Examining ozone effects on the tropical C_4 crop *Sorghum bicolor*

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ABSTRACT

Ozone (O_3), a major air pollutant, can negatively impact plant growth and yield. While O_3 impacts have been widely documented in crops such as wheat and soybean, few studies have looked at the effects of O_3 on sorghum, a C_4 plant and the fifth most important cereal crop worldwide. We exposed grain sorghum (*Sorghum bicolor* cv. HAT150843) to a range of O_3 concentrations (daytime mean O_3 concentrations ranged between 20 and 97 ppb) in open-top chambers, and examined how whole plant and leaf morphological traits varied in response to O_3 exposure. Results showed no significant impact of realistic O_3 exposure on whole plant biomass and its partitioning in sorghum. These findings suggest that sorghum is generally resistant to O_3 and should be considered as a favourable crop in O_3 polluted regions, while acknowledging further research is needed to understand the mechanistic basis of O_3 tolerance in sorghum.

Subjects Plant Science, Atmospheric Chemistry, Environmental Impacts
Keywords Biomass, Leaf mass per area, Stomatal properties

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INTRODUCTION

Tropospheric ozone (O_3) is one of the most important air pollutants produced naturally by a series of photochemical reactions between the precursors: nitrogen oxides (NO_x), volatile organic compounds (VOCs), methane (CH_4) and carbon monoxide (CO) (*Ainsworth et al., 2012*; *Schneider et al., 2017*). Since the start of the industrial revolution, ground-level O_3 concentrations have significantly increased globally due to increasing abundance of O_3 precursor emissions (*Monks et al., 2015*; *Young et al., 2013*), and the influence of changing atmospheric chemistry under contemporary climate change (*Brown et al., 2022*). Ozone is well known to have a detrimental impact on plant growth, yield, and productivity (*Ainsworth et al., 2012*; *Emberson et al., 2018*). Indeed, it has been suggested that current O_3 exposure reduces global yields of wheat, soybean, maize, and rice by 227 Tg annually (*Mills et al., 2018*). Even accounting for the positive CO_2 fertilization effect, *Tai et al. (2021)* estimated global yield losses of 3.6 ± 1.1% for maize, 2.6 ± 0.8% for rice, 6.7 ± 4.1% for soybean, and 7.2 ± 7.3% for wheat. Such substantial crop losses due to O_3 are a threat to global food production and security.

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Generally, O_3 from the atmosphere enters plant leaves through stomata (*Ainsworth et al., 2012*). When inside the leaf, O_3 reacts rapidly with molecules in the liquid surrounding leaf cells and forms reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), and hydroxyl ($OH^\bullet$) radicals (*Ainsworth et al., 2012*). O_3 and its ROS derivatives can initially attack the cell membranes with primary targets being membrane lipids, susceptible amino acids in membrane proteins, and a variety of organic metabolites contained in the cell wall (*Fiscus, Booker & Burkey, 2005*). Due to these reactions within the cell membrane, O_3 may produce new reaction products causing further cellular changes including oxidation of membrane lipids, alterations of protein function, and formation of ozonolysis products (*Emberson et al., 2018*; *Fiscus, Booker & Burkey, 2005*). This metabolic dysfunction and possible cell death causes a reduction in leaf photosynthesis and stomatal conductance, increased respiration rates, accelerated leaf senescence and visible injury (*Ainsworth et al., 2012*; *Emberson et al., 2018*). These physiological changes ultimately reduce plant growth, yield, and biomass accumulation (*Emberson et al., 2018*).

The reductions of biomass accumulation and yield represent integrated responses, useful in O_3 risk assessments (*Mills et al., 2018*). Although leaf visible injury has commonly been used as an O_3 response indicator, it is biologically less significant than the integrated measure of impacts on growth (*Feng et al., 2014*). The reduction in photosynthesis is also a functional O_3 response variable that corresponds with the O_3 impact on plant productivity (*Li et al., 2016*); however, the response of biomass has received more attention, as growth integrates internal and external processes that operate at different plant organizational levels (*Uddling et al., 2004*). These response variables in relation to O_3 exposure metrics have been used to examine O_3 effects on many plant species across a range of studies (*Büker et al., 2015*; *Calatayud et al., 2011*; *Li et al., 2016*; *Zhang et al., 2012*).

Different O_3 metrics have been developed for assessing O_3 risk (*Emberson et al., 2018*). At present, the most commonly used O_3 metrics are the concentration-based AOT_{40} (accumulated O_3 concentrations over a threshold of 40 ppb) and the flux-based POD_y (Phytotoxic O_3 Dose-the accumulated stomatal O_3 flux above a threshold of y) (*Shang et al., 2017*). The AOT_{40} metric only considers the O_3 concentrations in air that are above the 40 ppb threshold during daylight hours (*Assis et al., 2015*). However, as O_3 uptake is mediated through the stomata, POD_y which quantifies the effective stomatal flux of O_3 entering the leaves, and considers the biological and climatic factors influencing $g_{s,}$ is a more rational metric than AOT_{40} (*Assis et al., 2015*; *Peng et al., 2019*). Studies also have indicated that POD_y represents a better predictor than AOT_{40} to estimate the negative O_3 impacts on plants (*Büker et al., 2015*; *Karlsson et al., 2007*; *Mills et al., 2011*). Therefore, the development of O_3 flux-response relationships for specific species in different climatic conditions is a central step for assessing the O_3 risk at regional, national, and global scales (*Feng et al., 2015*).

