http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5 (9): 974-979, 2002

Periodical Ecophysiological Changes in *Glycine max* in Response to O_3 and CO_2 Singly and in Combination

[1]Akram A. Ali, [2]Ibrahim A. Hassan, [1]Yehia A. El-Zawahry and [3]Scott J. Angle
[1]Department of Botany, Faculty of Science, University of Zagazig, Zagazig, Sharkia, Egypt
[2]Department of Botany, Faculty of Science, University of Alexandria, Alexanderia, Egypt
[3]University of Maryland, Department of NRSLA, College Park, MD 20742, USA

Abstract: This research was conducted to determine the effect of increased tropospheric CO_2 and O_3 on responses of soil basal respiration rates (BR) and microbial population (MP) in soybeans (*Glycine max*) rhizosphere soil. Significant changes in BR, qCO_2 and MP were observed for all treatments. The greatest increases in BR and MP and the least in qCO_2, were found at early grainfill stage treatments. The deterrence in BR and MP values were stimulated by the increase in CO_2 singly, or in combination with high O_3 exposures compared with CF control. Reductions in BR and MP observed for NF + (30± 5 nL O_3 L^{-1}) treatments during flowering and early grainfill stages, thus suggests that O_3 injury can reduce the BR by decreasing the activity of microbes in soil. This study suggests that carbon dioxide could decrease the harmful effect of ozone on soybean plants.

Key words: Carbon dioxide, ozone, ecophysiology, respiration, microbes, soybeans

Introduction

Plants like soybeans are in an atmosphere that supplies CO_2 for carbon fixation and the production of carbon-containing compounds to maintain the structure and function of cells; and O_2 to oxidize these carbon compounds, producing cellular energy. In recent human history, human activities have produced increases in both CO_2 and O_3, (a highly reactive form of O_2). Singly, O_3 and CO_2, generally have either "detrimental" or "beneficial" effects on plants. However, the combined impact of both gases on vegetation is uncertain despite their likely co-occurrence in the future. Since the end of the 18th century, the industrial revolution, increase in the human population and need for energy and raw materials, has resulted in increasing levels of atmospheric pollutants. Previously, the positive feedback between a growing human population and release of pollutants has had significant impact on a regional level due to highly reactive pollutants such as O_3, but now has reached a global level of importance due to large increases in other greenhouse gases, especially CO_2.

On a global basis, over the last few decades, O_3 concentrations have increased between 1 to 2% per year (Fishman, 1991) and are expected to continue to increase. Based on patterns of expected emission of precursors, Hough and Derwent (1990) suggested that concentrations would increase 20-50% between 1990 and 2020 in lower latitudes (36 to 42° N) while at high latitudes (48-56° N) concentrations would increase 10-28%. Regional levels of O_3 are likely to continue increasing in major cities (e.g. Mexico City and likely Beijing, Bombay and Cairo) that continue to show rapid population growth and use of fossil fuels in automobiles and industry (Yunus *et al.*, 1996). Furthermore, the geographic extent of the O_3 effect on terrestrial ecosystems is likely to increase as more countries, particularly developing ones, become more industrialized or have more managed agriculture (Chameides *et al.*, 1994).

The atmospheric CO_2 concentration has been increasing dramatically for over a century. Other greenhouse gases (e.g. CH_4, N_2O, O_3) are increasing in concentration at the rates specific to each gas. Associated with the increases in CO_2 and other greenhouse gases, the IPCC (Intergovernmental Pannel on Climate Change) estimate that global air temperatures will increase (Houghton *et al.*, 1996). The potential increase in global temperature and likely associated changes in precipitation amounts, type and pattern; humidity and possibly other climatic factors that could have significant impacts on the world's ecosystems and, consequently, human health and welfare.

Individual species do not exist in isolation, but as members of an ecosystem, i.e., all organisms and their nonliving environment in a given area. To date most studies on air pollutant effects have focused on responses of individual components, almost entirely on a single species level and not in terms of ecosystem function. While this level of analysis has allowed for a "snapshot" of the status of an ecosystem at one point in time, it cannot provide for estimation of long-term responses. Thus we need to understand implications of pollutants for ecosystem health more fully.

