Carbon sequestration potential of selected grasses - an assessment using Carbon dioxide-controlled systems

Thesis submitted to the
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DOCTOR OF PHILOSOPHY IN BOTANY

By

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CERTIFICATE

This is to certify that the Ph.D. thesis titled **"Carbon sequestration potential of selected grasses - an assessment using Carbon dioxide-controlled systems"**, submitted to the University of Calicut in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Botany by Ms. Sashna N.C. is a bonafide record of research work carried out by her under my guidance and supervision in the Division of Environmental Science, Department of Botany, University of Calicut.

No part of the present work has previously formed the basis for the award of any other Degree, Diploma, Fellowship, or similar title, to any candidate in any University. It is also affirmed that the modifications suggested by the Research Advisory Committee (Botany) of the University of Calicut have been incorporated into the thesis.

Dr. C. C. Harilal
(Research Supervisor and Guide)

Dr. HARILAL C.C
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Calicut University Campus
Dated: 11-04-2025

DECLARATION

I hereby declare that the research work presented in the thesis entitled **"Carbon sequestration potential of selected grasses - an assessment using Carbon dioxide-controlled systems",** is based on the original research work done by me under the guidance and supervision of Prof. (Dr.) C.C. Harilal, Head, Department of Botany (Division of Environmental Science), University of Calicut, and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone plagiarism check using iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit.

I also declare that the thesis is free from AI-generated content.

SASHNA N.C.

Calicut University Campus
Date: 11-04-2025

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SASHNA N.C.

Dedicated to

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"Your laughter, love, and presence have been my strength. In moments of exhaustion and doubt, you reminded me why perseverance matters."

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

%	-	Percentage
°C	-	Degree Celsius
BECCS	-	Bioenergy carbon capture and storage
BSA	-	Bovine Serum Albumin
CC	-	Control chamber
CHNS	-	Carbon Hydrogen Nitrogen Sulfur
Ci	-	Intercellular CO_2 concentrations
CO_2	-	Carbon dioxide
COP	-	Conference of the parties
D1	-	Day 1
D15	-	Day 15
DF (N)	-	Net day flux
DF	-	Day flux
DMSO	-	Dimethyl sulphoxide
DOT	-	Day of treatment
E	-	Transpiration rates
EDTA	-	Ethylenediaminetetraacetic acid
ET	-	Evapotranspiration
FACE	-	Free Air Carbon dioxide Enrichment
FAS	-	Ferrous ammonium sulphate
FTIR	-	Fourier- Transform Infrared
g_s	-	Stomatal conductance
Gt	-	Gigatonnes
H_2SO_4	-	Sulphuric acid
HCl	-	Hydrochloric acid
IPCC	-	International Panel on Climate Change
IRGA	-	Infrared gas analyzer
$KMNO_4$	-	Potassium permanganate
LDPE	-	Low Density Polyethylene
Mg g^{-1}	-	Milligram per gram
Mg/Kg	-	Milligram per kilogram

N	-	Nitrogen concentration
Na_2CO3	-	Sodium carbonate
NaOH	-	Sodium hydroxide
NDIR	-	Non-Dispersive Infrared
NF	-	Night flux
NOAA	-	National Oceanic and Atmospheric Administration
NPP	-	Net primary productivity
OD	-	Optical density
OTC	-	Open top chamber
Pa	-	Pascal
PAR	-	Photosynthetically active radiation
Pg	-	Petagrams
pH	-	Potential of hydrogen
PMMA	-	Polymethyl Methacrylate
Pn	-	Photosynthetic rate
PPFD	-	Photosynthetic photon flux density
ppm yr^{-1}	-	Part per million per year
ppm	-	Parts per million
PVC	-	Polyvinyl chloride
SACC	-	Screen Aided Carbon dioxide Control
SCADA	-	Supervisory Control and Data Acquisition
SLA	-	Specific leaf area
SPAR	-	Soil-plant-atmosphere-research
STDEV	-	Standard deviation
STP	-	Standard temperature and pressure
t- SNE	-	t-Distributed Stochastic Neighbor Embedding
TC	-	Treatment chamber
TNC	-	Total non-structural carbohydrates
TOC	-	Total organic Carbon
UNFCCC	-	United Nations Framework Convention on Climate Change
WUE	-	Water use efficiency

ABSTRACT

The primary aim of the study is to assess the changes in microclimate brought about by the growth and metabolism of particular grass species in environments with elevated carbon dioxide levels. Two chambers, each with a size 6.32 m^3 were installed with Polyvinyl chloride (PVC) pipes of 40mm diameter as frames and 1mm thick PVC sheet as sidewall material. The facilities associated with the chambers were a CO_2 cylinder for the supply of air mixed with elevated concentrations of CO_2, an air compressor, and a nebulizer (a mixing chamber where the concentrated CO_2 gas from the cylinder was mixed with ambient air from the 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 within the chambers was made through an automated CO_2 analyzer. For regular monitoring of temperature and humidity, both chambers were fitted with a Hygro-thermometer. Among two chambers one is supplied with a CO_2-air mixture (Treatment chamber, TC), and the other one is supplied with ambient air (Control chamber, CC).

