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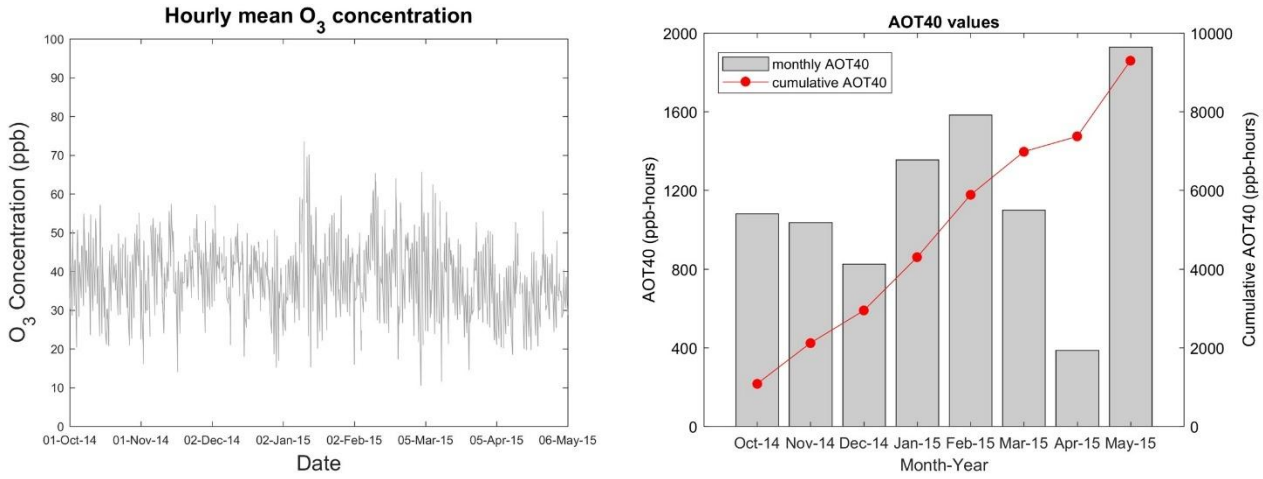
Calculated AOT40 values for conditions in the field

To estimate the potential risk of damage by O_3 on vegetation, the most commonly known indicators applied are the seasonal cumulative exposure over a threshold such as 60 ppb or 40 ppb (SUM06 and AOT40, respectively).[1-3] To obtain an approximate AOT40 for the ambient surroundings of the open-top chamber facility, monthly AOT values were calculated using continuous in situ O_3 data from the Welgegund Atmospheric Research Station. This site was chosen because it is approximately 30 km from Potchefstroom and provides continuous monitoring data for a wide suite of meteorological parameters. The station is a regionally representative background site for O_3, i.e. with few local anthropogenic sources of air pollution. Monthly averages for temperature and cumulative totals for precipitation were calculated.

O_3 concentrations measured at the Welgegund station, presented in Supplementary figure 1a, were taken at a sampling height of about 4–5 m. The AOT40 was calculated during the daylight period (08:00–17:00) for the sugarcane growing season, i.e. October to May, which differs from the AOT40 calculation method in Europe that uses only 3 months (May to July) and the daylight period might also be shorter.[4] Although AOT40 was calculated differently in this study, Supplementary figure 1b shows that the European AOT40 critical level of 3000 ppb h for crops is reached in the first 3 months at the South African background site. This indicates that if the AOT40 set by the United Nations Economic Commission for Europe[3] for crop damage was applied in southern Africa, there would likely be considerable exceedances of the 3000 ppb h critical level, particularly as interannual variability in weather conditions also influences ambient O_3 levels. Higher concentration thresholds have been suggested for defining critical levels in southern Africa. However, European open-top chamber experiments demonstrated that the 40 ppb threshold provided a much better linear fit to the exposure-response data than the use of higher thresholds.[5] It remains to be investigated through experiments if linear exposure-response relationships can be established at higher critical levels for locally adapted crops. As a comparison, the AOT40 inside the chamber (considering the start and end dates of fumigation) was calculated to be ~60 000 ppb h whilst the AOT40 in ambient air was calculated to be ~6 000 ppb h for the same period.