Terrestrial ecosystems provide many goods and services for humans. Agro-ecosystems provide food and fibre whereas natural ecosystems, especially forests, provide timber, fuel and non-timber products; protection of soils; sources of water; conservation of biodiversity and recreation. There is much debate about how the various roles of Agro-ecosystems and natural terrestrial ecosystems in global phenomena will be affected by future changes in climate and a changed atmospheric composition (Solomon *et al.*, 1996). Global vegetation models that have been used to simulate the transient response of the terrestrial biosphere to climatic changes and CO_2 have generally shown an eventual increase in C storage on the land after a substantial loss during the transient phase (Schimel *et al.*, 1994). None of these studies have included effects of other atmospheric pollutants such as O_3, which may be the counter effects of a changed climate.

As O_3 and CO_2 increase globally, changes in the flux of C to and from soils (respiration) and cycling of C and N will occur. This has important implications for the functioning of ecosystems because soil C and nutrient cycles are closely associated. Since terrestrial soils contain about 71% of total terrestrial C stocks, any change in the net flux of carbon into or out of soils may have major repercussions on atmospheric CO_2 concentrations and the potential for global change. As the most biologically active portion of soil, the rhizosphere (the soil immediately adjacent to plant roots) is likely to be affected most by environmental stresses. Therefore, it is essential to understand how specific stressors will affect the rhizosphere, which acts as an interface between primary carbon processes and primary nutrient and water processes. To study the rhizosphere, one needs to consider its biology and ecology as an integrated system. The interaction of biology, ecology, chemistry and physics within the mineral soil matrix creates the habitats found in soil (Rygiewicz and Ingham, 1998). The biotic community below-ground includes bacteria, fungi, etc., which serve various functions in maintaining biological, physical and chemical characteristics of the soil and all are dependent on energy inputs from plant residues for their maintenance (Kuikman, *et al.*, 1990).

The main objective of this study is to answer the following question through focusing on the microbial respiration in rhizosphere soil: will rising global atmospheric CO_2 counteract the regional or local detrimental effects of atmospheric O_3 on soybean plants?

Materials and Methods

Research facility: The research, using open–top chambers, was conducted at the United States Department of Agriculture (USDA) Beltsville Agricultural Research Center (BARC), Beltsville, MD, USA. The site was located on a Codorus silt loam soil containing about 40% sand, 21% clay and 39% silt, with a pH of 6.2. The treatments were begun as soon as plants were visible. Using Diazinon 50 W at 0.7 kg/ha in solution, the soybean plants were sprayed one or more times during its growth to control weeds.

Soybeans (cv. Essex and Forrest) were planted in June 2000 in 4 x 5 m^2 plots in rows spaced 0.5 m apart with seeds spaced 10 cm apart for over 200, 000 ha. The plots were covered in late June with 3 m diameter open–top chambers (Heagle *et al.,* 1973) as soon as uniform plant stands were assured and sprinkler irrigation units had been installed in the center of each chamber. Atmospheric treatments consisted of charcoal-filtered (CF) air as a control at 350 ± 5 μL CO_2 L^{-1}, CF (350 ± 5 μL CO_2 L^{-1})+ 155 ± 5 μL CO_2 L^{-1}, non-filtered (NF) air (25 ± 5 nL O_3 L^{-1})+ 35 ± 5 nL O_3 L^{-1} and NF + 35 ± 5 nL O_3 L^{-1} + 155 ± 5 μL CO_2 L^{-1} in a complete factorial design for a total of four atmospheric environments.

The treatments were arranged in a randomized complete block design and replicated twice for a total of 16 chambers (4x2x2). Half of these chambers were equipped with moveable rainfall shelters to exclude rainfall (sheltered soil moisture). Another half (irrigated plots) received natural rainfall and was irrigated to maintain moisture (wet −0.05 MPa x dry −1.5 Mpa). The CO_2 treatments were applied 12 h a day (05:00-17:00 h EST), 7days a week and the O_3 treatments were imposed 7 h a day (09:00-16:00 h EST), 5 days a week for 12 weeks. The CO_2 was supplied from cylinder CO_2 and the O_3 was synthesized from cylinder O_2 using a Griffin O_3 Generator (Griffin Technics Crop. Lodi, NJ). Both gases were injected into the chamber blowers immediately upstream from the fans. The flow of CO_2 to each chamber was monitored through glass flow meters and the individual rates were checked daily. The CO_2 was monitored in the stream prior to contacting the plants using a Beckman Model 315 B Infrared Analyzer. The CO_2 monitor calibrated using certified CO_2 standards in N_2 gas purchased from Air Products and Chemical Co., Washington, DC. The air stream CO_2 concentrations were measured on a biweekly basis and adjustments to flow rates were made if necessary. Chamber O_3 concentrations were monitored hourly during the treatment periods using a Thermo Electron Model 49, UV photometric O_3 Analyzer (Thermo Electron Corp., Hopkinton, MA). The O_3 meters were calibrated using a Dasibi Model 1003PC (Dasibi Environmental Corp., Glendale, CA). The chamber air samples were collected using Telflon tubing attached through a switching device to a central vacuum system. Sample lines in the chambers were adjusted weekly to about 0.2 m above the canopy throughout the growing season.