There are many studies about the adverse effects of O_3 on C_3 plant species (*e.g.*, wheat, rice, soybean). However, relatively little work has been done on C_4 species (*Li et al., 2022*, *2023*; *McGrath et al., 2015*). Sorghum (*Sorghum bicolor*), a C_4 tropical crop, is the fifth most important cereal crop in the world and has a high potential for bioenergy and ethanol production (*Regassa & Wortmann, 2014*; *Yuan et al., 2008*). Sorghum is highly adaptable and can be cultivated on marginal land where water deficit stress, alkalinity, salinity, and other limitations exist (*Regassa & Wortmann, 2014*; *Yuan et al., 2008*). Sorghum originated in Africa and is widely planted in tropical and sub-tropical regions (*Hossain et al., 2022*). At present, the tropics are experiencing high levels of O_3 pollution due to the combined pressures of an increase in population, industrialization, and energy consumption (*Kunhikrishnan et al., 2004*; *Leventidou et al., 2018*). Though some studies have investigated the effects of O_3 on sorghum in temperate environments (*Li et al., 2021*, *2022*), studies under tropical conditions with local cultivars are still absent. As regional variation in the O_3 susceptibility of crop cultivars is often present due to differences in breeding strategies and different climatic conditions (*Hayes et al., 2020b*), it is essential to investigate the impact of O_3 pollution on sorghum under tropical climatic conditions.

Therefore, we carried out this study to evaluate the effects of a range of O_3 exposures on sorghum using a cultivar grown in tropical Australia. Our objectives were (1) to examine the effects of O_3 on sorghum biomass under various O_3 concentrations and (2) to assess the leaf morphological trait responses to O_3 to understand mechanisms of impact and/or tolerance.

MATERIALS AND METHODS

The effects of O_3 on *Sorghum bicolor* were examined in open top chambers (OTC) built at the UK University of Exeter's TropOz Research facility (www.tropoz.org) located at James Cook University's Environmental Research Complex (ERC) on the Nguma-bada campus in northeast Queensland, Australia. The experiment was conducted using nine independently controlled and monitored OTCs employing a gradient design (daytime mean O_3 concentrations ranged between 20 and 97 ppb, Table 1). This gradient-design offers a better approach than replicating a limited number of exposure points, especially when attempting to identify a possibly nonlinear treatment response (*Kreyling et al., 2018*). Details of the OTC experimental set up are described in *Farha et al. (2023)*. However, in brief, pure O_3 generated on site (AirSep Onyx Plus O_2 generator and OZ-T4600 O_3 generator; Oxyzone International, Somersby, Australia) was provided between 08:00 and 17:00 h to OTC's (internal volume 22.2 m^3) receiving continuous ventilation with activated carbon filtered air (AirFlow-VC; Airpure Australia North St Marys, North St Marys, NSW, Australia). An ultraviolet (UV) absorption O_3 analyser (Model 205, 2B technology, Boulder, CO, USA) was used to continuously monitor O_3 concentrations in each chamber. At the same time environmental variables including air temperature (T), relative air humidity (RH), and photosynthetically active radiation (PAR) were recorded in the central OTC using a single meteorological monitoring station (Campbell Scientific, Logan, UT, USA) with an assumption that environmental conditions other than O_3 concentrations were the same between OTC's.

Plant material

Seedlings of grain sorghum (*Sorghum bicolor* cv HAT150843, commonly used in Queensland) were used in this experiment. A total of 2 months after germination, three

Table 1 **Daytime mean O_3 concentrations, and POD_6 per chamber during the experimental period from 13/07/2020 to 16/10/2020.**

Chamber ID	Daytime mean O_3 (ppb)	POD_6 (mmol m^{-2})
1	20.7 ± 4.8	0.1
2	21.2 ± 5.0	0.1
3	28.3 ± 6.2	2.0
4	34.3 ± 8.7	5.1
5	37.8 ± 10.0	7.2
6	48.4 ± 15.5	13.8
7	60.1 ± 22.8	21.5
8	76.0 ± 28.7	31.7
9	97.0 ± 39.3	44.9

seedlings established in 20-L pots filled with a locally sourced 'garden mix' topsoil were thinned to result in 27 homogeneous seedlings in individual pots. Each of these replicate seedlings were transferred into one of nine OTCs for O_3 exposure ($n = 3$). The plants were grown under O_3 fumigation for 3 months (from 13/07/2020 to 16/10/2020) until grain filling stage with a daily maximum exposure of 9 h (from 08:00 to 17:00). Plants were maintained in well-watered conditions using an automated drip irrigation system.

Stomatal conductance measurements and DO_3SE model parameterization

At various points throughout the experiment, stomatal conductance to water vapor (g_s) was measured on the newest fully expanded mature leaf using an SC-1 Leaf Porometer (Decagon Devices, Pullman, WA, USA). Point measurements of g_s on both abaxial and adaxial leaf surfaces were conducted over a range of conditions for a total of 270 measurements of g_s. These data were used to parameterize the Deposition of O_3 for Stomatal Exchange (DO_3SE) model (www.sei.org/tools/do3se-deposition-ozone-stomatal-exchange/) based on an empirical model of g_s (*Emberson et al., 2000*; *Jarvis, 1976*), allowing for the calculation of the O_3 flux-based metric POD_6 (phytotoxic O_3 dose-the accumulated stomatal O_3 flux above a threshold of 6 nmol O_3 m^{-2} projected leaf area (PLA) s^{-1}) according to *Convention on Long-range Transboundary Air Pollution (CLRTAP) (2017)*.

Prior to fitting DO_3SE empirical parameters (Table 2), all g_s data were transformed to relative stomatal conductance (RSC, g_s/g_{max}), with the maximum stomatal conductance (g_{max}) calculated as the measurement's 95th percentile of abaxial and adaxial data and final g_s computed on a projected leaf area basis (PLA) using the abaxial: adaxial g_s ratio. Parameterization of DO_3SE was carried out according to *Holder & Hayes (2022)* using the plotting of RSC against light, leaf temperature and VPD independently. In order to fit the physiologically relevant curves, the x-axis was divided into parts and for each part, the 90th percentiles of the observed g_s data were calculated (Fig. 1). To convert the g_{max} to $g_{max}O_3$, a conversion factor of 0.663 was used. The DO_3SE model estimates the stomatal O_3 uptake

Table 2 **Stomatal conductance model parameterization of *Sorghum bicolor* cv HAT150843.** The g_{max} is the maximum conductance, f_{min} is the fraction of g_{max} at minimum stomatal conductance (g_{min}). L_m is the effective leaf blade width, f_{temp}, f_{light}, f_{VPD} are the functions of g_s response to air temperature (T, °C), photosynthetically active radiation at the leaf surface (PAR, µmol m^{-2} s^{-1}), and vapour pressure deficit (VPD, kPa), respectively.

