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Assessment of the CO_2 uptake capabilities and growth responses of selected grass species under elevated CO_2

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Abstract

Screening plants for their CO_2 uptake efficiency could contribute to better species, which could be an asset to future CO_2 mitigation programs. This study evaluated the flux in CO_2 within a CO_2-controlled chamber having grass species, *Megathyrsus maximus*, *Saccharum arundinaceum*, *Cymbopogon flexuosus*, *Chrysopogon zizanioides*, *Arundo donax* and *Pennicetum pedicellatum*. The day flux of CO_2 (related to photosynthesis) and night flux of CO_2 (related to respiration) within the chambers were estimated. The net CO_2 exchange of grass species was also calculated to assess the actual CO_2 uptake capability for carbon sequestration. Regarding net day flux, *S. arundinaceum* (-338.64±65.01ppm) showed better performance, followed by *M. maximus*, *A. donax*, *P. pedicellatum*, *C. flexuosus* and *C. zizanioides*. However, *A. donax* showed a higher net CO_2 exchange (-27.63±11.42%) due to its lower respiratory contribution of CO_2 at night. While concerning overall morphology *A. donax* was on the last order, whereas *M. maximus* and *S. arundinaceum* were on the top. An equation was derived to estimate the CO_2 uptake per plant biomass, especially for chamber studies, in which *S. arundinaceum* showed more efficiency in CO_2 uptake per plant dry weight (8.008g CO_2 per Kg), followed by *A. donax*, *C. flexuosus*, *P. pedicellatum*, *M. maximus,* and *C. zizanioides*. Broad understanding from the study is that *M. maximus* and *S. arundinaceum* were more efficient in curbing CO_2 and since *A. donax* had higher CO_2 uptake capability and net CO_2 exchange it could also be employed in CO_2 mitigation with proper supplementation of nitrogen.

Ker words: *elevated CO_2, CO_2 uptake, day flux, night flux, net CO_2 exchange*

Introduction

Globally, several strategies are being developed to reduce atmospheric CO_2 levels. These include the confiscation of geological and oceanic resources, reductions in energy consumption, development of low- or no-carbon fuels, carbon sequestration through engineering methods, and other forestry/agro-forestry practices (Dhyani et al., 2020). Among the many strategies, carbon sequestration in terrestrial biomass is a significant and economical way to lessen the negative consequences of climate change. The process of removing carbon from different sources and storing it in reservoirs with varying longevity is known as carbon sequestration (Nogia et al., 2016). During photosynthesis, plants absorb CO_2 and water and convert them into low-carbon compounds like cellulose and starch. With a capacity to sequester about 100 Gt of carbon annually, biological sequestration through photosynthesis in biomass or soil is an attractive option (Park, 2008).

The biomass of grasses is a major sink of atmospheric carbon. Well-managed grasslands can sequester considerable amounts of carbon with ideal growing conditions, which makes them essential for reducing the effects of climate change (Bai & Cotrufo, 2022). Grass species are potentially more successful than tree species

as terrestrial carbon sinks due to a few of their unique properties. Grasses have a higher growth rate, which favours them for higher CO_2 accumulation. Mahanta et al. (2020) reported that over 26% of the world's land surface is covered with grasslands and that these soils store a significant quantity of carbon, with global carbon stocks estimated to be around 343 Pg carbons. Considering what they say, the estimated annual carbon sequestration capability of soils worldwide is between 0.4 and 1.2 Pg carbons with grasslands accounting for 0.01 to 0.30 Pg carbon. Fisher et al. (1994) previously reported the potential of grasses to shift carbon into the soil. Most grasses have an annual root system, with the majority of the tiny roots growing from the plant's base. As a result, a lot of grasses discharge significant amounts of fiber (mostly carbon) into the soil every year when they almost completely shed their root systems.

Specific plant functional traits could react to new environmental conditions to balance off increased CO_2 levels (Bhattacharya et al., 2023). Morphological, physiological, and phenological features are among the functional traits of plants that influence the performance of particular species. These characteristics enable long-term storage of carbon in plant biomass, or the majority of the plant, and subsequent transport of that carbon into soils. Many studies have previously examined the photosynthetic and growth capabilities of plants or ecosystems under rising CO_2 and temperature, either jointly or independently (Eller et al., 2013; Souza et al., 2016; Kimbal, 2016; Runion et al., 2016). Moreover, climate change may also alter the chemical composition of plant tissues (IPCC, 2013; Myers et al., 2014). Perennial biomass crops including grasses are more energy-producing, require less input, and help reduce greenhouse gas emissions more than other crops (Jansson et al., 2010). According to Harmon et al. (1990), perennial grasses are potentially a better option for sequestering carbon because short rotation woody crops like poplar (*Populus* spp.), willow (*Salix* spp.), mesquite (*Prosopis* spp.), etc. require more time to close their canopy and increasing the chances of soil organic carbon loss.

Agroforestry systems may be a better option for mitigating climate change than oceans and other terrestrial options because they have additional benefits for the environment, such as food security, secure land tenure, increased farm income, maintenance of watershed hydrology, restoration and maintenance of above- and below-ground biodiversity, and soil conservation (Pandey, 2002). According to Do et al. (2020), the requirement for grasses for livestock fodder can be met by agroforestry practices that include grass strips. Furthermore, as a rich source of lignocellulosic biomass, perennial grasses can serve as a substitute for fossil fuels and a second-generation alternative energy source (Leo et al., 2016).

Several methodologies have been developed worldwide since the 1970s to simulate and evaluate the effects of elevated CO_2 concentrations in plants. In the present study, cost-effective and easily replicable carbon dioxide-controlled chambers were constructed to perform CO_2 enrichment studies with six selected grass species such as *Megathyrsus maximus*, *Saccharum arundinaceum*, *Cymbopogon flexuosus*, *Chrysopogon zizanioides*, *Arundo donax* and *Pennicetum pedicellatum*. This study aimed to assess the relative CO_2 uptake potential of these grass species, for which fluxes of CO_2 inside the chambers were analyzed with each species. The reduction of carbon dioxide in the microclimatic environment due to the growth and metabolism of specific grass species was compared to determine which species would work best for carbon offset planting and agro-forestry initiatives. Additionally, relative responses of morphological, biomass, and biochemical characteristics to elevated CO_2 were also examined to identify the species that are better suited for carbon storage and resilience in changing climate.

Materials and Methods

Experimental chambers and Plant materials

Installation of experimental chambers and standardization of the experimental system were conducted for trees and grasses as part of a simultaneous research work. A general outline of the installation was provided in Sreekumar (2024). Two chambers, each with a size of 6.32 m^3 were installed using the materials and facilities mentioned in Table 1. The shape of the chamber is similar to a cuboid (5.78 m^3), with the top portion volume further reduced (0.54 m^3). They were then made airtight and checked for leakages, if any. The chambers were then fitted with the facility for CO_2–air mixed supply using a CO_2 cylinder, air compressor, and a nebulizer (a mixing tube where the concentrated CO_2 gas from the cylinder was mixed with ambient air from an air pump). An exhaust with a control facility was also attached to the top of the chamber to adjust the outflow of gases if required. A water supply facility was attached to both chambers to facilitate the irrigation of plants during experimentation. Monitoring of carbon dioxide concentration (in ppm) within the chambers was made through an automated CO_2 analyzer (NDIR type Infrared Gas Analyzer, Fuji Electric, Japan). For regular monitoring of temperature and humidity, both chambers were fitted with a Billion Bag digital wireless electronic Hygro-thermometer. Experimental chambers and associated facilities were shown in Fig 1.

Fig. 1 CO_2 controlled experimental chambers and associated facilities in which a) experimental chambers, b) CO_2 analyser, c) hygro-thermometer, d) CO_2 cylinder connected to a nebulizer, e) nebulizer and air pumb

Six grass species such as *Megathyrsus maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs, *Saccharum arundinaceum* Retz., *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson, *Chrysopogon zizanioides* (L.) Roberty, *Arundo donax* L. and *Pennisetum pedicellatum* Trin. were selected for the study owing to their greater biomass, suitability for tropical climatic conditions, and dearth of studies on their CO_2 sequestration capabilities. The healthy plantlets were grown in poly bags (40 x 24 x 24 cm) containing a potting mixture made with soil, dried cow dung, and sand in a ratio of 3:1:1. For each species plants were multiplied by using plantlets from the initial raring process and were then grown for 6-7 months to attain sizable biomass for experimentation.