Soil collection: Soil samples were collected in late 1999 to a depth of 15 cm using a 1.9 cm i.d. soil probe. Six cores were randomly collected from each site of the plot or from each soil cultivars. The cores were pooled and mixed in the field immediately after a site was sampled and placed in tightly sealed plastic bags. Soils were transported from the field site in plastic bags kept on ice in a dark cooler. The soil cores were gently sieved to pass through a 4 mm mesh to remove stones, roots and large organic residues.

Basal respiration rates (BR): The BR (M CO_2-C m^{-3} d^{-1}) was measured as the average CO_2 evolution of soil after an incubation period of 10 days. The BR values were determined during four growth stages (pre-cultivation, flowering, early grainfill and late grainfill) on three times through the light hours of the day [morning (10am), noon (12 pm) and afternoon (2 pm)] for each stage. The BR rates were calculated as follows:

$$BR = (CO_2\text{-}C_{UNFUM} - CO_2\text{-}C_{AIR})/10 \text{ days}$$

Where CO_2-C_{UNFUM} is the evolution of CO_2 during 10 days incubation of non-amended soil and CO_2-C_{AIR} is the CO_2 in a blank mason jar.

The BR method of Van de Werf and Verstrate (1987) was modified as follows: About 20 g oven-dried equivalent (ODE) of 2 mm sieved non-amended homogenized soil adjusted to 60% water-filled porosity (WFP) was placed in each of two 50 mL glass beakers. The soil in one beaker was amended with a glass vial containing 10 mL of distilled water to maintain humidity and a plastic vial containing 10 mL of 1 M NaOH to trap evolved CO_2 in a 1 L mason jar. Another group of jars were used as a control without using soil samples. The jars was sealed and incubated in the dark for 10 days at 25± 1°C. Following the incubation period, the Na_2CO_3 formed in the vial containing 1 M NaOH was precipitated as $BaCO_3$ by addition of 1 M $BaCl_2$. The remaining NaOH in each vial was titrated by phenolphthalein to endpoint with a standardized 1 M HCl solution (Islam *et al.,* 2000).

The specific maintenance respiration rates (qCO_2): The qCO_2 is the CO_2 release per unit of microbial biomass in soil. They were calculated as mean basal respiration rates over total microbial biomass (BR/C_{TMB}) (M CO_2-C m^{-3} d^{-1} C_{TMB}^{-1}) using method of Anderson and Gray (1991).

Microbial population (MP): Microbes number for variable soil rhizosphere in soybean was enumerated by spiral plating assay (Spiral System Inc., Model D, Cincinnati, OH). Microbial populations (MP) of rhizosphere soil collected once for all growth stages through mid of the day. Bacterial and fungal enumeration by the spiral plating method involve the counting of colonies in the outer region of the plate, where the colonies are well separated and dividing these counts by the volume of sample deposited within the counting region. Agar plate medium was used to prepare the plates. The same quantity of medium was poured into all plates so that the same height of agar was presented to the spiral plate stylus tip to maintain contact angle.

Soil samples were collected from the root area after shaking firmly. Ten grams of soil were added to 90 mL of buffer and homogenized in blender at 22000 rpm for one min and allowed to settle for one min. Sterile buffered solution tubes, containing 9 mL, were prepared to make a series of dilutions. One mL of soil dilutions amended with bacterial and fungal organisms was used to inoculate 9 mL buffered solution, creating a 1:10 dilution.

