Distribution of microorganisms and fractionation of sulphur in anthropogenic wetlands under long-term elevated CO_2 soil

ABSTRACT

The fate of extra carbon accessing soil under elevated CO_2 levels, as well as the repercussions for plant nutrition, is primarily determined by soil microbe activity. However, most increased CO_2 research has reported changes usually increases in soil organic carbon and reduction in the pH of the soil which is merely the first step in understanding how soil processes are altered. We analyzed these variables by assessing enzyme activities and identifying the individual components impacted by high CO_2 and those that reflect changes in soil organic matter pools. The majority of the microbial variables studied showed a significant increase under eCO_2 conditions, The rise in dehydrogenase activity suggests that the increased biomass of bacteria coincided with an increase in their activity. The rise in phosphatase activities implies that organic matter breakdown is being stimulated overall. The sulphur fractions had a significantly increased number of substrates consumed by soil microorganisms under increasing CO_2. Moreover, direct examination of data from these perspective steep shifts in soil biological activity points to possible areas of investigation

Keywords: Elevated CO_2, Soil organic matter, Sulphur fractions, Microbial distribution, Soil dehydrogenase.

1. INTRODUCTION

The world population is predicted to reach 9.1 billion by 2050, requiring a 60-110 % increase in the food supply [1]. Which would demand agricultural productivity enhancement given the restricted scope of arable land growth. Food security is a serious concern in nations with an increasing population and shrinking cultivable land space. Rice (*Oryza sativa* L.) is one of the most important staple crops, feeding almost half of the world population. Rice supplies more than half of the dietary requirements for 1.3 billion people, while rice provides 25-50 percent of the overall food requirements for 400 million individuals [2]. It is also a key source of calorie intake and nutrition, accounting for more than one-fifth of the calories ingested by humans globally. Rice is India's most significant staple food, consumed by around 65 percent of the population [3]. It is grown in almost all states, but West Bengal (13.79 percent), Uttar Pradesh (13.34 percent), Andhra Pradesh including Telangana (12.84 percent), Punjab (11.01 percent), Odisha (6.28 percent), Chhattisgarh (5.61 percent), Tamil Nadu (5.54 percent), Bihar (5.19 percent), Assam (4.41 percent), Haryana (3.88 percent), and Madhya Pradesh are the major rice producing states (3.86 percent).and it provides two-thirds of a person's calorie supply and one-half of total protein consumption [4]. Rice is special in that it can be produced in damp conditions when many other crops cannot. Such damp areas are common across Asia. However, soil fertility and fertilizer control are critical to a strong rice crop. Carbon and nutrient dynamics, which vary throughout agricultural ecosystems, are critical in soil fertility and crop yield [5]. As global temperatures rise due to greenhouse gas (GHG) emissions, sustainable agricultural output will be a major challenge in the future [6]. CO_2 is a significant GHG, accounting for approximately 60% of the entire greenhouse impact [7].

CO_2 emissions have increased in recent decades, elevating the atmospheric CO_2 level from 280 ppm at the start of the industrial revolution to 410 ppm now [8]. Human actions like fossil fuel combustion, indiscriminate land use, land cover, and so on are to blame for rising GHG concentrations in the atmosphere. In addition to water vapor and solar irradiance, well-mixed GHGs such as CO_2, nitrous oxide (N_2O), methane (CH_4), and halocarbons contribute to global temperature rises and climate change [9]. Global surface temperature is rising significantly, and crops may be unable to be cultivated in the future if such events continue [10]. According to an international team of 17 academics from 17 countries, temperature rises due to global warming could subsequently be double what climate models assess. Sustained CO2 increases in the atmosphere would have an effect on soil and plant nutrition. [11]

