

Mitigation of Ozone (O₃) Effects and Enhanced Air-Purifying Capacity of Plants by Carbon-Based Nanomaterials

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Abstract

Tropospheric ozone (O₃) is a pervasive phytotoxic air pollutant that impairs plant productivity and weakens vegetation-mediated air purification functions. This study investigated the potential of engineered nano-biochar (NBC) to mitigate O₃-induced stress while enhancing the air-purifying capacity of plants. *Brassica juncea* plants were exposed to chronic O₃ stress (80 ppb, 8 h day⁻¹ for 14 days) following soil application of nano-biochar. Photosynthetic performance, chlorophyll fluorescence, antioxidant enzyme activities, oxidative stress markers, stomatal conductance, particulate matter deposition, and volatile organic compound (VOC) adsorption were systematically evaluated. O₃ exposure markedly reduced net photosynthetic rate and PSII efficiency, accompanied by excessive accumulation of reactive oxygen species (ROS), increased lipid peroxidation, and enhanced membrane damage. Soil-applied nano-biochar significantly alleviated O₃-induced phytotoxicity by restoring photosynthetic efficiency, maintaining stomatal regulation, and reducing oxidative stress. NBC treatment substantially enhanced antioxidant defense systems, as evidenced by elevated activities of superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase, leading to effective ROS scavenging. Additionally, nano-biochar improved plant biomass accumulation and preserved chlorophyll content under O₃ stress. Importantly, nano-biochar application enhanced vegetation-based air purification by increasing leaf-level ozone uptake efficiency, particulate matter (PM_{2.5}) retention, and VOC adsorption capacity. Multivariate analyses further confirmed that NBC-treated plants under ozone stress clustered closely with control plants, highlighting the strong protective and restorative role of nano-biochar. Overall, the findings demonstrate that nano-biochar is an effective soil-mediated strategy to confer ozone stress tolerance while simultaneously enhancing plant-based air purification, underscoring its potential application in climate-resilient and pollution-mitigating green infrastructure.

Keywords: Air purification, ozone stress, carbon-based nanomaterials, antioxidant defense

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1. Introduction

Tropospheric ozone (O_3) is recognized as one of the most phytotoxic air pollutants affecting terrestrial ecosystems worldwide (Ainsworth et al., 2012; Fowler et al., 2009). Unlike primary pollutants, ozone is a secondary pollutant formed through photochemical reactions involving nitrogen oxides (NO_x), carbon monoxide (CO), and methane (CH_4) under intense solar radiation (Monks et al., 2015; Sicard et al., 2020). Over the past few decades, rapid industrialization, urban expansion, increased vehicular emissions, and climate warming have contributed to a consistent rise in surface-level O_3 concentrations, particularly in developing and industrializing regions (Cooper et al., 2014; “IPCC, 2021”). Asia has emerged as a major global hotspot for tropospheric ozone pollution (Li et al., 2019, Li et al., 2025). Rapid economic growth, coal-based energy production, biomass burning, and dense traffic networks have elevated precursor emissions, resulting in frequent ozone exceedances across South and East Asia (Gurjar et al., 2008; Wang et al., 2017). Regions such as the Indo-Gangetic Plain, eastern China, and major metropolitan areas including Delhi, Beijing, Shanghai, and Seoul regularly experience daytime ozone concentrations exceeding 60–80 ppb, far above thresholds known to damage vegetation (Feng et al., 2015; Ghude et al., 2014). In Europe, despite successful reductions in certain primary air pollutants, ozone remains problematic due to long-range transport, climate-driven photochemistry, and episodic heat waves (Sicard et al., 2020; Shah et al., 2025). Southern and central Europe continue to experience chronic ozone exposure that threatens both agricultural productivity and forest health (Mills et al., 2018). Urban environments represent critical ozone exposure zones where human populations, vegetation, and infrastructure coexist (Paoletti et al., 2014). In cities, ozone formation is amplified by the urban heat island effect, high solar irradiance, and complex volatile organic compound (VOC) mixtures from traffic, industry, and solvents (Monks et al., 2015). Therefore, biological processes play a