Stock A: 50.0 g of $MgSO_4.7H_2O$/liter. For buffered H_2O, 5.0 mL stock was used per liter.

Stock B: 34.0 g of KH_2PO_4/litre. For buffered H_2O, 1.25 mL stock was used per liter.

The platter was connected to appropriate electric and vacuum sources and set in the automatic model. A marked 5 mL disposable cup was filled with ethanol and another cup containing sterile distilled water was placed in the post mounted cup holder. The stylus was sanitized by immersing the stylus tip three times into the alcohol with the pinch valve open (valve switch on). The stylus was then rinsed by immersing the tip into the water. The water was pulled through the system once the valve was closed. The serial dilution of each treatment was poured into a disposable cup and placed in the cup holder. The stylus was lowered into the sample and the valve was opened until there was a continuous column of liquid sample without bubbles in the sight glass (about 2-3 sec.). The valve was turned off, the stylus was raised and the sample holder was moved out of the way. The dish was placed on the turntable, the stylus was lowered onto the medium and pressing the start switch to start the platter. When the plating cycle was completed, the stylus was raised automatically and the plate was removed and its lid was replaced.

The plates were inverted and incubated at 28°C in the dark for 4 days. After incubation, sector was chosen and counting of colonies was begun from the outer edge of the first segment

towards the center until 20 colonies have been counted. A similar area on the opposite side of the plate was counted and the sample volume deposited in those two areas divided the colonies counted from both sides. The volumes of samples associated with each portion were given in sectors (8, 9, 10, 11, 12, 13 and complete plate) as 100 mm plate size μL deposited i.e. 1.145, 2.798, 5.186, 8.634, 13.618, 24.151 and 48.302 respectively. The volume constants found for segments and then multiply by 1000 for cfu/mL divided the number of counted colonies from both sides of the plate.

Statistical procedure: Data were analyzed using analysis of variance (ANOVA) procedures for factorial design 4x2x2. The least Significant Difference (LSD) evaluated the mean differences between the four air quality treatments. Statistical Analysis System (SAS) was performed for all data statistical analysis (SAS Institute, 1990).

Results

Climatic parameters changes including temperature, wind velocity, precipitation and radiation values during the day for collecting samples obtained from a location adjacent to the experimental site are summarized in Table 1.

The effects of atmospheric CO_2, O_3 and moisture regimes were determined on BR rates for soil supporting soybean at four growth stages of plant development [pre-cultivation, flowering, early podfill (early grainfill) and grainfill (podfill) (Table 2). Moisture regimes produced significant effects at the pre-cultivation stage and at the early pod stage of development with lower values under dry treatments. Air quality treatments caused significant differences during the day at all four growth stages with elevated CO_2 producing higher CO_2 flux compared to carbon-filtered controls and elevated O_3 concentrations giving lower values compared to high CO_2 level. Moreover, the afternoon data showed much higher levels than the early morning ones.

The effects of cultivars of soybean on respiration rates were not significant in the major cases. The Forest cultivar exhibited slightly higher rates of respiration than samples collected from Essex. The effect of soil moisture on CO_2 flux rates in soil supporting soybean showed significant increases in wet conditions during all times of the early grainfill stage, but especially in the morning of pre cultivation stage (Table 2). Generally, elevated CO_2 treatments increased the rates of flux while O_3 treatments exhibited reduction in all levels. The BR data showing the highest values for all growth stages were recorded afternoon. Soils under high O_3 treatments typically showed higher levels of respiration rates than carbon-filtered controls, with only two instances where the differences were not significant, morning and afternoon of pre-cultivation. The combination of both CO_2 and O_3 at high concentrations slightly increased the levels of respiration at flowering and early grainfill stages, especially under high moisture concentrations.