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, multiplied, and grown for 6-7 months to attain sizable biomass for experimentation. For the experiment with each species, two sets of 3 plants each were selected and maintained in CC and TC respectively.

At the beginning of the experiment, the chambers were properly sealed. Afterward, the TC was supplied with CO_2+ ambient air mixture at 9.00 a.m. A concentration of 900-1000 ppm CO_2 was ensured inside the chamber by monitoring through a CO_2 gas analyzer. This range of CO_2 is attained in about 15 minutes. Similarly, ambient air was supplied to CC for 15 minutes in the morning (9 a.m.). After the supply of air/CO_2+air, the levels of CO_2 (ppm) in CC and TC respectively were monitored. Subsequent levels of temperature (°C) and humidity (%) were also noted. Monitoring of CO_2 concentration, temperature, and humidity was repeated at 6 p.m. Day flux of CO_2, and the amount of CO_2 assimilated by the plants in the chamber was then calculated. Night CO_2 flux and respiratory contribution of grass species were also calculated.

Growth attributes including morphological parameters such as plant height and tiller height, the number of tillers, number of leaves, leaf length, leaf breadth, leaf area, culm

diameter, and plant biomass were estimated. The biochemical parameters analyzed include pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids), metabolites (carbohydrate, protein, and phenol), and plant nutrients (carbon, nitrogen, calcium, magnesium, sodium, potassium). The soil characteristics analyzed include moisture, pH, total organic carbon (TOC), and nitrogen.

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. All procedures done in the experiment with grass species were repeated and day and night fluxes associated with ideal conditions of the chambers were calculated. The data obtained in the standardization study determines the pattern of CO_2 flux, temperature, and humidity with rising CO_2 concentrations in the chamber at ideal conditions without plants (chamber effect). The elimination of this chamber effect regarding CO_2 concentrations while analyzing the CO_2 flux or CO_2 intake potential of individual grass species gives the actual flux/actual intake of CO_2 by the respective grass species.

All the grass species exhibited higher and statistically significant day flux (day variations of CO_2) in TC compared to CC and standardization experiments. Morphological and biochemical results were statistically validated with Hedge's g (effect size) values. 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. It enables for the comparison of effect sizes between samples. Higher effect sizes were noticed to be associated with TC. The best grass species for mitigating atmospheric CO_2 were identified by analyzing a range of attributes and from these aspects, four matrices were considered including net day flux (DF (N)), net CO_2 exchange, overall morphology, and CO_2 uptake per plant biomass. An equation was derived for estimating CO_2 uptake per plant biomass. By analyzing all four matrices it is revealed that *S. arundinaceum* and *M. maximus* could be strongly suggested for CO_2 mitigation programs since the plants were superior in CO_2 uptake and growth attributes. Better net CO_2 exchange and more CO_2 uptake per plant biomass were observed in *A. donax*. While the plant exhibited a negative response regarding overall morphology, due to the poor nitrogen cycling of the plant. Adequate supply of nitrogen fertiliser may benefit the plant to grow well in high CO_2 environments and their high capability for CO_2 uptake makes them more suitable for carbon mitigation projects.

Keywords: *CO_2 controlled chambers, elevated CO_2, carbon sequestration, CO_2 flux, net CO_2 exchange, overall morphology, biochemical parameters, biomass*

സംഗ്രഹം

കാർബൺ ഡൈ ഓക്സൈഡ് (CO_2) സാന്ദ്രത കൂടിയ അന്തരീക്ഷത്തിൽ പരിപാലിച്ച തെരഞ്ഞെടുത്ത പുൽവർഗ്ഗ സസ്യങ്ങൾക്ക് വളർച്ചയിലും ഉപാപചയ പ്രവർത്തനങ്ങളിലും ഉണ്ടായ മാറ്റങ്ങളും അതുവഴി അവയുടെ മൈക്രോക്ലൈമേറ്റിൽ ഉണ്ടായ വ്യതിയാനങ്ങളും വിലയിരുത്തുക എന്നതാണ് ഈ പഠനത്തിന്റെ പ്രാഥമിക ലക്ഷ്യം.