Table 1 Materials used and facilities associated with CO_2-controlled experimental system

Chamber construction materials	Chamber associated facilities
○ PVC pipes (Chamber frame) (PVC Pipe 40mm, UPVC* Elbow 40mm, UPVC/PVC Cross Tee 40mm, UPVC/PVC Threaded Tee 40mm) ○ Polyvinyl chloride (PVC) sheet of 1mm thick ○ Foam sheet (2.5mm), Multi wood (8 mm) ○ Sand and green sheet (Chamber floor)	○ CO_2 cylinder - 7kg (Das Air Products, Calicut) ○ CO_2 analyzer (NDIR** type Infrared Gas Analyzer, Fuji Electric, Japan) ○ Mixing tube/nebulizer made of PVC pipe (PVC Pipe 140mm & UPVC End Cap 140mm) ○ Air compressor ○ Exhaust fan (Usha) and associated electrical wiring facility ○ Hygrothermometer (Billion bag digital wireless electronic) ○ Irrigation facility with plumbing pipes (PVC 20mm) and tap

* Unplasticized PVC, **Non-dispersive infrared

Monitoring of microclimatic conditions of control and treatment chambers

Mature plants of *Megathyrsus maximus*, *Saccharum arundinaceum*, *Cymbopogon flexuosus*, *Chryzopogon zizanioides*, *Arundo donax,* and *Pennicetum pedicellatum* were employed for experimentation, separately. For each study, 2 sets of 3 plants were selected and maintained in the control chamber (CC) and treatment chamber (TC) respectively. Ambient air was supplied to CC for 15 minutes in the morning (9 a.m.). Similarly, through the mixing tube/nebulizer elevated CO_2 was supplied to the TC for 15 minutes (9 a.m). After the supply of air/CO_2+air mixture, the levels of CO_2 (ppm) in CC and TC respectively were monitored using an automated CO_2 analyzer (Fuji Electric NDIR type Infrared Gas Analyzer). Subsequent levels of temperature (°C) and humidity (%) were monitored by a Billion Bag digital wireless electronic Hygrothermometer. Monitoring of CO_2 concentration, temperature, and humidity was repeated at 6 p.m. Accordingly the day flux of CO_2, day variations in temperature, and humidity were calculated by subtracting evening measurements from morning measurements after CO_2 supply. The amount of CO_2 retained in the chamber in the evening from that of the amount of CO_2 supplied in the same day morning gives the day CO_2 flux and is the amount of CO_2 assimilated by the plants in the chamber. Monitoring is repeated in the same way on the next day's morning and the night CO_2 flux is estimated by subtracting morning measurements from that of the previous day's evening. The night variations in the temperature and humidity were calculated. The monitoring was continued for fifteen days.

Standardization studies were undertaken in empty chambers for 15 days to assess the flux of CO_2 associated with the chambers as a result of retention or dissipation of CO_2 at ideal conditions of chambers in the absence of plants. Procedures undertaken for the experiment with plants were repeated for the standardization study. The schematic representation of the entire experimental setup is given in Fig 2.

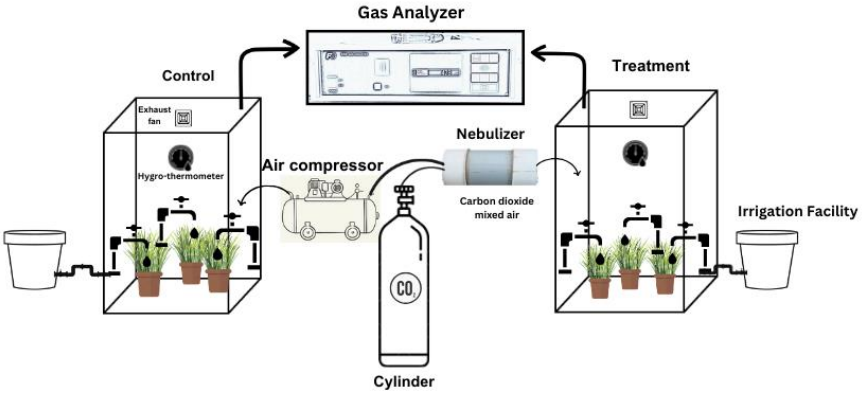

Fig. 2 Schematic representation of the experiment including CO_2-controlled chambers and associated facilities

Monitoring of Growth and Biochemical attributes

Growth attributes analysed on the first and final days include morphological parameters such as plant height and tiller height, number of tillers, number of leaves, leaf length, leaf breadth, leaf area, culm diameter, and plant biomass. Leaf samples collected on the 1st, 5th, 10^{th,} and 15th days were subjected to the analysis of pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids), metabolites (carbohydrate, protein, and phenol) and nutrients (carbon, nitrogen, calcium, and magnesium). Biomass of above-ground and belowground portions was estimated on a dry weight basis. Leaf pigments were quantitatively estimated using the DMSO method. Estimates of carbohydrates were made following Dubois et al. (1956). For this 0.1g of leaf samples from each treatment were hydrolyzed for three hours in a boiling water bath with 0.5 ml of 2.5N HCl. Centrifuged samples were added with anthrone reagent for further quantitative estimation of carbohydrates. The Lowry et al. (1951) method was employed for protein estimation. The phenol content was determined using the method of Malick and Singh (1980), which involved homogenizing 0.5 g of leaf tissue in 5 ml of 80% ethanol. Folin-Ciocalteau reagent was added to centrifuged and pooled samples for the quantitative estimation of phenol content using a UV-visible spectrophotometer.

Acid-digested leaf samples were subjected to the estimation of calcium and magnesium (EDTA titration). A CHNS organic elemental analyzer, FLASH 2000 (Thermo Scientific), was used to determine the carbon and nitrogen contents of each sample.

Matrices considered for identifying species with higher carbon sequestration efficiency

The best grass species for mitigating atmospheric CO_2 were identified by analyzing a range of aspects and from these aspects, four matrices were considered including net day flux, net CO_2 exchange, overall morphology, and CO_2 uptake per kilogram plant biomass.

***Net Day Flux of CO_2* /DF (N)**: The day flux (difference in CO_2 between morning and evening) and the night flux (difference in CO_2 levels between the previous day's evening and the next day's morning) of CO_2 were calculated. To eliminate the flux of CO_2 by other factors and to obtain the actual contribution of grass the net flux in CO_2 is calculated by subtracting the CO_2 flux values of the standardization study from the respective gross fluxes of each species under study.

***Net CO_2 exchange*:** In terms of carbon sequestration, both day flux (related to photosynthesis) and night flux (related to respiration) are considered and net CO_2 exchange over the experimental period is calculated. The balance between the amount of CO_2 that plants absorb during photosynthesis and the amount of CO_2 that they release during respiration is known as net CO_2 exchange. The net exchange reveals the actual CO_2 concentration decreased over the experimental period in the enclosed chamber associated with each grass species.

Overall plant morphology:

To analyze the effect of elevated CO_2 on the overall morphology of grass species, the t-SNE algorithm (t-Distributed Stochastic Neighbor Embedding) with Hedges g values was plotted. The t-SNE algorithm is initiated by changing the high dimensional Euclidean distances between data points into conditional probabilities that represent similarities. Then the similarities were mapped onto a lower dimensional space. Hedges g values (effect size) of overall morphological parameters associated with the grass species were also depicted in t-SNE.

CO_2 uptake per Kg of plant biomass:

To know the efficiency of each grass species for CO_2 uptake relative to their biomass, gram CO_2 uptake per kilogram plant biomass (dry weight) was calculated. An equation was derived for the calculation of gram CO_2 uptake per kilogram of plant biomass as follows (specifically for closed chamber studies). To derive an equation for the estimation of gram CO_2 uptake per kilogram of plant biomass when we know:

C = Average CO_2 concentration taken up by the plants from the chamber (in parts per million, ppm). In the present study, net day CO_2 flux (DF (N)) is considered.

V = Total volume of the chamber (in cubic meters, m^3)

W = Total dry weight of plants inside the chamber (in kilograms)

The following assumptions were considered for the derivation of the equation for the amount of CO_2 uptake per biomass.

A1-The volume of the chamber is filled with air at standard temperature and pressure (STP). In normal conditions, STP is 1 atm ((101,325 Pa). In the present study, an additional 400 - 500 ppm of CO_2 was supplied to the treatment chamber, and the temperature range was around 35 - 40 °C in the chamber. Approximately 10.13 Pa (Pascals) would be the increase in pressure within the chamber when the CO_2 content rises from 400 ppm to 500 ppm (Atkins et al., 2023). Considering that the overall pressure is normally about 101,325 Pa (1 atm), this is an equally minimal pressure rise (Borgnakke & Sonntag, 2020). Moreover, a slight rise in pressure may result from temperature variations (35–40°C), but the overall pressure will remain around 1 atm (Yunus & Michael, 2002; Monteith & Unsworth, 2013).

A2- 1 mole of an ideal gas occupies 22.4 L at STP.

