Some studies on gas [nitrogen and oxygen] concentrations in the rhizosphere

Bronwyn Townsend

Centre for Plant and Water Science, CQUniversity, Rockhampton.

Abstract

To determine at what stage there was a measurable, positive effect of aeration it was necessary to determine the optimal rate of oxygen concentration to be applied, and to see if they would be detectable at different, randomised oxygen concentration levels. The application levels were based on 20% oxygen concentration being the ambient concentration. The effect of enriched oxygen appeared to directly effect an increase of root respiration, whilst a decline in oxygen concentrations induced a decline of root respiration. The rate of exhaust followed similar trends at the oxygen concentrations between 25% to 10% which is the region at which glycolosis was suspected to have become an active reaction to declining oxygen levels. Below 10% oxygen respiration dropped more sharply, but it never ceased, even at zero oxygen concentration. The rate of flow was determined to not have been a factor as optimum rate of flow was 500mL/min with respiration declining as rate of flow increased, however maximum respiration levels peaked at 250mL/min. Soil moisture was not adversely affected by rate of flow, The overriding trend throughout two experiment was that oxygen enriched concentrations do increase plant ability to respire coupled with the rate of flow which was seen to affect the respirative ability and the moisture uptake function of a plant. It was assumed that enriched to ambient concentrations at a minimal flow rate was the optimal conditions for plant growth.

Introduction

Cotton is an important export commodity to Australia, comprising $1 billion of Australia's export revenue in a non-drought year; however, current irrigation and crop practices have seen this facet of the broad acre industry become unsustainable in terms of water efficiency and land use.

Sub-surface drip irrigation (SDI) has quickly become an ideal way to combat water efficiency issues that are facing our sustainable primary industries, particularly in Australia. Despite the innovations and obvious benefits of this particular method in terms of water efficiency and costs, there are limitations to this practice, particularly in regions that are characterized by heavy clay soils (vertisols). This has led to a reduction in efficiency by way of declining soil structure, as associated with increases in salt accumulation, increase of clay content and exchangeable sodium percentage. The overall result is a decrease in the lateral spread of water from the emitter during irrigation, a smaller effective root volume, and perhaps most importantly the occurrence of root hypoxia, as heavy clay soils and soils exposed to extreme drought become when saturated. As almost all of Australia's cotton in the south east is grown on such soil, innovative methods are required to combat this problem, particularly in the face of such a variable climate as is today's.

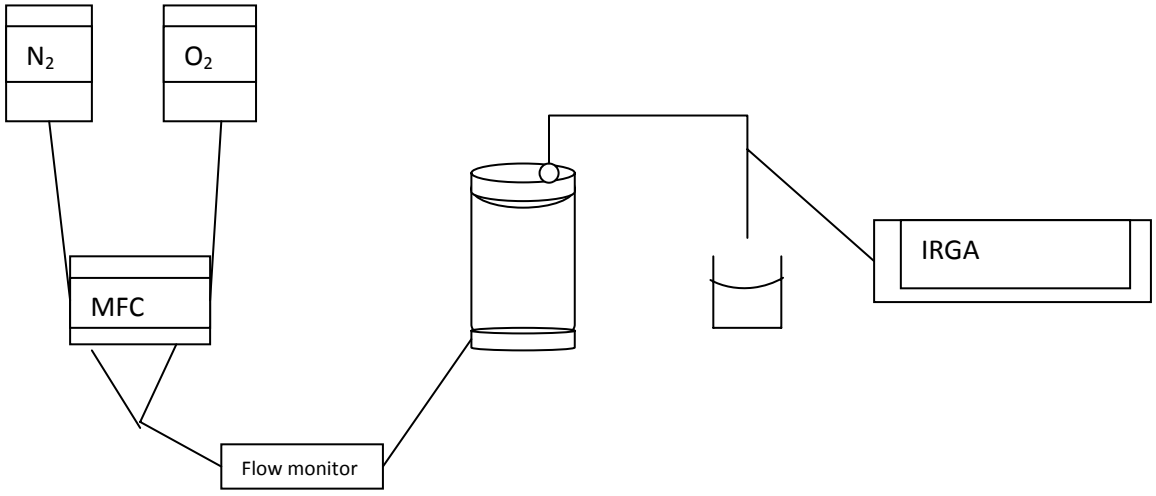
Root hypoxia is the common problem with SDI in the cotton industry particularly as cotton is predominantly gown in vertisols. Root hypoxia constrains xylem and phloem transport which

consequently affects the entire physiology of the plant, reducing phloem exports and consequently limiting leaf and canopy growth (*Smit et al 1990*). Previous studies conducted by the Centre for Plant and Water Science at Central Queensland University have examined the potential of oxygation, i.e., aeration of irrigation water, to reduce the stresses of flooding that commonly occurs in vertisol that are exposed to SDI (Pendergast & Midmore 2006) Central Queensland University has further targeted the afore mentioned issues that face the cotton industry, investigating the impacts of root hypoxia on cotton in grown in vertisols and the effects of aerating irrigation water with measurable positive results – field trials showing significant gains in yields and water use efficiencies compared to non-oxygated cotton (Bhattarai *et al.* 2004) Other studies in this area have also included oxygation of rhizosphere with subsurface aerated irrigation water improves lint yield and performance of cotton on saline heavy clay (Bhattarai et al. 2006).