പരീക്ഷണ പ്രവർത്തനങ്ങൾക്കായി 6.32 ഘന മീറ്റർ വ്യാപ്തമുള്ള രണ്ട് ചേംബറുകൾ നിർമ്മിച്ചു. ചേംബറിന്റെ ചട്ടക്കൂട് നിർമ്മിക്കുന്നതിനായി 40 എം.എം. വ്യാസമുള്ള പോളി വിനൈൽ ക്ലോറൈഡ് (PVC) പൈപ്പുകളും പാർശ്വഭിത്തി നിർമ്മിക്കുന്നതിനായി 1 എം.എം. കനമുള്ളതും, സുതാര്യവുമായ PVC ഷീറ്റും ഉപയോഗിച്ചു. ഉയർന്ന അളവിലുള്ള CO_2 വിതരണം ചെയ്യുന്നതിനുള്ള CO_2 സിലിണ്ടർ, വായു വിതരണത്തിനുള്ള എയർ കംപ്രസ്സർ, നെബ്യുലൈസർ (സിലിണ്ടറിൽ നിന്നുള്ള സാന്ദ്രീകൃത CO_2 വാതകം എയർ കംപ്രസ്സറിൽ നിന്നുള്ള വായുവുമായി കലർത്തുന്നതിനുള്ള ഒരു മിക്സിംഗ് ട്യൂബ്), ഒരു എക്സോസ്റ്റ് ഫാൻ (ആവശ്യമെങ്കിൽ അനിയന്ത്രിത അളവിലുള്ള CO_2 പുറന്തള്ളി സാന്ദ്രത ക്രമീകരിക്കുന്നതിന്) എന്നിവ ചേംബറുകളുമായി ഘടിപ്പിച്ചിരുന്നു.

പരീക്ഷണ വേളയിൽ ചെടികൾക്ക് ജലസേചനം സുഗമമാക്കുന്നതിന് രണ്ട് ചേംബറുകളിലും ജലവിതരണ സൗകര്യം ഏർപ്പെടുത്തിയിരുന്നു. ഒരു ഓട്ടോമേറ്റഡ് CO_2 അനലൈസർ വഴിയാണ് ചേംബറുകൾക്കുള്ളിലെ CO_2 സാന്ദ്രത (ppm - parts per million) നിരീക്ഷിക്കുന്നത്. താപനിലയും (Temperature - °C) ഈർപ്പവും (Humidity - %) പതിവായി നിരീക്ഷിക്കുന്നതിന് രണ്ട് ചേംബറുകളിലും ഹൈഗ്രോ തെർമോമീറ്റർ ഘടിപ്പിച്ചിരുന്നു. രണ്ടു ചേംബറുകളിൽ ഒന്നിൽ CO_2 + വായു മിശ്രിതം (ട്രീറ്റ്മെന്റ് ചേംബർ – TC) രണ്ടാമത്തേതിൽ വായു (ആംബിയന്റ് എയർ – കൺട്രോൾ ചേംബർ, CC) എന്നിവ നൽകി.

പരീക്ഷണങ്ങൾക്കായി ആറ് സ്പീഷീസ് പുൽവർഗ്ഗ സസ്യങ്ങൾ തെരഞ്ഞെടുത്തു. *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs, *Saccharum arundinaccum* Retz., *Cymbopogon flexuosus* (Nees ex steud.) W. Watson, *Chryzopogon zizanioides* (L.) Roberty, *Arunde donax* L., *Pennisetum pedicellatum* Trin. എന്നിവയാണ് തെരഞ്ഞെടുത്ത സ്പീഷീസുകൾ. ഇവ ആവശ്യമായ വളർച്ച കൈവരിക്കുന്നതിനും ഗണ്യമായ ബയോമാസ് ഉണ്ടാകുന്നതിനുമായി ആറു മുതൽ ഏഴ് മാസം വരെ വളർത്തി. ഓരോ സ്പീഷീസ് ഉപയോഗിച്ചുള്ള പരീക്ഷണത്തിനുമായി യഥാക്രമം CC, TC എന്നിവയിൽ മൂന്ന് ചെടികൾ വീതമുള്ള 2 സെറ്റുകൾ തെരഞ്ഞെടുക്കുകയും പരിപാലിക്കുകയും ചെയ്തു.

പരീക്ഷണത്തിന്റെ തുടക്കത്തിൽ ചേംബറുകൾ ശരിയായി സീൽ ചെയ്തിരുന്നു. തുടർന്ന് രാവിലെ 9ന് TCയിൽ CO_2+ വായു മിശ്രിതം വിതരണം ചെയ്തു. ഓട്ടോമേറ്റഡ് അനലൈസറിൽ ചേംബറിനകത്തുള്ള CO_2 നിരീക്ഷിച്ചുകൊണ്ട് ചേംബറിനുള്ളിൽ 900–1000 ppm CO_2 സാന്ദ്രത ഉറപ്പാക്കി. ഏകദേശം 15 മിനുട്ടിനുള്ളിൽ CO_2 സാന്ദ്രത ഈ പരിധി കൈവരിക്കുന്നു. അതു

