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Research Article

Response of Soil Enzyme and Root Activities of Tea Cultivars Under Elevated Temperature and Carbon Dioxide

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ABSTRACT

Aim: The experiment aimed to investigate the root activity of the tea plant as well as the Nitrate Reductase (NR) and Dehydrogenase Activity (DHA) of the tea rhizosphere soil. **Materials and Methods:** Two Open Top Chamber (OTC) facilities of diameter eight meter each were used in the experimental setup to elevate the CO_2 and temperature. With a CO_2 concentration of 550 ppm, OTC-1 (eTemp+eCO_2) had both a higher temperature and CO_2 than OTC-2 (eTemp), which just had a higher temperature. The temperature was 1.5-2°C warmer than the surrounding air. Inside the OTCs, four tea cultivars (TV1, TV20, TV22, and TV23) were planted, and soil samples from the rhizosphere were taken regularly. **Results:** The findings showed that the overall soil nitrate reductase activity after 300 hr of enrichment, showed no significant difference ($p>0.05$) in both treatments. In contrast, soil DHA showed a substantial increase ($p<0.001$) in eTemp+eCO_2 treatment after 300 hr of enrichment. Similarly, after 300 hr of enrichment, the overall root activity showed significant increase ($p<0.001$) in eTemp+eCO_2 treatment. Similarly, when the data were analysed cultivar-wise, after 300 hr of treatment, each of the cultivars TV1, TV20, TV22, and TV23 showed a significant variation in root activity under eTemp+eCO_2 treatment. **Conclusion:** The experimental investigation indicates that while soil nitrate reductase activity did not change, higher temperature and CO_2 did change the overall soil dehydrogenase activity as well as the root activity of the tea plant.

Keywords: Climate change, Dehydrogenase activity, Enzyme, Nitrate reductase, Rhizosphere, Tea.

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INTRODUCTION

One of the new globally concerned issues is climate change, which has profound effects on ecosystems, human health, and economic growth. According to a recent study, the atmospheric CO_2 concentration has increased from approximately 315 ppm to 390 ppm in the last 57 years.[1] North East India has also been witnessing the effects of climate change rising minimum temperatures, and rising carbon dioxide levels. Studies suggested a gradual increase in atmospheric CO_2 throughout the years with an average increase of 17 ppm of CO_2 in the South bank region of Assam.[2] Assam occupies a majority of the proportion of tea cultivation. For thousands of people, the tea industry in the state creates significant direct and indirect employment opportunities,[3] and this tea plant growth is directly related to its climatic parameters and the soil. Despite being of great significance, little is known about how climate change affects microorganisms and their extracellular enzymes.[4] Enzyme activity depends on temperature; up to a certain limit, higher temperatures usually result in faster enzymatic processes. Enzyme activities primarily the nitrate reductase activity and Dehydrogenase Activity (DHA) can be used as an indicator of a plant's nitrogen status[5] and soil microbial activity. The reactions of mass cycle-related soil enzyme activity to simulated climate change are poorly understood. More frequent and severe drought events associated with climate change can limit soil moisture, reducing microbial activities and root activity.[6] Understanding the influence of climate change on root activity is essential for assessing its impacts on soil health, nutrient cycling, and ecosystem functioning.[7] It also underscores the importance of adopting sustainable land management practices and adaptation strategies to maintain soil health in a changing climate. In current research, we wanted to understand how climate change affects the tea rhizosphere soil enzyme and its root activities. We aim to give light on the potential repercussions of climate change on the tea

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sector and the steps that could be taken to mitigate those effects by assessing the relationship between climate change and the enzyme activities of the tea rhizosphere soil.

MATERIALS AND METHODS

Experimental site

The experimental area of Tocklai Tea Research Institute, Assam, India (26º43˝N and 94º13˝E) served as the study's site. Three treatments and four clonal types, each with five replications, were included in the factorial RBD experimental design. To create localized conditions with elevated temperature and CO_2, two Open Top Chamber (OTC) facilities were used. OTC1 (eTemp+eCO_2) received an injection of both temperature and CO_2 at a concentration of 550±13 ppm, whereas OTC2 (eTemp) only received an increase in temperature. The ambient (control) temperature was 1.5-2º Celsius (ºC) lower.