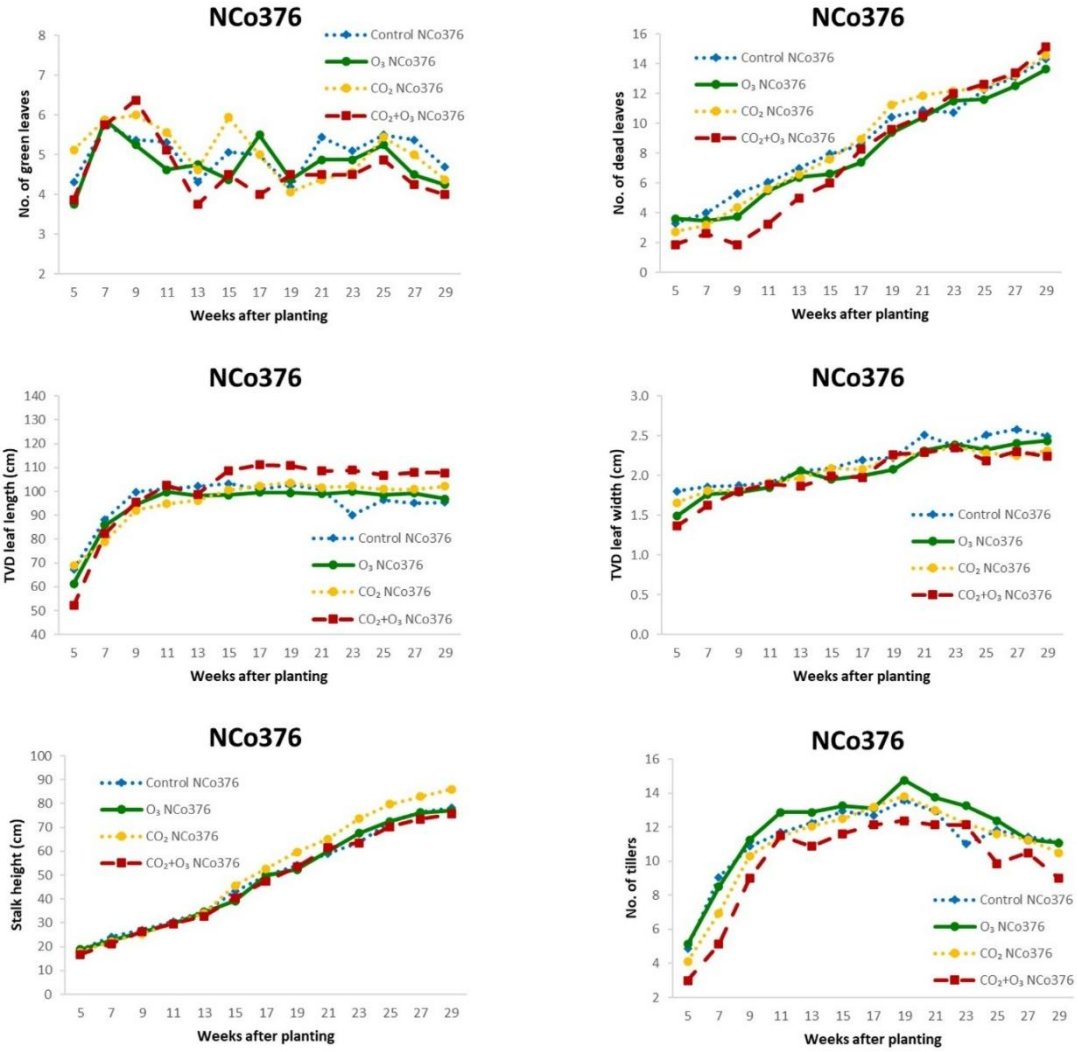
Growth parameters

Sugarcane yield is influenced by various plant growth responses as well as physiological traits. Indicators of plant growth over time and under the different treatments are presented in Supplementary figure 2a and 2b for each variety. The parameters measured include the numbers of green and dead leaves, TVD leaf length, TVD leaf width, stalk height and number of tillers.

