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Assessing Tropospheric Ozone Influence on Garlic (*Allium sativum L.)* Rhizosphere Microbial Activity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study conducted at the Climate Change Observatory, Woodhouse Farm, HRS, Ooty, during 2018-2019 focused on investigating the impact of ozone and ozone protectants on rhizosphere microbial activity in garlic cultivation. Specifically, the experiment evaluated the response of the commercial local variety Ooty-1 garlic to varying ozone levels and ozone protectants. Results from the study demonstrated notable variations in microbial activity, including bacteria, fungi, and actinomycetes, within the rhizosphere of garlic plants under different experimental conditions. Notably, the highest increase in bacterial activity was observed under ambient conditions with panchagavya spray, recording a significant log10 cfu/g of 8.9. Similarly, fungal and actinomycetes

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activity exhibited higher levels under ambient conditions, with respective log10 cfu/g values of 4.8 and 4.7. Conversely, elevated ozone levels at 150 ppb and 200 ppb led to a decline in bacterial, fungal, and actinomycetes activity within the rhizosphere. The reductions were substantial, with bacterial activity dropping to 2.5 log10 cfu/g and 1.9 log10 cfu/g, fungal activity to 1.1 log10 cfu/g and 0.9 log10 cfu/g, and actinomycetes activity to 2.4 log10 cfu/g and 2.1 log10 cfu/g at the respective ozone levels. However, the application of ozone protectants, including 3% panchagavya, 3% neem oil, and 0.1% ascorbic acid, demonstrated a mitigating effect on the negative impacts of tropospheric ozone on rhizosphere microbial diversity. This finding suggests that escalating concentrations of tropospheric ozone have a detrimental effect on the soil microbial activity of garlic. Still, the use of ozone protectants can significantly alleviate these effects by promoting microbial growth. Overall, the study underscores the importance of understanding the complex interactions between ozone levels, ozone protectants, and rhizosphere microbial activity in garlic cultivation, offering valuable insights for sustainable agricultural practices in the face of climate change challenges.

Keywords: Elevated ozone; microbial count; panchagavya; neem oil; ascorbic acid.

1. INTRODUCTION

Tropospheric ozone (O3) stands as a formidable environmental stressor, exerting profound impacts on terrestrial plant productivity and human health. As a potent greenhouse gas, O3 significantly contributes to global warming and exacerbates climate change, amplifying environmental challenges on a global scale [1]. However, its deleterious effects extend beyond climate impacts alone. Recent studies have highlighted O_3's detrimental impact on terrestrial vegetation productivity and net primary productivity, particularly in regions like China, where major crops such as maize, wheat, rice, and soybeans have experienced yield losses ranging from 4.4% to 12.4% [2,3]. Consequently, O3 emerges not only as a significant contributor to climate change but also as a pervasive global air pollutant, posing substantial risks to both vegetation and human well-being. In India, yield reduction was observed in turnip [4], mustard [5], and garlic [6] due to elevated tropospheric ozone levels of 150 ppb and 200 ppb levels

Root exudates, comprising a diverse array of compounds released by plant roots, play a pivotal role in mediating interactions between plants and soil microbiomes, thereby shaping the composition and dynamics of rhizospheric microbial communities. Under conditions of increased O3 levels, alterations in root exudation patterns, soil environment, and plant function can profoundly impact nitrogen (N) cycling processes, particularly N mineralization [7]. N mineralization, a key indicator of soil nutrient availability, is intricately linked to the abundance and activity of soil microorganisms [8]. Thus, disruptions in microbial diversity induced by O_3 pollution have the potential to disrupt N mineralization processes, resulting in cascading effects on soil fertility and nutrient cycling dynamics.

Microbial diversity serves as a cornerstone in the rhizosphere of soil ecosystems, where microorganisms utilize root exudates as energy sources for growth and development. Through activities such as organic matter decomposition and nutrient turnover, soil microbes play pivotal roles in improving soil organic content, enhancing nutrient availability, and optimizing nutrient use efficiency, ultimately contributing to enhanced crop productivity and soil health. Consequently, the activity and composition of rhizospheric microbial communities emerge as integral determinants of soil quality and fertility.