Plant and soil experimental setup

Four numbers of Tocklai-released tea cultivars (saplings) were collected and planted in pots during the month of September, 2020. Cultivars were namely TV1, TV20, TV22, and TV23. A total of approximately 7.5 kg of sieved soil that was collected from the Borbhetta Tea Estate in Jorhat, Assam, was placed into each of the 60 pots that were 17 cm in height and 23 cm in diameter.

Carbon dioxide and temperature elevation

In January 2020, the potted cultivars were set up in the two chambers (OTC1 and OTC2) and let to grow. CO_2 from a carbon dioxide cylinder was injected to one of the chambers (OTC1) in March 2020 at a concentration of 550±13 ppm, after the plants had been acclimated to the OTCs. The OTCs' temperature, humidity, and CO_2 levels as well as the ambient were measured every day. The CO_2 enrichment was carried out for 5 hr/day up to 375 hr.

Soil sampling

Following a 300 hr treatment period, rhizospheric soils were collected from each cultivars. Sixty number of soil samples were collected (20 samples from each treatment: OTC1 (eTemp+eCO_2), OTC2 (eTemp) and control). Soil samples from a depth of 0 to 15 cm were taken using an auger. Using the technique described by Pandey and Palni, soil that stuck tenaciously to the tea roots was acquired for the study.[8] After that, the soil's enzyme and plant root activity were examined under elevated carbon dioxide and temperature.

Analysis of soil enzyme and root activities

Anaerobic incubation of the soil was used to measure the potential nitrate reductase activity, and the Abdelmagid and Tabatabai approach was followed to express the activity.[9] Optical Density (OD) of supernatant was measured at 540 nm wavelength in a UV-visible spectrophotometer (Varian 50Bio Model no. UV1002M081). The nitrate reductase activity was then assessed from the standard graph of $NaNO_3$. Soil DHA was estimated using 2,3,5-Triphenyl Tetrazolium Chloride (TTC) reduction technique following the method given by Casida Jr.[10] For estimation of DHA, 1 g moist soil sample was taken in a 50 mL centrifuge tube with 2 mL of distilled water along with 2 mL of 1% TTC solution. OD was then measured at 484 nm in UV-visible spectrophotometer. Root activity of tea cultivars was estimated following the procedure using the 2,3,5-Triphenyl Tetrazolium Chloride (TTC) reduction technique given by Liu and his co-workers.[11] After immersing a 0.5 g root sample in 0.4% TTC and 66 mmol L^{-1} phosphate buffer solution for 3 hr at 37ºC, 1 mol L^{-1} sulfuric acid was added to terminate the reaction. To extract Triphenyl Tetrazolium Formazan (TTF), the roots were carefully removed, cleaned, and ground with 2 mL ethyl acetate. After washing the residues with a small amount of ethyl acetate, the solution was poured into the test tube and volumed up. The OD was measured at 485 nm in UV-visible spectrophotometer.

Statistical analysis

Statistical techniques were used to analyze the data. Dunnett's and Tukey's multiple comparisons tests were used after factorial one-way Analysis of Variance (ANOVA) was used to test for differences in soil enzyme activities. Analyses were performed with the GraphPad Prism ver. 9.3.1. and the histograms were plotted by R software, version 4.1.0.

RESULTS

It was observed that the overall soil nitrate reductase activity after 300 hr of enrichment, showed no significant difference in both treatments. However, the highest nitrate reductase activity was observed in the eTemp treatment with an average of 7.47±1.85 Units g^{-1} hr^{-1}, which was 9.17% higher than the control (6.84±1.41 Units g^{-1} hr^{-1}) followed by the eTemp+eCO_2 treatment with 7.00±0.67 Units g^{-1} hr^{-1} which was 2.28% higher than control [Figure 1(A)]. Similarly, none of the cultivars (TV1, TV20, TV22, and TV23) showed any significant variations when compared to the control [Figure 1(B)].