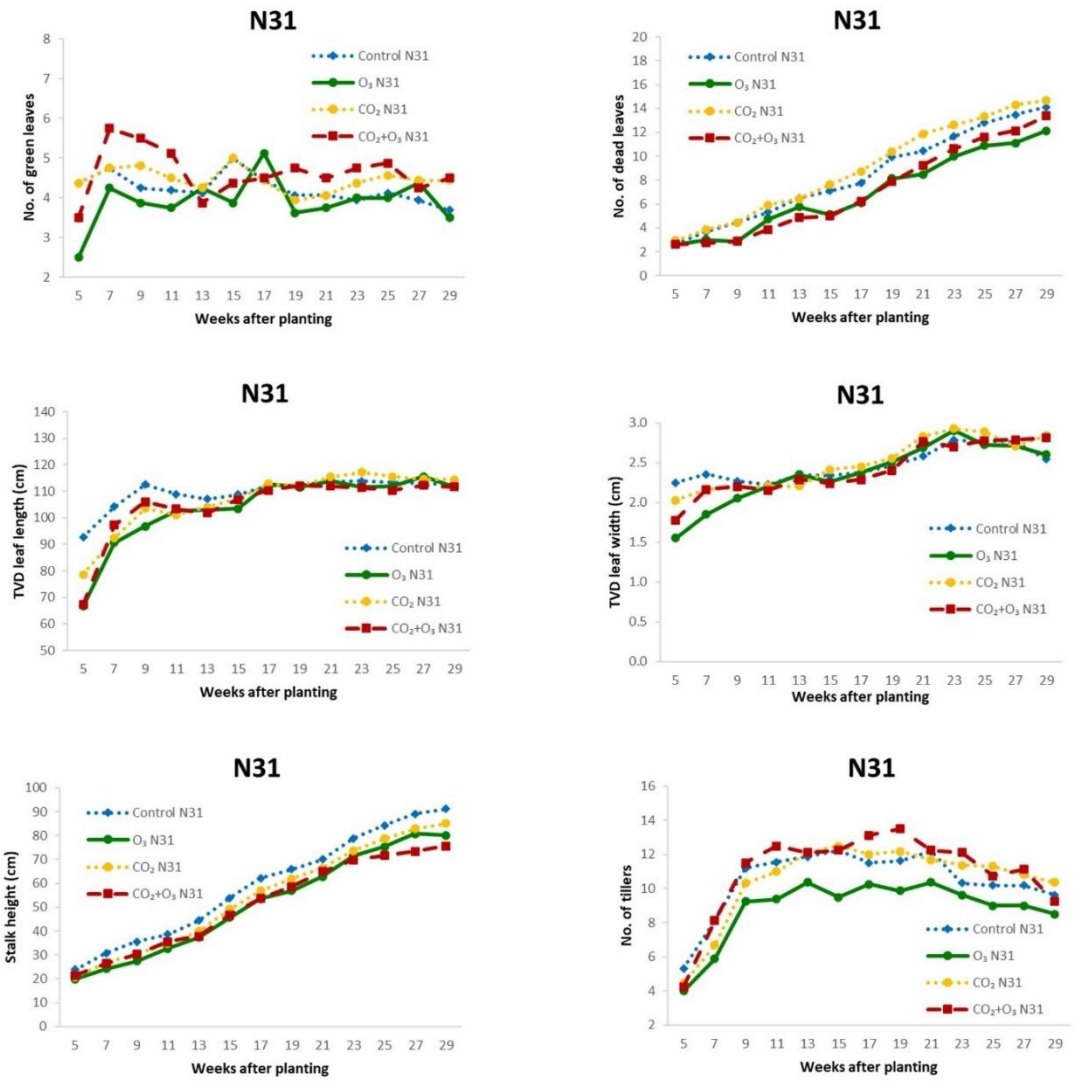


(a) **(b)**

Supplementary figure 1: (a) Average hourly O_3 concentration and (b) cumulative values of AOT40 at the regional background site Welgegund during the period from 1 October 2014 to 31 May 2015. Only measurements for daylight hours (8:00 to 17:00) were used.



