

Metagenomics study of tea rhizosphere soil in elevated carbon dioxide and temperature

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ABSTRACT

One of the most pressing environmental issue of our time is climate change. Additionally, the atmospheric carbon dioxide concentrations and the global temperature are also increasing. North East India is widely known for tea. Tea cultivation is largely dependent on its distinct soil fertility and its associated microorganisms. However, little is known about how high elevated CO₂ and temperature affect the rhizosphere of tea plants. This study was conducted in order to comprehend the general microbial diversity of the tea soil rhizosphere in response to rising temperatures and CO₂. The study was performed using two Open top chambers where four different tea cultivars were exposed to elevated CO₂ and temperature, and only elevated temperature where microbial dynamics was studied using metagenomics of 16s rRNA analysis. The results indicated that in the treatments highest abundance was found in the phylum Proteobacteria, 32.11%, which was followed by Acidobacteria, 14.32% and Actinobacteria, 9.13%. Significant variations were observed in both treatments when compared to the control. The results of this study would thus help in understanding the culturable as well as unculturable bacteria associated with tea plant rhizosphere, which may be beneficial in planning future adaptation and mitigation strategies under climate change scenarios.

Key words: Climate change, Tea, Rhizosphere, Metagenomics, Microorganisms

Introduction

The increasing atmospheric carbon dioxide (CO₂) is of global concern and affects various regions, including North Eastern parts of India. Recent studies have suggested that there has been a gradual increase in atmospheric CO₂ throughout the years. An

average increase of 17 ppm of CO₂ is observed yearly (Roy *et al.*, 2020). The atmospheric CO₂ concentration increased from approximately 315 ppm to 390 ppm in the last fifty-seven years (Bhagat *et al.*, 2016); it is predicted that the average atmospheric CO₂ concentration will reach about 550ppm by 2050 (CHANGE-IPCC, 2007). The minimum temperature

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for the year also exhibited an increasing trend. According to reports, the average minimum temperature has risen by 1-1.3°C during the past 50 years (Bhagat *et al.*, 2016).

The North East region of India is known for its rich biodiversity, dense forests, and diverse ecosystems (Chatterjee *et al.*, 2006). As a result of the favourable subtropical temperature and sufficient rainfall, both macroflora and microflora exhibit a similar diversity, and it is essential for the growth of tea plants to have fertile soil, which is made possible by this wide microbial variety. Tea is an important cash crop of Assam, occupying 3.47×10^5 hectares of agricultural area, according to the Tea Board of India. Changes in microbial diversity of tea-associated soil can harm tea productivity (Singer, 2008).

Understanding the impact of change in soil microflora due to the increasing CO₂ and temperature is crucial. Climate variables influence soil microbial diversity and community composition. Climate variables, including temperature and precipitation greatly influence soil microbial activity, diversity, and community composition (Bahram *et al.*, 2018; Nottingham *et al.*, 2018). The microbial taxonomic diversity and community composition are influenced mainly by temperature, precipitation, soil pH, plant production, or their interactions along altitudes (Bahram *et al.*, 2018; Borchetia *et al.*, 2017; Siles and Margesin, 2016). Only a small percentage of environmental bacteria can be isolated into pure cultures, necessitating culture-independent approaches to investigate the true richness of bacteria in the tea soil (Borchetia *et al.*, 2017). Multiple species of microorganisms inhabit a particular niche, and the dominant species that can adapt to elevated carbon dioxide and temperature needs to be understood. A thorough understanding of the microbial community structure surrounding the *Camellia sinensis* var. *assamica* root system, including the rhizosphere regions, was anticipated to not only give better insight into how they impact the health and productivity of the plants but also to aid in the exploitation of beneficial microorganisms with traits that promote plant growth (Fernández-González *et al.*, 2019).

Metagenomics can help to address the gaps and provide insights into the in situ microbial community, including knowledge of community members, their identification, and potential metabolic capabilities (Maphosa *et al.*, 2012). The phylogenetic, genomic, and biochemical characterization of tea soil

microorganisms from the environment is crucial to better understand adaptation and mitigation strategies under elevated temperature and CO₂. Hence, the present study was aimed to elucidate the overall rhizosphere microbial diversity of the soil bacterial community in the CO₂-enhanced tea soil with the help of metagenomics and provide new insights into the tea soil rhizosphere microbial community.

Materials and Methods

Experimental site and set-up

The study was conducted in the experimental area of Tocklai Tea Research Institute Jorhat, Assam, India (26°43′N and 94°13′E) using two Open Top Chamber (OTC1 and OTC2). In OTC1, both CO₂ and temperature were elevated (eCO₂ + temp), CO₂ was injected at a concentration of 550 ppm, and enrichment was done for five hours per day up to 300 hours. While, in OTC2, only temperature (eTemp) was elevated at 1.5-2 °C higher than the ambient temperature. An open region with ambient temperature and CO₂ set up the control.