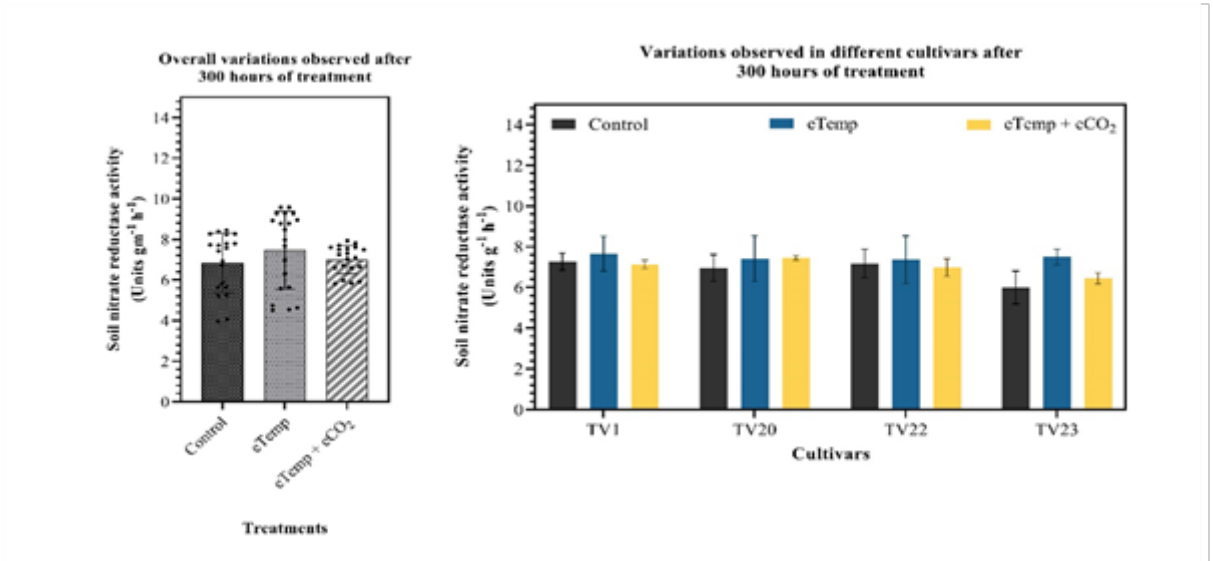
In terms of soil DHA, after 300 hr of enrichment, DHA had significant increase (p<0.001) in eTemp+eCO_2 condition when compared to control. The highest soil DHA was observed in eTemp+eCO_2 treatment with an average of 5.42±0.33 Units g^{-1} hr^{-1} which was 23.14% higher than control (4.40±0.51 Units g^{-1} hr^{-1}), followed by eTemp (4.58±0.41 Units g^{-1} hr^{-1}), which was 3.95% higher than control [Figure 2(A)]. Similarly, cultivar wise when it was observed, after 300 hr of treatment, under eTemp+eCO_2 the four experimental cultivars TV1, TV20, TV22, and TV23 showed significant increase (p<0.05) in soil dehydrogenase activity. The highest mean dehydrogenase activity was observed in cultivar TV23 (5.57±0.20 Units g^{-1} hr^{-1}), which was 21.69%

higher than its control; while in eTemp it showed 5.12% higher activity. In eTemp, the rhizosphere soil of cultivar TV1, TV20, TV22 and TV23 showed 4.42±0.17 Units g^{-1} hr^{-1}, 4.52±0.25 Units g^{-1} hr^{-1}, 4.28±0.05 Units g^{-1} hr^{-1} and 5.12±0.43 Units g^{-1} hr^{-1} of dehydrogenase activity [Figure 2(B)].