Sulphur, one of the most essential components for all plants and animals, is regarded as the fourth most important nutrient for agricultural crop development after nitrogen, phosphorus, and potassium. The relevance of sulphur in Indian agriculture is growing due to the recognition of its function in improving crop yield, not only for oil seeds, pulses, legumes, and forages but also for numerous kinds of cereal [12]. The sulphur deficit in crops is becoming more common as a result of the continued use of sulphur-free fertilizers, high-yielding crop types, intense multiple cropping systems, and increased productivity. Sulphur deficiency affects around 41% of India's farmed land. In India, crops remove around 1.26 million tons of sulphur, while fertilizers restore approximately 0.76 million tonnes [13]. Furthermore, the recovery of additional sulphur from external sources is approximately 8-10%. The continued depletion of local sulphur deposits throughout the post-green revolution period has resulted in a sulphur deficit in many sections of the country [14]. Sulphur exists in both inorganic and organic forms in soils, and the proportion of inorganic to organic sulphur varies greatly depending on the composition of the soil, its depth, and the management variables to which the soil is subjected. In most arable land top soils, total soil sulphur, which includes inorganic and organic binding forms, varies between 250 and 2500 kg/ha. Water soluble and adsorbed SO_4^{2-} composes inorganic “ S "which is widely assumed to be the direct source of plants. In general, it accounts for less than 5% of total soil S. SO_4^{-2} is present in soil solutions only present in trace amounts that vary continually, and its concentration at any one moment is determined by the balance of plant uptake, and fertilizer input, mineralization, and immobilization. Understanding the S transformations in connection to crop availability in soil is required to utilize S fertilizers more efficiently.

Recent studies reveal that if temperatures rise by more than 0.5°C, the effects will be disastrous for millions of the world's poorest people due to reduced food output. As eCO_2 causes, an increase in global temperature in the future, soil health, nutrient availability, and crop output are all expected to suffer. Adaptation studies are being conducted all around the world, although they are often insufficient. India is far behind in terms of studies on the effects of climate change on agriculture. As a result, the current study was carried out to investigate the impacts of eCO_2 on the dynamics of microbes and sulphur fractions in the soil.

2. MATERIALS AND METHODS

2.1 **Site description**

The study was conducted in the A1 field in the Wetland Farms, Tamil Nadu Agricultural University, Coimbatore, India (11^{o}005”13” N, 76^{o} 93”045” E) which is under long-term elevated (eCO_2) 550±30 for a decade. Soil sampling was conducted on a randomized quadrant basis during the year 2018. A total of three samples with three replications have been collected from (i) Long-term elevated CO_2 open-top chamber (OTC) (eCO_2) (ii) OTC without any CO_2, ambient (aCO_2), and (iii) Soil samples from a normal low-land rice ecosystem in the ambient bulk soil (ABS).

2.2. Enumeration of functional guilds

Enumeration of different functional bacterial groups. The media employed for the isolation of sulphur-oxidizing bacteria is mineral salt thiosulphate medium [15]. For the enumeration of methanogens, the [16] method was followed. For the enumeration of sulphur-reducing bacteria by [17] method. Methanotrophs enumerated by NMS medium. Diazotrophs and heterotrophs were enumerated by the method given by [18].

2.3. Soil enzyme analysis

All enzyme activities can be carried out by processing the soil size to < 2mm and following the colorimetric method for the analysis of the dehydrogenase [19], acid and alkaline phosphatase [20] beta-glucosidase [21] in the soil was estimated by the standard methods -, aryl sulphatase by [22]. and florescent diacetate hydrolysis was carried out by the standard method [23].

2.4. Sulphur fractions in soil

Fractionation of Sulphur

Less than 2 mm of soil samples were used in the sequential extraction of soil sulphur fractions [24].The smaller fractions (water soluble)were extracted with demineralized water for the first time at (w/v) 1:10. After 30 minutes of shaking, each sample was analyzed. The sample was spun at 10,000 rpm for 10 minutes, and the homogenate was assessed using the turbidity method. Exchangeable sulphur fractions were extracted at a 1:10 ratio with 0.032M NaH_2PO_4 (w/v) proportion after 30 minutes of shaking, each sample was centrifuged at 10,000 rpm for 10 minutes and the supernatant was analyzed by turbidity method. For precipitated sulphur, the remainder of the soil sample was extracted by1M HCl. The ratio of extraction was 1:20 (w/v). After 60 minutes of shaking, the samples were ready centrifuged at 10,000 rpm for 10 minutes, and analyzed by turbidity method.