Isolation and collection of samples

Tea soil was collected from the rhizosphere of the potted cultivars of OTC1, OTC2, and control. The soil within the 0-15 cm layer was collected using sterile spatulas from the three conditions and placed inside a sterile falcon tube. The tubes were then sealed tightly and kept in cool packs.

Metagenomic DNA Extraction, Qualitative and Quantitative analysis

Metagenomic DNA was extracted from the soil samples using Nucleospin Soil kit (Soliman *et al.*, 2017) per the manufacturer's instruction. The quality of the extracted metagenomic DNA sample was analyzed using Nano-Drop by determining A260/280 ratio (Xie *et al.*, 2012). The extracted metagenomic DNA was then sequenced using the Illumina MiSeq platform using 16S rRNA primers (Garg *et al.*, 2021) (Table 1).

Bioinformatics Analysis

QIIME2 (Quantitative Insights Into Microbial Ecology version 2) is a microbiome analysis package which can translate the sequence data into interpretable visualizations along with statistical results (Kumar *et al.*, 2020). Data denoising, featuring taxo-

nomic assignment, phylogenetic reconstruction, diversity analysis and visualizations are all included in QIIME.

To process the data of three 16S samples, the following steps were performed. Trimmomatic v0.38 was used to remove adapter sequences, genomic ambiguous reads (reads with unknown nucleotides "N" more significant than 5%), and low-quality sequences (reads with more than 10% quality threshold (QV) 25 Phred scores). The minimum length of the reads was 100 bp, with a sliding window of 20 bp. (Kumar *et al.*, 2021). Using FLASH(v1.2.11), stitching of PE data into single-end reads (Hailu *et al.*, 2021) was done. High-quality clean reads and chimeric sequences were filtered through DADA2. Taxonomic classification of amplicon sequence variants were performed with g2-feature-classifier using a pre-trained classifier based on SILVA database (Ramakodi, 2022).

Results and Discussion

Soils were collected from the rhizosphere of potted tea plants and treated with two different treatments. A metagenomic study was performed with the two rhizosphere tea soil samples treated with eCO₂ +temp and eTemp. These samples were compared against the control soil sample collected from ambient condition. Metagenomic studies showed that the

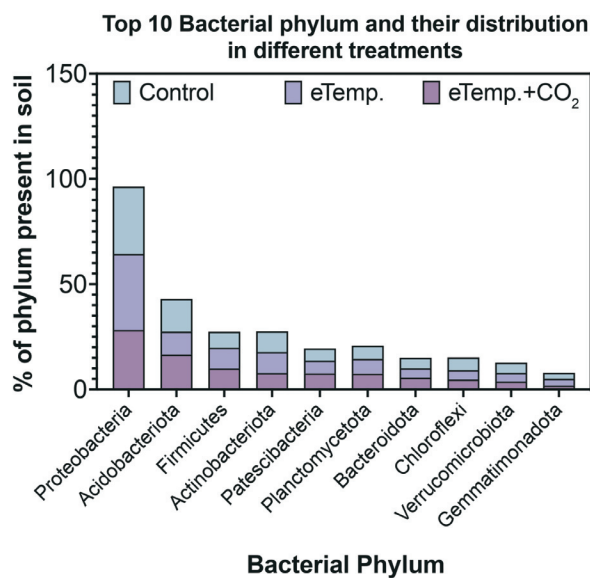


Fig. 1. Distribution of top 10 bacterial phylum in different treatments

soil contained 32 different phyla of bacteria. In all the treatments, the highest number of bacterial species belong to the phylum Proteobacteria (~32.11%), which was followed by Acidobacteriota (~14.32%) and Actinobacteriota (~9.13%) (Figure 1). Among the lowest recorded species majority of them belonged to Halobacterota (~0.01%), followed by MBNT15 (~0.009%), a phylum distantly related to Desulfobacterota (Begmatov *et al.*, 2022). The least number of species were recorded from the phylum WS4 (~0.002%), a candidate phylum of uncultured bacterium often reported in soil (Serkebaeva *et al.*, 2013) (Figure 2).