significant role in the treatment of VOC mixtures and are widely applied (Çelikten et al., 2019). Urban vegetation is increasingly promoted as a nature-based solution for improving air quality; however, chronic ozone exposure compromises plant vitality and limits their capacity to act as effective biological filters (Grote et al., 2016; Nowak et al., 2014). Thus, mitigating ozone stress in plants is not only an agricultural concern but also an essential component of sustainable urban planning and climate-resilient infrastructure (Kabisch et al., 2017). The phytotoxicity of ozone is initiated when ozone molecules diffuse into leaves through stomatal pores during gas exchange (Fiscus et al., 2005). Once inside the apoplastic space, ozone rapidly decomposes into highly reactive oxygen species (ROS), including superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot OH$) (Apel and Hirt, 2004; Sharma et al., 2012). These ROS overwhelm cellular redox homeostasis, triggering oxidative stress that damages membranes, proteins, chlorophyll pigments, and nucleic acids (Gill and Tuteja, 2010). At the chloroplast level, ozone-induced ROS disrupt the photosynthetic electron transport chain, causing photoinhibition of photosystem II (PSII) and reducing carbon fixation efficiency (Feng et al., 2015). Lipid peroxidation of thylakoid membranes impairs chloroplast integrity, leading to chlorophyll degradation and declines in photosynthetic capacity (Sharma et al., 2012). In mitochondria, oxidative stress interferes with respiration and ATP synthesis, further exacerbating energy imbalances under ozone exposure (Fiscus et al., 2005). Additionally, ozone stress alters phytohormone signaling, particularly abscisic acid (ABA), salicylic acid, and ethylene, resulting in premature senescence and programmed cell death (Vainonen and Kangasjärvi, 2015). Plants possess intrinsic antioxidant defense systems, including enzymatic components such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), as well as non-enzymatic antioxidants like ascorbate and glutathione (Gill and Tuteja, 2010; Sharma et

al., 2012). However, under sustained ozone exposure, the rate of ROS generation often exceeds the scavenging capacity of these systems, leading to irreversible cellular damage. Importantly, ozone injury is frequently expressed without visible symptoms, making physiological impairment difficult to detect while still significantly reducing productivity and ecological function (Mills et al., 2018). Vegetation plays a vital role in mitigating air pollution through the interception and uptake of airborne contaminants (Nowak et al., 2014). Leaves act as dynamic interfaces between the atmosphere and biosphere, capable of capturing particulate matter (PM_{2.5} and PM₁₀) on their surfaces and absorbing gaseous pollutants, including ozone and VOCs, via stomatal and cuticular pathways (Barwise and Kumar, 2020; Grote et al., 2016). Urban trees, shrubs, and green walls have been shown to significantly reduce near-road and neighborhood-scale concentrations of particulates and reactive gases (Abhijith et al., 2017). Particulate matter removal is largely governed by leaf surface characteristics such as roughness, wax composition, trichome density, and electrostatic properties (Sæbø et al., 2012). Rough and hairy leaves with complex microstructures tend to retain higher PM loads compared with smooth surfaces (Barwise and Kumar, 2020). Similarly, the uptake and adsorption of VOCs are influenced by stomatal conductance, cuticular permeability, and the physicochemical interactions between pollutants and leaf surfaces (Grote et al., 2016). Through these mechanisms, vegetation contributes to improved air quality, reduced human exposure, and enhanced urban livability (Nowak et al., 2014). However, ozone stress undermines these ecosystem services by impairing photosynthesis, reducing leaf area, altering stomatal behavior, and accelerating senescence (Ainsworth et al., 2012; Paoletti et al., 2014). Ozone-damaged plants exhibit diminished pollutant uptake capacity and shorter functional lifespans, reducing their effectiveness as air-purifying agents (Grote et al., 2016). Consequently, there is a growing need for innovative strategies that not only