(a) NCo376 cultivar



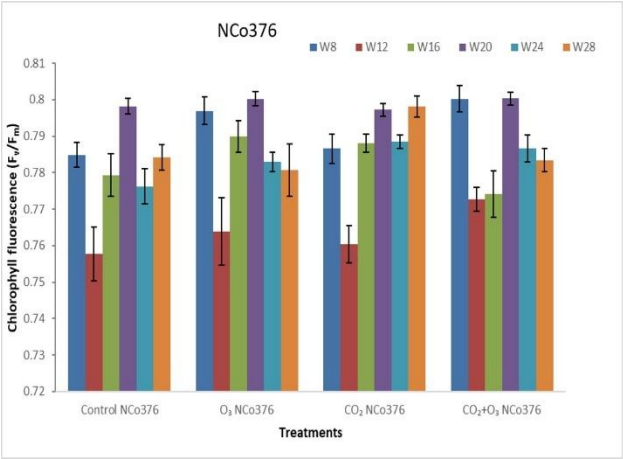
(b) N31 cultivar

Supplementary figure 2: Growth variables in two cultivars of sugarcane, (a) NCo376 and (b) N31, under the different treatments in open-top chambers: exposure to charcoal-filtered air (control), elevated O_3 (80 ppb), elevated CO_2 (750 ppm) and elevated O_3 plus CO_2 (80 ppb and 750 ppm).

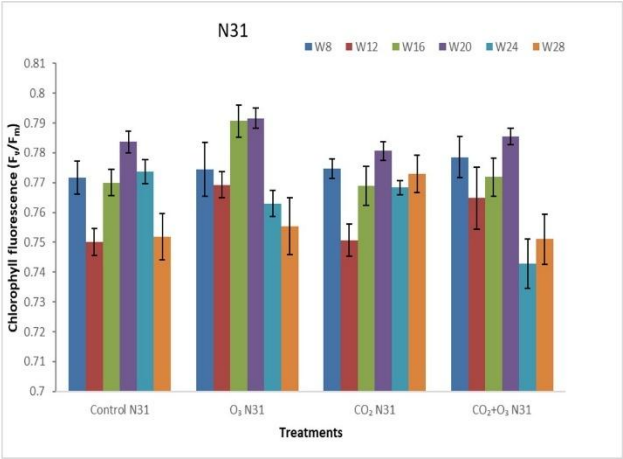
Chlorophyll fluorescence

Chlorophyll (Chl) *a* fluorescence is used to monitor photosystem II (PSII) activity and the processes that affect it.[6] The sensitivity of PSII activity to various biotic and abiotic factors can be beneficial in studying the effects of multiple stressors on the physiological state of plants.[7] The fast phase chlorophyll kinetics can provide insight into the impact of elevated O_3 and/or CO_2 exposure on photosynthetic performance and how plants respond to environmental change.[8]

The F_v/F_m parameter is an important indicator describing the efficiency of photosystem II (PSII). The F_v/F_m values for the four treatments were calculated and are plotted in Supplementary figure 3. The results demonstrate an overall decrease in F_v/F_m for elevated O_3-treated plants over time and generally higher F_v/F_m values for elevated CO_2-treated plants compared to elevated O_3-treated plants.



(a)

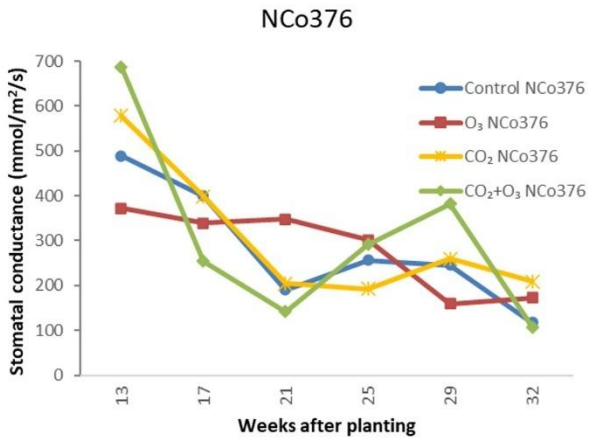


(b)