Parameter		Unit	*Sorghum bicolor* cv HAT150843
g_{max}		mmol O_3 m^{-2} s^{-1}	290
L_m		m	0.02
f_{min}		Fraction	0.13
f_{PAR}		Unitless	0.007
f_{VPD}	VPD_{min}	kPa	4.50
	VPD_{max}	kPa	2.60
f_{temp}	T_{min}	°C	14
	T_{opt}	°C	31
	T_{max}	°C	48

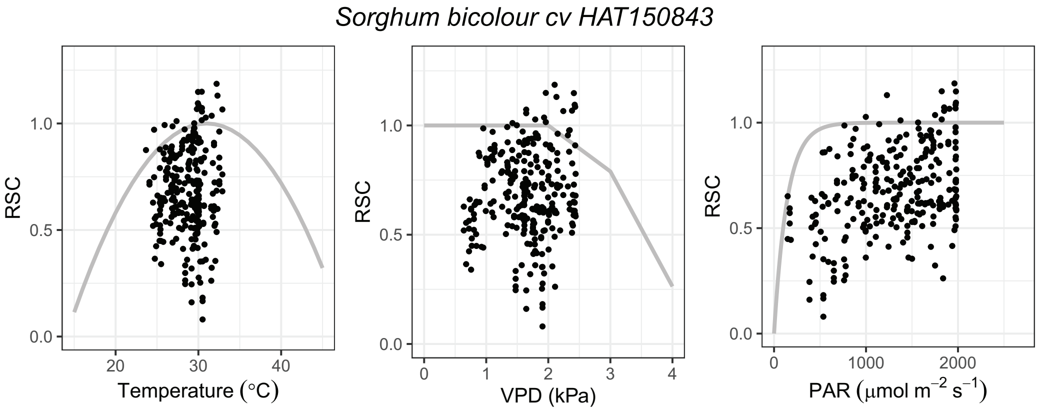

Figure 1 **Functions describing the dependence relationship between relative stomatal conductance (RSC, g_s/g_{max}) and air temperature (°C), vapour pressure deficit (VPD, kPa), and photosynthetically active radiation at the leaf surface (PAR, µmol m^{-2} s^{-1}) respectively in *Sorghum bicolor* cv HAT150843.** Data points represent the observed data, and the grey lines show the physiologically relevant curve derived from the functions (based on 90th percentiles of the observed data).

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using the hourly mean values of O_3 and meteorological conditions from the experiment (Table 2, Data set S1).

Net photosynthesis and chlorophyll concentration measurements

Additional gas exchange survey measurements using a portable photosynthesis system (Licor-6400; LI-COR Inc., Lincoln, NE, USA) were conducted at one point in time (14/08/2020) on all plants to determine the response of net photosynthesis (A_{net}) and g_s to O_3 exposure. During these survey measurements, carried out approximately halfway along the newest fully expanded leaf of each plant, block temperature was set to 28 °C, while

Table 3 Estimated values of the maximum chlorophyll concentration, a rate parameter, β, and optimal time, t_{opt}, to achieve the maximum concentration for each chamber and overall summary statistics.

Chamber ID	Maximum chlorophyll concentration (μmol m^{-2})	β	t_{opt} (day)
1	739.1	0.7	29
2	762.4	0.7	28
3	771.2	0.8	27
4	760.7	0.7	27
5	769.8	0.8	26
6	717.6	0.6	26
7	678.7	0.7	25
8	673.9	0.4	31
9	682.2	0.5	27
Mean	728.4	0.6	27
SD	41.1	0.1	2

PAR, and the CO_2 concentration and relative humidity in the cuvette were set at 1,500 μmol m^{-2} s^{-1}, 400 ppm and 70% to 80% to match ambient conditions at the time.

Changes in leaf chlorophyll content index (CCI) were measured in developing leaves from across the range in O_3 exposure using a portable chlorophyll meter (MC-100; Apogee instruments, Logan, UT, USA) (Data set S2). The CCI metric was determined along the entire leaf-bade (six measurements) of one leaf per plant every 2 to 3 days for a total of 2 months from leaf-initiation through full expansion and to the point that chlorophyll concentration declined (*i.e.*, 13 sampling points). For each leaf/sampling date measurements across the lamina were averaged, with CCI values converted to leaf chlorophyll concentration (μmol m^{-2}) according to the sorghum conversion equation of *Parry, Blonquist & Bugbee (2014)*. To estimate maximum chlorophyll concentration and the time since leaf-initiation that maximum concentration was reached, a quadratic equation was fitted to the averaged lamina data for each plant (Table 3).

Leaf morphological traits

At the end of the experimental period, three fully developed leaves were collected from each plant. Lamina subsections from each leaf were taken and measured for total fresh weight. After weighing, lamina sections were scanned (Cannon LiDE 400) and kept in a drying oven (70 °C, 72 h) for dry mass determination. Leaf dry mass and the lamina area, calculated using image analyser software (Image-J, NIH), were used to calculate leaf mass per unit area (LMA, g m^{-2}). Prior to drying, lamina sections were subjected to the leaf impression method to determine the stomatal density (SD, number of stomata per unit area) and stomatal pore length (SPL, the length of stomatal pore opening). This method utilizes clear nail polish to make an impression or cast of the leaf surface, with the cast removed with sticky tape and mounted on a microscope slide. These slides were scanned with an Aperio CS2 scanner (Leica, Wetzlar, Germany). Aperio ImageScope Slide Viewing Software was used to count stomata and measure SPL.