The effects of air quality and soil moisture levels on BR rate values were largely significant under soil moisture treatments. Respiration levels at all growth stages were lower under dry conditions during all times of the day. With regard to air quality treatments, exposure to high O_3 significantly reduced the levels of respiration in comparison with CO_2 enrichment, while high CO_2 increased the levels compared with carbon-filtered air (Table 2). The highest

Table 1: Mean values of climatic parameters changes during the day for collecting samples

Climatic parameters	Pre-cultivation	Flowering	Early grain-fill	Late grain-fill
Soil temperature (°C)	26.6	22.5	20.8	12.3
Air temperature (°C)	30.8	29.2	28.2	18.6
Wind velocity (ms^{-1})	1.1	1.4	1.3	1.6
Precipitation (mm)	17.0	44.0	40.0	32.0
Solar radiation (MJm^{-2})	666.0	610.0	515.0	511.0

Table 2: Summary of the effect of enriched CO_2 and O_3 stress on basal respiration rates (M CO_2-C m^{-3} d^{-1}) of soil supporting soybean plants from OTC during growth stages

	Growth Stages											
	Pre-Cultivation			Flowering			Early grain-fill			Late grain-fill		
Chamber treatments	M	N	A	M	N	A	M	N	A	M	N	A
CO_2 Mean												
Ambient	0.7	0.9	1.1	4.3	5.2	5.6	7.2	8.8	8.9	6.9	7.1	8.2
Am.+ 160μL L^{-1}	1.5	2.2	1.9	7.9	8.3	8.6	11.5	12.9	13.8	9.8	10.5	11.0
Statistical sign	*	***	NS	***	**	***	***	**	***	**	**	**
O_3 Mean												
CF	0.8	0.9	1.0	4.4	5.2	5.6	7.1	8.8	8.8	6.8	7.1	8.2
NF+ 40 nL L^{-1}	1.5	1.5	1.5	4.9	5.3	5.5	7.8	10.5	11.6	8.1	8.4	9.1
Statistical sign	*	NS	NS	NS	NS	NS	NS	*	**	*	NS	*
CO_2 + O_3 Mean												
CF	0.8	0.9	1.1	4.4	5.3	5.6	7.3	8.8	8.7	6.9	7.2	8.2
NF+ O_3 + CO_2	0.9	1.8	1.3	6.6	7.1	7.6	10.0	12.2	12.9	8.8	9.8	10.1
Statistical sign	NS	*	NS	*	**	*	**	***	***	*	*	*
Cultivar mean												
Essex	1.1	1.5	1.4	5.2	6.3	6.6	9.1	10.8	11.1	8.1	8.8	8.9
Forest	1.2	1.6	1.5	6.6	6.6	7.2	8.9	11.9	12.6	8.8	8.9	9.9
Statistical sign	NS	NS	NS	*	NS	*	NS	*	**	NS	NS	*
Moisture mean												
Irrigated	1.8	1.9	1.8	6.2	6.6	6.9	10.1	12.0	13.2	8.8	8.9	9.5
Non-irrigated	0.6	1.3	1.1	5.6	6.3	6.9	7.9	10.7	10.5	8.1	8.9	9.3
Statistical sign	*	NS	NS	NS	NS	NS	***	**	***	NS	NS	NS

Statistical significant: (*)= $P \leq 0.1$, (**)= $P \leq 0.05$, and (***) = $P \leq 0.01$ respectively and NS= Non-significant. M= Morning, N= Noon and A= Afternoon. CF= Carbon-filtered, NF= Non-filtered air, OTC = Open top chambers.

Table 3: Summary of the effect of enriched CO_2 and O_3 stress on specific maintenance respiration rates (qCO_2) (M CO_2-C m^{-3} d^{-1} C_{TMB}^{-1}) of soil supporting soybean plants from OTC during growth stages

	Growth stages											
	Pre-Cultivation			Flowering			Early grain-fill			Late grain-fill		
Chamber treatments	M	N	A	M	N	A	M	N	A	M	N	A
CO_2 Mean												
Ambient	0.26	0.22	0.15	0.22	0.18	0.15	0.16	0.12	0.09	0.16	0.14	0.13
Am.+ 160μL L^{-1}	0.23	0.19	0.12	0.19	0.15	0.12	0.13	0.09	0.07	0.12	0.11	0.10
Statistical sign	*	***	NS	***	**	***	***	**	***	**	**	**
O_3 Mean												
CF	0.27	0.21	0.16	0.24	0.19	0.15	0.17	0.11	0.09	0.17	0.14	0.13
NF+ 40 nL L^{-1}	0.28	0.26	0.18	0.25	0.23	0.19	0.22	0.14	0.11	0.18	0.17	0.16
Statistical sign	*	NS	NS	NS	NS	NS	NS	*	**	*	NS	*
CO_2 + O_3 Mean												
CF	0.26	0.22	0.16	0.22	0.20	0.15	0.16	0.12	0.09	0.16	0.14	0.13
NF+ O_3 + CO_2	0.23	0.23	0.16	0.23	0.16	0.14	0.16	0.11	0.09	0.14	0.14	0.14
Statistical sign	NS	*	NS	*	**	*	**	***	***	*	*	*
Cultivar mean												
Essex	0.28	0.25	0.14	0.25	0.21	0.16	0.11	0.10	0.09	0.16	0.15	0.13
Forrest	0.22	0.19	0.15	0.21	0.16	0.12	0.19	0.12	0.08	0.14	0.14	0.13
Statistical sign	NS	NS	NS	*	NS	*	NS	*	**	NS	NS	*
Moisture mean												
Irrigated	0.25	0.19	0.15	0.22	0.15	0.14	0.12	0.11	0.09	0.15	0.15	0.13
Non-irrigated	0.26	0.26	0.15	0.24	0.22	0.14	0.19	0.11	0.09	0.15	0.14	0.13
Statistical sign	*	NS	NS	NS	NS	NS	***	**	***	NS	NS	NS