Against this backdrop, the primary objective of this paper is to investigate the impact of O3 on soil microbial biodiversity across different concentration levels and to examine the responses of soil microbes to various ozone protectants. By elucidating how O3 influences soil microbial communities and exploring potential mitigation strategies using ozone protectants, this study aims to advance our understanding of the complex interactions between atmospheric pollutants, soil microorganisms, and agricultural ecosystems. Ultimately, this research endeavors to inform evidence-based strategies for preserving soil fertility, enhancing crop productivity, and mitigating the adverse effects of O3 pollution on agricultural sustainability and environmental health.

Recent studies corroborate the pressing need to address the multifaceted impacts of O3 pollution on terrestrial ecosystems and underscore the importance of understanding the intricate interplay between atmospheric pollutants and soil microbial communities [9,10]. By integrating recent findings and employing cutting-edge methodologies, this study aims to contribute to the ongoing discourse on sustainable agricultural practices and environmental stewardship in the face of escalating environmental challenges posed by O3 pollution.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted in the subtropical highland climatic region of Western Ghats at 11.4°N, 76.7°E at an altitude of 2520 m mean sea level from September to December 2018 in the experimental Woodhouse farm of Horticultural Research Station, Tamil Nadu Agricultural University, Ooty.

2.2 Treatment Details

The potted experiment of garlic was conducted with two factors, the first factor, treated with three different levels of ozone (EO) (Ambient (AO), 150ppb and 200ppb) and the second factor, treated with three foliar sprayed ozone protectants such as 3% panchagavya, 3% neem oil and 0.1% ascorbic acid under open top chambers (OTCs) with twelve treatments arranged in factorial completely randomized block design (FCRD) with three replications. The treatments were: T1 – AO, T2 – EO @ 150ppb, T3 EO @ 200 ppb, T4 – AO + 3%Panchagavya, T5 AO + 3%Neem oil, T6 – AO + 0.1%Ascorbic acid, T7 – EO @ 150ppb + 3%Panchagavya, T8 – EO @ 150ppb + 3%Neem oil, T9 – EO @ 150ppb + 0.1%Ascorbic acid, T10 – EO @ 200ppb + 3%Panchagavya, T11 – EO @ 200ppb + 3%Neem oil, T12 – EO @ 200ppb + 0.1%Ascorbic acid.

2.3 Microbial Enumeration Methodology

The microbial counts were determined using the pour plate method, a widely employed technique for enumerating microorganisms in soil samples. The procedure involved the following steps:

2.3.1 Sample collection and dilution

A representative soil sample weighing 1 gram was aseptically transferred into a test tube containing 10 ml of sterile blank water. This initial dilution (10-1) served as the starting point for serial dilution. The sample was then serially diluted, typically up to a dilution of 10-6, to achieve dilutions suitable for microbial enumeration.

2.3.2 Preparation of petriplates

From the serial dilutions, aliquots of 1 ml were pipetted out and evenly spread onto sterilized petriplates. For bacterial enumeration, dilutions of 10-6 were used, while dilutions of 10-4 and 10-3 were utilized for fungi and actinomycetes, respectively.

2.3.3 Inoculation and incubation

Each petriplate was filled with the appropriate selective growth medium: Nutrient Agar Media for bacteria, Rose Bengal Agar Media for fungi, and Kenknight's Media for actinomycetes. The plates were then gently rotated in clockwise and anticlockwise directions to ensure uniform distribution of the inoculum within the agar medium. Subsequently, the plates were incubated under controlled conditions: bacterial plates were incubated at 37.5°C for 24 hours, fungal plates at room temperature for 72 hours, and actinomycete plates at room temperature for one week.

2.3.4 Colony counting

Following the respective incubation periods, the plates were removed from the incubator, and the colonies that developed were carefully counted. Each visible colony represented a single viable microbial cell. The colony counts were recorded, and the results were expressed as colony-forming units (cfu) per gram of soil, indicating the density of viable microorganisms present in the soil sample.

By employing this standardized pour plate method and utilizing selective growth media tailored to specific microbial groups, the study ensured the accurate enumeration of bacteria, fungi, and actinomycetes within the garlic rhizosphere. This meticulous approach allowed for the assessment of microbial dynamics in response to elevated tropospheric ozone levels, providing valuable insights into the impact of environmental stressors on soil microbial communities.

2.4 Statistical Analysis

The above characters were statistically analyzed by using SPSS version 16 in two-way analysis of variance (ANOVA) and the significant differences between the means were determined with Duncan's multiple range test to assess the impact of tropospheric ozone on microbial count in garlic.

3. RESULTS AND DISCUSSION

The enumeration of microorganisms (bacteria, fungi and actinomycetes) significantly differed under different levels of tropospheric ozone and ozone protectants as depicted in Fig. 1.