പോലെ CC ൽ 15 മിനുട്ട് സമയം വായു (ആംബിയന്റ് എയർ) വിതരണം ചെയ്തു (9 a.m) വായു/CO_2+ വായു മിശ്രിതം വിതരണം ചെയ്തതിനു ശേഷം യഥാക്രമം CC, TC എന്നിവയിലെ CO_2 (ppm) സാന്ദ്രത നിരീക്ഷിച്ചു. തുടർന്ന് താപനില, ഈർപ്പം എന്നിവയും നിരീക്ഷിച്ചു. CO_2 സാന്ദ്രത, താപനില, ഈർപ്പം എന്നിവയുടെ നിരീക്ഷണം വൈകുന്നേരം 6ന് ആവർത്തിച്ചു. തുടർന്ന് പകൽ സമയത്ത് ചേംബറിനുള്ളിൽ CO_2 സാന്ദ്രതയിലുണ്ടായ വ്യതിയാനം അഥവാ CO_2 flux (day flux സസ്യങ്ങൾ ഉപയോഗിച്ച CO_2) കണക്കാക്കി. അതുപോലെ രാത്രി സമയത്തെ CO_2 സന്ദ്രതാ വ്യതിയാനവും (സസ്യങ്ങളുടെ ശ്വസന പ്രവർത്തനത്തിന്റെ ഫലമായി ഉണ്ടാകുന്ന വ്യതിയാനം - Night flux) കണക്കാക്കി. ഈ പരീക്ഷണ പ്രവർത്തനങ്ങൾ 15 ദിവസം തുടർന്നു.

സസ്യങ്ങളുടെ രൂപശാസ്ത്രപരമായ ഘടകങ്ങൾ അഥവാ മോർഫോളജി (ചെടിയുടെ ഉയരം, ടില്ലറിന്റെ ഉയരം, ടില്ലറുകളുടെ എണ്ണം, ഇലകളുടെ എണ്ണം, ഇലയുടെ നീളം, ഇലയുടെ വീതി, ഇലയുടെ വിസ്തീർണ്ണം, തണ്ടിന്റെ വ്യാസം) ബയോമാസ്സ് എന്നീ വളർച്ചാ സവിശേഷതകൾ പരീക്ഷണവേളയിൽ കണക്കാക്കി. ബയോകെമിക്കൽ ഘടകങ്ങളായ പിഗ്മെന്റുകൾ (ഹരിതകം എ, ഹരിതകം ബി, ആകെ ഹരിതകം, കരോട്ടിനോയിഡുകൾ), മെറ്റബോളൈറ്റുകൾ (ധാന്യകങ്ങളുൾ, മാംസ്യം, ഫിനോൾ), സസ്യ പോഷകങ്ങൾ (കാർബൺ, നൈട്രജൻ, കാൽസ്യം, മഗ്നീഷ്യം, സോഡിയം, പൊട്ടാസിയം) എന്നിവയും വിശകലനം ചെയ്തു. മണ്ണിന്റെ സവിശേഷതകളായ ഈർപ്പം, pH, മൊത്തം ജൈവ കാർബൺ (Total organic carbon - TOC) എന്നിവയും കണക്കാക്കി.

സസ്യങ്ങൾ ഇല്ലാത്ത സാഹചര്യത്തിൽ ചേംബറുകളിൽ ഉയർന്ന CO_2 വിതരണം ചെയ്യുമ്പോൾ ചേംബറിനുള്ളിലെ വായുവിൽ ഉണ്ടാകുന്ന പ്രത്യേകതകൾ വിലയിരുത്തുന്നതിനായി standerdization പഠനങ്ങൾ നടത്തി. സസ്യങ്ങൾ ഉപയോഗിച്ച് നടത്തിയ പഠനങ്ങളിൽ അനുവർത്തിച്ച എല്ലാ നടപടിക്രമങ്ങളും ഇവിടെയും ആവർത്തിച്ചു. ഇത്തരത്തിൽ ചേംബറിനുള്ളിൽ തനത് അവസ്ഥയിൽ ഉണ്ടാകുന്ന day flux, night flux എന്നിവ അടയാളപ്പെടുത്തി. ഈ ഡാറ്റ വർദ്ധിച്ച CO_2സാന്ദ്രതയിൽ ചേംബറുകളുടെ തനത് അവസ്ഥ (ചേമ്പർ എഫക്ട്) ആയി മനസ്സിലാക്കി. ഓരോ സ്പീഷീസിന്റെയും പരീക്ഷണത്തിന്റെ ഫലമായി അടയാളപ്പെടുത്തിയ CO_2 fluxൽ നിന്നും standardisation പഠനത്തിൽ ലഭിച്ച CO_2 flux കുറയ്ക്കുമ്പോൾ അതത് സ്പീഷീസുമായി ബന്ധപ്പെട്ട യഥാർത്ഥ CO_2 സ്വാംശീകരണ തോത് ലഭിക്കുന്നു.