A3- Molecular weight of CO_2 is 44 g / mol

Derivation of equation for CO_2 uptake per plant biomass

CO_2 concentration in **ppm** is a dimensionless unit. Thus it is to be converted to volume of CO_2 in the chamber:

The volume of CO_2 (vCO_2) uptake in the chamber (m^3) is given by:

$$vCO_2 = \frac{C}{10^6} \times V \qquad (1)$$

Where C is the CO_2 concentration in ppm and V is the Chamber volume in m^3. The volume vCO_2 is the average volume of CO_2 (mean net day flux) taken by the plants from the chamber. Using this volume of CO_2 could be converted to moles. According to A1, the volume of the chamber is filled with air at standard temperature and pressure (STP). At STP, 1 mole of CO_2 occupies 22.4 L=0.0224 m^3 (A2).

$$nCO_2 = \frac{vCO_2}{0.0224} \quad (2)$$

Substituting vCO_2:

$$nCO_2 = \frac{\frac{C}{10^6} \times V}{0.0224}$$

Simplifying:

$$nCO_2 = \frac{C \times V}{10^6 \times 0.0224}$$

The molar mass of CO_2 is 44g/mol (A3), so to convert the moles of CO_2 to grams, multiplying moles of CO_2 by the molar mass:

$$\text{Mass of } CO_2 = nCO_2 \times 44 \quad (3)$$

Substituting the value of nCO_2:

$$\text{Mass of } CO_2 = \frac{C \times V}{10^6 \times 0.0224} \times 44$$

Simplifying:

$$\text{Mass of } CO_2 = \frac{C \times V \times 44}{10^6 \times 0.0224} \text{ g}$$

$$\text{Mass of } CO_2 = \frac{C \times V \times 44}{22400} \text{ g}$$

To calculate CO_2 uptake per kilogram of biomass (U), divide the mass of CO_2 by the plant dry weight in Kilograms (denoted by W):

$$U = \frac{Mass\ of\ CO_2}{W} \quad (4)$$

Substituting the value of Mass of CO_2:

$$U = \frac{\frac{C \times V \times 44}{22400}}{W}$$

Simplifying:

$$U = \frac{C \times V \times 44}{22.4 \times 10^3 \times W}$$

Now this equation gives the gram CO_2 uptake per kilogram of plant biomass

Final equation for the gram CO_2 uptake per kilogram of plant biomass (in closed chamber studies) is:

$$U = \frac{C \times V \times 44}{22.4 \times 10^3 \times W}$$

Where U is CO_2 uptake per kilogram of plant biomass, C is CO_2 concentration (mean plant uptake) in ppm, V is chamber volume in cubic meters and W is plant dry weight in kilograms. The constant 44 is the molecular weight of CO_2. The constant 22.4 is applicable only when the volume of the chamber is filled with air at standard temperature and pressure.

Statistical analysis

All statistical analyses of the CO_2 flux data were performed using *SPSS version 26*. T test was carried out to determine the significance of the difference in day and night CO_2 flux between the control and treatment groups. One-way ANOVA was performed to determine whether there is a significant difference between the species

under experimentation in the day and night CO_2 fluxes. It was followed by post-hoc testing to compare the day and night CO_2 flux of standardization experiments to the day and night CO_2 flux attributed by each species under experimentation. Paired samples T-test was conducted using Jamovi ver.2.3.28 to validate the significance of morphological and biochemical outcomes. In certain cases even if there are visible changes in plant traits, the T-test results were insignificant. In this context, for expressing the size of the difference between two groups (first and final days of the experiment) Hedges g statistical measure was carried out. Hedges g is an effect size measure that quantifies the difference between two groups in terms of their means, standardized by their pooled standard deviation. Analysis was done using the *'esc'* package in R. Furthermore to analyze the effect of elevated CO_2 on the overall morphology of each grass species, the t-SNE algorithm (t-Distributed Stochastic Neighbor Embedding) with Hedges g values was plotted. Analysis was done using *'Matplotlib'*, *'seaborn'* libraries in Python.

Results

Changes in microclimatic CO_2 flux associated with grass species under study

The variable day flux (DF), which is determined by calculating the difference in CO_2 levels in the experimental chamber between morning and evening, embodies the amount of CO_2 that each plant assimilates (Fig. 3). Similarly the night time variations in the chamber CO_2 levels (night flux, NF) are the respiratory contribution. In comparison to the standardization study, grass plants attributed a higher day flux. Furthermore, T-test results show that, in all plants, day flux differs significantly between treatment and control groups ($p<0.05$). One-way ANOVA univariate analysis of day flux data confirms greater day flux ($P<0.001$) associated with grass species under TC. Multiple comparisons of the mean difference of day flux regarding all grass species with the standardization study show significant day flux for all species.

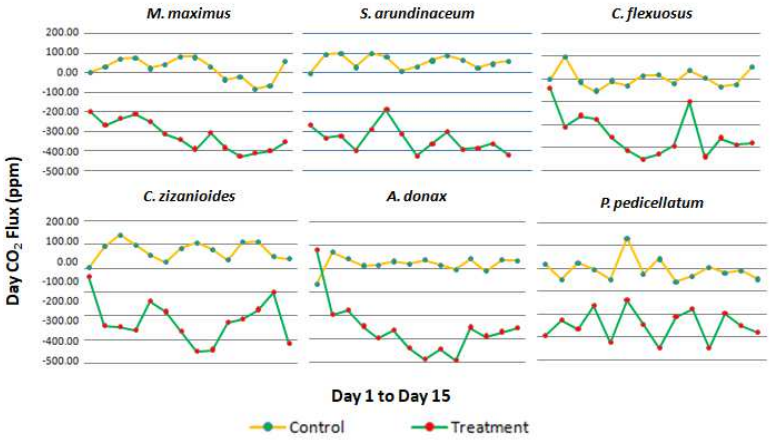

Fig. 3 Day CO_2 flux in experimental chambers

Many factors may influence the gas concentration in a closed chamber. To eliminate the flux of CO_2 by other factors and to obtain the actual contribution of grass species, the CO_2 fluxes of standardization study were taken into account. Accordingly, the net flux in CO_2 (DF (N) and NF (N)) is obtained by subtracting the CO_2 flux values of the standardization study from respective gross fluxes (DF (G) and NF (G)) of each species (Table 2). Percentage change in CO_2 flux during the experiment is also worked out to understand the relative efficiency of

plants in carbon assimilation. The net percentage change in CO_2 flux was obtained by subtracting percentage changes in CO_2 flux regarding standardization study from that associated with each plant species.

Standardization values of DF and NF associated with CC were -29.14±16.41ppm and 36.93±12.50 ppm respectively. Similarly, respective DF and NF of TC were -77.07±16.83 ppm and 23.21±16.16 ppm. DF (G) and NF (G) regarding *M. maximus* in TC were -398.14±85.05 ppm and 49.71±94.33ppm respectively. Consequently, DF (N) and NF (N) associated with *M. maximus* in TC is -321.07±78.16 ppm and 26.50±92.34 ppm respectively. Likewise in TC regarding *S .arundinaceum* respective DF (N) and NF (N) were -338.64±65.01ppm and 55.07±47.75ppm. DF (N) noticed in TC of the species *C. flexuosus* is -240.86±93.26 ppm and here the NF (N) is 38.43±140.87. In *C. zizanioides* net flux of CO_2 in TC is -222.93±89.46ppm and -38.86±74.55ppm respectively at day and night. *A. donax*-associated DF (N) in TC is -257.86±115.64 and the NF (N) is -28.07±77.05. The DF (N) attributed by *P. pedicellatum* is -245.50±67.13 and the associated NF (N) is -2.29±70.17. Thus based on DF (N) *S. arundinaceum* is superior followed by *M. maximus*, *A. donax*, *P. pedicellatum*, *C. flexuosus* and *C. zizanioides*.

Table 2 Gross and net CO_2 fluxes concerning experiment with grass species

CO_2 fluxes (ppm)	CC (DF)	TC (DF)	CC (NF)	TC (NF)
Standardization (CC)	-29.14±16.41	--	36.93±12.50	--
Standardization (TC)	--	-77.07±16.83	--	23.21±16.16
M. maximus (G)	-10.64±53.37	-398.14±85.05	110.29±112.25	49.71±94.33
M. maximus (N)	19.57±53.85	-321.07±78.16	73.36±111.27	26.50±92.34
S. arundinaceum(G)	28.00±27.81	-415.71±63.92	34.00±31.00	78.29±46.58
S. arundinaceum(N)	58.21±33.54	-338.64±65.01	-2.93±35.89	55.07±47.75
C. flexuosus(G)	26.21±38.72	-317.93±93.55	49.21±53.01	61.64±145.83
C. flexuosus(N)	4.00±39.58	-240.86±93.26	12.29±53.00	38.43±140.87
C. zizanioidesG)	40.50±35.23	-300.00±86.91	-14.50±39.25	-15.64±68.09
C. zizanioides(N)	70.71±39.18	-222.93±89.46	-51.43±43.78	-38.86±74.55
A. donax(G)	-8.57±20.19	-334.93±123.16	3.64±29.55	-4.86±74.73
A. donax(N)	20.57±31.76	-257.86±115.64	-33.29±35.86	-28.07±77.05
P. pedicellatum(G)	-31.50±54.86	-322.57±61.54	-5.64±62.64	20.93±70.13
P. pedicellatum(N)	-2.36±49.38	-245.50±67.13	-42.57±60.40	-2.29±70.17

Where, DF- day flux; NF- night flux; G- gross flux, and N- net flux.

Day and night fluxes differ from their gross values, as mentioned above and the removal of CO_2 by other factors was omitted in net values. However multiple criteria were taken into consideration to determine which species had the highest potential for CO_2 assimilation. The present study is not intended to examine the photosynthetic or respiratory process of plants. However, in terms of carbon sequestration, both DF (related to photosynthesis) and NF (related to respiration) were considered and net CO_2 exchange over the experimental period was

calculated. The balance between the amount of CO_2 that plants absorb during photosynthesis and the amount of CO_2 that they release during respiration is known as net CO_2 exchange. The net exchange reveals the actual CO_2 concentration decreased over the experimental period in the enclosed chamber associated with each grass species (Table 3).