3.1 Bacteria

The analysis of bacterial counts in soil revealed a highly significant difference among all treatments, indicating the profound influence of elevated tropospheric ozone exposures on soil bacterial communities. Specifically, the highest bacterial counts were recorded in treatment T4, where ambient ozone levels were maintained. This finding suggests that under typical atmospheric conditions, bacterial populations thrive, possibly due to the availability of optimal environmental conditions and resources. Following this, treatment T1 exhibited the next highest bacterial counts, indicating that even in the presence of elevated ozone, bacterial populations could persist, albeit to a lesser extent than under ambient conditions. Subsequently, treatments T5 and T6 showed intermediate bacterial counts, suggesting a moderate impact of elevated ozone on bacterial populations. In contrast, the lowest bacterial counts were observed in treatments T3, T9, and T8, indicating a substantial decline in bacterial populations under more severe ozone exposure conditions.

These findings align with previous research conducted by Islam et al. [11], which reported a decline in soil microbial biomass, including bacteria, following exposure to elevated ozone levels over an entire growing season in wheat and soybean soils. Similarly, studies by Phillips et al. [12] have demonstrated alterations in the total microbial population under elevated ozone exposure, suggesting a consistent trend across different plant species and environmental conditions.

3.2 Fungi

Significant differences were observed among all treatments concerning fungi, highlighting the diverse responses of fungal communities to elevated tropospheric ozone exposures. The highest fungal count was recorded in treatment T1, indicating that fungal populations may exhibit resilience or even proliferation under elevated ozone conditions. This resilience could be attributed to certain fungal species' ability to tolerate or even benefit from ozone exposure, potentially due to their unique physiological characteristics or metabolic pathways. Following treatment T1, treatments T4, T6, and T7 exhibited progressively lower fungal counts, suggesting a gradual decline in fungal populations with increasing ozone exposure levels. In contrast, treatments T12, T11, and T9 showed the lowest fungal counts, indicating a significant suppression of fungal populations under the most severe ozone exposure conditions.

These results are consistent with findings by Scagel and Andersen [13] and Yoshida et al. [14], which indicated variable responses of microbial and fungal biomass under elevated ozone stress conditions. Additionally, the observed increase in fungal and bacterial populations in the rhizosphere of sorghum in response to elevated tropospheric ozone, as noted by Shafer [15], highlights the complex interactions between ozone exposure and soil microbial dynamics. Conversely, the decline in soil microbial biomass due to heat stress, with fungal biomass exhibiting greater sensitivity than bacterial biomass, as reported by Riah-Anglet et al. [16], underscores the multifaceted nature of environmental stressors on soil microbial communities.

3.3 Actinomycetes

The significant differences observed among all treatments concerning actinomycetes underscore the diverse responses of actinomycetes populations to elevated tropospheric ozone exposures. The highest count was observed in treatment T1, suggesting that actinomycetes may exhibit resilience or even proliferation under certain ozone exposure conditions. This resilience could be attributed to the unique metabolic capabilities or ecological niches occupied by actinomycetes, which may confer a degree of tolerance to ozone-induced stress. Following treatment T1, treatment T4 exhibited the next highest actinomycetes count, indicating that under ambient ozone levels, actinomycetes population can persist, albeit to a lesser extent than under optimal conditions. Conversely, treatments T3, T12, and T2 showed the lowest actinomycete counts, suggesting a significant suppression of actinomycetes population under more severe ozone exposure conditions.