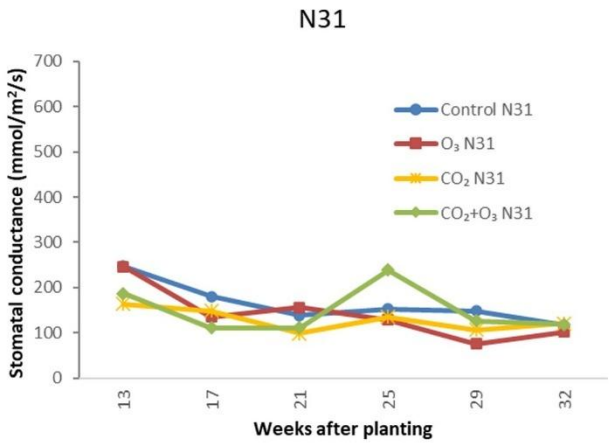
Supplementary figure 3: Chlorophyll fluorescence (expressed as Fv/Fm) during the growing season for (a) NCo376 and (b) N31 leaves after exposure to charcoal-filtered air (control), elevated CO_2 (750 ppm), elevated O_3 (80 ppb) and elevated O_3 plus CO_2 (80 ppb and 750 ppm) in open-top chambers. Each bar represents the mean value±SE.

Stomatal conductance

Stomatal closure by plants in response to O_3 stress can be an avoidance mechanism to prevent the uptake of O_3 by the leaves. However, by closing their stomata (pores for exchanging CO_2 and water vapour) to limit uptake of O_3 also limits CO_2 uptake, which leads to decreased photosynthetic activity.[9] The evolution of stomatal conductance through the growing season is presented in Supplementary figure 4.



(a)

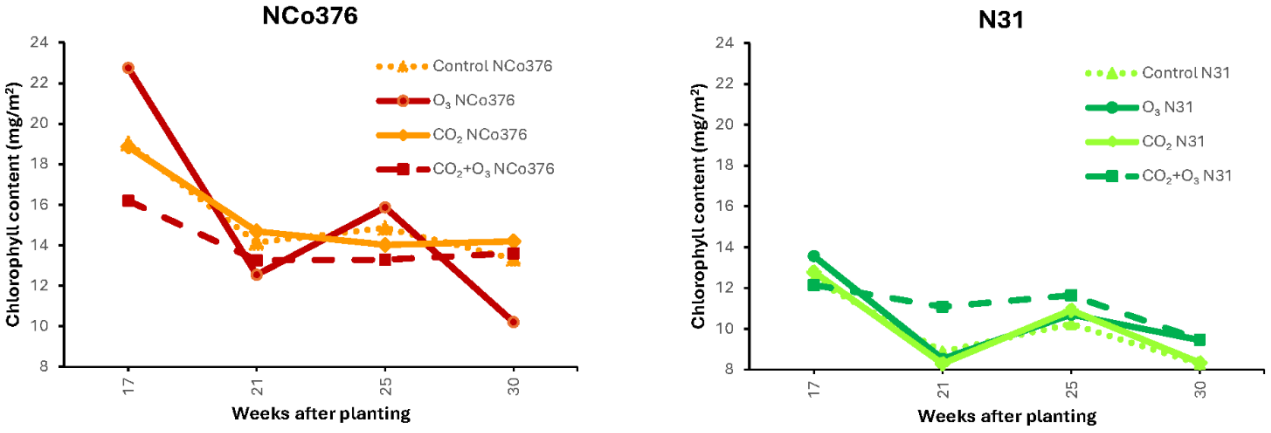


(b)

Supplementary figure 4: Mean values of stomatal conductance of (a) NCo376 and (b) N31 leaves after exposure to charcoal-filtered air (control), elevated O_3 (80 ppb), elevated CO_2 (750 ppm) and elevated O_3 plus CO_2 (80 ppb and 750 ppm) in open-top chambers.

Chlorophyll content

Leaf chlorophyll content presented in Supplementary figure 5 provides valuable information about the physiological status of plants and is related to photosynthesis. The primary function of chlorophyll molecules is radiation absorption, which includes energy essential for photosynthesis. Reduced chlorophyll content has been linked to a decline in photosynthesis and growth in forest trees.[10]



(a) (b)

Supplementary figure 5: Mean values of chlorophyll content (in mg/m^2) of (a) NCo376 and (b) N31 leaves after exposure to charcoal-filtered air (control), elevated O_3 (80 ppb), elevated CO_2 (750 ppm) and elevated O_3 plus CO_2 (80 ppb and 750 ppm) in open-top chambers.

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