Biomass

At the end of the experiment, leaves, stems, roots, and grain were each harvested separately. All plant materials were dried in an oven at 70 °C until constant weight and finally, dry biomasses were determined.

Statistical analyses

We examined the impact of O_3 on response variables using a gradient design (*Kreyling et al., 2018*) wherein a linear regression of averaged data at the chamber level ($n = 3$ plants) was used to investigate the relationship of plant biomass, maximum chlorophyll concentration and leaf traits with the O_3 flux metric, POD_6. Pearson's correlation test was performed to identify correlations between the leaf traits. Statistical analyses were conducted using the software R version 4.1.2 (*R Core Team, 2021*) utilizing base R and the packages *plyr* (*Wickham, 2011*), *ggplot2* (*Wickham, 2016*), *scales* (*Wickham, Pedersen & Seidel, 2023*), *nlme* (*Pinheiro et al., 2021*), *plantecophys* (Duursma, 2015), and *drc* (*Ritz et al., 2015*).

RESULTS

Sorghum (*Sorghum bicolor* cv HAT150843) was exposed to nine different concentrations of O_3 in OTCs. Over the experimental period, the average values of daytime (*i.e*., between 8 am and 5 pm) O_3 concentration ranged between 20.7 ± 4.8 and 97 ± 39.3 ppb (Table 1) and the average daytime air temperature, PAR, and RH were 28.1 ± 3.2 °C, 1137.7 ± 670.3 $\mu mol\ m^{-2}\ s^{-1}$, and 57.5 ± 12.9%, respectively (Data set S1). Parameterization of g_s functions in the DO_3SE model (Table 2, Fig. 1) were carried out in accordance with *Holder & Hayes (2022)*, with calculated POD_6 ranging between 0.1 and 44.9 mmol m^{-2} (Table 1).

Biomass accumulation in sorghum was found to be not significantly impacted by exposure to O_3. Specifically, there was no significant change in above ground (leaves and stems) biomass (Adj-R^2 = −0.14, P = 0.86), below ground biomass (Adj-R^2 = −0.01, P = 0.38) or grain biomass (Adj-R^2 = −0.14, P = 0.85) with increasing O_3 flux metric, POD_6 (Fig. 2).

Our quadratic model provided an estimate of the optimal time (t_{opt}) for maximizing chlorophyll concentration and a rate parameter beta (β) as well as the mean and standard deviation of each parameter across all chambers (Table 3). Parameter beta indicates how quickly chlorophyll concentration decreases as time moves away from t_{opt}. The maximum chlorophyll concentration showed a slight but significant decline with increasing POD_6 (Adj-R^2 = 0.39, $P < 0.05$) (Fig. 3). However, leaf morphological traits such as LMA, SD, and SPL were not affected by O_3 flux in sorghum. The LMA across the O_3 exposure ranged between 44.8 and 48.8 g m^{-2} (Adj-R^2 = −0.07, P = 0.52), SD ranged between 173 and 206 mm^{-2} (Adj-R^2 = −0.10, P = 0.62) and SPL ranged between 19.4 and 20.6 μm (Adj-R^2 = −0.13, P = 0.80) (Data set S1).

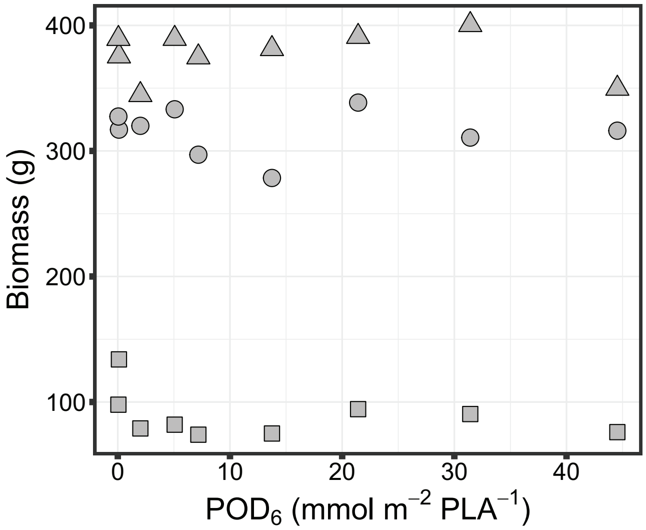

Figure 2 Variation in biomass of grain sorghum (*Sorghum bicolor* cv HAT150843) grown under various O_3 concentrations. Grain biomass (triangle), above ground biomass (circle) and below ground biomass (square) are shown in relation to the O_3 metric POD$_6$. No significant relationship was found for grain biomass (Adj-R^2 = −0.14, P = 0.85), above ground biomass (Adj-R^2 = −0.14, P = 0.86), or below ground biomass (Adj-R^2 = −0.01, P = 0.38) with increasing O_3 flux metric POD$_6$.
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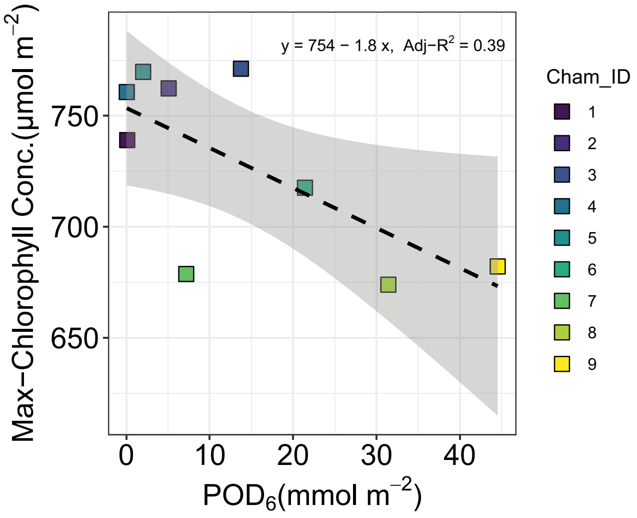