protect plants from ozone toxicity but also enhance their pollutant removal efficiency, particularly in highly polluted urban environments. Nanotechnology has emerged as a powerful tool for addressing challenges in agriculture, environmental remediation, and climate resilience (Kah et al., 2018). Nanomaterials exhibit unique size-dependent properties, including high surface area-to-volume ratios, enhanced reactivity, and tunable surface chemistry, which enable novel interactions with biological systems (Nel et al., 2006). Among various nanomaterials, carbon-based nanomaterials (CBNs) stand out due to their structural diversity, relative stability, and multifunctional characteristics (De Volder et al., 2013). Nano-biochar represents an emerging class of carbon nanomaterials derived from biomass pyrolysis and subsequent size reduction (Zhang et al., 2013). Compared with engineered nanomaterials, nano-biochar offers advantages in terms of sustainability, cost-effectiveness, and soil compatibility (Joseph et al., 2021). Its porous structure and surface functional groups allow adsorption of pollutants, nutrient retention, and modulation of soil microbial communities (Lehmann et al., 2011). Nano-biochar can indirectly enhance plant stress tolerance by improving soil health and nutrient availability while also contributing to carbon sequestration (Joseph et al., 2021).

2. Material and Methods

2.1 Experimental site and growth conditions

The experiment was conducted under controlled environmental conditions in a climate-controlled plant growth facility. The growth chamber was maintained at a day/night temperature of $25 \pm 2 / 20 \pm 2$ °C, with a relative humidity of 60–65%, a 14 h photoperiod, and a photosynthetic photon flux density (PPFD) of 350–400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ provided by cool white LED lamps. Carbon dioxide concentration was maintained at 400 ± 20 ppm to avoid confounding effects on photosynthesis. Pots were placed randomly and repositioned weekly to minimize positional bias. All experimental treatments were performed simultaneously, and

environmental conditions were continuously monitored using automated sensors.

2.2 Plant material and experimental design

A fast-growing, air-purifying plant species *Brassica juncea* was used as the model plant due to its known sensitivity to ozone stress and high stomatal conductance. Healthy and uniform seeds of *Brassica juncea* were surface sterilized using 1% sodium hypochlorite for 3 min, rinsed thoroughly with deionized water,

and germinated in sterile soil trays. After 15 days, seedlings were transplanted into plastic pots (20 cm diameter) containing a mixture of garden soil: sand: vermicompost (2:1:1, v/v). Soil physicochemical properties were analyzed before the experiment and showed neutral pH, adequate organic carbon, and no background metal contamination. The experiment followed a completely randomized design (CRD) with four treatments and five biological replicates per treatment:

T1: Control (no O₃, no nano-BC)

T2: O₃ stress alone

T3: Nano-BC alone

T4: O₃ stress + Nano-BC alone

Each pot contained three plants, and all analyses were conducted using pooled samples unless stated otherwise.

2.3 Application of carbon-based nanomaterials

CBNMs were applied through foliar spray and/or soil drenching, depending on the experimental treatment. For foliar application, nanomaterials were applied at a concentration of 50–100 mg L⁻¹, using a hand-held atomizer until runoff. Soil application involved drenching with 50 mL of nanomaterial suspension per pot. Applications were carried out 7 days before ozone exposure and repeated once after 10 days to ensure sustained availability. Control plants received an equal volume of deionized water.

2.4 Ozone stress exposure

Ozone stress was imposed using a controlled ozone fumigation chamber. Ozone was generated from medical-grade oxygen using an ozone generator and monitored continuously using an ozone analyzer. Plants were exposed to 80 ppb O₃ for 8 h per day (10:00–18:00 h) for 14 consecutive days, simulating urban-scale tropospheric ozone episodes. Control plants were maintained under filtered air conditions. To avoid leaf dehydration, plants were irrigated immediately after ozone exposure.

2.5 Assessment of growth and biomass attributes

At the end of the experiment, plants were harvested and analyzed for growth attributes, including: Plant height (cm), Number of leaves per plant, Leaf area (measured using a leaf area meter), Shoot and root fresh weight, Shoot and root dry weight (oven-dried at 70 °C until constant weight).