This experiment specifically was based on research undertaken by The Centre for Plant and Water Science, Central Queensland University, project 1.02.18 Oxygation - Enhancing Water & Nutrient Efficiency specifically to answer "Under what conditions will there be a measurable positive effect of aeration?". Based on this Question, this experiment was designed to target the constraints to date of this research, specifically the modification of soil oxygen concentration independent of the irrigation rate. The aim of the experiment was to control precisely the amount of oxygen in the soil atmosphere by using a gas exchange system and delivering nitrogen and oxygen in known proportions to cotton plants.

Method and materials

Figure 1. The experimental set-up.



MFC – mass flow controller, IRGA – infra-red gas analyzer

Seedling establishment

Forty cotton seedlings were germinated in vermiculite in a growth chamber, set at 28°C - 30°C and a 12:12 hr day: night period. At 3 weeks 20 seedlings were selected at random and were then transplanted into polyurethane pots of 90 mm x 100 mm (dimensions width x Length) in a coarse perlite medium and relocated to a growth chamber where growing conditions could be regulated to allow for optimal health and growth of the plants. Once the seedlings had been transferred an additional 40 seedlings were germinated and followed the same regime. The light intensity was measured at 655 µmol m^{-2} s^{-1} in the growth chamber

Crop details

The plants were then maintained in an alternating 14:10 hr day:night regime with a humidity range of 85 -100%, a day temperature of 30 °C and a night temperature of 25°C-28 °C. This was intended to mimic the optimal growth parameters for this type of cotton. The plants were watered with Manutec, a soluble fertilizer containing all major trace elements and rich in Nitrogen to encourage foliar growth, mixed at full strength and applied twice weekly to ensure good nutrition, the plants were used at ages 10-12 weeks, 12-14 weeks and 13 weeks respectively, for each experiment.

Experimental designs and treatments

Experiment 1- The effect of different oxygen concentrations on soil respiration

The first experiment involved 10 potted cotton plants (designed as per Layzell et al., 1989) selected randomly from group 1 seedlings, aged 10-12 weeks over the duration of the experiment, into which known proportions of N_2 and O_2 were delivered into the root system. The mixtures were delivered at a constant rate of 500 mL/min. Exhaust gas was measured for CO_2 using a Li-Cor Biosciences LI- 6262 IRGA system and rates of net respiration quantified in order to determine a respiration response curve to soil oxygen concentration. The gas ratios were changed, ranging from oxygen enriched at 30% and ramped down in succession 30%, 25%, 20%, 17.5%, 15%, 12.5%, 10%, 7.5%, 5%,4%,3%,2%,1% and 0% (anoxic). Plants were mainatend iat a steady 23-25oC in the laboaratoryu with low light (c. 100 µmol m^{-2} s^{-1}). To determine the effect of oxygen, obtained from the rate of respiration, each plant was subjected to the successive decrease in oxygen, as lack of oxygen availability catalyses the production of ethanol and potentially causes damage to root structures. On average, plants were left for 75 minutes at a given oxygen:nitrogen ratio before steady state CO_2 was measured, and the successive ratio was imposed. The experiment was run over 10 days and each replicate plant was subjected to the same treatment, we then expected to have a base line of steady state ratios of N_2:O_2, 10 replicates for each proportion. After subjecting plants to the complete set of gas ratios, the roots were separated from the media and weighed fresh, scanned and weighed dry. The net CO_2 output was referred back to the size of the root

system, and to the soil oxygen concentration, to gain insight into the response of soil respiration to soil oxygen concentration.

Experiment 2- The effect of different oxygen concentrations on soil respiration and photosynthesis

The treatment consisted of 10 replicate 10 to 14 week old plants for the duration of the experiment, in pots (designed as per Layzell et al, 1989) which were filled with a coarse perlite, into which known proportions of N_2 and O_2 were delivered at random to each replicate at a steady flow rate application of 500 mL/min for an average of 75 min to allow a steady state of exhaust to be established. The replicates were acclimated to laboratory conditions for 48 hrs to ensure that temperature and humidity were uniform before imposition of treatments. Heat and light emitting lamps were employed to this end providing 895 µmol m^{-2} s^{-1} irradiance, a fan was employed to reduce the amount of sensible heat on leaves to induce normal stomatal function, and temperature at the leaf level was measured and ranged from 25.7°C to 31.8°C. A hand held IRGA (ADC LC-4) was used to monitor the leaf transpiration and photosynthesis. The proportions of oxygen and nitrogen delivered ranged from oxygen enriched at 30%, through 25%, 20%, 17.5%, 15%, 12.5%, 10%, 7.5%, 5%,4%,3%,2%,1% and 0% (anoxic). The ratios were randomised for application to the root systems to determine the optimal rate at which oxygen is applied to a root system to promote maximum growth.

The replicates soil medium was kept at a reduced moisture content, measured using a Campbell Scientific Hydrosense probe that ranged between 12% to 8% to reduce any variability that may occur with a reduction of soil moisture content. Exhaust gas was measured for CO_2 concentration using an Li-Cor Li-6262 IRGA system and rates of net respiration quantified in order to determine a respiration response curve to soil oxygen concentration. Leaf photosynthesis and transpiration was measured with a handheld IRGA at the end of each session and the root systems were then extracted from each experimental unit, fresh and dry weights were quantified and the above ground components were separated and dry weighted. Root respiration was expressed on a per plant basis, and on a per fresh and dry root weight basis.