Figure 3 Changes in maximum chlorophyll concentration of grain sorghum (*Sorghum bicolor* cv HAT150843) grown under various O_3 concentrations. Full-size DOI: 10.7717/peerj.18844/fig-3

In addition, we tested for correlations among leaf traits in sorghum. There was a strong correlation between A_{net} and log of g_s as measured by the portable photosynthesis analyzer (Adj-R^2 = 0.88, $P < 0.001$), but the slope of the relationship was not affected by increasing POD$_6$ (Fig. 4). Between the morphological traits the Pearson's correlation test showed a positive correlation between LMA and SD (r = 0.41, $P < 0.05$) and a negative correlation between SD and SPL (r = −0.41, $P < 0.05$).

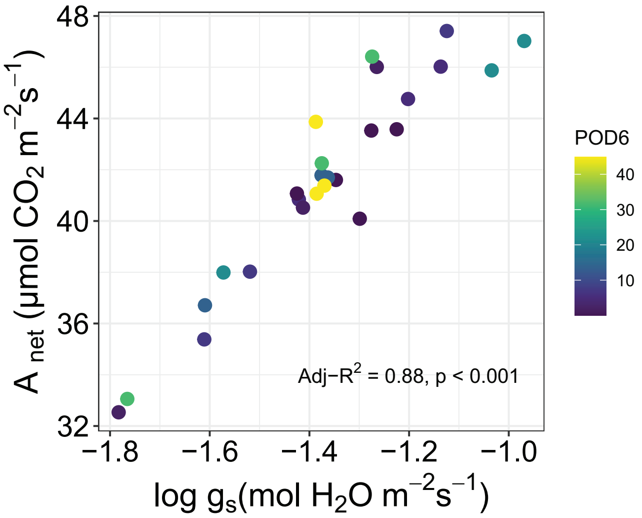

Figure 4 **Relationship between net photosynthesis, A_{net}, and log of stomatal conductance, log g_s, for sorghum grown under various O_3 concentrations.** Full-size DOI: 10.7717/peerj.18844/fig-4

DISCUSSION

Many studies have highlighted the interspecific and cultivar-level variability in negative impacts of O_3 on plant biomass and yields in C_3 crops (*Emberson et al., 2018*; *Wittig et al., 2009*). However, while C_4 plants are generally more O_3 tolerant there is also evidence that elevated O_3 has a variable impact on C_4 plants (*Li et al., 2022*) with some species such as switchgrass (*Li et al., 2019*) and sorghum (*Hayes et al., 2020a*; *Li et al., 2021*) showing a low suceptibility to O_3 exposure. In the first experiment for sorghum grown under tropical conditions, we found that in the cultivar examined (cv. HAT150843) there was no significant change in biomass or yield with increasing O_3 flux, although maximum chlorophyll concentration was found to be reduced with increasing POD_6. As sorghum has evolved in the tropics, environmental adaptations to cope with environmental stresses such as high temperature, drought, and light intensity (*Regassa & Wortmann, 2014*) have resulted in high water-use efficiency and antioxidant capacity, which may confer a capacity to also cope with the oxidative stress as a result of O_3 exposure (*Li et al., 2021*).

Leaf mass per area is a good indicator of plant functional type that can explain the differences in O_3 susceptibility among species (*Feng et al., 2018*), whereby leaves with high LMA tend to be less susceptible to O_3 (*Li et al., 2016*). However, there can be an impact of O_3 on LMA itself, as demonstrated by *Shang et al. (2017)* who found a significant negative relationship between LMA and O_3 metrics in C_3 poplar clones. Our results show no significant relationship between LMA and O_3 flux in sorghum, consistent with a recent study of maize (*Peng et al., 2020*) and other C_4 bioenergetic crop species (*i.e.*, switchgrass, sorghum, maize, and miscanthus) (*Li et al., 2022*). This difference may be the result of the unique leaf anatomical features of C_4 species, including mesophyll cells surrounding bundle sheath cells in which the photosynthetic carbon reduction cycle occurs (*Montes et al., 2022*).

As O_3 uptake from the atmosphere occurs through leaf stomata, stomatal properties play an important role in shaping plant responses to O_3, at the same time that O_3 exposure can impact stomata through disrupting leaf development. Previous work in other species has reported that O_3 exposure increases SD (*Fusaro et al., 2016*; *Paoletti & Grulke, 2005*). In our study, neither SD nor SPL were found to be significantly correlated with O_3 flux, POD_6, with similar findings reported in switchgrass with respect to foliar anatomy (*Li et al., 2019*). However, in our study on sorghum, there was a negative correlation between SD and SPL, and a positive correlation between SD and LMA. Across various species, SD shows a negative relationship with SPL (*Franks & Beerling, 2009*; *Hetherington & Woodward, 2003*; *Roth-Nebelsick et al., 2012*), with plants with a higher density of small stomata able to open and close them more rapidly in response to changes in leaf water status (*Hetherington & Woodward, 2003*). This has been shown to contribute in varying degrees to drought tolerance in grasses (*Xu & Zhou, 2008*) and olive cultivars (*Bosabalidis & Kofidis, 2002*). It may also contribute to tolerance of O_3 induced stress. However, more studies on a range of sorghum genotypes would be required to understand the relationship among leaf traits across the breadth of genetic variation observed within the species.

As might be expected we found a strong correlation between A_{net} and log of g_s as measured by leaf-level gas-exchange in sorghum, however the proportionality between the two did not respond to increasing O_3 exposure. This is perhaps inconsistent with a recent study in which this proportionality was shown to have responded to increasing O_3 concentrations (*Cernusak, Farha & Cheesman, 2021*), and provides yet another indication that the physiology of sorghum was largely resistant to impacts of O_3 across the range of exposure that we applied in our experiment. In this context, it is interesting that we could detect an influence of increasing level of O_3 exposure on the maximum chlorophyll concentrations observed in leaves of the experimental plants (Fig. 3). However, it seems that the approximately 10% decline in maximum chlorophyll concentration from lowest to highest O_3 exposures was not of large enough magnitude to markedly impact leaf-level gas exchange (Fig. 4).