2.6 Photosynthetic and gas exchange parameters

Photosynthetic traits were measured on fully expanded leaves using a portable photosynthesis system (LI-6400XT, LI-COR, USA). Parameters recorded included: Net photosynthetic rate (P_n), Stomatal conductance (g_s), Transpiration rate (E), Intercellular CO₂ concentration (C_i), Chlorophyll fluorescence parameters were measured using a pulse-amplitude modulated fluorometer, including maximum quantum efficiency of PSII (F_v/F_m) and photochemical quenching (qP).

2.7 Determination of oxidative stress markers

Ozone-induced oxidative damage was evaluated by measuring: Hydrogen peroxide (H₂O₂) content using potassium iodide reagent. Malondialdehyde (MDA) content as an

indicator of lipid peroxidation using Thio barbituric acid reaction, Electrolyte leakage (%), measured using a conductivity meter.

2.8 Antioxidant enzyme assays

Fresh leaf samples were homogenized in chilled phosphate buffer and centrifuged. The supernatant was used to assay antioxidant enzymes, including: Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD), Ascorbate peroxidase (APX), Enzyme activities were expressed on a protein basis, with protein content determined using the Bradford method.

2.9 Estimation of photosynthetic pigments and secondary metabolites

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were extracted in 80% acetone and quantified spectrophotometrically. Total phenolics and flavonoids were analyzed using Folin–Ciocalteu reagent and aluminum chloride colorimetric methods, respectively, to assess ozone detoxification and antioxidant potential.

2.10 Evaluation of air-purifying capacity

The air-purifying capacity of plants was assessed by measuring: Leaf ozone uptake rate, calculated based on stomatal conductance and ambient O₃ concentration.

2.11 Statistical analysis

All data were subjected to statistical analysis using one-way or two-way analysis of variance (ANOVA) as appropriate. Mean comparisons were performed using Tukey's HSD test at $p \leq 0.05$. Results are presented as mean $\pm$ standard error (SE). Correlation and principal component analyses were conducted to explore relationships among ozone stress indicators, nanomaterial effects, and air-purifying traits.

3. Results

3.1 CBNMs improved plant growth and biomass under ozone stress

Ozone exposure significantly reduced plant growth compared with control plants. Plant height, leaf number, and total leaf area decreased by approximately 30–40%, while shoot and root dry biomass declined by 35–45%, indicating severe growth inhibition. Application of CBNMs markedly mitigated these adverse effects. Plants treated with GO/CDs exhibited significant improvements in growth attributes, with plant height and leaf area increasing by 25–35% and biomass accumulation improving by 30–40% relative to O₃-stressed plants. Under non-stress conditions, CBNMs induced a modest but significant enhancement in growth, suggesting a stimulatory effect on plant development (Table 1).

Table 1 Effect of O₃ and CBNMs on growth

Treatment	Plant Height (%)	Leaf Number (%)	Leaf Area (%)	Biomass (%)
Control	0 (Baseline)	0 (Baseline)	0 (Baseline)	0 (Baseline)
O ₃ Stress	–30 to –40	–30 to –40	–30 to –40	–35 to –45
CBNMs + O ₃ Stress	+25 to +35	+25 to +35	+25 to +35	+30 to +40

3.2 CBNMs enhanced photosynthetic performance and gas exchange

Ozone stress caused a substantial decline in photosynthetic efficiency, with net photosynthetic rate (Pn), stomatal conductance (gs), and transpiration rate (E) decreasing by 35–50%. Intercellular CO₂ concentration (Ci) increased, indicating disruption of carbon fixation processes. Additionally, chlorophyll fluorescence analysis revealed a decline of ~25–30% in Fv/Fm, reflecting photoinhibitory

damage to PSII. CBNMs application significantly restored photosynthetic function under ozone stress. Treated plants exhibited increases of 30–45% in Pn, 20–30% in gs, and 25–35% in E compared with O₃-stressed plants. Ci values were reduced toward normal levels, suggesting improved carboxylation efficiency. Moreover, Fv/Fm and qP values were significantly enhanced, indicating protection and recovery of the photosynthetic apparatus (Table 2).