Table 3 Net CO_2 exchange concerning grass species

Species	Net CO_2 exchange (%)	
	CC	TC
M. maximus	20.51±25.09	-26.15±17.26
S. arundinaceum	10.88±8.36	-21.46±5.39
C. flexuosus	3.78±12.19	-15.87±13.89
C. zizanioides	4.14±11.52	-26.55±12.60
A. donax	-2.16±5.00	-27.63±11.42
P. pedicellatum	-6.59±11.57	-23.28±9.73

Carbon sequestration occurs when plants have a net absorption of CO_2, meaning that they take up more CO_2 through photosynthesis than they expel through respiration. Thus in terms of carbon sequestration, all species are potential ones with higher net CO_2 exchange at elevated CO_2 (TC) while *A. donax* is identified as having higher potential followed by *C. zizanioides*, *M. maximus, P. pedicellatum, S. arundinaceum,* and *C. flexuosus.* The net CO_2 assimilations of *S. arundinaceum* (-31.69±6.00%) and *M. maximus* (-31.22±7.34%) were higher during the day than *A. donax* (-25.00±10.81%) and *C. zizanioides* (-22.15±8.66). Despite having superior CO_2 assimilation during the day, these species cannot be regarded as the most efficient species for sequestering carbon due to their increased CO_2 emissions at night (*M. maximus*: 5.07±14.82%; *S. arundinaceum*:10.23±7.90%). Although *C. zizanioides* is a species with a lower efficiency in terms of absorbing CO_2 during the day, its emission during the night is minimal and even lowers the CO_2 levels (-4.41±10.24) at night in TC. Consequently with higher net CO_2 exchange *A. donax and C. zizanioides* become the most potential species.

Changes in morphological traits and biomass of grass species under elevated CO_2

Visible changes were noticed in the morphological traits and biomass of plants when treated under elevated CO_2 levels. Elevated CO_2 concentration had a considerable positive effect on the plant height of *M. maximus*, *S. arundinaceum,* and *C. zizanioides* since the effect size of TC is higher than CC. Regarding tiller height higher effect size with TC is shown only by *C. zizanioides* and *P. pedicellatum*. The number of leaves declined in all grass species except *M. maximus* where new leaves were developed. Leaf number was highly dropped in *A. donax* at elevated CO_2. Regarding *S. arundinaceum*, the elevated CO_2 effect is a relative augmentation of the leaf length and breadth than other grasses. Elevated CO_2-induced increase in the leaf area was shown only by *S. arundinaceum* and leaf area was significantly decreased in *A. donax*. Considering the species *P. pedicellatum*, regarding leaf area, the direction of effect size was different and magnitude was highly varied between TC and CC where the lowered leaf area in TC compared to CC was assumed to be a result of the effect of elevated CO_2. Elevated CO_2 environment-induced enhancement in the culm diameter was observed in all species while regarding *M. maximus* and *S. arundinaceum* negligible change was noticed.

t-SNE algorithm (t-distributed Stochastic Neighbor Embedding) with Hedges g values were plotted to analyze the effect of elevated CO_2 on overall morphology (Fig 4 and Fig 5). Concerning with CC of each species,

diagonal points indicate the species had not considerably changed its position except *P. pedicellatum*, thus there is only negligible change has happened in the overall morphological traits of control plants. While regarding the t-SNE plot of TC, a considerable change in the position of diagonal points of *M. maximus* and *S. arundinaceum* was evident. As far as the overall effect size of morphological traits is concerned, CO_2 treatment exhibits a higher positive effect size in all plants except *A. donax,* with a higher effect size noticed in *M. maximus* and *S. arundinaceum*. A notable result obtained from this plot was the higher negative effect size of *A. donax* in TC compared to a negligible effect size in TC. Likewise in *P. pedicellatum* a smaller effect size was noted in TC compared to a higher effect size in CC.

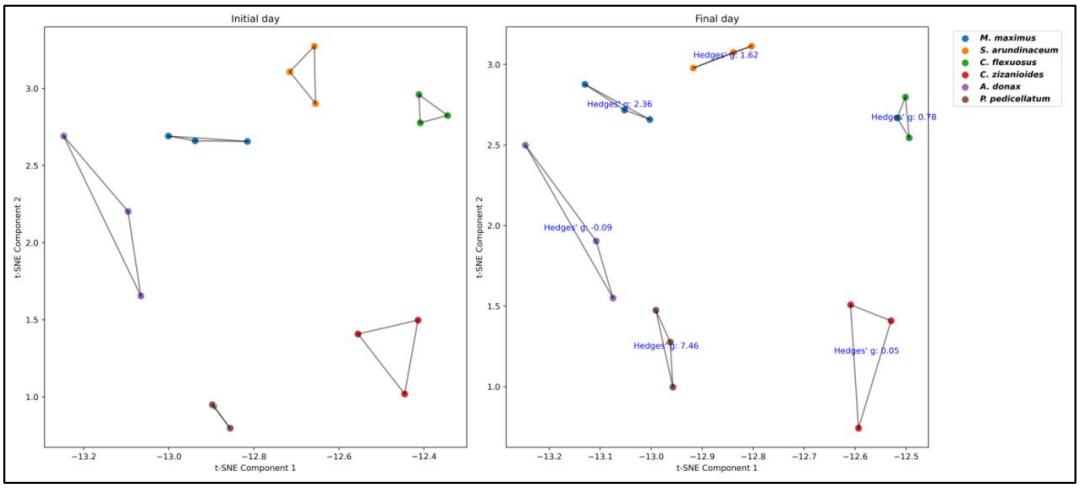

Fig. 4 t-SNE plot of morphological parameters in CC

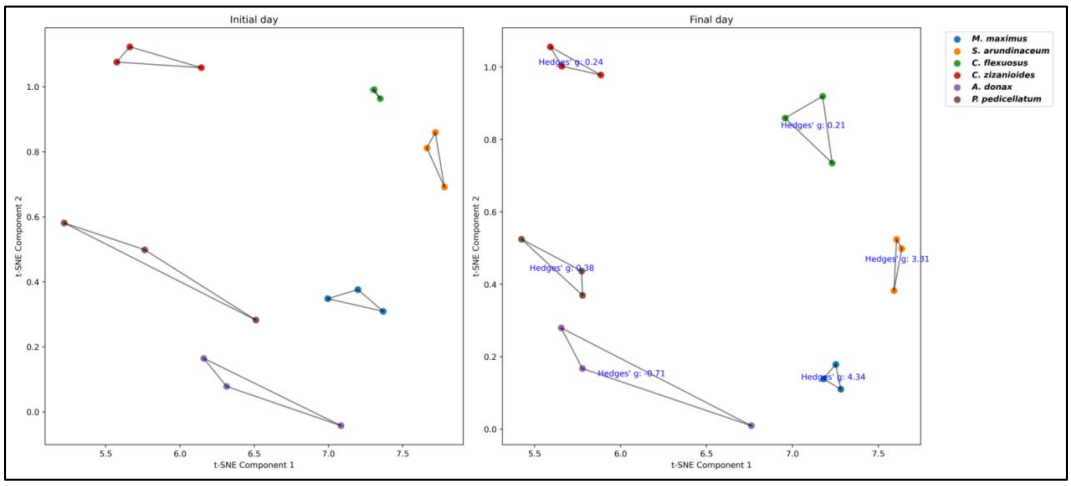

Fig. 5 t-SNE plot of morphological parameters in TC

The biomass of the plants was measured both on a dry and fresh weight basis. Concerning biomass, *M. maximus* and *C. zizanioides* were superior with 825g on a dry weight basis followed by *S. arundinaceum* (525g) and *P. edicellatum* (525g) and the least was *C. flexuosus* (450g) and *A. donax* (450g). The calculation of CO_2 uptake per biomass of a plant is a useful method for enumerating and comparing the carbon sequestration abilities of plants. This metric offers insights into the efficiency of plants in converting atmospheric CO_2 into biomass. Using the new equation derived, gram CO_2 uptake per kilogram plant dry weight was calculated (Table 4). *S.*

arundinaceum was more efficient in CO_2 uptake per biomass, followed by *A. donax*, *C. flexuosus*, *P. pedicellatum*, *M. maximus,* and *C. zizanioides*.

Table 4 CO_2 uptake efficiency of grass species with respect to biomass

Grass species	DF(N) ppm	Total Biomass of TC plants (Dry weight in kg)	CO_2 uptake potential per kg of plant dry weight (g)
M. maximus	321.07	0.825	4.831
S. arundinaceum	338.64	0.525	8.008
C. flexuosus	240.86	0.45	6.645
C. zizanioides	222.93	0.825	3.355
A. donax	257.86	0.45	7.114
P. pedicellatum	245.5	0.525	5.805

Elevated CO_2 effect on leaf pigments, metabolites, and nutrients

Changes were noticed in the concentration of pigments. Chlorophyll a content was increased in *S. arundinaceum* and significantly decreased in *A. donax*. As far the chlorophyll b is concerned a reduction was noticed in all plants except *S. arundinaceum*, where an increase was reported. Regarding total chlorophyll, an augmentation was noticed only in *S. arundinaceum* and *P. pedicellatum*, in all other species it was decreased in both CC and TC. A significant decrease in the carotenoid content was noticed in *C. flexuosus* and *A. donax*. Significance values and effect sizes are represented in Table 5.

M. maximus, *C. zizanioides,* and *A. donax* show higher positive effect sizes regarding carbohydrate levels in TC. Among these three species *A. donax* exhibited an extremely high positive effect size in TC compared to a smaller negative effect size in CC. Regarding protein concentration noticeable differences between CC and TC were shown only by *S. arundinaceum* and *C. zizanioides*, with extremely lower effect sizes in TC than CC. Considering the concentration of total phenol significant change due to elevated CO_2 was shown only by *C. Zizanioides*, which was a drop in levels of total phenol.