2.5. Available sulphur

Five grams of soil was taken in a polythene shaking bottle and put it in a shaker container. 25 ml 0.15 percent $CaCl_2$ solution, shake for 30 minutes, filter with Whatman no.42 filter paper, pipette out 5 ml extract in 25 ml volumetric flask, then add 10 ml sodium acetate and acetic acid buffer after that, add 1 gram of barium chloride crystals and mix for 1 minute. After that, add 1 ml of 0.25 percent gum acacia solution and dilute to 25 ml with distilled water and shake until the barium chloride crystals dissolve 2-8 minutes after 420nm absorption. Calculate the concentration of accessible sulphur in the soil sample by plotting the graph with a standard curve using K_2SO_4.

2.6. Statistical analysis

All the data sets recorded were analyzed by SPSS version 16.0. means of values compared by the least significant difference (LSD) at a 5 % level of probability

3. RESULTS AND DISCUSSION

3.1 Effect of eCO_2 concentrations on soil chemical properties. Soil pH, electrical conductivity, and Organic carbon in soil.

The pH of the soil was decreased as CO_2 concentration rise (Table 1). The lowest pH value (7.9) was noticed with 550 ppm eCO2, while the highest pH value (8.2) was observed in ambient bulk soil. According to [25], eCO2 is anticipated to raise CO_2 concentration in soil via the generation of H_2CO_3, and hence pH in the soil is likely to drop. The majority of plant nutrients become accessible when soil pH varies from 6.5 to 8.0. When submerged, the pH of the soil normally reaches 7.0. According to [26], an increase in CO_2 concentration affects soil pH. However, investigations have revealed that when a large quantity of CO_2 was added to the soil system, the pH changed insignificantly. It is also insignificant when compared to the depth of the soil. This may be due to the solubility of carbon dioxide (CO_2) in water, which results in the formation of undissociated carbonic acid. Acidic root exudates from plants and other microorganisms, on the other hand, lower pH and alter the solubility relationship of many plant nutrients. Acetic acid is the most common anaerobic respiration intermediate. During the decomposition process, all organic matter, including fats, amino acids, and carbohydrates, is converted to acetic acid. [27–29]. An increase in the eCO_2 increases the soil's organic carbon. The soil organic carbon was found higher in the eCO_2 range of 6.9 mg/kg while the lowest was observed in the ambient bulk soil range of 5.7 mg/kg. These findings support [30]. The suggestion is that physical fractionation techniques can aid in identifying changes in the soil carbon pool under higher CO_2 conditions, even in trials with high intrinsic variability and beginning C content, as in our experiment. The increased sensitivity of SOC to changes in C dynamics with rising CO_2 than total C is most likely due to considerably quicker turnover in SOM pools (Table-1 & 3). The greater proportion of new SOC relative to total new soil versus the proportion of total SOC related to total new soil C indicates a quicker turnover of SOC. SOC content primarily through boosting photosynthate synthesis and allocation to the rhizosphere, hence increasing C intake into the soil [31]. Soil organic matter is a key factor that affects all physical, chemical, and biological activity in soils. It is soil's essence that enhances soil health, agricultural productivity, and soil aggregates. More C sequestration in soils equals lower CO_2 levels in the atmosphere, reducing the potential for global warming [32].

Table 1. Soil physical properties from three different soils elevated, ambient, and ambient bulk soil. All the values are means of ±SD

Soil parameters	Elevated (eCO2)	Ambient	Ambient bulk soil

pH	7.9±0.1^{a}	8.1±0.3^{b}	8.2±0.4^{b}
EC	0.76±0.03^{b}	0.75±0.02^{b}	0.8±0.01^{a}
Bulk density(mg/m^{3})	1.49 ±0.1^{a}	1.49±0.1^{a}	1.49±0.2^{a}
SOC (g/kg)	6.9±0.1^{a}	5.8±0.4^{b}	5.7±0.2^{b}