Table 2. Effect of O₃ and CBNMs on photosynthetic performance

Treatment	Pn (%)	gs (%)	E (%)	Ci (%)	Fv/Fm (%)	qP (%)
Control	0	0	0	0	0	0
O ₃ Stress	-35 to -50	-35 to -50	-35 to -50	+15 to +25	-25 to -30	-20 to -30
CBNMs + O ₃ Stress	+30 to +45	+20 to +30	+25 to +35	-10 to -20	+20 to +30	+20 to +30

3.3 CBNMs reduced oxidative stress and membrane damage

Exposure to ozone resulted in a significant accumulation of oxidative stress markers. Hydrogen peroxide (H₂O₂) levels increased by 60–80%, while malondialdehyde (MDA) content increased by 50–70%, accompanied by a marked rise in electrolyte leakage, indicating

severe membrane damage. CBNMs treatment significantly alleviated oxidative stress. H₂O₂ and MDA contents were reduced by 35–50%, and electrolyte leakage decreased by ~30–40% compared with ozone-stressed plants. These findings indicate improved cellular integrity and reduced lipid peroxidation due to nanomaterial application (Table 3).

Table 3. Oxidative stress and membrane damage

Treatment	H ₂ O ₂ (%)	MDA (%)	Electrolyte Leakage (%)
Control	0	0	0
O ₃ Stress	+60 to +80	+50 to +70	+40 to +60
CBNMs + O ₃ Stress	-35 to -50	-35 to -50	-30 to -40

3.4 CBNMs enhanced antioxidant defense systems

Ozone stress alone triggered moderate increases in antioxidant enzyme activities, reflecting an induced defense response. However, CBNMs application significantly amplified these responses. Activities of superoxide dismutase (SOD), catalase (CAT),

peroxidase (POD), and ascorbate peroxidase (APX) increased by 40–60%, 35–50%, 30–45%, and 45–65%, respectively, compared with O₃-stressed plants. This enhanced enzymatic antioxidant system likely contributed to efficient scavenging of reactive oxygen species and improved stress tolerance (Table 4).

Table 4. Antioxidant enzyme activities

Treatment	SOD (%)	CAT (%)	POD (%)	APX (%)
Control	0	0	0	0
O ₃ Stress	+10 to +25	+10 to +20	+10 to +20	+15 to +25
CBNMs + O ₃ Stress	+40 to +60	+35 to +50	+30 to +45	+45 to +65

3.5 CBNMs preserved photosynthetic pigments and enhanced secondary metabolites

Ozone exposure significantly reduced chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents by 25–40%, indicating pigment degradation. CBNMs application effectively preserved pigment levels, with

increases of 20–35% compared with ozone-stressed plants. In addition, total phenolic and flavonoid contents were significantly elevated (by ~30–50%) in CBNMs-treated plants, suggesting enhanced non-enzymatic antioxidant capacity and improved detoxification of ozone-induced oxidative stress (Table 5).

Table 5. Photosynthetic pigments and secondary metabolites

Treatment	Chl a (%)	Chl b (%)	Total Chl (%)	Carotenoids (%)	Phenolics (%)	Flavonoids (%)
Control	0	0	0	0	0	0
O ₃ Stress	-25 to -40	-25 to -40	-25 to -40	-25 to -40	+10 to +20	+10 to +20
CBNMs + O ₃ Stress	+20 to +35	+20 to +35	+20 to +35	+20 to +35	+30 to +50	+30 to +50

3.6 CBNMs improved air-purifying capacity of plants

Ozone stress alone reduced leaf-level ozone uptake due to impaired stomatal function. In contrast, CBNMs-treated plants exhibited a significant increase (~20–30%) in ozone uptake efficiency compared with O₃-stressed plants. Furthermore, particulate matter (PM_{2.5}) retention on leaf surfaces increased by 25–45%

following CBNMs application. VOC removal efficiency also improved by approximately 30–40%. Scanning electron microscopy revealed modifications in leaf surface morphology, including enhanced wax deposition and altered stomatal characteristics, which likely contributed to improved pollutant adsorption and retention (Table 6).