Standardisation, Control എന്നീ പരീക്ഷണങ്ങളിൽ ലഭിച്ച day flux മായി താരതമ്യപ്പെടുത്തുമ്പോൾ എല്ലാ പുൽവർഗ്ഗ സ്പീഷീസും TC യിൽ ഉയർന്ന day flux പ്രകടിപ്പിച്ചു. ഇത് അന്തരീക്ഷ വായുവിലെ CO_2 കുറക്കുന്നതിനുള്ള ഈ പുൽവർഗ്ഗ സസ്യങ്ങളുടെ കഴിവിനെ സാധൂകരിക്കുന്നു. രൂപശാസ്ത്രപരമായ ഘടകങ്ങളുടെയും, ബയോകെമിക്കൽ ഘടകങ്ങളുടെയും ഡാറ്റ പരിശോധിച്ചപ്പോൾ, ഉയർന്ന CO_2 സാന്ദ്രതയിൽ വ്യതിയാനങ്ങൾ കാണിക്കുന്നതായി കണ്ടെത്തി പരീക്ഷണങ്ങൾക്ക് അടിസ്ഥാനമാക്കിയ സ്പീഷീസുകളിൽ നിന്നും അന്തരീക്ഷ വായു വിലെ CO_2 സാന്ദ്രത ലഘൂകരിക്കുവാൻ ഉപയോഗപ്പെടുത്താനുള്ള മികച്ച സ്പീഷീസ് വിവിധ വശങ്ങൾ വിശകലനം ചെയ്തുകൊണ്ട് തിരിച്ചറിഞ്ഞു. വിശകലനം ചെയ്ത വശങ്ങളിൽ നിന്ന് Net day

flux [DF(N)], Net CO_2 exchange, overall morphology, ബയോമാസ് അനുസരിച്ചുള്ള CO_2 ആഗിരണ ശേഷി എന്നീ നാല് മെട്രിക്സുകൾ പരിഗണിച്ചു. ഒരു ചെടിയുടെ ബയേമാസ് അനുസരിച്ചുള്ള CO_2 ആഗിരണശേഷി കണക്കാക്കുന്നതിന് ഒരു സമവാക്യം (ചേംബർ പരീക്ഷണങ്ങളിൽ മാത്രം) ഉരുത്തിരിഞ്ഞു. നാല് മെട്രിക്സുകളും വിശകലനം ചെയ്യുമ്പോൾ മെച്ചപ്പെട്ട CO_2 ആഗിരണശേഷി, മെച്ചപ്പെട്ട വളർച്ച എന്നിവ *S. arundinacuum , M. maximus* എന്നീ സ്പീഷീസുകൾ കാഴ്ചവെയ്ക്കുന്നതായി മനസ്സിലാക്കി. അതിനാൽ ഈ സ്പീഷീസുകളെ CO_2 ലഘൂകരണ പരിപാടിക്കായി നിർദ്ദേശിക്കാം എന്ന് വെളിപ്പെടുത്തുന്നു.

മികച്ച Net CO_2 എക്സ്ചേഞ്ച്, ബയോമസ് അനുസരിച്ചുള്ള CO_2 ആഗിരണശേഷി എന്നിവ *A. donax* ൽ നിരീക്ഷിക്കപ്പെട്ടു. എന്നാൽ പൊതുവിൽ ഉയർന്ന CO_2 സാന്ദ്രതയുള്ള അന്തരീക്ഷത്തിൽ C_3 സസ്യങ്ങളിൽ ഉണ്ടാകുന്ന നൈട്രജൻ വിതരണത്തിലെ കുറവുകൾ ഈ സസ്യത്തിലും വളർച്ചാശോഷണം ഉണ്ടാക്കി. ആയതിനാൽ ഈ സ്പീഷീസിന്റെ മെച്ചപ്പെട്ട CO_2 ആഗിരണ ശേഷി/ലഘൂകരണശേഷി പരിഗണിച്ചുകൊണ്ട് ഉചിതമായ രീതിയിൽ നൈട്രജൻ വളങ്ങൾ കൊടുക്കുകയാണെങ്കിൽ ഈ സ്പീഷീസിനെയും CO_2 ലഘൂകരണ പരിപാടികൾക്കായി ഉപയോഗിക്കാവുന്നതാണ്.

സൂചകപദങ്ങൾ : CO_2 നിയന്ത്രിത ചേംബറുകൾ, വർദ്ധിത CO_2, കാർബൺ സെക്വസ്ട്രേഷൻ, CO_2 ഫ്ളക്സ്, നെറ്റ് CO_2 എക്സ്ചേഞ്ച്, ഓവറോൾ മോർഫോളജി, ബയോകെമിക്കൽ പാരാമീറ്ററുകൾ, ബയോമാസ്സ്