3.2. Microbial distribution

The development of bacteria has shown an increasing tendency as CO_2 levels rise (Fig 1). However, insignificant increase in microbial populations of bacteria, fungi, actinomycetes, heterotrophs, and diazotrophs. There was a significant difference observed in methanotrophs, Sulphur oxidizers, methanogens, and phosphorous solubilizing bacteria at *($p<0.01$)* and *($p<0.05$)* levels of significance among the three soils. Insignificant decrease of SRB under eCO_2 is observed this may be due to the proliferation of methanogens by utilizing CO_2 as a substrate and limiting the substrates required for the sulphidogenesis. The highest microbial populations were seen in soil treated with eCO_2 followed by ambient CO_2 soil, respectively. Variations in the microbial population may have altered the breakdown of organic molecules. Furthermore, bacteria account for around one-quarter of all living biomass on the planet, and their biomass is employed as an early indication of changing physical and chemical features of soils. Increased microbial growth was associated with higher levels of plant-derived C in the rhizosphere under eCO_2. Which is consistent with previous research using other plant species [33–35]. Increase in the eCO_2 increases the soil temperature and thus influences the total the microbial communities of bacteria, diazotrophs, fungi, actinobacteria, methanotrophs, and diazotrophs (Lou et al.,2006). In present soil hike in total anaerobic population under eCO_2 and decrease in methanotrophic population compared bulk soil as shown in fig 1. Similar results were obtained by [36]. Increases in the enzyme activities in soils boost up the carbon pools and diazotrophic population which was observed in our study (Fig 1 & Table 2). Similar results were observed by [37]. Significantly higher microbial ratios under enhanced CO_2 treatments than under ambient CO_2 imply that CO_2 amplification of C inputs may still play a significant role in defining community structure in N-rich agroecosystems, as seen in many forests and grasslands [38]. Organic matter concentration, water availability, and soil physical and chemical qualities all have an impact on microbial biomass.

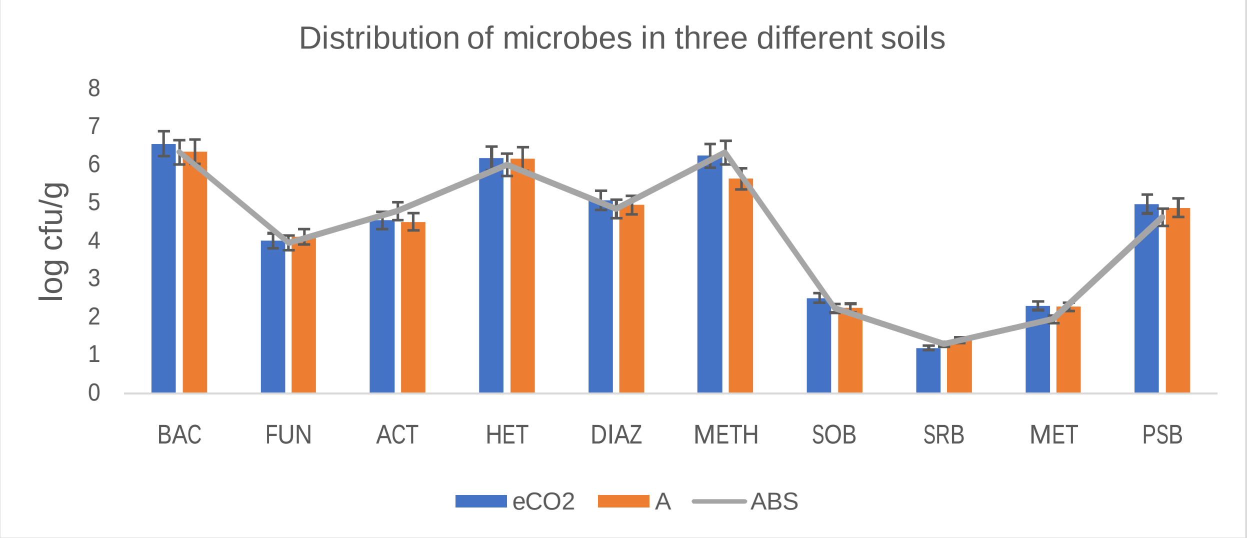

Fig.1. Distribution of different groups of microbes. Bacteria, fungi, actinomycetes, heterotrophs, diazotrophs, methanotrophs, sulphur-oxidizing bacteria, sulphur reducing bacteria, methanogens, and phosphate solubilizing bacteria from three different soils elevated(eCO_2), ambient(A), and ambient bulk soil (ABS).