Experiment 3- The effect of flow rate on respiration and water loss

Five replicates from group 1 seedlings at 13 weeks of age were potted in coarse perlite in 90 mm x 100 mm pots, and were subjected to three flow rates for 60 min whilst a steady state ratio of N_2:O_2 (20%-ambient) was administered. Flow rates were applied steadily for a 60 minute period to allow the plants to establish a steady respiration rate, the plant being allowed to rest for one hour intervals so natural root respiration could be re-established before the application of a different flow rate to the roots. Flow rate was based on 500 ml/min as the intermediate flow rate, and alternated between 750 mL/min and 250 mL/min. Water loss was measured over time, (control with soil medium only) so as to differentiate plant water uptake compared to water loss due to gas flow. Temperature in the root medium was also monitored, as any large variance in root temperature would confound the effects of

flow rate and the root respiration. The field capacity of each plant was established prior to the beginning of the experiment; the weight and therefore the degree of soil saturation of the unit were recorded at the end of each treatment to determine water loss over the course of the varied flow applications for each separate treatment.

Instrumentation

Class G' Oxygen Cylinder- food grade

Class G' Nitrogen cylinder- food grade

Sierra instruments Top Trak 820- Flow monitor
Installed in the gas flow line to monitor the gas, mass flow rate.

MKS Mass Flow Controller-110 series, Type (1159B)
Mass flow controller with type D connector, is a laminar flow device that measures and controls mass flow rate of gases.

MKS type (247C) 4 channel read out
Power supply, read out and set point for the four channel mass flow controller, monitors and provides set point levels and ratioed set points for multiple gas input control.

Dynavac-serial 40668, WISA-113 165600
115V -60Hz capacity,

Infra red gas analyser- Li-Cor 6262
Effluent gas (CO_2) enters the soda lime CO_2 Scrubber and then proceeds to the magnesium percholate drying column which draws a reference gas stream through the reference cell. Output is measured and displayed digitally and the value is recorded on a pen chart recorder.

Hand held Infra red gas analyser- ADC LC-4
The Leaf is held in narrow leaf chamber where a reference (ambient) gas source passes over the leaf surfaces and back (called the analytical) to the infrared analysis unit where the reference and analytical gases are measured alternately for CO_2 absorption (for net photosynthesis) and H_2O release (for transpiration) by the known leaf area. Values are recorded digitally.

RESULTS-

Experiment 1- The effect of different oxygen concentrations on soil respiration

Oxygen Saturation	1	2	3	4	5	6	7	8	9	10
30%	16.62	15.78	14.58	10.56	4.44	10.8	8.04	4.92	5.58	7.2
25%	14.1	11.82	13.26	9.72	4.56	10.74	7.92	4.14	4.8	7.02
20%	14.22	10.62	13.32	8.94	4.38	10.14	7.44	3.72	4.62	6.96
18%	13.26	9.9	13.86	8.76	4.08	9.66	7.32	3.6	4.68	11.4
15%	13.02	8.34	13.5	8.64	3.96	9.84	7.56	3.54	3.84	11.4
13%	12.54	9.96	13.92	8.7	3.96	9.96	7.92	3.54	4.74	11.4
10%	12	10.92	13.8	8.64	3.9	10.2	7.86	3.48	5.1	7.02
8%	10.98	10.92	13.62	8.52	3.96	10.14	8.16	3.48	5.04	6.78
5%	10.92	9.84	13.32	8.16	3.66	10.14	8.22	3.36	4.62	6.42
4%	11.04	10.26	13.02	7.86	3.66	9.84	8.1	3.3	4.44	6.12
3%	10.86	8.76	12.78	7.5	3.48	8.64	7.74	3.06	4.32	6.06
2%	10.98	8.46	12.18	7.08	3.24	9.48	7.38	2.94	4.08	5.82
1%	10.86	8.28	11.1	6.54	3.12	9.24	7.08	2.7	3.84	5.22
0%	8.04	5.94	8.34	3.66	2.52	7.44	4.8	1.68	2.94	4.02

Table 1. The data in this table are represented in linear succession of different oxygen concentrations and the rate of CO_2 efflux for each plant. Measured as µmol CO_2/g DW/hr

Table 2. The mean and range for standard deviation for the rate of respiration (µmol CO_2/g DW/hr).

Oxygen Saturation	Mean	STD min	STD max	STD
30%	9.852	5.295177	14.40882	4.556823
25%	8.808	5.136074	12.47993	3.671926
20%	8.436	4.759349	12.11265	3.676651
17.5%	8.652	4.963119	12.34088	3.688881
15%	8.364	4.662519	12.06548	3.701481
12.5%	8.664	5.045691	12.28231	3.618309
10%	8.292	4.808806	11.77519	3.483194
7.5%	8.16	4.816469	11.50353	3.343531
5%	7.866	4.549803	11.1822	3.316197
4%	7.764	4.438318	11.08968	3.325682
3%	7.32	4.16038	10.47962	3.15962
2%	7.164	3.977629	10.35037	3.186371
1%	6.798	3.727098	9.868902	3.070902
0%	4.938	2.548013	7.327987	2.389987