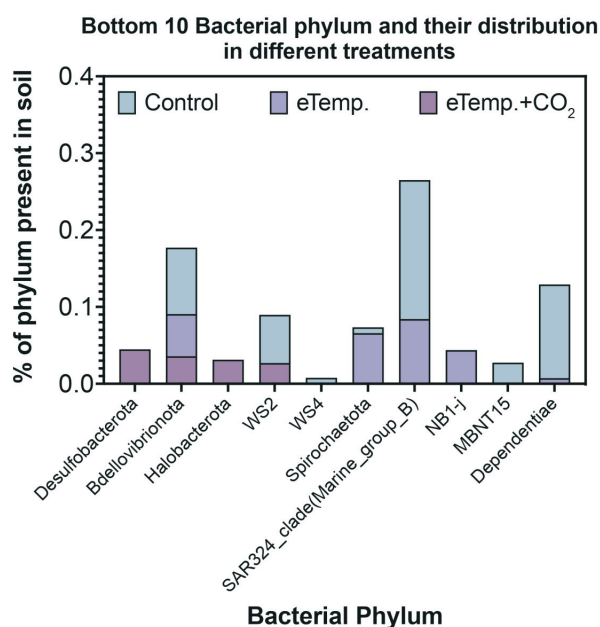


Fig. 2. Distribution of bottom 10 bacterial phylum in different treatments

Proteobacteria is a phylum comprising majority of Gram-negative bacteria. Proteobacteria widely include pathogenic genera, such as *Escherichia*, *Salmonella*, *Legionella*, *Yersinia*, *Vibrio*, and many others. Lipopolysaccharide is generally the outermost layer. Usually, they are motile, and the movement is achieved by using flagella, whereas some are found to be non-motile. Metabolically they are mostly facultative or obligately anaerobic, heterotrophic, and chemolithoautotrophic. In our study, we observed a higher concentration of Proteobacterium species in eTemp treatment, showing a significantly higher abundance of 36.14% (9.88×10^3 nos., $p < 0.001$), followed by control with 32.02% abundance ($8.134 \times$

10^3 nos.). The least abundance of Proteobacterial species was observed in eCO₂ + temp treatment with only 28.19% (6.29×10^3 nos.) abundance, which is significantly different from the control ($p < 0.001$). Proteobacteria are widely distributed in soil and associated with various processes involved in carbon, nitrogen, and sulphate cycling. The relative richness of Proteobacteria increases with high organic carbon content in soils (Mhete *et al.*, 2020). A higher presence of Proteobacteria in high-temperature treatment can result from higher respiration rates and high sequestration rates of tea plants.

Following Proteobacteria, the second highest species were from Acidobacteriota, a phylum comprising Gram-negative bacteria. Although its members are numerous and diverse, they have limited cultural representation. In our study, we observed a higher concentration of Acidobacteriota species in eCO₂ + temp treated tea rhizosphere soils, showing a significantly higher abundance of 16.6% (3.7×10^3 nos., $p < 0.001$), followed by control with 15.51% abundance (3.94×10^3 nos.). The least abundance of Acidobacteriota species was observed in the eTemp treatment, with only 10.87% (2.97×10^3 nos.) abundance, which is significantly lower than the control ($p < 0.001$). Increasing CO₂ concentrations benefit Acidobacteriota, especially mild acidophilic strains. Potential environment functions of several members of this phylum aspect of carbon usage and nitrogen assimilation (Kielak *et al.*, 2016). Hence, increasing CO₂ may have led to the increase in soil acidity, contributing to higher diversity of acidophilic Acidobacteriota strains in our sample.

Actinobacteria are aerobic saprophytes in nature, a phylum encompassing Gram-positive bacteria, with terrestrial and aquatic habitats. Actinobacteria widely includes the dominant genera *Gaiella*, *Solirubrobacter*, *Nocardioides*, *Mycobacterium*, and *Pseudonocardia*. In our study, we observed a higher concentration of Actinobacteria species in eTemp treated tea rhizosphere soils, showing a significantly increased abundance of 9.96% (2.72×10^3 nos., $p < 0.001$), followed by control with 9.85% abundance (2.5×10^3 nos.). The lowest abundance of Actinobacteria species was observed in eCO₂ + temp treatment with only 7.77% (1.73×10^3 nos.) abundance, which is significantly lower than the control ($p < 0.001$). These bacteria play an essential role in the cycling of carbon, nitrogen, and phosphorus in the soil.

Among the lowest recorded bacteria,

Halobacteria were the third least. These bacteria are photoheterotrophs that comprise of numerous species of Archaea. They are considered 'extremophiles,' an organism that can survive under extreme conditions, such as high temperature, salinity, and acidity. Halobacteria (~0.01%) in our samples was observed only in eCO₂ + temp treatment with 0.03% abundance (7 nos.). No such count was observed in eTemp treatment and control, indicating that the increased CO₂ and temperature provide a favourable environment for the growth and diversification of such extremophilic bacteria. As Halobacteria grows best at an optimum temperature of ~ 42 °C in the background (Robinson *et al.*, 2005), a future increase in the temperature of soil may favor the growth of these bacteria resulting in a decrease of beta-diversity of soil which may impact tea soil rhizosphere.