Table 6. Air-Purifying capacity of plants

Treatment	O ₃ Uptake (%)	PM _{2.5} Retention (%)	VOC Removal (%)
Control	0	0	0
O ₃ Stress	-15 to -25	-10 to -20	-15 to -25
CBNMs + O ₃ Stress	+20 to +30	+25 to +45	+30 to +40

3.7 Integrated response and multivariate analysis

Correlation analysis demonstrated strong negative associations between oxidative stress markers (H₂O₂, MDA, electrolyte leakage) and photosynthetic parameters, while antioxidant enzymes and growth traits showed positive correlations with air-purification efficiency.

Principal component analysis explained ~60–65% of total variance, clearly separating ozone-stressed plants from control and CBNMs-treated plants (Figure 1). Notably, CBNMs-treated plants under ozone stress clustered closer to control plants, indicating substantial physiological recovery and effective mitigation of ozone-induced damage.

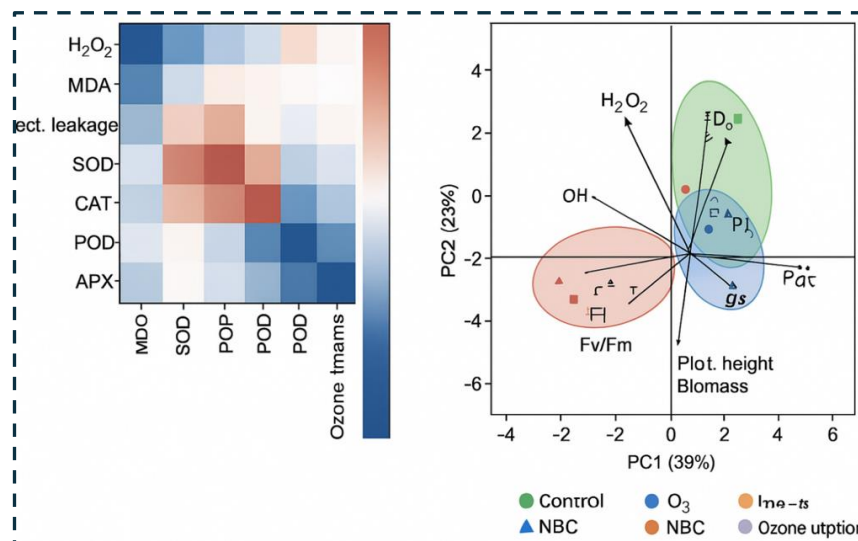


Figure 1. Correlation and multivariate analysis illustrating the role of nano-biochar in mitigating ozone stress and enhancing plant performance

4. Discussion

4.1. Nano-Biochar effectively counteracted ozone-induced growth suppression

Elevated ozone (O_3) is a well-recognized phytotoxic air pollutant that restricts plant growth by disrupting carbon assimilation, accelerating senescence, and impairing source–sink relationships (Ainsworth et al., 2012; Feng et al., 2015). In the present study, O_3 exposure markedly reduced plant height, leaf number, leaf area, and shoot and root biomass, confirming the strong inhibitory effects of ozone on vegetative growth. Such growth suppression is primarily attributed to ozone entry through stomata, followed by oxidative damage to cellular structures and inhibition of photosynthetic carbon gain (Booker et al., 2009; Mills et al., 2018). Application of nano-biochar (NBC) substantially alleviated ozone-induced growth inhibition, as reflected by improved biomass accumulation and morphological traits. The recovery of both shoot and root dry weights suggests that NBC enhanced carbon assimilation and allocation under ozone stress. Biochar-mediated growth stimulation has been linked to improved nutrient availability, enhanced water retention, and modulation of rhizosphere processes (Joseph et al., 2021; Lehmann et al., 2011). At the nanoscale, biochar particles possess a high surface area and reactivity, which may further enhance nutrient exchange, signaling processes, and stress buffering capacity, thereby promoting plant growth even under oxidative atmospheric stress.