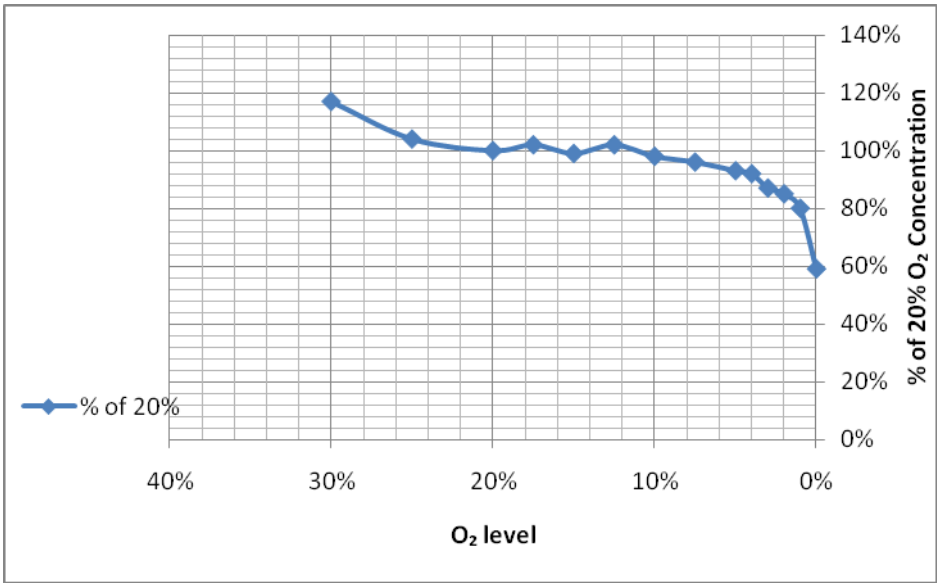


Figure 2: Data represent the proportion of respiratory CO_2 evolution as a % of that at 20% O_2 concentration using the mean from each treatment.

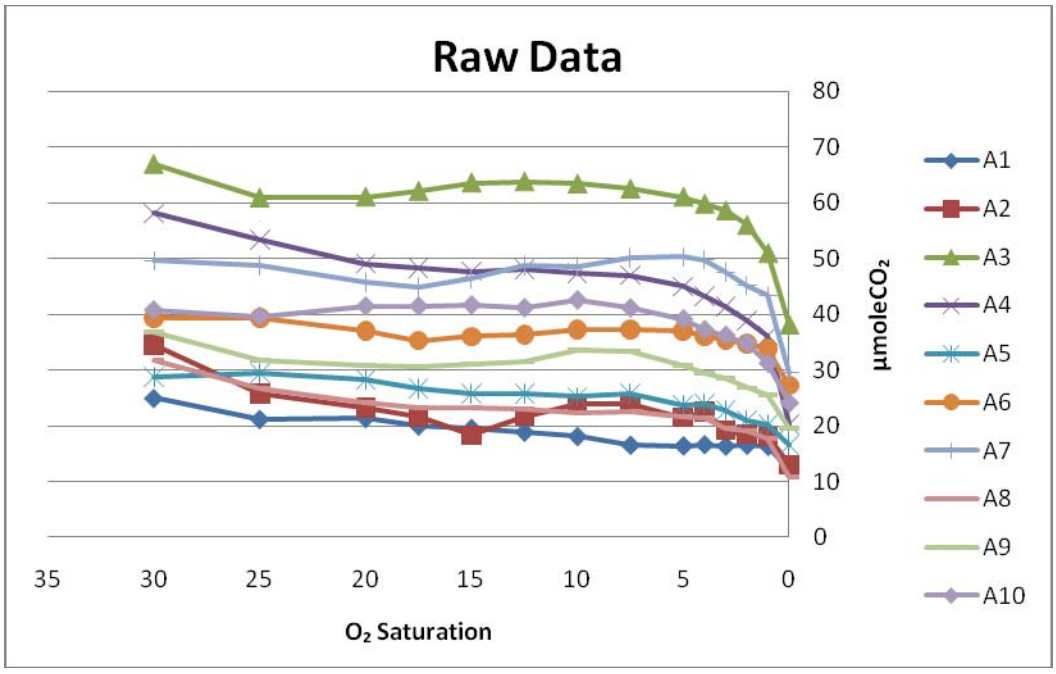


Figure 3. This is representative of the raw data from each successive replicate within treatment 1, measured in $\mu molCO_2$ per pot.

The first experiment was a pilot experiment to establish a base respiration rate at each oxygen saturation level; as such each replicate was subjected to a systematicramping down of oxygen ratios from 30% to 0%, each replicate took approximately an hour to acclimate to each saturation ratio and to sustain a steady state of respiration – each successive decrease in oxygen was accompanied by a

reduction of measurable exhaust gas CO_2. Typically a decline began at 5% oxygen slowing considerably, but at 0%, respiration was maintained for the duration of the period, despite the lack of supplied oxygen. The data are not included, however it was noticed that outside of the one hour measurable time period that the level of exhaust did decline, but no more than 1 µmol CO_2/g DW/hr in the hour it was left outside of the experimental time period.
Data in Table 2 emphasise that the reduction of O_2, resulted in a steady decline in respiration over the treatments; a marked drop occurring at 25% which was the medium between oxygen rich and ambient levels. Figure 2 represents the decline in measurable exhaust CO_2, the data is the percentage for each mean from each treatment based on the ambient oxygen concentration of 20%, this reflects the overall reduction in the plant ability to respire at varying levels of oxygen availability.