GENERAL INTRODUCTION

GENERAL INTRODUCTION

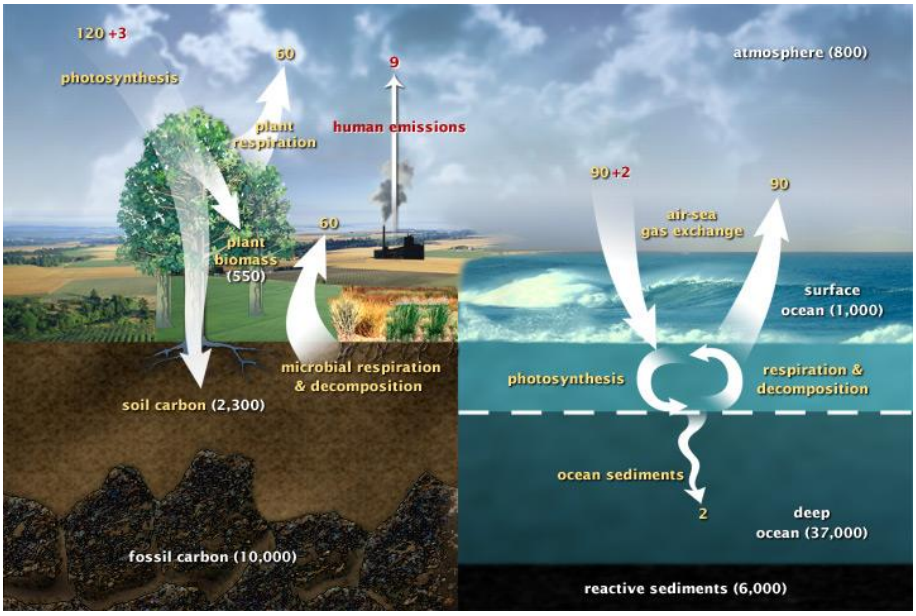
As per the Intergovernmental Panel on Climate Change (IPCC), the observed rise in global average temperatures since the mid-20th century is presumed to be caused by an increase in anthropogenic greenhouse gas concentrations, which warm the Earth's surface and lower atmosphere. The Kyoto Protocol lists greenhouse gases as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and fluorinated gases, including sulphur hexafluoride (SF_6), hydrofluorocarbons (HFCs), and perfluorocarbons (PFCs). The uptake of outgoing infrared radiation by carbon dioxide (CO_2), methane, and other atmospheric gases raises the temperature, leading to greenhouse effect. Since CO_2 is the most significant anthropogenic greenhouse gas, it is essentially accused of being the primary cause of the greenhouse effect (IPCC, 2007). Soil, ocean, atmosphere, biomass, and fossil fuels are the main sources of carbon in nature. These reservoirs continue to exchange carbon. The altered carbon concentration of one reservoir immediately impacts the other (Mehmood et al., 2020). The global temperature rises when there is a greater CO_2 concentration in the atmosphere.

It is well established that in the middle of the 19th century, industrialization and urbanization contributed to increased CO_2 levels, which in turn caused global warming and climate change (Yaashikaa et al., 2019). The Earth's natural greenhouse effect attributed to CO_2 is sufficient to maintain an average global surface temperature above freezing. Without an atmosphere, solar radiation would be directed straight into space, resulting in an average surface temperature of minus 18°C (Hashimoto & Hashimoto, 2019). The natural greenhouse effect is being intensified by human activity, which is raising the atmospheric concentration of carbon dioxide and raising global temperatures. According to the observations made by the NOAA Global Monitoring Lab, about two-thirds of the total heating effect of all greenhouse gases released by humans in 2021 came from carbon dioxide alone. The earth's total carbon content remains constant, and until the emergence of the industrialized civilization era, its distribution among the lithosphere, atmosphere, and biosphere was largely balanced (Yang et al., 2008). This stable distribution of carbon had been maintained by natural processes such as photosynthesis, respiration, decomposition, weathering, and oceanic absorption.

The carbon dioxide monitoring began in Mauna Loa Observatory (Hawaii) in 1958. Before the 1970s, the carbon dioxide concentration was less than 1 part per million per year (ppm yr^{-1}), but it has since risen to more than 2 ppm yr^{-1} in recent years. The average carbon dioxide concentration in March 2025 is 429.44 ppm.

The industrial development and rapid economic growth of developed nations during the 1870s and the 1970s led to a constant increase in atmospheric CO_2 of approximately 0.28 ppm yr^{1} (Hashimoto & Hashimoto, 2019). The emissions of carbon dioxide from developed countries have caused a significant acceleration since 1970, with a rate of 1.85 ppm yr^{-1}. These emissions are too high to be assimilated by Earth's atmosphere. Due to the high industrial activity of developed countries along with the economic development of developing countries, the atmospheric carbon dioxide concentration increased at a much faster rate between January 2007 and January 2018 to nearly 2.36 ppm yr^{1} (Hashimoto & Hashimoto, 2019). Resultingly, the global surface temperature increased on average by 1.1°C between 2011 and 2020.