Following Halobacteria, reduced abundance was recorded for the uncultured bacteria of the proposed phylum MBNT15, which is distantly linked to Desulfobacterota and is found in organically rich aquatic environments (Begmatov *et al.*, 2022). MBNT15 bacteria are obligate anaerobes that couple molecular hydrogen (H₂) and acetate oxidation to nitrate reduction. Based on phylogenetic and genomic analyses, MBNT15 is classified as *Candidatus* *Deferrimicrobium borealis* within the candidate phylum Deferrimicrobiota. In our study, MBNT15 was observed only under control with an abundance of 0.027% (7 nos.). However, bacteria of this phylum were not observed in either of the two treatments indicating higher sensitivity of bacteria from this phylum to environmental conditions. Changes in the atmospheric CO₂ and temperature may lead to decreased diversity of this phylum in soil and even may lead to the extinction of many of these strains (Figure 3).

Finally, the least number of recorded bacteria belonged to the phylum WS4. Like the phylum MBNT15, the members of this phylum were only recorded in the control soil with an abundance of 0.007%. Hence like MBNT15, the members of this group are also susceptible to changes in environmental conditions. Therefore, it becomes crucial to understand the complex interactions that are at interplay in the soil and how changes in the atmospheric conditions may impact this delicate balance.

In addition to the above-observed phylum, it was found that Hydrogenedentes, FCP426, and Armatimonadota showed abundance in both eCO₂ +

temp and eTemp treatments. Phylum Hydrogenedentes are candidature phylum considered putative degraders of organic carbon, hydrolyzing carbon compounds such as lipids, glycerols, and phthalates (Momper *et al.*, 2018). FCPU426 is also a candidature phylum, which is known to be potentially related to cellulose degradation (Whitehead *et al.*, 2022). The presence of such bacteria in the two treatments indicates that there may be an increase in soil organic carbon content. Phylum Armatimonadota is a Gram-negative bacterium. It is

aerobic in nature, saccharolytic, and obligately thermophilic, and thus its abundance in elevated temperature may facilitate its growth. RCP2-54, Methylomirabilota, and Desulfobacterota showed abundance only in eCO₂ + temp treated rhizosphere soil. RCP2-54 is a candidature phylum whose abundance negatively correlates with soluble Nitrogen (Muhammad *et al.*, 2022). Methylomirabilota is methanophilic bacterial phylum that can oxidize carbon from methane for growth (Zhu *et al.*, 2022). Hence, such bacteria indicate a higher carbon con-