After 300 hr, overall root activity showed significant increase (p<0.001) in eTemp+eCO_2 treatment. The highest root activity was observed in eTemp+eCO_2 with an average of 0.029±0.002 Units g^{-1} hr^{-1}, which showed 52.79% higher activity than control (0.019±0.04 Units g^{-1} hr^{-1}) [Figure 3(A)]. After 300 hr of treatment, cultivars TV1, TV20, TV22, and TV23 showed a significant variation in root activity under eTemp+eCO_2 treatment. Cultivar TV1 showed significant increase (p<0.05), and its root activity was 0.027±0.005 Units g^{-1} hr^{-1}, which was observed to be 43.94% higher than control. Considering all four cultivars, the most increased root activity was observed for cultivars TV20 and TV23 in eTemp+eCO_2 treatment [Figure 3(B)]. Whereas under eTemp treatment, after 300 hr no significant variations were observed for either DHA or root activity.

DISCUSSION

Climate change is an unavoidable feature of contemporary, developing societies, as it has caused global warming and rising CO_2 concentrations, which have changed the ecology. Despite being quantitively minute, soil enzymes are an essential part of the soil ecology for plants. Likewise, the enzyme nitrate reductase is involved in the process of denitrification, however, the relative significance of atmospheric temperature and CO_2 rise and biogeochemical restrictions on dissimilatory nitrate reductase mechanisms are less known, especially in tea rhizosphere. In our study, the tea soil nitrate reductase activity after 300 hr showed no significant difference in both treatments. Our findings were in line with that of the works done by Marhan with his co-workers[12] under elevated CO_2, the rhizosphere soil of oilseed rape showed no potential influence on nitrate reductase activity. Similarly, another work performed by Deiglmay,[13] in their study observed that the composition of the nitrate reducing community, responsible for nitrate reductase activity in the rhizosphere of *L. perenne, T. repens* was unaffected by increased atmospheric CO_2. Further, in our study it was observed that after 300 hr, none of the cultivars, TV1, TV20, TV22, and TV23, showed any significant variations (p>0.05) in nitrate reductase activity in both the treatments. The total nitrogen content in the rhizosphere soil of these cultivars suggested that this might be because of low nitrogen supply. Hu and his co-workers[14] reported that the rhizosphere soil nitrate reductase activity was much reduced in the ripening stages of wheat under elevated tropospheric ozone. Dehydrogenases are one of the most important enzymes in the soil environment and are used to measure overall soil microbial activity since they are found intracellularly in all living organisms.[15] In our findings, it was found that the overall soil Dehydrogenase Activity (DHA) showed significant variations (p>0.001) after 300 hr in eTemp+eCO_2. Wolinska and Stepniewska's research corroborated our results.[16]. They reported an increasing, linear trend for DHA in surface layer of gleysols as temperature increased from 5°C to 30°C (p<0.01). Additionally, they discovered in the fluvisol sample that the highest amount of soil DHA, 0.0087 g TPF g^{-1} min^{-1}, was recorded in May. In contrast, the same soil type showed a 42.5% decrease in DHA in October. DHA was



(A) (B)

Figure 1: No significant (A) Overall variations were observed in nitrate reductase activity after 300 hr in the two different treatments when compared to control. (B) Cultivar-wise variations were observed after 300 hr in the two different treatments when compared to control.