Statistical significant: (*)= $P \leq 0.1$, (**)= $P \leq 0.05$, and (***)= $P \leq 0.01$ respectively, and NS= Non-significant. M= Morning, N= Noon, and A= Afternoon. CF= Carbon-filtered, NF= Non-filtered air, OTC= Open top chambers.

Table 4: Summary of the mean values for microbial count of soil supporting soybean plants during afternoon from OTC under atmospheric CO_2 or O_3 enrichments and soil moisture regimes

	Microbial count								
	Bacteria # cfu/g ode (x 10^6)					Fungi # cfu/g ode (x 10^3)			
Chamber treatments	P	F	E	L	Growth stages	P	F	E	L
CO_2 Mean									
Ambient	0.22	0.35	0.84	0.54		1.21	2.42	5.22	2.44
Am.+ 160μL L^{-1}	0.33	0.48	0.88	0.58		1.35	2.52	6.66	3.69
Statistical sign	**	***	NS	NS		**	**	***	***
O_3 Mean									
CF	0.24	0.35	0.88	0.54		1.20	2.42	5.22	2.44
NF+ 40 nL L^{-1}	0.18	0.34	0.64	0.48		1.02	2.34	4.91	2.15
Statistical sign	NS	NS	***	NS		*	NS	NS	NS
CO_2 + O_3 Mean									
CF	0.22	0.36	0.84	0.54		1.21	2.42	5.22	2.44
NF+ O_3 + CO_2	0.26	0.40	0.72	0.54		1.25	2.40	5.52	2.36
Statistical sign	NS	NS	*	NS		NS	NS	*	NS
Cultivar mean									
Essex	0.25	0.45	0.67	0.52		1.01	2.31	3.54	2.36
Forrest	0.25	0.36	0.85	0.55		1.23	2.51	6.95	3.32
Statistical sign	NS	NS	**	NS		NS	NS	***	*
Moisture mean									
Irrigated	0.31	0.53	0.86	0.55		1.25	2.34	6.75	3.13
Non-irrigated	0.20	0.31	0.68	0.54		1.15	2.50	4.61	2.22
Statistical sign	*	**	**	NS		NS	NS	*	NS

Statistical significant: (*) = $P \leq 0.1$, (**) = $P \leq 0.05$, and (***) = $P \leq 0.01$ respectively, and NS = Non-significant, CF = Carbon-filtered, NF = Non-filtered air, OTC = Open top chambers, cfu = Colony forming unit, and ode = Oven dry equivalent, NS = Non-significant. P = Pre-cultivation, F = Flowering, E = Early grain-fill, and L = Late grain-fill.

respiration rates for all growth stages were observed during early pod development. With respect to the combination of elevated CO_2 and O_3; respiration levels throughout the day were comparable if not slightly larger, than those observed in the carbon-filtered control treatments.

In terms of air quality treatment effects, the results were typically significant for CO_2, showing decreases for qCO_2 of soils under soybean roots for all growth stages and at all times during the day (Table 3). The effects of O_3 treatments were generally significant compared with carbon-filtered air controls otherwise non-significant in pre-cultivation and flowering stages. The effects of combined high CO_2 and high O_3 included a decrease in qCO_2 levels for all growth stages and especially in the flowering, early podfill stages and late podfill compared with control.