The breakdown as per Figure 3 is that 30%, 20% and 10% show a similar trend in respiration, irrespective of the variations that are applicable at each level. At 0% we see that respiration is substantially lower than even at 1% O_2, but in turn it was still appreciable.

Experiment 2- The effect of different oxygen concentrations on soil respiration and photosynthesis

Table 3-5: The data in these three tables describe the raw data obtained from the IRGA trends in root respiration and photosynthesis at different levels of Oxygen concentrations.

Respiration µmol CO_2

plants	0%	1%	2%	3%	4%	5%	7.50%	10%	12.50%	15%	17.50%	20%	25%	30%
1	7.12	14.56	14.87	14.87	16.07	13.65	15.87	9.16	13.24	12.87	11.46	14.32	14.6	9.16
2	9.3	8.22	10.29	5.65	8.42	8.1	6.87	6.74	8.32	6.8	7.65	8.49	8.17	9.2
3	11.3	13.09	14.13	14.7	14.82	13.9	12.79	17.24	13.65	9.64	16.04	13.7	17.75	20.62
4	12.32	13.06	14.7	14.2	13.9	15	10.71	15.20	14.21	22.6	13.9	14.98	15.10	22.14
5	28.52	20.94	49.30	18.92	28.20	17.86	28.90	17.82	29.86	32.48	32.39	44.18	40.78	31.25
6	14.02	19.69	24.6	22.55	17.68	28.5	20.12	16.87	21.72	17.88	22.52	25.74	27.86	27.86
7	11.3	23.84	18.63	18.69	20.87	18.73	19.93	26.5	21.03	20.98	21.08	20.52	21.16	22.15
8	4.9	1.9	2.7	4.23	3.64	6.6	3.4	6.09	2.52	3.9	4.16	3.64	3.87	18.03
9	26.4	26.88	19.08	21.05	24.93	17.6	35.4	32.6	26.8	23.21	25.21	31.05	23.14	29.13
10	14.82	14.56	15.21	14.21	14.13	12.67	13.91	13.67	13.3	17.24	17.37	14.23	15.12	17.98
Average	12.771	14.371	16.884	13.49	14.879	13.765	15.721	14.672	15.047	14.505	15.791	17.59	17.25	18.54

Respiration rate µmol CO_2/g DW/hr

plants	0%	1%	2%	3%	4%	5%	7.50%	10%	12.50%	15%	17.50%	20%	25%	30%
1	1.74	0.029	1.8	2.04	1.86	1.68	1.92	1.14	1.62	1.5	1.44	1.74	1.8	1.32
2	2.28	1.98	2.34	1.38	1.92	1.98	1.68	1.68	2.04	1.68	1.68	1.98	1.86	2.22
3	0.9	1.08	1.14	1.2	1.2	1.14	1.02	1.38	1.14	0.78	1.2	1.14	1.5	1.68
4	1.5	1.8	1.8	1.8	1.74	2.1	1.32	1.98	1.74	2.76	1.74	1.86	1.92	2.94
5	2.7	2.46	3.9	2.28	3.48	2.28	3.54	2.1	3.66	3.9	5.34	4.68	5.46	3.78
6	3.3	4.68	5.88	5.52	4.26	6.9	4.92	4.14	5.34	4.38	5.52	6.3	5.4	6.72

7	0.9	1.98	1.5	1.5	1.68	1.5	1.62	1.8	1.74	1.74	1.74	1.68	1.74	1.8
8	1.08	0.48	0.6	0.96	1.26	0.9	0.72	1.5	0.9	0.96	1.02	0.9	0.78	4.2
9	6.36	6.36	4.62	5.16	6.06	4.26	8.52	7.92	6.48	5.7	6.18	7.62	5.64	7.08
10	0.9	0.9	0.96	0.9	0.96	0.78	0.84	0.84	0.84	1.08	0.96	0.9	0.9	1.18
Average	**2.166**	**2.1749**	**2.454**	**2.274**	**2.442**	**2.352**	**2.61**	**2.448**	**2.55**	**2.448**	**2.682**	**2.88**	**2.7**	**3.292**

Photosynthesis µmol/m/s

plants	0%	1%	2%	3%	4%	5%	7.50%	10%	12.50%	15%	17.50%	20%	25%	30%
1	0.72	1.13	0.92	0.12	0.18	0.53	0.21	0.44	0.18	0.02	1.12	0.47	0.47	1.12
2	0.16	0.84	0.22	1.24	1.31	0.47	0.29	1.26	0.93	0.72	0.35	0.14	2.41	0.5
3	2.34	0.86	0.5	0.15	0.23	0	0.21	0.19	0.64	0.13	0.87	1.28	0.34	0.11
4	1.98	0.23	0.79	0.28	0.21	0.10	0.21	0.13	0.75	0.15	0.15	0.87	0.42	0.10
5	0.53	0.15	0.49	0.31	0.34	0.14	0.42	0.36	0.56	0.46	0.20	0.26	0.93	0.35
6	0.03	0.08	1.26	0.53	0.24	0.86	0.1	0.76	0.76	0.31	0.68	1.71	0.1	1.05
7	0.03	0.5	1.14	0.18	1.12	0.2	0.87	0.22	0.32	0.23	0.08	0.37	0.42	0.87
8	0.01	0.13	0.13	0.03	0.08	0.54	0.06	0.1	0.07	0.05	0.08	0.07	0.03	0.27
9	1.28	1.12	0.53	1.42	0.12	0.23	0.27	0.65	0.98	0.61	0.32	0.67	0.02	1.41
10	2.34	0.29	0.34	0.38	0.42	0.67	0.21	0.59	0.98	0.23	0.78	0.54	0.81	0.64
Average	0.942	0.533	0.632	0.464	0.425	0.374	0.285	0.47	0.617	0.291	0.463	0.638	0.595	0.642