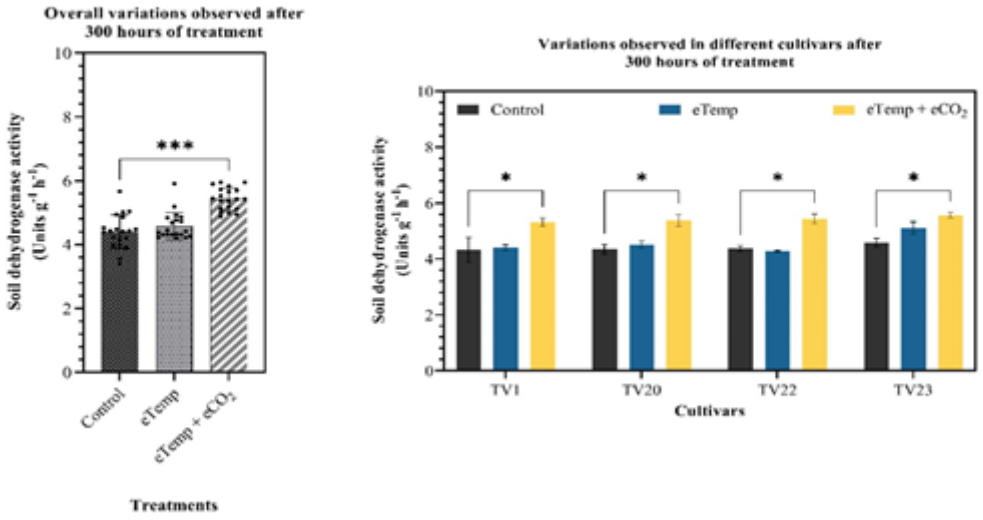


Figure 2: Significant (A) Overall variations observed in soil DHA after 300 hr in the two different treatments when compared to control (B) Cultivar-wise variations observed after 300 hr in the two different treatments when compared to control.

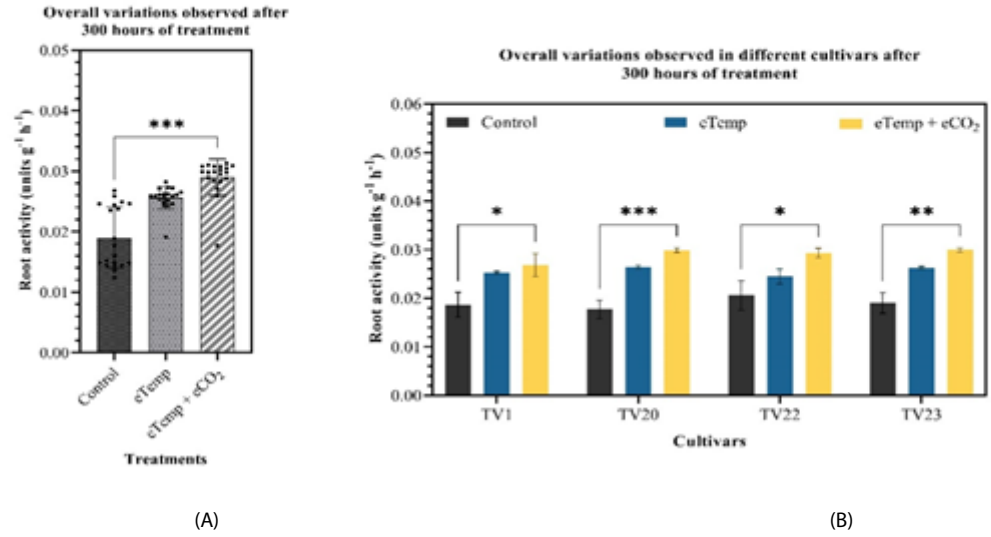


Figure 3: Significant (A) Overall variations were observed in plant root activity after 300 hr in the two different treatments when compared to control. (B) Cultivar-wise variations were observed after 300 hr in the two different treatments when compared to control.

significantly higher (p<0.05) during the cropping season of rice plant under elevated CO_2 and temperature.[17] Moreover, it was found that the soil dehydrogenase activity of *P. minor* enhanced under CO_2 enrichment, attaining 35.9 Units g^{-1} day^{-1}; similarly, it was found that the dehydrogenase activity of *E. crusgalli* was higher at CO_2 enrichment, with 39.8 Units g^{-1} day^{-1}, followed by elevated CO_2 and temperature, with 34.8 Units g^{-1} day^{-1}.[18] Thus, from our findings, it can be related that higher DHA at increasing CO_2 and temperature might be attributed to higher organic matter content, leading to increased soil microbial dehydrogenase enzyme activity as Nayak and his co-workers observed.[19]