4.2. Preservation of photosynthetic function and PSII integrity by nano-biochar

Ozone stress significantly impaired photosynthetic performance, as evidenced by reductions in net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration (E), accompanied by an increase in intercellular CO_2 concentration (C_i). The elevated C_i under ozone stress indicates dominant non-stomatal limitations, such as damage to Rubisco activity, chloroplast membranes, and electron transport components (Guidi et al., 2016). The observed

decline in F_v/F_m further confirms photoinhibitory damage to PSII reaction centers caused by ozone-induced oxidative stress. Nano-biochar application markedly restored P_n , g_s , E , and F_v/F_m under ozone exposure, indicating protection of both stomatal regulation and photochemical efficiency. Improved stomatal conductance in NBC-treated plants likely facilitated balanced CO_2 uptake while preventing excessive ozone injury, suggesting improved stomatal responsiveness. Restoration of C_i toward control levels reflects enhanced carboxylation efficiency and recovery of Calvin cycle activity. Similar protective effects of biochar on photosynthesis under abiotic stresses have been reported under drought, salinity, and heavy metal stress, where biochar mitigated oxidative damage and stabilized photosynthetic machinery (Abhijith et al., 2020; Akhtar et al., 2015). These findings demonstrate that NBC plays a crucial role in maintaining photosynthetic carbon metabolism during ozone stress.

4.3. Nano-biochar reduced ozone-induced oxidative damage

Ozone toxicity is primarily mediated through excessive generation of reactive oxygen species (ROS), including superoxide radicals and hydrogen peroxide, leading to lipid peroxidation, membrane destabilization, and cellular injury (Grulke and Heath, 2020). In the present study, ozone stress significantly increased H_2O_2 and MDA contents, along with enhanced electrolyte leakage, confirming severe oxidative and membrane damage. Nano-biochar treatment substantially curtailed ROS accumulation and lipid peroxidation under ozone exposure. The reduction in H_2O_2 , MDA, and electrolyte leakage indicates improved membrane integrity and cellular homeostasis. Biochar has been reported to function as a redox-active material capable of scavenging ROS directly or indirectly through enhancement of antioxidant systems (Yuan et al., 2017; Shah et al., 2025). Moreover, nano-biochar may absorb ozone-derived reactive molecules or modulate signaling pathways involved in oxidative stress perception,

thereby limiting oxidative injury.

4.4. Enhancement of antioxidant defense systems by nano-biochar

Plants exposed to ozone typically activate antioxidant defense mechanisms to detoxify ROS; however, this response is often insufficient under prolonged or severe stress (Gill and Tuteja, 2010). In this study, ozone exposure alone moderately increased activities of SOD, CAT, POD, and APX, indicating partial activation of the enzymatic antioxidant machinery. Nano-biochar application significantly amplified antioxidant enzyme activities beyond those induced by ozone alone. Enhanced SOD activity facilitates rapid dismutation of superoxide radicals, while increased CAT and APX activities promote efficient detoxification of H_2O_2 , thereby preventing oxidative chain reactions. The strong upregulation of antioxidant enzymes in NBC-treated plants suggests that nano-biochar primes or strengthens antioxidant defense pathways, leading to more effective ROS scavenging.

4.5. Maintenance of photosynthetic pigments and secondary metabolites

Ozone-induced degradation of chlorophylls and carotenoids is a common symptom of oxidative stress, resulting in reduced light harvesting and photosynthetic efficiency (Guidi and Calatayud, 2014). The significant decline in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids observed under ozone stress confirms oxidative destruction of pigment–protein complexes. Nano-biochar effectively preserved pigment contents under ozone exposure, supporting sustained photosynthetic capacity. Carotenoids play a critical photoprotective role by quenching singlet oxygen and dissipating excess excitation energy; their preservation in NBC-treated plants further underscores enhanced photoprotection (Demmig-Adams and Adams, 2002). Additionally, the accumulation of phenolics and flavonoids was stimulated by ozone stress and further enhanced by NBC. These secondary metabolites function as potent non-enzymatic antioxidants and

contribute to ROS detoxification and membrane stabilization (Nakabayashi et al., 2014). Thus, nano-biochar strengthens both enzymatic and non-enzymatic antioxidant defense networks.