According to the CHNS analysis leaf carbon content was increased at elevated CO_2 conditions in all grass species except *A. donax* (Fig. 6 A). Concerning the nitrogen content, the direction of change varied with species and treatment. It was increased in *C. flexuosus* and *C. zizanioides* while greatly decreased in *A. donax* at elevated CO_2 treatment (Fig. 6 B).

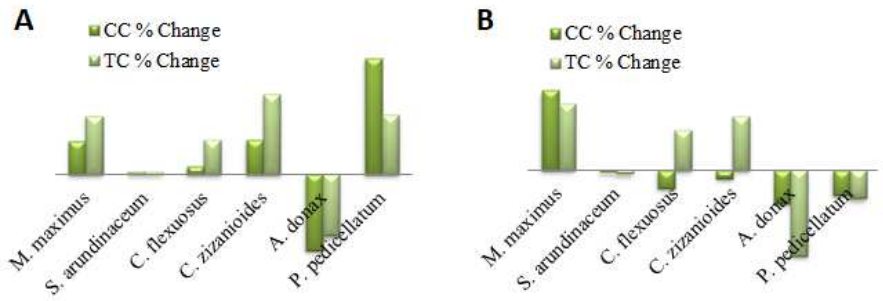

Fig.6 Percentage change in the leaf carbon (A) and nitrogen (B) of CC and TC samples

Table 5 T-test significance values and Hedges g values of plant pigments and metabolites

Pigments & metabolites	Species	Paired samples T-test (p-value)		Hedges g (effect size)	
		CC	TC	CC	TC
Chlorophyll a	*M. maximus*	0.256	0.305	-1.119	-1.219
	S. arundinaceum	0.421	0.442	0.670	0.827
	C. flexuosus	0.223	0.008*	-1.591	-3.672
	C. zizanioides	0.012*	0.094	-2.695	-2.514
	A. donax	0.228	0.008*	-0.959	-1.506
	P. pedicellatum	0.026*	0.025*	2.958	5.248
Chlorophyll b	*M. maximus*	0.003*	0.153	-4.119	-1.569
	S. arundinaceum	0.263	0.406	0.824	0.943
	C. flexuosus	0.202	0.011*	-1.719	-2.551
	C. zizanioides	0.057	0.031*	-2.632	-3.996
	A. donax	0.012*	0.012*	-1.110	-1.146
	P. pedicellatum	0.035*	0.050*	-4.326	-3.891
Total Chlorophyll	*M. maximus*	0.047*	0.181	-3.046	-1.551
	S. arundinaceum	1.194	0.418	1.115	0.913
	C. flexuosus	0.218	0.008*	-1.618	-3.435
	C. zizanioides	0.020*	0.066	-2.780	-3.217
	A. donax	0.153	0.008*	-1.030	-1.420
	P. pedicellatum	0.094	0.105	1.470	2.528
Carotenoids	*M. maximus*	0.573	0.185	-0.573	-0.365
	S. arundinaceum	0.012*	0.023*	4.477	3.262
	C. flexuosus	0.317	0.010*	-1.191	-2.259
	C. zizanioides	0.044*	0.080	-2.288	-3.035
	A. donax	0.220	0.010*	-0.917	-1.685
	P. pedicellatum	0.031*	0.030*	2.959	4.401
Carbohydrates	*M. maximus*	0.307	0.168	0.662	0.918
	S. arundinaceum	0.062	0.238	-1.508	-1.499
	C. flexuosus	0.099	0.422	-1.924	-0.624
	C. zizanioides	0.584	0.084	0.292	0.957
	A. donax	0.672	0.131	-0.266	1.909
	P. pedicellatum	0.003*	0.533	6.303	0.210
Protein	*M. maximus*	0.050*	0.033*	-1.915	-1.206
	S. arundinaceum	0.019*	0.994	-1.257	-0.005
	C. flexuosus	0.010*	0.008*	-2.484	-1.060
	C. zizanioides	0.176	0.885	-1.342	0.096
	A. donax	0.731	0.566	0.353	0.375
	P. pedicellatum	0.387	0.209	0.764	0.867
Phenol	*M. maximus*	0.053	0.115	-2.389	-1.821
	S. arundinaceum	0.157	0.957	-2.033	0.031
	C. flexuosus	0.005*	0.043*	-6.196	-2.037
	C. zizanioides	0.094	0.011*	-2.711	-2.588
	A. donax	0.027*	0.070	3.422	2.403
	P. pedicellatum	0.121	0.089	2.079	2.150

*significance at $p<0.05$; (-), decrease; NaN (Not a Number), Zero variance between samples

Calcium content was decreased in TC of all grass species under elevated CO_2 treatment, except *P. pedicellatum*. Magnesium content was decreased in all species at elevated CO_2 treatment. *A. donax* treated with elevated CO_2 exhibited a significant decrease in the magnesium content. The significance of the changes in calcium and magnesium at elevated CO_2 is represented in Table 6.

Table 6 T-test significance values of calcium and magnesium

Plant Nutrients	Species	Paired samples T-test (p value)	
		CC	TC
Calcium	*M. maximus*	0.038*	0.083
	S. arundinaceum	0.423	0.529
	C. flexuosus	1.000	0.423
	C. zizanioides	0.742	0.184
	A. donax	0.464	0.118
	P. pedicellatum	0.667	0.423
Magnesium	*M. maximus*	0.031*	0.054
	S. arundinaceum	0.529	0.074
	C. flexuosus	0.116	0.072
	C. zizanioides	0.621	0.423
	A. donax	0.057	0.023*
	P. pedicellatum	0.423	0.423

Relative performances of grass species to selected matrices

The current study aims to assess the effectiveness of selected grass species in carbon mitigation. Net day flux attributed by each species was considered as CO_2 uptake potential and to get the best species based on carbon sequestration, the net CO_2 exchange of each grass species was calculated. Overall morphology and CO_2 uptake per plant biomass were also considered for selecting grass species with better efficiency in terms of CO_2 sequestration. Thus concerning overall performance, *S. arundinaceum* and *M. maximus* both offer better results based on these matrices. The grass species were ranked based on these matrices (Fig. 6).

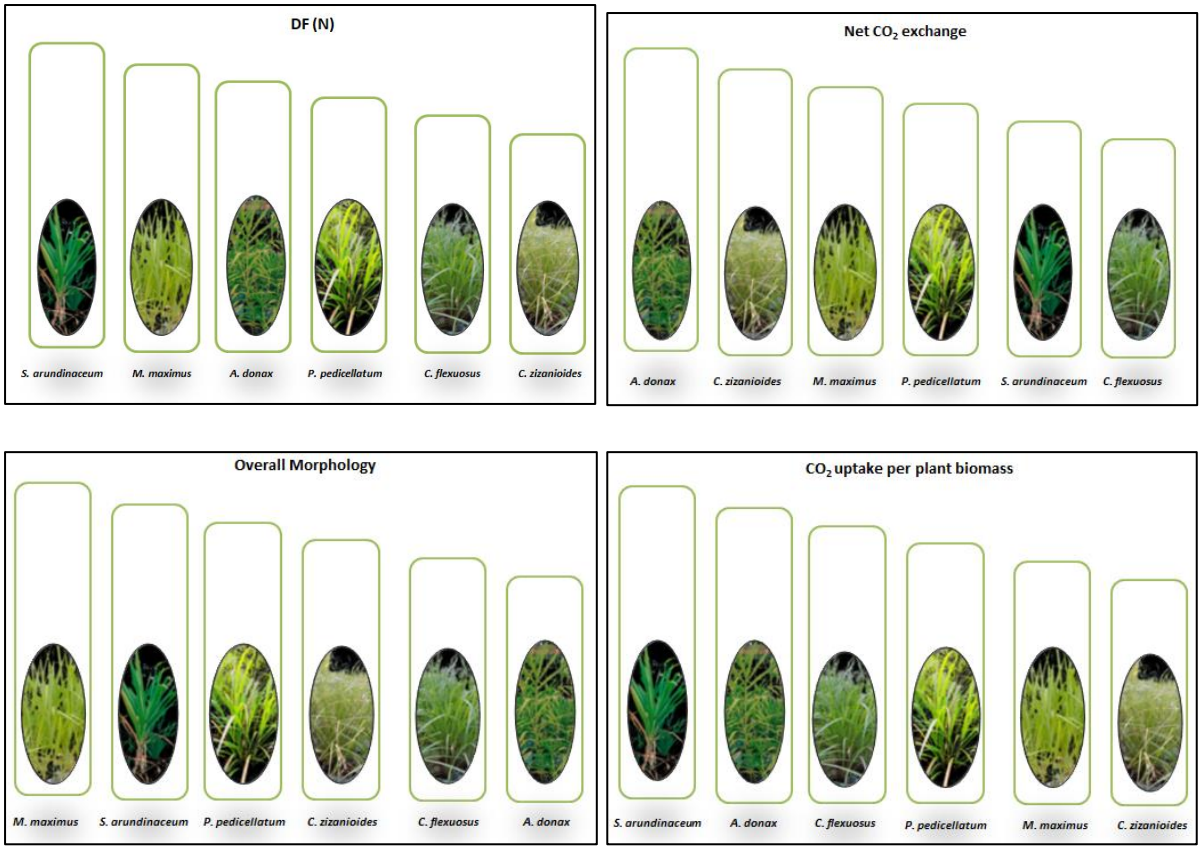

Fig.6 Relative performances of grasses based on matrices such as DF (N), Net CO_2 exchange, overall morphology, and CO_2 uptake per plant biomass