CONCLUSION

Based on plant biomass, leaf-level gas exchange and the morphological trait responses studied, our results showed little significant effect of O_3 on grain sorghum. Thus, it can be concluded that sorghum is not susceptible to elevated O_3, even when grown under tropical field conditions. Hence, sorghum could be an advantageous crop in tropical regions with high levels of O_3 pollution to help combat tropical food insecurity challenges (*Hayes et al., 2020a*). However, it should be noted that in this study, only one genotype was examined. To more broadly understand O_3 responses, additional experiments with more genotypes, and a broader range of functional traits and conditions are required.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Mst Nahid Farha conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Flossie Brown conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Lucas A. Cernusak conceived and designed the experiments, authored or reviewed drafts of the article, supervision, Resources, and approved the final draft.
- Stephen Sitch conceived and designed the experiments, authored or reviewed drafts of the article, funding Acquisition, and approved the final draft.
- Alexander W. Cheesman conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, project administration, Funding acquisition, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw data are available in the Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.18844#supplemental-information.

REFERENCES

Ainsworth EA, Yendrek CR, Sitch S, Collins WJ, Emberson LD. 2012. The effects of tropospheric ozone on net primary productivity and implications for climate change. *Annual Review of Plant Biology* **63**:637–661 DOI 10.1146/annurev-arplant-042110-103829.

Assis PI, Alonso R, Meirelles ST, Moraes RM. 2015. DO$_3$SE model applicability and O$_3$ flux performance compared to AOT40 for an O$_3$-sensitive tropical tree species (*Psidium guajava* L.'Paluma'). *Environmental Science and Pollution Research* **22**:10873–10881 DOI 10.1007/s11356-015-4293-1.

Bosabalidis AM, Kofidis G. 2002. Comparative effects of drought stress on leaf anatomy of two olive cultivars. *Plant Science* **163**:375–379 DOI 10.1016/S0168-9452(02)00135-8.

Brown F, Folberth GA, Sitch S, Bauer S, Bauters M, Boeckx P, Cheesman AW, Deushi M, Vieira IDS, Galy-Lacaux C. 2022. The ozone-climate penalty over South America and Africa by 2100. *Atmospheric Chemistry and Physics* **22**:12331–12352 DOI 10.5194/acp-22-12331-2022.

Büker P, Feng Z, Uddling J, Briolat A, Alonso R, Braun S, Elvira S, Gerosa G, Karlsson PE, Le Thiec D. 2015. New flux based dose-response relationships for ozone for European forest tree species. *Environmental Pollution* **206**:163–174 DOI 10.1016/j.envpol.2015.06.033.

Calatayud V, Cerveró J, Calvo E, García-Breijo F-J, Reig-Armiñana J, Sanz MJ. 2011. Responses of evergreen and deciduous Quercus species to enhanced ozone levels. *Environmental Pollution* **159(1)**:55–63 DOI 10.1016/j.envpol.2010.09.024.

Cernusak LA, Farha MN, Cheesman AW. 2021. Understanding how ozone impacts plant water-use efficiency. *Tree Physiology* **41(12)**:2229–2233 DOI 10.1093/treephys/tpab125.

Convention on Long-range Transboundary Air Pollution (CLRTAP). 2017. Mapping critical levels for vegetation, chapter III of manual on methodologies and criteria for modelling and mapping critical loads and levels and air pollution effects, risks and trends. In: *UNECE Convention on Long-Range Transboundary Air Pollution*. Geneva: UNECE.

Duursma RA. 2015. Plantecophys-An R package for analysing and modelling leaf gas exchange data. *PLOS ONE* **10(11)**:e0143346 DOI 10.1371/journal.pone.0143346.

Emberson L, Ashmore M, Cambridge H, Simpson D, Tuovinen J-P. 2000. Modelling stomatal ozone flux across Europe. *Environmental Pollution* **109(3)**:403–413 DOI 10.1016/S0269-7491(00)00043-9.

Emberson LD, Pleijel H, Ainsworth EA, den Berg MV, Ren W, Osborne S, Mills G, Pandey D, Dentener F, Büker P. 2018. Ozone effects on crops and consideration in crop models. *European Journal of Agronomy* **100**:19–34 DOI 10.1016/j.eja.2018.06.002.

Farha MN, Daniells J, Cernusak LA, Ritmejerytė E, Wangchuk P, Sitch S, Mercado LM, Hayes F, Brown F, Cheesman AW. 2023. Examining ozone susceptibility in the genus *Musa* (bananas). *Functional Plant Biology* **50(12)**:1073–1085 DOI 10.1071/FP22293.

Feng Z, Büker P, Pleijel H, Emberson L, Karlsson PE, Uddling J. 2018. A unifying explanation for variation in ozone sensitivity among woody plants. *Global Change Biology* **24**:78–84 DOI 10.1111/gcb.13824.

Feng Z, Paoletti E, Bytnerowicz A, Harmens H. 2015. Ozone and plants. *Environmental Pollution* **202**:215–216 DOI 10.1016/j.envpol.2015.02.004.

Feng Z, Sun J, Wan W, Hu E, Calatayud V. 2014. Evidence of widespread ozone-induced visible injury on plants in Beijing, China. *Environmental Pollution* **193**:296–301 DOI 10.1016/j.envpol.2014.06.004.

Fiscus EL, Booker FL, Burkey KO. 2005. Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. *Plant, Cell & Environment* **28**:997–1011 DOI 10.1111/j.1365-3040.2005.01349.x.

