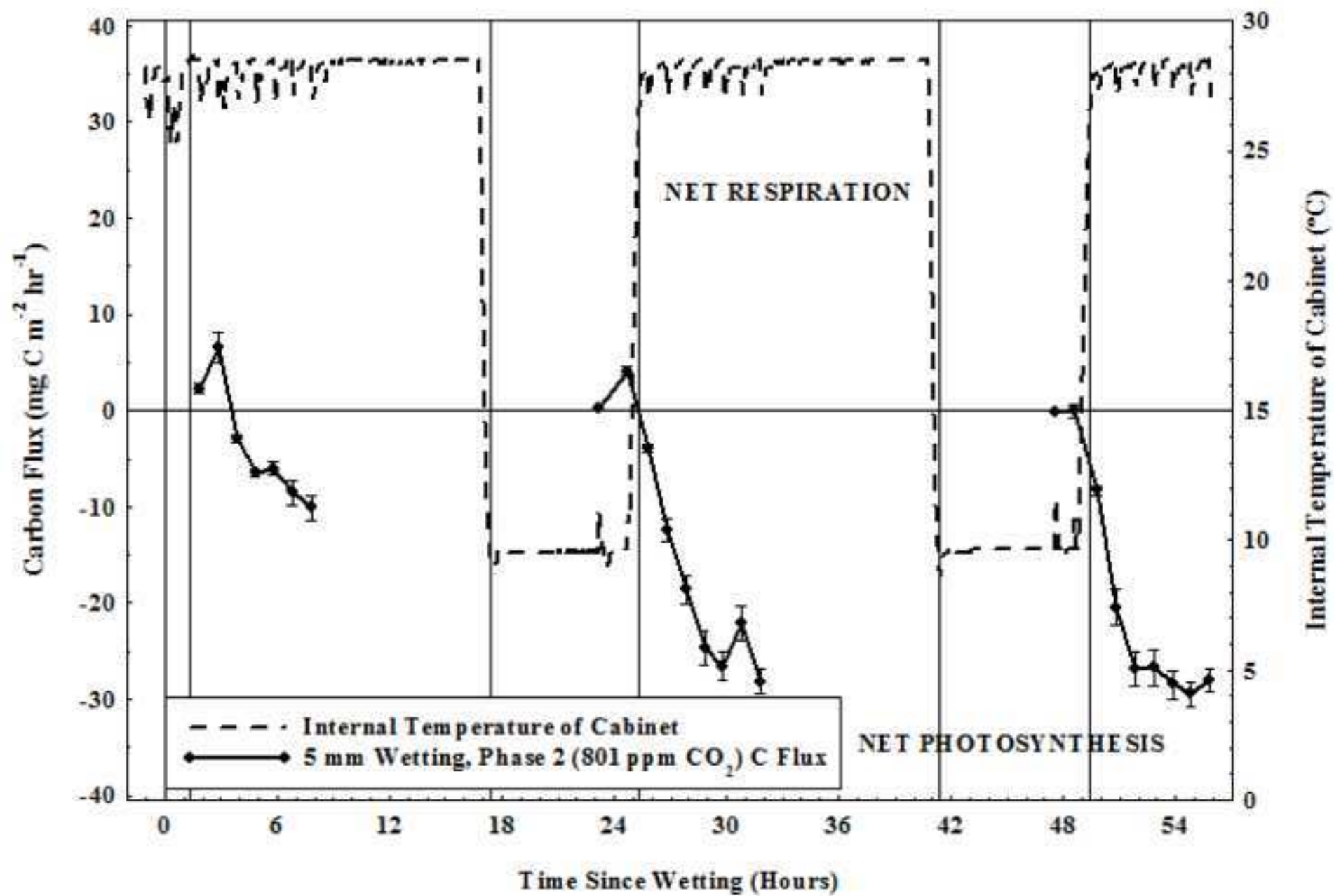




Figure5b  
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## TABLES

**Table 1.** Carbon balances for each chamber during Phase 1 (392 ppm CO<sub>2</sub>). Chambers 1.2-1.3 had no additional water, chambers 2.1-2.3 each had a 2 mm wetting treatment and chambers 3.1-3.3 each had a 5 mm wetting treatment.

Carbon Balance (mg C) (+ve = efflux, -ve = sequestration)					
Chamber	Day 1	Day 2	Day 3	Net	Average for each chamber group (±SE)
1.1	0.119	0.021	-0.098	0.041	
1.2	0.030	0.013	-0.024	0.020	
1.3	0.033	0.046	-0.082	-0.003	0.02 ± 0.01
2.1	0.431	-0.163	-0.330	-0.062	
2.2	0.775	-0.300	-0.421	0.055	
2.3	0.354	-0.324	-0.450	-0.420	-0.14 ± 0.14
3.1	0.120	-0.307	-0.420	-0.607	
3.2	0.085	-0.199	-0.428	-0.541	
3.3	0.068	-0.293	-0.487	-0.712	-0.62 ± 0.05

**Table 2.** Carbon balances for each chamber during Phase 2 (801 ppm CO<sub>2</sub>). Chambers 1.2-1.3 had no additional water, chambers 2.1-2.3 each had a 2 mm wetting treatment and chambers 3.1-3.3 each had a 5 mm wetting treatment.

Carbon Balance (mg C) (+ve = efflux, -ve = sequestration)					
Chamber	Day 1	Day 2	Day 3	Net	Average for each chamber group (±SE)
1.1	-0.109	-0.096	-0.163	-0.369	
1.2	-0.174	-0.190	-0.102	-0.466	
1.3	-0.125	-0.114	-0.082	-0.321	-0.39 ± 0.04
2.1	0.045	-0.703	-0.880	-1.538	
2.2	0.317	-1.067	-1.217	-1.966	
2.3	0.304	-0.284	-0.468	-0.449	-1.3 ± 0.5
3.1	-0.145	-0.621	-0.845	-1.611	
3.2	-0.076	-0.811	-1.115	-2.001	
3.3	-0.215	-0.946	-1.194	-2.356	-2.0 ± 0.2