In case of soybean there were significant differences for all growth stages except the pre-cultivation stage. The moisture treatments exhibited minimal significant difference between the two regimes except the early podfill. In terms of air quality treatment effects and moisture, high CO_2 concentrations caused decreases in qCO_2 in both pre-cultivation and flowering stages as compared with carbon-filtered air controls. Elevated O_3 treatments showed a significant increase of qCO_2 (Table 3). The combination of elevated CO_2 and O_3 produced significantly higher levels of qCO_2 compared with high CO_2 treatments. There were no observed interaction effects between the four air quality treatments and soil moisture regimes with similar patterns of response being found under both moisture treatments for early podfill and late podfill stages.

The MP counts in the soybean rhizosphere soils from the open-top chamber under atmospheric CO_2 or O_3 enrichments and two moisture regimes over afternoon periods are shown in Table 4. Fungal populations were not significantly affected by the moisture treatments while bacterial results were significantly different. Atmospheric CO_2 treatments typically increased the MPs in the soil while ozone enrichment tended to inhibit the growth of both types of microorganisms in the soil rhizosphere. The combination treatment of CF + CO_2 and high O_3 treatments showed trends for increased populations, with few samples appearing significantly higher than the ozone treatments.

The combination of air quality treatments with moisture regimes generally showed increased MPs under CF + CO_2 for both moisture conditions; however, NF + O_3 treatment results were consistently lower than the charcoal filtered air controls although were normally not significantly different. Although the data from both moisture regimes were somewhat varied over the periods of growth, the patterns of results appeared generally similar under the two moisture regimes. The interaction of air quality treatments vs. moisture treatments was significant in most cases for bacterial counts and fungal numbers in soils (Table 4). Under the wet treatments, fungal counts for the elevated CO_2 treatments were significantly higher than CF controls; however, results for high O_3 treatments were all comparable to CF controls, except for a few results during the late grainfill stages. Additionally, the combination of high O_3 and CO_2 treatments had fungal counts larger than CF controls under dry treatments. The high concentration of O_3 and CO_2 treatments stimulated the fungal counts compared with CF controls in the combined results, while the results for NF + O_3 + CO_2 treatments were generally insignificant. Bacterial counts were also stimulated by the elevated CO_2 treatments but high O_3 and NF + O_3 + CO_2 treatment effects were largely non-significant in pre-cultivation and late podfill stages under dry conditions.

Discussion

The present study investigates the dependence of BR, MP and qCO_2 on climatic conditions. The data showed that strong responses to BR and MP under the four air quality treatments gradually from pre-cultivation to early grain-fill stages and decreased through the late grainfill stage. The treatments of tropospheric O_3 decreased CO_2 evolution in wet moisture regimes. Significant interactions of CO_2 x O_3 enrichments with moisture were observed for qCO_2 during all growth stages. The results for MP exhibited similar patterns to that for BR, where significant increases were found in both bacteria and fungi in rhizosphere soil subjected to elevated CO_2 effects and effective decreases under high O_3 concentrations. These results agree with those obtained by Insam (1990), Edward (1991), Koizumi *et al.* (1991), Tingey *et al.* (1995), Cheng *et al.* (1996), Schortemeyer *et al.* (1997).

Generally, significant relationships were found between the effects of CO_2 and O_3 treatments and CO_2 fluxes and microbial numbers. The functional relationship between BR and MP is not fully understood. Sparling (1991) considered BR to be representative of the active part of MP. Anderson and Domsch (1985), however, viewed the BR as reflecting the activity of whole microbial activity. Typically, a high qCO_2 is found in soils with a recent input of easily degradable substrate. Such substrates would induce a micro flora that usually respired more CO_2 per unit degradable C (Islam *et al.*, 2000). The relationship between BR and qCO_2 were found to be linked to climatic conditions. Part of the climatic effect may be explained by an altered quantity of metabolizable substrates due to an influence on primary production or substrate allocation to the roots and decomposition as such in response to climatic conditions. Both factors affected the mediator of decomposition, MP; BR. Significant relationships between qCO_2 and the stimulation of microbial activity may be attributed to the translocation of photosynthetic compounds under ground. The suitable time for detecting BR and MP is the period after 12pm because higher temperatures correlate with soil surface CO_2 fluxes and accelerate the development of a soil (Jensen *et al.*, 1996).