Franks PJ, Beerling DJ. 2009. Maximum leaf conductance driven by CO_2 effects on stomatal size and density over geologic time. *Proceedings of the National Academy of Sciences of the United States of America* **106**:10343–10347 DOI 10.1073/pnas.0904209106.

Fusaro L, Gerosa G, Salvatori E, Marzuoli R, Monga R, Kuzminsky E, Angelaccio C, Quarato D, Fares S. 2016. Early and late adjustments of the photosynthetic traits and stomatal density in *Quercus ilex* L. grown in an ozone-enriched environment. *Plant Biology* **18**:13–21 DOI 10.1111/plb.12383.

Hayes F, Harmens H, Sharps K, Radbourne A. 2020a. Ozone dose-response relationships for tropical crops reveal potential threat to legume and wheat production, but not to millets. *Scientific African* **9**:e00482 DOI 10.1016/j.sciaf.2020.e00482.

Hayes F, Sharps K, Harmens H, Roberts I, Mills G. 2020b. Tropospheric ozone pollution reduces the yield of African crops. *Journal of Agronomy and Crop Science* **206**:214–228 DOI 10.1111/jac.12376.

Hetherington AM, Woodward FI. 2003. The role of stomata in sensing and driving environmental change. *Nature* **424**:901–908 DOI 10.1038/nature01843.

Holder AJ, Hayes F. 2022. Substantial yield reduction in sweet potato due to tropospheric ozone, the dose-response function. *Environmental Pollution* **304(2)**:119209 DOI 10.1016/j.envpol.2022.119209.

Hossain MS, Islam MN, Rahman MM, Mostofa MG, Khan MAR. 2022. Sorghum: a prospective crop for climatic vulnerability, food and nutritional security. *Journal of Agriculture and Food Research* **8(2)**:100300 DOI 10.1016/j.jafr.2022.100300.

Jarvis P. 1976. The interpretation of the variations in leaf water potential and stomatal conductance found in canopies in the field. *Philosophical Transactions of the Royal Society B: Biological Sciences* **273(927)**:593–610 DOI 10.1098/rstb.1976.0035.

Karlsson P, Braun S, Broadmeadow M, Elvira S, Emberson L, Gimeno B, Le Thiec D, Novak K, Oksanen E, Schaub M. 2007. Risk assessments for forest trees: the performance of the ozone flux versus the AOT concepts. *Environmental Pollution* **146(3)**:608–616 DOI 10.1016/j.envpol.2006.06.012.

Kreyling J, Schweiger AH, Bahn M, Ineson P, Migliavacca M, Morel-Journel T, Christiansen JR, Schtickzelle N, Larsen KS. 2018. To replicate, or not to replicate-that is the question: how to tackle nonlinear responses in ecological experiments. *Ecology Letters* **21(11)**:1629–1638 DOI 10.1111/ele.13134.

Kunhikrishnan T, Lawrence MG, von Kuhlmann R, Richter A, Ladstätter-Weißenmayer A, Burrows JP. 2004. Analysis of tropospheric NO_x over Asia using the model of atmospheric transport and chemistry (MATCH-MPIC) and GOME-satellite observations. *Atmospheric Environment* **38**:581–596 DOI 10.1016/j.atmosenv.2003.09.074.

Leventidou E, Weber M, Eichmann K-U, Burrows JP, Heue K-P, Thompson AM, Johnson BJ. 2018. Harmonisation and trends of 20-year tropical tropospheric ozone data. *Atmospheric Chemistry and Physics* **18**:9189–9205 DOI 10.5194/acp-18-9189-2018.

Li P, Calatayud V, Gao F, Uddling J, Feng Z. 2016. Differences in ozone sensitivity among woody species are related to leaf morphology and antioxidant levels. *Tree Physiology* **36**:1105–1116 DOI 10.1093/treephys/tpw042.

Li S, Courbet G, Ourry A, Ainsworth EA. 2019. Elevated ozone concentration reduces photosynthetic carbon gain but does not alter leaf structural traits, nutrient composition or biomass in switchgrass. *Plants* **8**:85 DOI 10.3390/plants8040085.

Li S, Leakey AD, Moller CA, Montes CM, Sacks EJ, Lee D, Ainsworth EA. 2023. Similar photosynthetic but different yield responses of C3 and C4 crops to elevated O_3. *Proceedings of the National Academy of Sciences of the United States of America* **120**:e2313591120 DOI 10.1073/pnas.2313591120.

Li S, Moller CA, Mitchell NG, Lee D, Ainsworth EA. 2021. Bioenergy sorghum maintains photosynthetic capacity in elevated ozone concentrations. *Plant, Cell & Environment* **44**:729–746 DOI 10.1111/pce.13962.

Li S, Moller CA, Mitchell NG, Lee D, Sacks EJ, Ainsworth EA. 2022. Testing unified theories for ozone response in C_4 species. *Global Change Biology* **28**:3379–3393 DOI 10.1111/gcb.16108.

McGrath JM, Betzelberger AM, Wang S, Shook E, Zhu X-G, Long SP, Ainsworth EA. 2015. An analysis of ozone damage to historical maize and soybean yields in the United States. *Proceedings of the National Academy of Sciences of the United States of America* **112**:14390–14395 DOI 10.1073/pnas.1509777112.

Mills G, Pleijel H, Braun S, Büker P, Bermejo V, Calvo E, Danielsson H, Emberson L, Fernández IG, Grünhage L. 2011. New stomatal flux-based critical levels for ozone effects on vegetation. *Atmospheric Environment* **45**:5064–5068 DOI 10.1016/j.atmosenv.2011.06.009.

Mills G, Sharps K, Simpson D, Pleijel H, Frei M, Burkey K, Emberson L, Uddling J, Broberg M, Feng Z. 2018. Closing the global ozone yield gap: quantification and cobenefits for multistress tolerance. *Global Change Biology* **24**:4869–4893 DOI 10.1111/gcb.14381.