Discussion

Factors influencing Net CO_2 exchange of C3/C4 species

Physiological mechanisms and genetic traits determine how different species absorb, assimilate, and release CO_2. In the present experiment, the C3 species *A. donax* exhibited a superior CO_2 exchange than other species (C4). According to several previous experiments, it is identified that CO_2 enrichment is more beneficial to C3 plants (Waggoner, 1984; Kirkham, 2016; Wang et al., 2019). Under a doubled CO_2 environment, CO_2 enrichment reduces energy-losing photorespiration in C3 plants, stimulating growth to a range of 40–45%, while C4 plants only experience growth to a range of 10–20% (Ghannoum et al., 2000). The initial stage of carbon fixation, in which plants convert atmospheric carbon dioxide into glucose, requires RuBisCO, as an essential component. Long et al. (2006) related the increased CO_2-driven photosynthesis to competitive inhibition of the RuBisCO oxygenase activity and acceleration of carboxylation because the CO_2 binding site is not saturated at the current CO_2 levels. As the C4 pathway is CO_2-saturated and is not competitively hindered by O_2, C4 plants exhibit little photosynthetic response to high CO_2 (Ghannoum et al., 2000). In C3 plants increased photosynthesis occurs when RuBisCO is saturated by elevated CO_2, which also significantly reduces photorespiration (Long, 1992). Sashna et al. (2022) compiled the physiological and growth responses of C3 and C4 grasses at high CO_2 recorded over the last three decades and found that 84.62% of C3 grasses exhibit this photosynthetic advantage.

To enable plant responses to varying climates, plant stomata play an important regulatory function between the plant and its surroundings (Xu & Zhou, 2008). In plants, CO_2-regulated stomatal development and movements jointly control stomatal conductance and, consequently, CO_2 exchange (Driesen et al., 2020). The effects of CO_2 concentration on transpiration and stomatal aperture rates in five distinct species including C3 and C4 species were investigated by Pallas in 1965. According to the author, maize and sorghum (C4) show complete stomata closure at 0.2 and 0.3% CO_2, respectively, while stomata in C3 species did not close fully even at 0.4% CO_2. Subsequently, many scientists shared the view that C4 species exhibit greater sensitivity to CO_2 than C3 species (Akita & Moss, 1972; Ludlow & Wilson, 1971 and Osmond et al., 1982). According to Akita and Moss (1972) leaf stomata of C4 species are highly responsive to environmental changes. They found that when the amount of light was reduced or the concentration of CO_2 increased, the stomata of C3 species were less likely to close than those of C4 species. The stomatal sensitivity described in the studies mentioned above may have contributed to variations in day and night fluxes associated with each grass species.

Leaf area and the number of stomata per plant contribute to stomatal conductance and determine CO_2 exchange. Owensby et al. (1993) reported modest increases in leaf area index (LAI) in response to elevated CO_2 in large, open-top field chambers. The gas exchange rates may be impacted by this decrease in stomatal density, which may affect transpirational water loss and CO_2 intake. The leaf area of each species measured in the initial (1DOT) and final (15DOT) days of the experiment is depicted in the following table. Leaf area is obtained by multiplying leaf length, leaf breadth, and Kemp's constant (0.9 for grass species).

Species	Leaf Area (m^2)			
	CC		TC	
	1DOT	15DOT	1DOT	15DOT
M. maximus	0.88±011	1.01±0.04	0.90±0.18	1.04±0.32
S. arundinaceum	0.94±0.15	0.93±0.25	0.79±0.03	1.10±0.49
C. flexuosus	1.44±0.22	1.45±00.28	1.60±0.41	1.60±0.20
C. zizanioides	1.86±0.64	2.15±0.55	2.19±0.46	2.36±0.62
A. donax	0.82±0.34	0.79±0.32	0.93±0.28	0.75±0.25
P. pedicellatum	1.29±0.31	1.66±0.19	1.58±0.10	1.52±0.52

A. donax exhibited the least leaf area (in both CC and TC). Nevertheless, the C3 photosynthetic mechanism of the species contributed to the greater CO_2 exchange. According to the studies of Akita & Moss, 1972 stomata of C3 species are less responsive to atmospheric CO_2 enrichment; accordingly, the species *A. donax* with the least leaf area *(*0.75±0.25 m^2) and a minimal number of stomata exhibited a higher CO_2 exchange *(*-27.63±11.42) in TC than other C4 species. According to Pritchard et al., (1999), plants that are exposed to higher CO_2 levels may develop larger leaves with fewer stomata per unit area. Consequently species such as *M. maximus*, *S. arundinaceum,* and *C. flexuosus* with larger leaf areas than *A. donax* exhibit day CO_2 assimilation similar to this species. In addition, the negligible respiratory release during the night (-2.62±9.88%) also explains the higher CO_2 exchange. Followed by *A. donax, the species C. zizanioides* (C4) is best in terms of carbon sequestration. LA of *C. zizanioides* (C4) is 2.36±0.62 m^2, the highest among the 6 species. Thus the species is assumed to have more stomata. The day flux associated with the species is -22.15±8.66ppm, the least among the 6 species but the species have negligible respiratory release and even lower CO_2 concentration in the chamber during the night. This explains the higher CO_2 exchange and higher carbon sequestration exhibited by the species. Regarding other C4 species respiratory release during the night is evident to be higher, except for *P. pedicellatum (*-0.74±10.14%).

Overall morphology and Resource allocation pattern of grasses at elevated CO_2

From the t-SNE plot with Hedges g, the overall morphological change that happened in each species was established. A considerable change in the position of diagonal points of *M. maximus* and *S. arundinaceum* was evident in the t-SNE plot. This indicates considerable changes in the overall morphology of these plants at elevated CO_2. As far as the overall effect size of morphological traits is concerned, CO_2 treatment exhibits a higher positive effect size in all plants except *A. donax,* with a higher effect size noticed in *M. maximus* and *S. arundinaceum*. Since *A. donax* is a C3 species, the morphological responses of the species differ from the C4 species under study. The CO_2 exchange potential of *A. donax* was established while Hedges g values indicate a fall in the overall morphology of *A. donax*. Changes in resource allocation may cause C3 grasses to respond negatively in terms of morphology at high CO_2 (Reich et al., 2018). Elevated CO_2 can improve the plant's ability to absorb CO_2, however, these plants frequently give priority to growing biomass above ground rather than developing structural traits like tiller number or leaf area. This trade-off may result in a less complex overall structure by reducing morphological features even when CO_2 intake is enhanced. The morphological response of C3 grasses to elevated CO_2 is often greater than that of C4 grasses, however, this

response is not necessarily positive and can rely on exposure time, acclimatization processes, and interactions with other environmental factors (Pinto et al., 2014; Reich et al., 2018). To completely comprehend the intricate reactions of C3 and C4 grasses to elevated atmospheric CO_2 levels, long-term research is required. In the present study, the C4 grasses *M. maximus* and *S. arundinaceum* exhibited higher effect size concerning overall morphology in TC, 4.34 and 3.31 respectively. *M. maximus* allocated the resources from improved photosynthesis at elevated CO_2 towards increasing the leaf number and *S. arundinaceum* despite having fewer leaves overall, increases the efficiency of photosynthesis by investing in larger leaves i.e. resource allocation towards leaf enlargement.

CO_2 uptake capacity of grass species based on biomass

A relation between the average net day flux and biomass of the plant was established in the present study. For this an equation for the estimation of gram CO_2 uptake per kg dry weight of plant was derived using the variables such as net day CO_2 flux (average CO_2 uptake by plant species), plant dry weight, and volume of the chamber were considered. By using this equation, CO_2 uptake (in grams) per kg of plant biomass (dry weight basis) was calculated. Major insights into CO_2 uptake per plant biomass have been understood from these results. Increasing atmospheric CO_2 concentrations have been shown to promote biomass accumulation and plant development, mostly through the CO_2 fertilization effect (Zheng et al., 2018). According to their research, with total biomass growing by 60%, 15%, and 30% for tall fescue, perennial ryegrass, and Kentucky bluegrass, respectively, the results showed that the ideal CO_2 concentrations for aboveground biomass were 945, 915, and 1151 ppm for the corresponding species. Due to its higher optimal CO_2 threshold, Kentucky bluegrass may be more resilient to changes in the climate in the future. Various grass species have various effects, and these variations are affected by their functional categories. Likewise in the present study, each species was provided with a similar range of elevated CO_2 while the CO_2 uptake per biomass was different. Here the DF (N) of *C. zizanioides* with higher biomass (825g) was only 222.93 ppm, while *A. donax* with 450g has a DF (N) of 257.86 ppm. *S. arundinaceum* with only 525g biomass was the efficient species with a DF (N) of 338.64 ppm and the highest CO_2 uptake potential per kg of plant biomass (8.008g CO_2). When compared to C4 grasses, C3 grasses typically respond to higher CO_2 conditions more robustly. The C3 species often show considerable increases in biomass and photosynthesis in relation to rates of carbon uptake (Pendall et al., 2011). Numerous studies have demonstrated that C4 plant species have lower CO_2 sensitivity than C3 plants (Hager et al., 2016). Reich et al. (2018), however, discovered that during prolonged exposure, C4 species generated higher biomass and became more sensitive to elevated CO_2 than C3 species. Research using tropical C4 plants, such as *Saccharum officinarum* (De Souza et al., 2008), *Panicum maximum* (Habermann et al., 2019), and *Sorghum bicolour* (Prasad et al., 2009), have demonstrated increased leaf photosynthesis in the presence of elevated CO_2. The patterns of biomass allocation affect the efficiency with which grasses use CO_2 for growth and energy. Grasses that emphasize aboveground biomass might not be as good at absorbing CO_2. Likewise, grasses that put more of an emphasis on root development might be better at storing carbon below ground. The total carbon dynamics in grassland ecosystems are largely determined by these allocation patterns (Hungate, 1997). This might be also true with the individual grass species.