Soil surface CO_2 evolutions can originate from any ecosystem carbon. The majority of CO_2 fluxes that reach plants could originate from the soil and be related to daily net photosynthetic rates. The respiration be produced in soil by the activity of roots and microbes and affected by soil and air temperatures, soil moisture content, wind velocity, is precipitation and solar radiation (Rochette *et al.*, 1997). Also, Kassim *et al.* (1982) suggested that lower metabolic efficiency is mean high maintenance respiration. Basal soil respiration values are inversely proportional to qCO_2 due to efficient assimilation of organic C by higher proportions to activity of microbes (Islam *et al.*, 2000).

Plant and soil-associated biota such as bacteria, fungi, etc. play important roles in nutrient cycling and plant productivity in natural and managed ecosystems. Penetration of O_3 into soil supporting soybean plants is believed to be limited essentially to the soil surface. In *Acersaccharum*, a decrease in the number of arbuscules, an increase in the number of vesicles in roots and decreases in the numbers of rhizosphere bacteria and non-arbuscular mycorrhizal fungi in soil were associated with increased levels of tropospheric O_3, increased UV-B radiation and increased levels of flavonoids, tannins and lignins (Rozema *et al.*, 1997). Edwards (1991) concluded that decreased respiration rates in the soil surrounding roots of the O_3-exposed plants imply that root-derived organic materials available for microbial proliferation may have reduced as an indirect result of the O_3-exposure.

Increases in global atmospheric CO_2 will not only directly affect the growth of plants, but might also alter the living conditions for soil biota. Elevated CO_2 has been reported to affect on population sizes of bacteria in the rhizosphere widely. It is included that reports of increases in total bacterial counts in the rhizosphere of *Gossypium hirsutum* exposed in Free Air CO_2 Enriched (FACE) rings. Increases in microbial biomass in the rhizosphere soil surrounding *Populus grandidentata* roots of plants exposed in open top chambers to CO_2 treatments have also reported (Zak *et al.*, 1996). It is reported that an increase in arbuscular mycorrhizal (AM) fungi infection in *Trifolium*, which was attributed to increased root mass and AM fungal entry points, rather than to changes in root exudate levels or composition. Generally, Schortemeyer *et al.* (1997) indicated that in legume crops, at least in terms of inoculums quality in the rhizosphere soil, symbiotic nitrogen-fixing organisms might be favored by elevated atmospheric CO_2 concentrations.

The effects of CO_2 and O_3 interactions on soil biota are few. It is studied that the effects of joint exposures of CO_2 and O_3 on mycorrhizal infection of Scots pine saplings. High O_3 reduced mychorrizal infection of roots; however, there was no CO_2 and O_3 interaction, as elevated CO_2 had no effect on mychorrizal infection at either O_3 level.

Given the broad range of responses both within and between plant genera and species, differences in sampling methodology, differences in the exposure regimes and length of studies, it is clear that more research is needed to clarify the nature and mechanism of the effects of O_3 and CO_2 on plant and soil associated microbiota. Assessment of the effects of O_3 and CO_2 on non-symbiotic N_2 fixation, on non-symbiotic, pathogenic and saprophytic fungi and on soil invertebrates and on the quality, quantity and rates of decomposition of litter are urgently needed. Collectively, availability of these diverse types of data will enhance our ability to identify and develop the measures of ecosystem status and function. The data will also be useful to develop and validate the predictive models of effects of elevated CO_2 and O_3 or other stressors on ecosystem integrity. With increased industrialization and concomitant increases in levels of tropospheric O_3, information on O_3 effects on plant and soil associated biota is needed to help the identification of potential indicators of O_3 damage. The information may also be useful to understand the basic mechanisms by which the damage occurs and to develop strategies to minimize or mitigate adverse effects of ozone exposure in natural and managed ecosystems. Effects of ozone on plant-associated microbes in rhizosphere soil indicate

sometimes-contrasting results and ones, which may be genus or even plant species specific.

Acknowledgments

I would like to express my appreciation to University of Maryland at College Park, Climate Stress Laboratory, USDA-Beltsville Agricultural Research Center, USA for field facilities. Special thanks for Prof. Dr. C. L. Mulchi, University of Maryland, for sending soil samples.

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