Table 6: Temperature variation at the root

temperature variation	1	2	3	4	5	6	7	8	9	10
	20.4	24.6	24.2	22.6	25.1	21	24.7	23.5	26.3	25.1
	20.3	18.6	17.8	17.5	23.6	20.7	23.5	19.8	22.3	20.1
	23.1	18.6	17.8	17.5	23.6	20.7	23.5	19.8	22.3	20.1
	23	20.4	18.7	18.1	20.7	19.8	21.6	19.6	21.6	20.9
	22.50	20.4	18.7	18.1	20.7	19.8	21.6	19.6	21.6	20.9
	18.40	19.8	18.3	18	18.2	19.2	18.4	19.2	21.2	20.7
	18.4	19.8	18.3	18	18.2	19.2	18.4	19.2	21.2	20.7
	20.4	20	20	18.7	17.8	19.2	18.6	19.2	21.4	19.4
	21.2	20	20	18.9	17.8	19.2	18.6	19.2	21.4	19.4
	21.4	21.1	20.2	19.5	17.2	18.9	19.1	19.3	21.8	19.2
	21.4	21.1	20.2	19.5	17.2	18.9	19.1	19.3	21.8	19.2
	21.4	21.7	20.1	19.5	18.1	19	19	19.1	22.1	19.8
	21.4	21.7	20.1	19.5	18.1	19	19	19.1	22.1	19.8
	22	18	19.8	18.3	19.6	19	19	18.8	22.3	19.7
	22	18	19.8	18.3	19.6	19	19	18.8	22.3	19.7
	21.7	19.1	20.1	18.9	20.7	19.2	19.4	18.8	22.1	19.7
	21.7	19.1	20.1	18.9	20.7	19.2	19.4	18.8	22.1	19.7
	21.9	19.6	20	18.4	19.2	18.9	20.1	18.8	21.5	19.9
	21.9	19.6	20	18.4	19.2	18.9	20.1	18.8	21.5	19.9
	22.2	20.1	20.3	18.9	20.3	19.3	20.2	18.9	21.2	20
	22.2	20.1	20.3	18.9	20.3	19.3	20.2	18.9	21.2	20
	22.5	19.8	20.5	19.3	18.3	19.6	20.1	19.4	20.6	20.1
	22.5	19.8	20.5	19.3	18.2	19.6	20.1	19.4	20.6	20.1
	22.3	20.6	20.6	19.5	18.2	19.9	20.8	19.6	19.8	19.9

	22.3	20.6	20.6	19.5	18.2	19.9	20.8	19.6	19.8	19.9
	21.9	20.1	20.3	19.2	18.2	19.9	22.1	19.5	20.7	19.8
	21.9	20.1	20.3	19.2	18.6	19.9	22.1	19.5	20.7	19.8
	21.7	20.2	20	18.9	18.8	20.2	21.9	19.5	20.9	20.2
Variation	**4.7**	**6.6**	**6.4**	**5.1**	**7.9**	**2.2**	**6.3**	**4.7**	**6.5**	**5.9**

Table 7: Soil Moisture Change

	rep1	rep2	rep3	rep4	rep5	rep6	rep7	rep8	rep9	rep10
	7%	9%	7%	9%	11%	17%	11%	5%	15%	6%
	7%	8%	6%	9%	11%	17%	11%	5%	15%	6%
	7%	8%	6%	9%	11%	17%	11%	5%	15%	6%
	6%	8%	6%	8%	11%	17%	11%	5%	15%	6%
	7%	8%	6%	9%	11%	17%	11%	5%	15%	6%
	7%	8%	6%	9%	11%	17%	11%	5%	15%	6%
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	6%	6%	5%	7%	9%	15%	10%	4%	13%	5%
	6%	6%	5%	7%	9%	13%	10%	4%	13%	5%
	6%	6%	5%	7%	9%	13%	10%	4%	13%	4%
	6%	6%	5%	7%	9%	12%	10%	4%	13%	4%
total change	1%	2%	2%	2%	2%	5%	1%	1%	2%	2%

Values of respiration did not drop so markedly in this experiment at low O_2 as observed in Experiment 1. Indeed, respiration rates per unit root dry weight were in the experiment on average only 25% of those in Experiment 1.