Pigment levels and growth of grasses at elevated CO_2 conditions

Chlorophyll a content and photosynthetic efficiency can vary amongst grasses because of genetic variations and species-specific responses to increased CO_2 (Silva et al., 2020). C3 and C4 plants have a number of genes that are directly related to how the plants react to their surroundings (Wang et al., 2003). Amplified CO_2 was shown to activate 327 genes in soybeans, suggesting that enrichment in the atmospheric CO_2 promotes the breakdown of carbohydrates, hence enhancing energy and growth precursors and leaf expansion (Ainsworth et al., 2006). In the present study, *S. arundinaceum* exhibits increased carbon assimilation, which is responsible for its increased chlorophyll content under elevated CO_2 levels. Higher concentrations of chloroplast CO_2 are the result of elevated CO_2, and this enhances photosynthetic efficiency in plants, enabling a larger allocation of resources to chlorophyll synthesis (Sugiura et al., 2023). Moreover, *S. arundinaceum* allocated the photosynthates towards leaf enlargement (leaf length and breadth) thus this might be a reason for increased leaf area which might increase the number of chloroplasts and thus augmentation in chlorophyll content. This assumption was concomitant with the view of Dusenge et al, (2019). According to them, modifications in leaf structure in grasses exposed to high CO_2 levels may help in enhancing the production of chlorophyll. In particular, greater leaf area and thickness may result from high CO_2 leaf development, which may have an impact on light interception. By further increasing photosynthetic capacity, this structural adaptation can establish a positive feedback loop whereby higher levels of chlorophyll encourage even higher levels of photosynthetic activity.

In C3 species *A. donax* concentration of all pigments declined. C3 grasses generally undergo a brief reduction in total chlorophyll content, chlorophyll a, and chlorophyll b under high CO_2 environments in closed chambers. According to Taub (2010), this decrease is brought on by the impacts of nitrogen dilution due to higher carbohydrate synthesis. According to the author, relative nitrogen content drops when carbohydrates build up from improved photosynthesis, which restricts the synthesis of chlorophyll and lowers pigment concentrations in C3 species. As well *A. donax* exhibited an enhanced carbohydrate production (25.58% increase) and declined concentration of leaf nitrogen (40.01% decrease). Under certain growth conditions, C4 plants can make trade-offs in the allocation of resources. In situations of reduced light levels or specific genetic limitations, C4 species may exhibit a preference for reallocating resources from pigment production to growth and survival strategies (Lara & Andreo, 2011). In these situations, their growth response can resemble that of C3 plants, which naturally prioritize comparable strategies for survival in undesirable environments (Anderson et al., 2021). This may happen in the photosynthetic pigment content of other C4 species such as *M. maximus*, *C. flexuosus,* and *C. zizanioides*. According to Sun et al. (2023), the Biosynthesis pathway of chlorophyll and that of carotenoids are closely related. For example, geranylgeranyl pyrophosphate (GGPP), which is produced by the methylerythritol 4-phosphate (MEP) route, is a common precursor to both pigments. This link emphasizes how carotenoid and chlorophyll biosynthesis are interdependent, with changes in the pathway influencing the total amount of both pigments that accumulate. The similar reduction in both pigments in *M. maximus*, *C. flexuosus*, *C. zizanioides,* and *A. donax* might happened due to this interdependence of biosynthetic pathways of chlorophyll and carotenoids.

Factors affecting plant metabolites under elevated CO_2 conditions

Due to unique physiological characteristics or genetic factors, certain species may not be able to maintain the initial rise in carbohydrate levels (Dusenge et al., 2019; Taub, 2010). In *S. arundinaceum*, *C. flexuosus* and *P. pedicellatum* carbohydrate concentration either decreased or negligible increase has occurred. Under conditions of elevated CO_2, *S. arundinaceum* exhibits a decrease in carbohydrate concentration, despite a rise in leaf area and pigments. The metabolic changes a plant goes through in response to increased production of carbohydrates help explain the phenomena (De Souza et al., 2008). For a short time, the plant prioritizes development (extension of the leaf area) over the storage of carbohydrates, which lowers its concentration. Growth and leaf area are enhanced by redirecting resources that would normally go toward carbohydrate storage. The plant prioritizes rapid development responses above the accumulation of carbohydrates, which leads to higher investment in structural components (leaf area) rather than in storage carbohydrates. Along with carbohydrate concentration, overall development, biomass, and pigment concentration were dropped at elevated CO_2 in *C. flexuosus*.

Experiments carried out in controlled settings, including open-top chambers, consistently show that C3 grasses exhibit lower nitrogen content and higher levels of carbohydrates when exposed to increasing CO_2 (Barbehenn et al., 2004). Moreover, Oijen et al. (1999) also reported that elevated CO_2-based increases in carbohydrate levels may be due to an indirect effect of declined nitrogen concentrations leading to lowered respiration rates. This is true with *A. donax* where leaf carbohydrate concentration increased by 25.58% and leaf nitrogen decreased by 40.01%. Furthermore, a lower night flux associated with *A. donax* was an indication of lowered respiration rates due to declined nitrogen concentration. Despite generally being less sensitive to increased CO_2 than C3 species, C4 grasses yet show notable physiological reactions (Ghannoum et al., 2000). Accordingly, C4 grasses such as *M. maximus* and *C. zizanioides* exhibited an increased carbohydrate concentration, whereas pigment contents were reduced.

Regarding the protein content changes occurred in both chambers, while notable differences between control and treatment groups were noticed only in *S. arundinaceum* and *C. zizanioides* while only a meager change from 1DOT to 15 DOT has happened in these species at TC. It was previously reported that elevated CO_2 levels have little effect on the protein content of grasses, especially C4 grasses (Barbehenn et al., 2004). Many plant species usually exhibit lower nitrogen concentrations when CO_2 rises, which always has an impact on protein content because proteins are nitrogenous molecules. This is commonly explained by an increase in the amount of carbohydrates in plants, which dilutes the concentrations of minerals and proteins in plant tissues (Taub et al., 2008). The effects of the closed chamber environment on temperature, humidity, and nutrient dynamics have a substantial impact on plant physiology. Due to the combined effects of environmental aspects and physiological reactions to elevated CO_2 levels, these factors have the potential to cause a significant reduction in protein concentrations in grasses over time (Taub, 2010; Burgess & Huang, 2016).

Phenolic compounds, the significant secondary metabolites in plants, often act as defense mechanisms against infections and herbivores (Bezemer et al., 2000). Variations in the environment, such as elevated CO_2 levels in the atmosphere, can cause substantial differences in their concentration (Castells et al., 2002). Variable responses are noticed in the concentration of phenol regarding the grass species under the present study. In grasses, the phenolic biosynthesis pathway can be considerably influenced by elevated CO_2 levels (Castells et

al., 2002). Enhanced carbon availability could improve the allocation of carbon to phenylpropanoids, an essential step in the phenolic biosynthesis process. Accordingly in *S. arundinaceum* phenol content was increased in CO_2 treatment while a considerable decrease has occurred in control. However, phenol content was decreased in *M. maximus*, *C. flexuosus* and *C. zizanioides* with a significant decrease in *C. zizanioides*. As a defense mechanism, certain species may increase their phenolic synthesis, while others may have adaptive features that limit their phenolic biosynthesis under high CO_2 environments. Inconsistent findings of phenolic concentrations in response to increased CO_2 in various grass species may be caused by this variability (Castells et al., 2002). The pattern of nitrogen allocation may also account for the reduced amounts of phenolic compounds (Vogt, 2010). The phenolic production pathway may be disrupted by the decline in nitrogen content that occurs in grass tissues when CO_2 levels are increased. While here in the present study, the grass species where phenol levels decreased (*M. maximus*, *C. flexuosus,* and *C. zizanioides*) exhibited an increase in nitrogen concentration. Since elevated CO_2 levels enable grasses to grow and accumulate biomass more often there causes a change in resource allocation from the production of secondary metabolites, such as phenolics, to primary processes like photosynthesis and biomass formation (Wang et al., 2023). As a result key enzymes in the phenolic biosynthesis pathway, such as phenylalanine ammonia-lyase (PAL), may not exhibit a comparable rise in activity despite higher nitrogen levels.