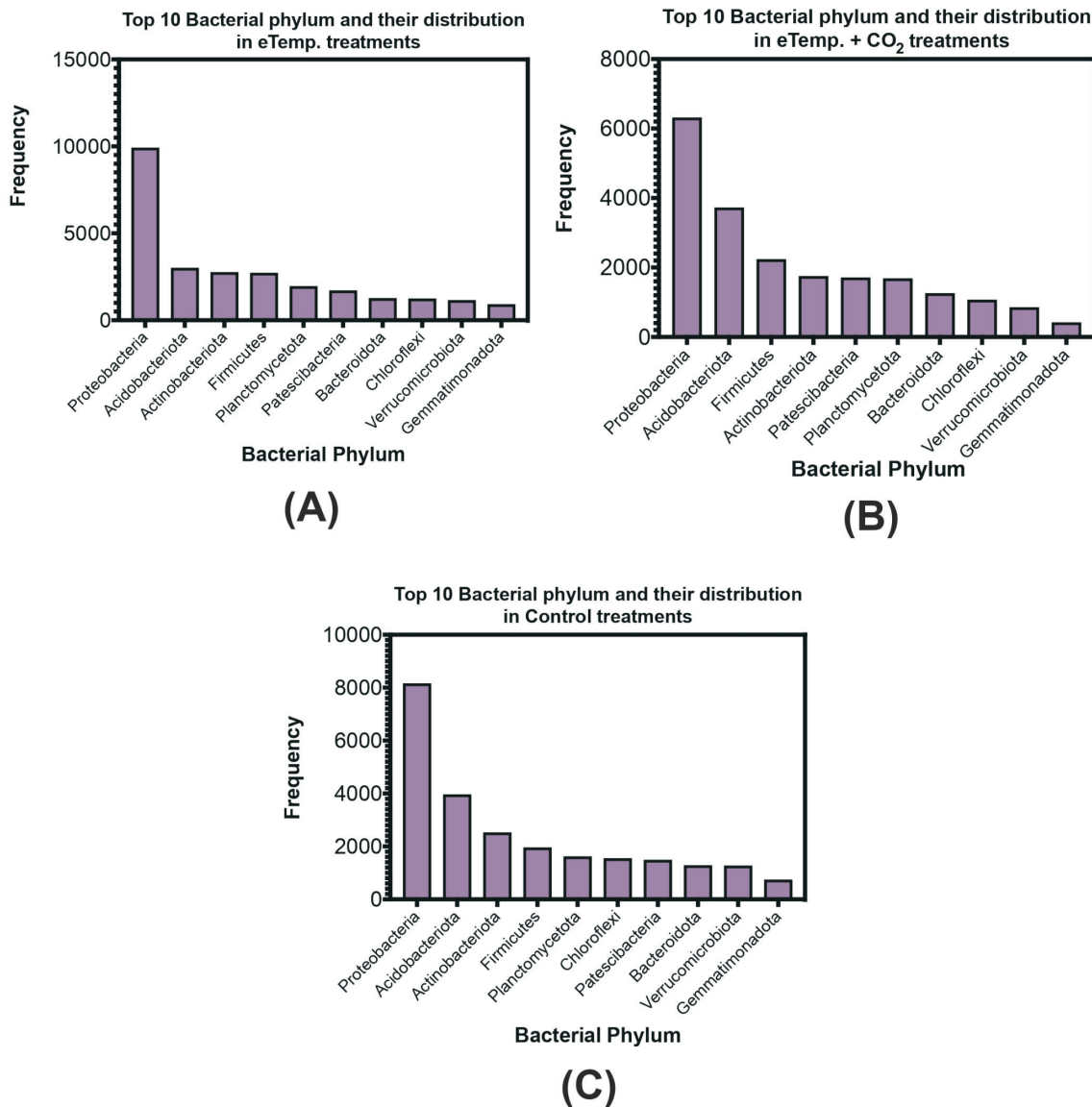


Fig. 3 (A) Distribution of top 10 bacterial phylum in elevated temperature treatment, (B) Distribution of top 10 bacterial phylum in elevated CO₂ and temperature treatments and (C) Distribution of top 10 bacterial phylum in control.

Table 1. Universal primer sequence for 16S rRNA of bacteria used in the metagenomic analysis.

Primer Name	Sequence
16S rRNA F	5'-GCCTACGGGNGGCWGCAG-3'
16S rRNA R	5'-ACTACHVGGGTATCTAATCC-3'

centration in soil. Similarly, *Desulfobacterota* is a thermophilic bacterium that may be facilitated by high-temperature treatment. Lastly, it was found that NB1-j showed abundance only in eTemp treated tea rhizosphere soil. These bacteria are known to be associated with nitrogen fixation and are found in soil with higher nitrogen content. The development of bio-mitigation strategies in regard to increasing scenarios of climate change concerning tea soil has not yet been understood. It requires knowledge of microbial populations, their relationships, and functions. Thus, proper research is required in this aspect for better understanding with respect to changes in climatic variables.

Conclusion

This study characterized two treated rhizosphere soil samples along with a control. The study also confirmed the presence of taxa previously found to inhabit the tea soil and identified many bacterial phyla not yet reported along with the metagenomic survey of the bacterial community. The identified phylum from the metagenome was reported to have the capability to survive in the presence of elevated CO₂ and temperature (eCO₂+ temp) and high temperature (eTemp). Metagenomic studies showed that out of the 32 different phyla of bacteria, 26 bacterial phyla were found in the eCO₂+ temp treated tea soil metagenome, which may have increased abundance in future climate scenarios in tea soil. Despite negative aspects of changes caused by climatic variations, such severe environmental conditions can induce intriguing adaptations in microbes and plants that allow them to survive and reproduce. Regarding bioprospecting, the undocumented microbiological relevance of such strains in tea soil demands immediate attention. These adaptations may result in establishing new functional groups in the ecosystem or act as a crucial tool for enhancing plant agricultural methods (da Silva *et al.*, 2013). This study will thus help to better understand the overall rhizosphere microbial diversity of the tea plant bacterial community under elevated tempera-

ture and CO₂ and may aid in formulating new adaptation and mitigation strategies for future high temperature and CO₂ scenarios.

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Declarations: The data used in this article is provided in the supplementary materials.

Conflict of Interest: The authors find no conflict of interest and would like to report the same.

Research involving human participants or animals: This study doesn't contain involvement of any human or animal participants.

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