To determine whether temperature flux in the rhizosphere impacted on the rate of respiration, temperature was measured (Table 6) at each successive change in oxygen ratios, soil moisture was also monitored (Table 7) to determine whether soil moisture had an effect on soil respiration. The change in temperature was of some concern as Table 6 shows, the initial temperature of replicates 2, 3, 4 5 8, 9 and 10 dropping more than 1°C is of some concern given the sensitivity of cotton to rapid temperature fluxes, however , beyond the initial drop of root temperature, each successive change in oxygen concentration was accompanied by a change in temperature which was generally less than 1 degree. Of note are the replicates 2, 3, 5, 7, 9 and 10 where the variation in temperature exceeds that of 5°C over the course of the treatment with a maximum of 7.9 in replicate 5.

Experiment 3 – The effect of flow rate on respiration and water loss

Tables 8-12 Respiration different flow rates at at 20% O_2 saturation

plant 1 Field capacity = 727.5g

flow mL/min	temp °C	weight g	% moisture	µmolCO_2 of exhaust gas	µmolCO_2/pot/hr
250	24.6	725.55	32	89.78	54.96
250	23.6	723	32	85.54	52.37
250	23.5	723	31	85.39	52.28
500	23.5	722.5	26	61.73	75.59
500	23.5	720	27	42.4	51.67
500	23.5	719	24	43.65	53.45
750	23.3	715.5	30	31.94	58.67
750	20.9	712.5	29	19.34	35.52
750	19.3	711	27	16.32	29.98

plant 2 field capacity = 574.5g

flow mL/min	temp °C	weight g	% moisture	µmolCO_2 of exhaust gas	µmolCO_2/pot/hr
500	20.1	563	32	50.17	61.43
500	19.8	558	28	44.3	54.244
500	19.8	557.5	24	42.98	52.62
250	25	530	21	58.53	35.83
250	24.8	528	20	56.7	34.71
250	24.1	525	20	54.12	33.13
750	22.7	523	21	25.75	47.3
750	23.7	523	20	12.6	23.14
750	25.3	521.5	20	12.21	22.43

plant 3 Field capacity = 553.5g

flow mL/min	temp °C	weight g	% moisture	µmolCO_2 of exhaust gas	µmolCO_2/pot/hr

500	20.8	547	34	53.54	65.56
500	20.3	536.5	30	26.5	32.45
500	21.3	533.5	28	23.5	28.78
250	20.3	534.5	27	76.61	46.9
250	20.6	534.5	22	76.63	46.91
250	20.8	533.5	24	72.67	44.5
750	20	533	24	4.6	8.45
750	19	531	22	3.89	7.14
750	18.6	530	21	3.23	5.93

plant 4 Field capacity = 640.0g

flow mL/min	temp °C	weight g	% moisture	µmolCO_2 of exhaust gas	µmolCO_2/pot/hr
750	21.8	636	40	9.9	18.18
750	21.6	634.5	40	11.58	21.27
750	22.2	632.5	39	11.1	20.39
500	21.8	631.5	34	41.8	51.18
500	22	630	35	30.5	37.35
500	21.5	628.5	33	30	36.73
250	23.3	607	30	55.2	33.8
250	22.5	597	32	56.7	34.71
250	23	592	27	58	35.51

Control(perlite only)Field capacity = 530

flow mL/min	temp °C	weight g	% moisture	µmolCO_2 of exhaust gas	µmolCO_2/pot/hr
750	22.3	530	36	0	0
750	21.6	530	36	0	0
750	20.4	530	36	0	0
500	20.2	530	36	0	0
500	20.1	529.5	35	0	0
500	20.1	529.5	35	0	0
250	20	529.5	35	0	0
250	19.9	529	35	0	0
250	20	529.5	35	0	0

This experiment was to determine whether respiration would be affected by flow rate [it was suspected that respiration would be affected by flow rate] and whether there was a relationship between flow and loss or uptake of moisture.

The control (without any plant) showed a minimal reduction of moisture that was not altered by varied flow, and it appears that plant moisture uptake, not necessarily flow, affected the rate of moisture uptake in each replicate despite the randomised flow rates. Interestingly it appears that increased flow hindered rate of respiration, as each table exhibits a reduction in measurable exhaust at the 750ml flow for each replicate in this treatment.

Temperature also appeared to respond across replicates to flow rate; the control showed the least change of temperature over the randomised rates of flow.

Discussion.

Experiment 1 - The effect of different oxygen concentrations on soil respiration

The data reveal a steady decline over the ramping down of concentration levels, with a peak at 17.5 % and again at 12.5% this is possibly a response from the plants in the form of increased anaerobic respiration as aerobic respirative function decreases, however from 10% the decline in measurable CO_2 becomes somewhat more rapid with the largest drop occurring between 1% and 0%

Data (not presented) on individual plant responses to oxygen concentrations show that the replicate 1 and 2 experienced increased respiration at enriched oxygen concentrations, however the remaining replicates exhibited minimal variation in exhaust levels between enriched and ambient and oxygen concentrations. This may be attributed to the plants oxygen uptake capacity, it is speculated that if the replicates were to stay in an oxygen enriched environment there would be an increase in lateral root mass to accommodate the excess available oxygen. As such the data is not included in this report, however the root system of each plant had thick tap roots and fibrous lateral root system, but the dry weight of each replicate did not exceed 2 gm this is unusually light for plants at 10-12 weeks maturity, the stunted growth of the root system indicates that the confinements of the pots inhibited root growth. This is possibly reflected in the low respiration rate of each replicate. On physical examination it was noted that vegetative branching was occurring, fruiting structures of at least three square per replicate was present and all had one visible bloom known as the early to mid season bloom, and in a healthy crop would occur normally at approximately 50-58 days after planting (Deterling and El-Zik 1982), however at 10 -12 weeks maturity this stage of development is a good indication that the replicates growth wasstunted due to the confinement of the pots. There is aslothe possibility that not enough nutrient was provided to assist in the early stages of development; this may be the cause of stunted growth, and not the confines of the pot size