All of the carbon on Earth does not appear to be able to enter the atmosphere or be completely stored in rocks since the carbon cycle appears to maintain a long-term equilibrium. Similar to a thermostat, this equilibrium serves to maintain Earth's temperature at a relatively constant level (Riebeek, 2011). According to NASA, the carbon cycle is an exchange that involves both fast and slow carbon movements between each reservoir. When the cycle is altered, more carbon is added to the other reservoirs and less carbon is removed from the other one. Earth's temperature rises when changes add carbon gases to the atmosphere. In the slow carbon cycle, tectonic activity and a series of chemical reactions cause carbon to flow slowly over 100–200 million years between rocks, soil, the ocean, and the atmosphere. Most of the fast carbon cycle is composed of the movement of carbon through Earth's biosphere or living beings. The following figure illustrates the fast carbon cycle on Earth.



(Source: U.S. DOE, Biological and Environmental Research Information System).

The continuous movement of carbon between the land, atmosphere, and oceans is depicted in this figure on the fast carbon cycle. Red numbers represent human contributions in gigatons of carbon annually, whereas yellow numbers represent natural movements. Carbon stored is indicated by white numbers.

Human activities are considered to be the sole reason behind the current alterations in the carbon cycle. With the overuse of fossil fuels and the removal of land cover, humans interfere with the carbon cycle. Plants that would normally absorb carbon from the atmosphere as they grow are eliminated when a forest is destroyed. Consequent to a multitude of activities, including changes in land use, humans currently release about one billion metric tonnes of carbon dioxide into the atmosphere annually (IPCC, 2021).

Primary sources of carbon dioxide emissions

The global trend in greenhouse gas emissions has been influenced by many factors including the unsustainable energy usage, land use-land change patterns, and the consumption and production among individuals, between countries, and across regions (IPCC, 2023). It has become the prime responsibility of mankind to examine all the processes that can affect the atmospheric concentration of CO_2, to comprehend what regulates its abundance in the atmosphere and, consequently, its

impact on the greenhouse effect. CO_2 is released into the atmosphere when fossil fuels (such as coal, natural gas, and oils), solid waste, trees, and other biological materials are burned (Yoro & Daramola, 2020). Moreover, most of the chemical processes, like that of the making of cement can release CO_2.

Human activities have drastically increased atmospheric CO_2 concentrations, reducing the ability of natural sinks like forests to absorb CO_2. Burning fossil fuels for energy and transportation is the main human activity that releases CO_2 into the atmosphere, however other changes in land use, including forest clearing, will also contribute to increasing the atmospheric concentration of CO_2 (Amaral et al., 2019). The CO_2 emissions (in billions of metric tons of CO_2 emissions annually) from different anthropogenic sources are as follows, as reported by Yoro and Daramola (2020). The sources include fossil fuel combustion engines (392), cement production plants (113), coal-fired power plants (279), transportation (191), industrial manufacturing (178), and land use changes (deforestation) (13). Non-anthropogenic sources include plant, animal, and human respiration (7), ocean-atmosphere exchange (7), soil respiration and decomposition (1.54), and volcanic eruptions (0.15). These staggering figures point to the urgent need to develop strategies for the capture and storage of atmospheric carbon dioxide, given the current levels and the catastrophic implications that it poses to ecosystems and climate. The IPCC (2019) released a report that emphasized how critical it is to limit global warming below 1.5°C to avoid forthcoming issues related to climate change.

CO_2 mitigation- initiatives

The matter of climate change was initially accepted as a reality in the first International Climate Conference, which took place in Geneva in 1979. The World Meteorological Organization and the United Nations Environment Programme (UNEP) established the Intergovernmental Panel on Climate Change (IPCC) in 1988 to provide governments and other official agencies with access to scientific data and knowledge that can be used to develop policies related to climate change. The United Nations Framework Convention on Climate Change (UNFCCC), which was adopted in 1992 and put into force in 1994, was the most significant move in response to climate change. Since then, the UNFCCC has been taking the lead role and providing support for global climate action. Stabilizing levels of greenhouse gases in

the atmosphere is the main objective of the convention, as it aims to prevent significant impacts on the climate system. The Kyoto Protocol was approved in 1997 at the third conference of the parties to the UNFCCC (COP-3), and it came into effect in 2005. As part of the Kyoto Protocol, developed nations committed to lowering their emissions in five years between 2008 and 2012. The main strategies for lowering emissions have been the use of renewable energy, energy efficiency, and afforestation/reforestation-related initiatives (Fawzy et al., 2020). Carbon taxes may be a useful instrument for lowering greenhouse gas emissions, according to emerging scientific data; nevertheless, public and industry resistance to such a mechanism is the primary reason that many countries have not yet adopted it (Wang et al., 2016). The Paris Agreement became effective in 2016 after being adopted at COP-21, the twenty-first conference of the parties to the UNFCCC in 2015. Limiting global temperature increases to 2°C by 2100 and aiming for 1.5°C is the primary goal of the accord. To attain equilibrium between sources of emissions caused by humans and sinks and reservoirs of greenhouse gases between 2050 and 2100, the agreement strives to bring about the global maximum of greenhouse gases as soon as possible. Studies verify that the temperature targets set by the Paris Agreement will not be met by current mitigation efforts alone or by future commitments to cut emissions (Nieto et al., 2018; Lawrence et al., 2018). If efforts are to be made to achieve these objectives, new mitigation strategies and supplementary measures need to be looked into.