3.3. Enzyme activities in soil

Soil enzymes profit from biological and metabolic activities in soil, which form the foundation of the terrestrial ecosystem. Elevated atmospheric CO_2 levels clearly have an effect on the soil enzyme activity that is influenced by the physical and chemical properties of soil, soil biological community, and plants either directly or indirectly. All the values are calculated by using a standard graph with an *($R^2>0.90$)*. There were significant changes observed in all enzyme activity in the soils at ($p<0.01$) and ($p<0.05$) levels of significance respectively. Where the highest values for all the samples were observed in the soil treated under long-term elevated CO_2 as shown in Table 2. Similar results were also obtained by [39]. The stimulation in enzyme activities by increasing CO_2 demonstrated that increased microbial biomass was accompanied by increased activity [40]. It is recognized that increased CO_2 levels can boost carbon input into the soil by improving the allocation of fixed carbon below ground [41]. This procedure can provide some information on future enzyme activity or the sum of active enzymes. However, because enzyme activities (particularly immobilized enzymes) are affected by edaphic circumstances, actual activity may differ from potential activity [42].

Table 2. Soil enzyme activities from three different soils elevated, ambient, and outside ambient. All the values are means of ±SD. Significant change in all the enzyme activities at 1% and 5% level of significance.

Soil parameters	**Soil Dehydrogenase (µg TPF g^{-1} soil 24 hr^{-1})**	**Beta Glucosidase (µg pNP g^{-1} soil)**	**Fluroscent Diacetate (µg fluorescein released g^{-1} soil)**	**Alkaline phosphatase(µg pNP g^{-1} soil)**	**Acid phosphatase(µg pNP g^{-1} soil)**	**Urease (µg NH_3 g^{-1} soil)**	**ArylSulphatase (nmol g^{-1}min^{-1})**
eCO_2	10.02 ± 0.23^{a}	132.35 ± 1.29^{a}	4.04 ± 0.04^{a}	57.69 ± 0.21^{a}	36.04 ± 0.12^{a}	48.16 ± 0.39^{a}	12.20 ± 0.11^{a}
A	5.38 ± 0.27^{c}	130.38 ± 1.54^{a}	3.92 ± 0.08^{b}	49.01 ± 0.15^{b}	31.41 ± 0.19^{a}	41.36 ± 0.34^{b}	9.55 ± 0.12^{b}
ABS	8.22 ± 0.26^{b}	118.92 ± 1.34^{b}	2.96 ± 0.09^{c}	45.93 ± 0.18^{b}	36.42 ± 0.26^{a}	34.85 ± 0.26^{c}	8.94 ± 0.16c

3.4. Sulphur fractions

The content of S and sulphur fractions in the soil increased significantly due to eCO2 in OTC, the available sulphur ranging from 13.49 to 17.13 mg kg^{-1} (Fig 2). The largest available S content was discovered in the 550 ppm CO_2 treated OTC 17.13 mg kg^{-1} followed by ambient soil 14.48 mg kg^{-1}) and the lowest value was reported in ambient bulk soil 13.49 mg kg^{-1}. Fractions of sulphur in the soils follow the order (AS≥WSS<ES<PS) Shown in Fig 2. The capacity of soils to adsorb sulfate varies greatly due to

the clay composition of soils containing different proportions of hydrous oxides of Al and Fe. However, [43] found that eCO_2 had little effect on soil S content. According to [44], diminished pH enhances sulphate adhesion in soil systems.