Monks PS, Archibald A, Colette A, Cooper O, Coyle M, Derwent R, Fowler D, Granier C, Law KS, Mills G. 2015. Tropospheric ozone and its precursors from the urban to the global scale from air quality to short-lived climate forcer. *Atmospheric Chemistry and Physics* **15**:8889–8973 DOI 10.5194/acp-15-8889-2015.

Montes CM, Demler HJ, Li S, Martin DG, Ainsworth EA. 2022. Approaches to investigate crop responses to ozone pollution: from O_3-FACE to satellite-enabled modeling. *The Plant Journal* **109**:432–446 DOI 10.1111/tpj.15501.

Paoletti E, Grulke NE. 2005. Does living in elevated CO2 ameliorate tree response to ozone? A review on stomatal responses. *Environmental Pollution* **137**:483–493 DOI 10.1016/j.envpol.2005.01.035.

Parry C, Blonquist JM Jr, Bugbee B. 2014. In situ measurement of leaf chlorophyll concentration: analysis of the optical/absolute relationship. *Plant, Cell & Environment* **37**:2508–2520 DOI 10.1111/pce.12324.

Peng J, Shang B, Xu Y, Feng Z, Calatayud V. 2020. Effects of ozone on maize (*Zea mays* L.) photosynthetic physiology, biomass and yield components based on exposure-and flux-response relationships. *Environmental Pollution* **256**:113466 DOI 10.1016/j.envpol.2019.113466.

Peng J, Shang B, Xu Y, Feng Z, Pleijel H, Calatayud V. 2019. Ozone exposure-and flux-yield response relationships for maize. *Environmental Pollution* **252**:1–7 DOI 10.1186/2193-1801-2-222.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2021. nlme: linear and nonlinear mixed effects models_. R package version 3.1-153. *Available at https://CRAN.R-project.org/package=nlme*.

R Core Team. 2021. *R: a language and environment for statistical computing*. Vienna, Austria: The R Foundation for Statistical Computing. *Available at https://www.R-project.org/*.

Regassa TH, Wortmann CS. 2014. Sweet sorghum as a bioenergy crop: literature review. *Biomass and Bioenergy* **64**:348–355 DOI 10.1016/j.biombioe.2014.03.052.

Ritz C, Baty F, Streibig JC, Gerhard D. 2015. Dose-response analysis using R. *PLOS ONE* **10(12)**:e0146021 DOI 10.1371/journal.pone.0146021.

Roth-Nebelsick A, Grein M, Utescher T, Konrad W. 2012. Stomatal pore length change in leaves of *Eotrigonobalanus furcinervis* (Fagaceae) from the Late Eocene to the Latest Oligocene and its impact on gas exchange and CO_2 reconstruction. *Review of Palaeobotany and Palynology* **174**:106–112 DOI 10.1016/j.revpalbo.2012.01.001.

Schneider GF, Cheesman AW, Winter K, Turner BL, Sitch S, Kursar TA. 2017. Current ambient concentrations of ozone in Panama modulate the leaf chemistry of the tropical tree *Ficus insipida*. *Chemosphere* **172**:363–372 DOI 10.1016/j.chemosphere.2016.12.109.

Shang B, Feng Z, Li P, Yuan X, Xu Y, Calatayud V. 2017. Ozone exposure-and flux-based response relationships with photosynthesis, leaf morphology and biomass in two poplar clones. *Science of the Total Environment* **603**:185–195 DOI 10.1016/j.scitotenv.2017.06.083.

Tai AP, Sadiq M, Pang JY, Yung DH, Feng Z. 2021. Impacts of surface ozone pollution on global crop yields: comparing different ozone exposure metrics and incorporating co-effects of CO_2. *Frontiers in Sustainable Food Systems* **5**:534616 DOI 10.3389/fsufs.2021.534616.

Uddling J, Günthardt-Goerg M, Matyssek R, Oksanen E, Pleijel H, Selldén G, Karlsson P. 2004. Biomass reduction of juvenile birch is more strongly related to stomatal uptake of ozone than to indices based on external exposure. *Atmospheric Environment* **38**:4709–4719 DOI 10.1016/j.atmosenv.2004.05.026.

Wickham H. 2011. The split-apply-combine strategy for data analysis. *Journal of Statistical Software* **40(1)**:1–29 DOI 10.18637/jss.v040.i01.

Wickham H. 2016. *ggplot2: elegant graphics for data analysis*. New York: Springer-Verlag.

Wickham H, Pedersen TL, Seidel D. 2023. scales: scale functions for visualization. R package version 1.3.0. *Available at https://CRAN.R-project.org/package=scales*.

Wittig VE, Ainsworth EA, Naidu SL, Karnosky DF, Long SP. 2009. Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: a quantitative meta-analysis. *Global Change Biology* **15**:396–424 DOI 10.1111/j.1365-2486.2008.01774.x.

Xu Z, Zhou G. 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of Experimental Botany* **59(12)**:3317–3325 DOI 10.1093/jxb/ern185.

Young P, Archibald A, Bowman K, Lamarque J-F, Naik V, Stevenson D, Tilmes S, Voulgarakis A, Wild O, Bergmann D. 2013. Pre-industrial to end 21st century projections of tropospheric ozone from the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP). *Atmospheric Chemistry and Physics* **13(4)**:2063–2090 DOI 10.5194/acp-13-2063-2013.

Yuan JS, Tiller KH, Al-Ahmad H, Stewart NR, Stewart CN Jr. 2008. Plants to power: bioenergy to fuel the future. *Trends in Plant Science* **13(8)**:421–429 DOI 10.1016/j.tplants.2008.06.001.

Zhang W, Feng Z, Wang X, Niu J. 2012. Responses of native broadleaved woody species to elevated ozone in subtropical China. *Environmental Pollution* **163**:149–157 DOI 10.1016/j.envpol.2011.12.035.