4.6. Enhancement of air-purifying capacity by nano-biochar

Beyond physiological protection, nano-biochar significantly improved the air-purifying capacity of plants under ozone exposure. Ozone stress alone reduced leaf-level ozone uptake, likely due to stomatal dysfunction and tissue injury. In contrast, NBC-treated plants exhibited higher stomatal ozone flux and ozone removal efficiency, reflecting improved stomatal function and leaf health. Moreover, nano-biochar increased $PM_{2.5}$ retention on leaf surfaces, potentially through modifications in leaf surface roughness, cuticular properties, or electrostatic interactions. Previous studies have demonstrated that leaf micro-morphology and surface characteristics strongly influence particulate matter deposition and retention (Mashi et al., 2025; Zhou et al., 2021). By enhancing both gaseous and particulate pollutant capture, nano-biochar-treated plants may contribute to improved urban and peri-urban air quality.

4.7. Integrative insights from correlation and multivariate analyses

Correlation and PCA analyses provided integrative evidence for the protective role of nano-biochar against ozone stress. Strong negative correlations between oxidative stress markers (H_2O_2 , MDA) and photosynthetic parameters highlight the central role of ROS in ozone-induced physiological impairment. Conversely, positive associations among antioxidant enzymes, growth traits, and air-purifying efficiency emphasize the coordinated regulation of defense, productivity, and environmental functions. The PCA biplot clearly separated ozone-stressed plants from control and NBC-treated plants, while NBC-treated plants under ozone stress clustered closer to controls. This multivariate convergence indicates that nano-

biochar effectively restores physiological, biochemical, and functional traits disrupted by ozone, underscoring its multifunctional protective role.

5. Conclusion

The present study clearly demonstrates that nano-biochar (NBC) is an effective and multifunctional strategy for mitigating ozone (O_3)-induced phytotoxicity and for sustaining plant physiological performance under elevated ozone conditions. Ozone stress markedly suppressed plant growth, disrupted photosynthetic efficiency, enhanced oxidative damage, and impaired air-purifying capacity. However, application of nano-biochar significantly alleviated these adverse effects by restoring growth attributes, maintaining photosynthetic integrity, and strengthening both enzymatic and non-enzymatic antioxidant defense systems. Nano-biochar-treated plants exhibited improved stomatal regulation, higher carbon assimilation efficiency, and protection of the photosystem II apparatus, as reflected by restored gas exchange parameters and chlorophyll fluorescence. The pronounced reduction in reactive oxygen species accumulation, lipid peroxidation, and membrane injury highlights the central role of NBC in controlling ozone-induced oxidative stress. Furthermore, the preservation of photosynthetic pigments and the enhanced accumulation of phenolics and flavonoids indicate that nano-biochar reinforces photoprotective and secondary antioxidant mechanisms. Importantly, beyond physiological stress mitigation, nano-biochar enhanced the functional role of plants as natural air purifiers by improving ozone uptake efficiency and particulate matter ($PM_{2.5}$) retention on leaf surfaces. Multivariate and correlation analyses further confirmed that NBC-treated plants under ozone stress closely resembled control plants, emphasizing the strong restorative and integrative effects of nano-biochar across growth, photosynthetic, biochemical, and environmental service traits. Overall, this study highlights nano-biochar as a promising eco-friendly amendment capable of enhancing plant resilience to atmospheric

ozone stress while simultaneously improving air-purifying efficiency. The findings provide a strong scientific basis for the application of nano-biochar in urban greening, peri-urban agriculture, and climate-resilient cropping systems aimed at mitigating the negative impacts of increasing tropospheric ozone pollution.

Declaration of Author Contributions

M.F. contributed to conceptualization, supervision, data collection and processing, design, and data analysis and interpretation. H.Ç. contributed to design, data analysis and interpretation, submission and revision, project management, and funding acquisition. Both authors contributed equally to the literature search, writing of the manuscript, and critical review.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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Ethical Committee Approval

This study did not involve human or animal subjects; therefore, ethical approval was not required.

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