Of initial concern was the effect of temperature on respiration rate, it was determined that external temperature was not of as much concern as temperature change in the rhizosphere, the length of time was useful in identifying any flux in root respiration that could be associated with the plants internal diurnal rhythms of which there was no recognizable pattern occurring. It is possible that the length of time required for each full run of each replicate may have hindered the ability of the plant to respond and respire within its natural limits, which unfortunately means that the data from each replicate may not be a true representation of any measurable effect of oxygation to the plants root system.

It would be worthwhile to apply a constant saturation level, bordering on Hypoxic such as 10% for a specified length of time to determine how long the plant can maintain a constant rate of respiration

Experiment 2 - The effect of different oxygen concentrations on soil respiration and photosynthesis

The results from this experiment appear to show the ability of the plants to substitute oxygen supply, as evidenced by a steady photosynthetic rate across root oxygen concentrations. Unfortunately the broad leaf chamber which is suitable for cotton leaf analysis was broken so a narrow leaf chamber was substituted. Due to the sensitivity of the stomata, it is believed that photosynthetic recordings were moderately affected by the use of the narrow leaf chamber as stomatal closure was precipitated by physical trauma; dot were not recorded digitally so we do not have data on stomatal conductance nor transpiration.

The data do however suggest that respiration of the root system was affected by the randomised levels of oxygen saturation and the plants maintained a decline in exhaust gas in the face of declining available oxygen at the root environment.

There is some speculation that photosynthesis became the main source of available oxygen for the continued function of the plants within the low O_2 treatment.

Tables 7 represents the change in soil moisture per replicate, replicate 6 exhibited a change of 5%, replicate 6 also had the highest soil moisture per the treatment, it was undecided as to whether flow rate impacted on soil moisture reduction or it was the result of the plant moisture uptake ability.

The plants were also subjected to a fungal pest within the growth chamber, a fungicide was applied and the cotton plants were used when all visible signs of the microbe disappeared. Again this impacted on the slow growth of the replicates as well as did the pot size, however the dfference in respiration rates between treatment 1 and 2 had too many variables to be able to compare the results and obtain an accurate overview of the variation in respiration.

This experiment emphasized the need to run a treatment for each oxygen saturation for a longer period of time, specifically to determine plant ability to respire normally in oxygen poor conditions and to provide a firm comparison to that of ambient and enriched environments.

Experiment 3- The effect of flow rate on respiration and water loss

This experiment indicates that despite the rate of flow, oxygen is required for the active uptake of moisture, The control (no plant) only showed a 0.5 g decline in weight and a 1% decrease in overall moisture content which is due to flow. The other five replicates, with plants, also exhibited a reduction in moisture uptake at 750 ml/min, compared to the change in moisture content at the reduced rates of flow of 500 ml/min and 250 ml/min. The exception to this is plant the change in flow rates may be partially responsible for the rate of uptake, and may be a response to the change in environment at the root level. What was also noticed was the alteration in respiration rates of each treatment, particularly when flow was increased to 750 ml/min. This is possibly a result of increased flow causing the plant to

reduce oxygen uptake as a response to trauma n the rhizosphere, and rapid flow of oxygen could be construed as this.

Conclusions

The task of experiment one was to determine if there was a measurable positive effect of aeration to the functioning of the rhizosphere, as such from the raw data it can be seen that there was increased exhaust of CO_2 at an oxygen enriched level, with a slight drop throughout the ramping down of the treatment. Further analysis shows that over each replicate the rate of exhaust was not dissimilar over the range of 30% to 10% oxygen concentration, with the variability being due to individual plant characteristics as opposed to a systematic response. Through the representation of the data as a percent of ambient concentrations the data do reflect a definite positive effect at oxygen enriched concentrations and a reduction in ability to respire aerobically, respiration via glycolosis may occur over the range of the treatments, but we were not able to show where the switch from aerobic to anaerobic respiration took place. We hope to study this in the future.

Experiment 2 showed an increase in the ability of the replicates to respire at oxygen enriched levels. The Treatment applied emphasized the vulnerability of the plants to changes in the environment surrounding the rhizosphere, increase in photosynthesis (minimal) occurred as soil O_2 concentrations decreased however the rate of photosynthesis did not obviously impact on the ability of the root system to produce exhaust CO_2. With the slight increases in respiration at lower saturation levels being attributed to the plants anaerobic functions, as the soil moisture was intended to remain relatively uniform.

It was concluded that 10% to 20 % oxygen concentrations allowed a measurable if slight positive effect of aeration, with oxygen enriched clearly increasing the rate of root respiration.

Experiment 3, determined that rate of flow impacted on the ability of the plants to take up moisture. As such it can be seen that the application of oxygation at different rates of flow affects the ability of a plant to respire and its moisture uptake function.

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