Plant nutrients and growth of grass species under elevated CO_2 conditions

Elevated CO_2 levels have significant effects on the accumulation of nutrients in grass leaves, especially on the dynamics of carbon and nitrogen. As discussed elevated CO_2 concentrations promote photosynthetic rates and the synthesis of carbohydrates, resulting in significant alterations to the nutritional composition of leaves. The carbon content of the grass species exhibited only a meager difference between CC and TC. Such responses might be influenced by the duration of the experiment (Taub, 2010 and Hager et al, 2016). According to them, plants may not differ significantly in the early stages of either treatment in the case of nutrient level as they become used to the new environment; this could eventually result in levels of carbon content that are similar in both treatments. This view is true with *S. arundinaceum* and *A. donax*. However, a marginal increase was there in the carbon content of *M. maximus*, *C. flexuosus,* and *C. zizanioides* under elevated CO_2 compared to control. In grasses, elevated CO_2 circumstances usually lead to higher growth rates and biomass as discussed earlier. According to Hungate et al. (1997), the extra carbon that is fixed during photosynthesis adds to the total biomass of leaves, which raises the carbon content of the leaves directly. The authors also proposed that plants may modify their internal carbon allocation methods in response to rising CO_2 levels. Leaf tissues receive a share of the increased carbon intake, which may result in a corresponding rise in carbon content as observed in *M. maximus*, *C. flexuosus* and *C. zizanioides*. Thus the change in allocation can also increase the amount of carbon stored in leaf tissues, which can support leaf structure and promote growth.

Studies show that increased CO_2 levels usually lead plant tissues to lose nitrogen, as seen in a variety of grass species exposed to elevated CO_2 environments (Hager et al., 2016). Different grass species may react differently to increased CO_2 in terms of leaf nitrogen concentration. Consequently, *S. arundinaceum*, *A. donax,* and *P. pedicellatum* exhibited a decrease in the concentration, in which *S. arundinaceum* showed a weak response (1.60% decrease). Likewise, a study by Zheng et al. (2018) reported that perennial ryegrass responded little to increased CO_2, but tall fescue and Kentucky bluegrass showed a substantially decreasing response to leaf and

root nitrogen. Under elevated CO_2, the nitrogen content in *Stipa grandis* reduced as a result of growth dilution and direct detrimental impacts on nitrogen absorption, which affected photosynthesis and feed quality (Shi et al., 2016). In a study by Davey et al. (1999), reduced leaf nitrogen content was seen in grass species under increasing CO_2, especially in *Agrostis capillaris* and *Lolium perenne*, suggesting adaptation to enhanced carbon uptake despite decreased nitrogen levels. As well in the present study *A. donax* exhibited a decrease of 40.01% in TC compared to 15.58% in CC. There are multiple physiological factors responsible for the decrease in nitrogen levels. Increased CO_2 harms nitrogen assimilation by preventing the uptake and assimilation of nitrate, which in turn influences the concentration of nitrogen in plant tissues (Feng et al., 2015). Furthermore, enhanced photosynthesis in high CO_2 environments can cause a dilution effect in which increased carbohydrate synthesis surpasses nitrogen uptake, lowering the total nitrogen status of plants (Gojon et al., 2023). Increased carbohydrate content might be a cause of the dilution effect on leaf nitrogen in the case of *S. arundinaceum*, *A. donax,* and *P. pedicellatum* especially in *A. donax* it was factual where carbohydrates increased by 25.58% while nitrogen decreased by 40.01% in TC. Carbohydrate dilution of nitrogen content (Wong, 1990; Kuehny et al., 1991; Gifford et al., 2000) at elevated CO_2 decreases nitrogen use efficiency of C_3 plants, thus C_3 leaves need to invest more nitrogen on RuBisCO. Such nitrogen requirements down-regulate C_3 plants at elevated CO_2 environments (Lara & Andreo, 2011). Increased CO_2 levels also cause difficulties for plants in terms of absorbing nutrients, mostly because of decreased stomatal conductance. When grown in elevated CO_2 conditions, this reduced transpiration hinders the uptake of important elements, such as nitrogen, causing the nitrogen deficit seen in many plant species (Taub, 2010; Igarashi et al., 2021). As a result, even if greater photosynthesis may lead to a rise in plant biomass, nitrogen availability may still decrease, which could limit the growth and health of the plants as a whole (Taub, 2010). This viewpoint put forward a potential explanation for the negative responses of *A. donax* to overall development. Contrary to the common phenomenon of the reduction in nitrogen levels at elevated CO_2 conditions, it increased in *C. flexuosus* and *C. zizanioides*. Despite overall tendencies in broader research suggesting nitrogen dilution, factors including improved growth rates and nutritional availability may lead to increasing nitrogen levels in certain experimental setups (Taub, 2010).

The calcium content of the leaves of various grass species has been found to generally decrease with elevated atmospheric CO_2 levels. Here calcium content was decreased in TC of all grass species, except *P. pedicellatum*. Increased biomass output surpasses the capacity of a plant to accumulate important minerals, such as calcium (Taub, 2010). The responses of various grass species to increased CO_2 in terms of calcium content can vary. According to a study by Baxter et al. (1994) on the nutrient uptake, allocation, and efficiency of use of three grass species at elevated CO_2, substantial declines in nutritional productivities, including calcium, magnesium, and potassium were recorded in *Poa alpina*. As a result of changes in growth dynamics and strategies for allocating nutrients, nutrient content may vary in different species. Research shows that increased CO_2 can help plants develop, but it can also cause important minerals like calcium to deplete at the same time, which could be detrimental to the long-term nutritional value of plants. While as mentioned *P. pedicellatum* exhibited an increase in calcium. In particular, an increased need for nutrients may result from the enhanced photosynthetic rate under increasing CO_2, which could make calcium absorption more effective in *P. pedicellatum.* A similar response was reported by Carvalho et al. (2020) in *Panicum maximum*, where the calcium concentration increased with warming at 23 days after treatment, independent of CO_2, and at 30 days after treatment, the calcium content increased more in the elevated CO_2 treatment.

Regarding the magnesium content, all species exhibited a decline at varying levels in the elevated CO_2 treatment, in which *A. donax* exhibited a significant decrease (57.14 %). Depending on the species, the magnesium concentration in grasses varied in experimental chambers with elevated CO_2 levels. According to a study by Baxter et al. (1994) on the nutrient uptake, allocation, and efficiency of use of three grass species at elevated CO_2, magnesium productivity was significantly decreased in *Poa alpina* and little effect was seen in *Agrostis capillaris* and *Festuca vivipara* in response to a doubling of atmospheric CO_2. Magnesium is an essential part of chlorophyll and it is necessary for light energy absorption and for the transformation of carbon dioxide and water into carbohydrates (Pet al., 2022). Here in the present study, chlorophyll a, chlorophyll b, and total chlorophyll were noticed to be decreased in *A. donax* as discussed previously. It is presumed that the main cause of this decline in pigments regarding *A. donax* is the notable decrease in magnesium content. Insufficient magnesium availability can severely impair photosynthetic efficiency, which has an immediate effect on the general health and yield of plants (Ye et al., 2019). Consequently, *A. donax* exhibited a negative response in the overall development. Further, under high CO_2 conditions, the total ionome of plants, which comprises the concentrations of several minerals, tends to change. When plants are subjected to higher CO_2 levels, magnesium and other vital minerals might decrease in plant tissues by 6.5% to 10% (Loladze, 2014). The author also validated that the increased production of carbohydrates is assumed to be connected to this systemic decline, which lowers the concentration of minerals like magnesium. As discussed earlier it has been observed that as stomatal conductance declines and plants absorb less water, elevated CO_2 levels may lead to a decrease in the uptake of minerals, including magnesium (Yilmaz et al., 2017). Conversely, plants with low magnesium levels showed a reduction in biomass when exposed to high CO_2 levels. This implies that while certain species might be able to sustain their magnesium levels, those that are magnesium deficient could not respond as well to rising CO_2 concentrations (Yilmaz et al., 2017). Lowered magnesium levels have also implications for the protein concentration of plants. Protein synthesis may be negatively impacted by a decrease in magnesium availability in environments with elevated CO_2 (Taub, 2010). Low magnesium also results in decreased uptake of nitrogen, which is essential for the synthesis of amino acids and, ultimately, the formation of protein (Beach et al., 2019). The lowered magnesium and associated decrease in the nitrogen was especially true with *A. donax*.

Conclusions

Multiple matrices were looked at, and it became clear that *M. maximus* and *S. arundinaceum* might be suggested for CO_2 reduction initiatives. *S. arundinaceum* has a higher DF (N). It was found that *A. donax* (C_3) was the best in terms of Net CO_2 exchange, which is the matric that deals with the balance between the amount of CO_2 that plants absorb during photosynthesis and the amount that they release during respiration. According to the t-SNE plot, *M. maximus* and *S. arundinaceum* had better overall morphology than the other species, which was evident in their leaf characteristics. In terms of CO_2 uptake per biomass, *S. arundinaceum* was shown to be superior. Thus it might be advised to use *M. maximus* and *S. arundinaceum* in CO_2 mitigation initiatives. Furthermore, despite showing a negative response in terms of overall morphology, *A. donax* had stronger CO_2 uptake per plant biomass and better net CO_2 exchange. As a result, *A. donax* can thrive in high CO_2 environments and be employed in CO_2 mitigation programs when nitrogen fertilizer is employed.

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Data availability

The additional datasets of our study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest: The authors declare no conflicts of interest.

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