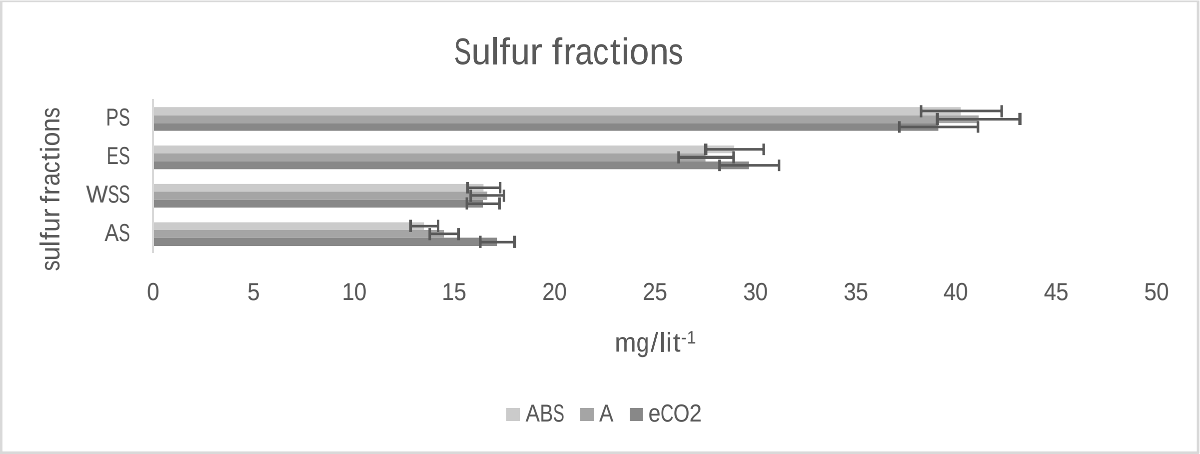

Fig 2. Distribution of sulphur fractions available sulphur (AS), water-soluble sulphur(WSS), exchangeable sulphur (ES), and precipitated sulphur(PS) from three different soils elevated, ambient, and ambient bulk soil.

3.5. Total Carbon, Nitrogen, Sulphur, and Available NPK in soil.

The quantity of CO_2 within the OTC had a detrimental influence on available soil N, P, and K content, which increased as CO_2 concentration increased significantly at 1% and 5 % levels of significance (Table-3). The maximum soil NPK concentration, was found with eCO_2, followed by ambient and ambient bulk soil respectively as shown in Table-3, Plant requirement for nitrogen (N) is predicted to grow as atmospheric CO_2 levels rise (eCO2). Nitrogen supplementation improves the impact of CO_2 on plant production. Furthermore, eCO2 greatly improved N absorption [45, 46]. As a result, the amount of the response in plant productivity is primarily determined by how well plant N absorption can keep up with eCO2-induced stimulation of carbohydrate formation and growth. Plants may positively control a number of physiological processes, such as enzyme production and root development, to boost plant nutrient acquisition capacity for optimum eCO_2 adaptation [47]. Significant increases in available P requirement by plants are predicted under increased CO_2 levels due to photosynthetic stimulation and subsequent growth responses. Elevated CO_2 affects P acquisition by altering root shape and increasing rooting depth. Furthermore, with high CO_2, the amount and content of root exudates are anticipated to alter due to changes in carbon fluxes through the glycolytic pathway and the tricarboxylic acid cycle. As a result, root exudates may induce P mobilization via alkylation of P from low solubility P complexes, shifts in the biochemical environment, and alters indigenous soil microbial activity.[48].Significant increases in the available potassium in the soil due to lowering of pH in soil due to the formation of carbonic acid and solubility of the ions increases in soil. Increases in the available potassium under elevated CO_2 have been reported by [49].In terms of total carbon nitrogen and sulphur, no significant difference was found among the three different treatments observed.

Table 3 Distribution of carbon, nitrogen, and sulphur in three different soils elevated, ambient, and ambient bulk soil. All the values are means of ±SD. Significant difference found among available N, P, K at (p<0.01) and (p<0.05) level of significance.

Soil parameters	Elevated CO_2	Ambient soil	Ambient bulk soil

Available N kg/ha	285.6±2.2^a	268.8±3.6^a	246.4±3.9^b
Available P Kk/ha	37.01±0.56^a	32.48±0.94^b	28.95±0.85^c
Available K kg/ha	490±12^a	470±14^b	454±18^c
Total N (%)	0.0784±0.005^a	0.0795±0.09^a	0.0756±0.06^a
Total S (%)	0.42±0.08^a	0.41±0.09^a	0.41±0.06^a
Total C (%)	0.88±0.02^a	0.87±0.02^a	0.87±0.01^a

Conclusion

Elevated CO_2 levels are responsible for rising global temperatures, which may have a significant influence on soil health, crop output, and plant nutrient usage efficiency. In the current experiment, the findings showed that the responses of soil microbes and their community structure to elevated CO_2 changed significantly in the rice low land ecosystem which is under long-term elevated. S fractionation in rice field is a powerful tool for understanding S dynamics and biogeochemistry in wetland ecosystems. eCO_2 greatly influences the fractionation of sulphur. The study found that all sulphur fractions, including available S, water soluble S, and exchangeable S, precipitated S and total S were significantly influenced. Precipitated sulphur was discovered to be the most dominant among the observed fractions. These changes may be largely related to CO_2-induced changes in soil C, N and S availability.

Ethical approval

Not applicable.

Consent for Publication

All authors have reviewed the manuscript and agree to its publication.

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