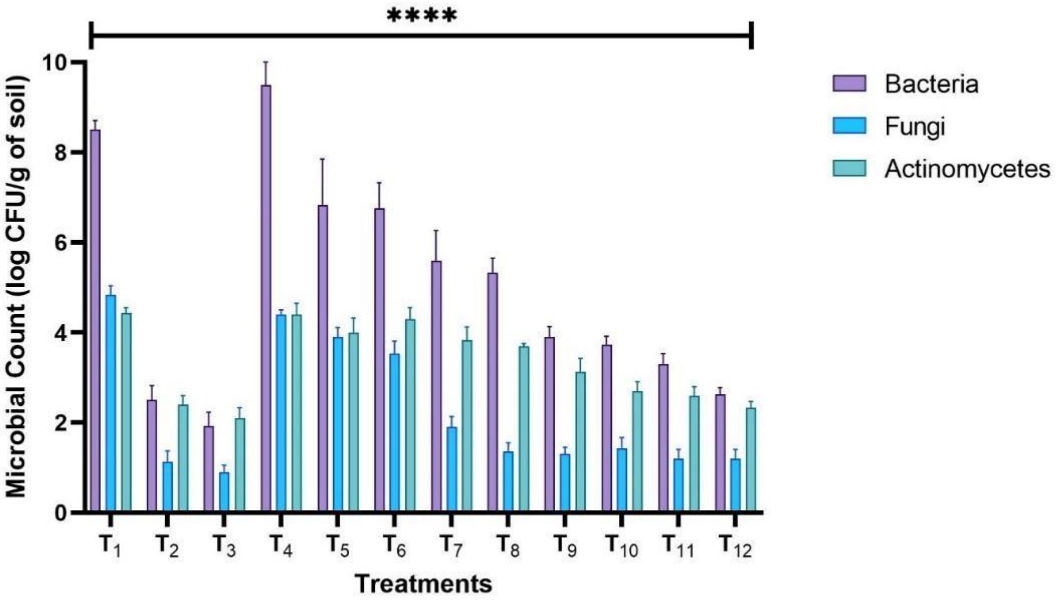

Fig. 1. Enumeration of microorganisms impacted by tropospheric ozone in rhizosphere soil of garlic

These findings are consistent with a study conducted in soybean (*Glycine max*) soil by Zhang et al. [17], which found that actinomycetes enumeration was sensitive to higher ozone concentrations. The observed sensitivity of actinomycete populations to elevated ozone levels further emphasizes the need to consider the broader ecological context and microbial community dynamics when assessing the impacts of environmental stressors on soil ecosystems.

4. SUMMARY AND CONCLUSION

The study investigated the impact of elevated tropospheric ozone exposures on soil microbial communities, focusing on bacteria, fungi, and actinomycetes, in the context of garlic cultivation. Results revealed significant differences in microbial populations across various ozone exposure treatments, highlighting the complex and nuanced responses of soil ecosystems to environmental stressors. Bacterial counts showed a distinct decline under elevated ozone levels, with the most severe suppression observed in treatments with higher ozone concentrations. Fungal populations exhibited variable responses, with some treatments showing resilience or even proliferation under elevated ozone conditions, while others experienced significant suppression. Actinomycete populations also displayed sensitivity to ozone exposure, with higher concentrations leading to decreased counts. These findings underscore the importance of considering the broader ecological context and microbial community dynamics when assessing the impacts of environmental stressors on soil health and agricultural productivity.

The conclusion of our study underscores the vital role of soil microorganisms in facilitating nutrient availability for plant growth, thereby holding immense economic and environmental significance. Elevated tropospheric ozone levels exerted a notable impact on microbial activity within the garlic rhizosphere, leading to diminished crop growth and yield. Our findings confirm that heightened ozone levels corresponded to a decline in soil microbial activity, adversely affecting garlic cultivation. However, the application of ozone protectants proved effective in bolstering microbial populations, thereby stimulating soil microbial activity and mitigating the detrimental effects of tropospheric ozone. These results underscore the potential of targeted interventions, such as the use of ozone protectants, to safeguard soil health and agricultural productivity in the face of environmental challenges. Moving forward, continued research and implementation of sustainable practices are essential to ensure the resilience and sustainability of agricultural systems amidst changing environmental conditions.

5. FUTURE RESEARCH FOCUS

Future research should aim to elucidate the underlying mechanisms driving the observed responses of soil microbial communities to elevated tropospheric ozone levels. Investigating the biochemical and physiological pathways involved in microbial responses to ozone stress can provide mechanistic insights into how soil ecosystems cope with environmental challenges. Long-term studies are essential to assess the cumulative effects of elevated ozone exposure on soil microbial communities and their implications for soil health and agricultural productivity over multiple growing seasons. Monitoring microbial dynamics over extended periods will enable researchers to identify trends and patterns that may not be apparent in short-term studies. Further investigation is needed to evaluate the efficacy of ozone protectants in mitigating the negative impacts of tropospheric ozone on soil microbial communities. Comparative studies examining different types and concentrations of ozone protectants, as well as their long-term effects on soil health and microbial diversity, can provide valuable insights into their potential application in agricultural systems. Genomic and metagenomic approaches to analyze the genetic and functional diversity of soil microbial communities under elevated ozone conditions, shedding light on microbial adaptation strategies and metabolic pathways involved in ozone detoxification.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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