Abiotic stressors strongly influence plant root growth and its activity, changing water and nutrient intake as well as root interactions with rhizosphere bacteria.[20] Under environmental stresses, roots undergo a sequence of structural and functional alterations to mitigate the adverse effects on plant development caused by a shift in this equilibrium.[21] In our study, it has been found that the overall root activity after 300 hr of treatment showed significant increase (p<0.001) in eTemp+eCO_2 treatment. Similarly, studies investigated that the effect of higher temperature and CO_2 on groundnut plant root length were higher, and total root length increased to 44% at higher CO_2 over control.[22]

In our study, we found that though there was no significant increase in eTemp treatment, however the average root activity was observed to be 0.026 Units g^{-1} h^{-1}, which was 34.77% higher than control. Likewise, high temperature increases the overall root activity that leads to an increase in water absorption in tomatoes.[23] Another study found that elevated temperature significantly increased the fine root activity of *P. asperata* seedlings with an average increase of 26.4%, thus they found that elevated temperature significantly increased the root exudation rates *P. asperata* seedlings. Shuping Huang found in his studies, that elevated temperature significantly stimulated the total root length of tea seedlings by 3.9%.[24] Greater root exudation may have come from warming-induced fluctuations in plant root morphological characteristics.[25] Additionally, in our work, it was found that after 300 hr of treatment, cultivar TV1, TV20, TV22, and TV23 showed a significant variation in their root activity in eTemp+eCO_2 treatment. Studies performed by Wang and his co-authors, observed that the rate of root exudation of *Pinus tabulaeformis* was significantly affected by experimental temperature rise, indicating that the warming significantly increased its root exudation rates compared to control plots during the whole sampling period.[26] Thus, in terms of tea cultivars, our findings suggest a better knowledge of potential changes in tea root activity during experimental elevated CO_2 and temperature, which is crucial for predicting biotic responses to climate change.

CONCLUSION

Tea is a valuable commodity for the economies of North East India, but the timing of tea pruning and the emergence of new leaves have changed significantly as a result of the recent climate shift. The practices used in tea cultivation have evolved during the past ten years. To sustain tea cultivation, tea soil requires ideal conditions. According to current climate change models, many ecosystems may become substantially nitrogen-limited in the future.[27] This is primarily due to increasing atmospheric CO_2 concentrations, which may result in increased plant nitrogen demands,[28] hence the study of enzyme activities of tea rhizosphere soil in elevated CO_2 and temperature will provide better insights into the microbial ecosystem. The purpose of the study was to determine how rising temperatures and CO_2 levels affected the tea soil enzyme activities along with its root activity. By monitoring the effects of temperature and CO_2 on the tea rhizosphere through evidence-based experimental work, this study aims to fill the research gap. From the study, it can be concluded that elevated temperature and CO_2 alter the overall tea soil dehydrogenase activity along with its root activity whereas no such alteration was found for soil nitrate reductase activity.

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FUNDINGNIL.CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DA: Dehydrogenase activity; **OTC:** Open Top Chamber; **TV:** Tocklai Vegetative; **TTF:** Triphenyl Tetrazolium Formazan.

FUTURE STUDY PERSPECTIVE

Additionally, this work will help future researchers by identifying new research questions and by studying more tea cultivars with novel ideas and purposes. It will also help develop a better understanding of the invisible impacts of rising temperatures and CO_2 in the rhizosphere of tea soil, crucial for sustainable tea cultivation.

ETHICAL APPROVAL

The authors want to declare that no animals were used for these experiments; hence, no ethical clearance is required.

CONTRIBUTION OF AUTHORS

ASR, JB and SRS designed the experiment; ASR performed the soil analysis. ASR wrote the manuscript draft while JB and SRS reviewed it. The statistical analysis, along with the graphs, was prepared by ASR, AB and RDB. ASR, JB and SRS finalized the manuscript.

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