Emission reduction and mitigation strategies

Globally, many 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). Conventional mitigation strategies and negative emissions technologies are the major approaches to CO_2 mitigation (Fawzy et al., 2020). The former method uses decarbonization technologies and methods like renewable energy, nuclear power, fuel switching and efficiency improvements, and carbon capture and storage, to lower CO_2 emissions. Renewable energy sources like wind and solar power are important approaches that have a larger potential to reduce carbon emissions. The

irregularity and intermittent nature of power generation is one of the primary technological obstacles involved. Combining these technologies with storage and other renewable base load and grid technologies can overcome the situation. Burning fossil fuels will generate the majority of the world's electricity in the present and the future (more than 60%), with little improvement predicted for 2040 (58%). The world is still not quite at the top of the global warming solution, despite a consistent growth in utilization of renewable energy sources. To take the place of fossil fuels as the primary energy source in our industry, we need to put in a lot more effort. Nuclear energy, although seen as a low-carbon solution to slow down global warming, has several serious disadvantages, including high initial and operating costs, the risk of radioactive pollution in the environment due to potential reactor accidents, and the dangers associated with disposing of radioactive waste (Abdulla et al., 2019). Another common technique for decarbonization is fuel switching, which is the process of substituting carbon-intensive fuels (like petrol or propane) with low- or zero-carbon alternatives. This technique is being considered as a possible strategy for the future economic shift to a low-carbon economy, and ideally a zero-carbon one (Wendling, 2019). Further, the efficiency gains are crucial for mitigating efforts. Thermal power plants need to increase both the efficiency of fuel combustion and the efficiency of turbine generators to increase overall power sector efficiency. According to Bustreo et al. (2019), a manageable amount of risk is associated with most of these well-established technologies. A similar opinion has been held by previous authors (Victor et al., 2018; Mathy et al., 2018).

The negative emission technologies have the potential to be used to sequester and capture carbon dioxide from the atmosphere. According to Lin (2019), these techniques should be used to eliminate emissions that are difficult to eliminate using traditional methods. It is crucial to remember that negative emissions should not be seen as a replacement for traditional decarbonization methods, but rather as an additional set of tools and strategies (Pires, 2019). These strategies include soil carbon sequestration, afforestation and reforestation, enhanced weathering, direct air carbon capture and storage, ocean fertilization, ocean alkalinity enhancement, bioenergy carbon capture and storage, and alternative techniques for utilizing and storing negative emissions, such as mineral carbonation and using biomass in construction (McLaren, 2012; McGlashan et al., 2012; Lawrence et al., 2018;

Lenzi, 2018; Yan et al., 2019; Goglio et al., 2020). Bioenergy carbon capture and storage (BECCS), is a well-known negative emission technology that has received a lot of attention in the literature. The IPCC also mainly relies on BECCS to meet temperature targets (IPCC, 2018). Although estimates vary, Fuss et al. (2018) predict that BECCS has the potential to remove 0.5–5 Gt CO_2 per year by 2050. Obtaining a sufficient quantity of biomass feedstocks to effectively reduce emissions is the primary obstacle linked with this technology (Fawzy et al., 2020). Forestation is a valuable tool for mitigating climate change due to its biogenic negative emissions. Forestation involves either establishing new forests (afforestation) or reforesting areas that have been deforested or degraded. By 2050, the forestation strategy could remove between 0.5 and 3.6 Gt CO_2 per year globally (Fuss et al., 2018).

Among the many strategies, carbon sequestration plays a crucial role in mitigating climate change by reducing the amount of CO_2 in the atmosphere. 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). Carbon sequestration in terrestrial biomass is a significant and economical way to check the causative factors of climate change. Photosynthesis is the most significant photochemical process associated with plants. During photosynthesis, CO_2 is captured and along with water, it is converted into 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).

Apart from trees, the biomass of grasses is a major sink of atmospheric carbon. Well-managed grasslands with ideal growing conditions can sequester considerable amounts of carbon, 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 favors them for higher CO_2 accumulation. Mahanta et al. (2020) reported that over 26% of the world's land surface is made up of grasslands and that these soils store a significant quantity of carbon, with global carbon stocks estimated to be around 343 Pg carbons. Considering this, 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 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.

The increasing demands for agricultural land and population growth have resulted in a significant reduction of the area accessible for free grazing, hence bringing up the need for cow fodder grasses (Atieno et al., 2020). According to Do et al. (2020), the requirement for grass for livestock fodder can be met by agroforestry practices that include grass strips. 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). 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).

Few studies have examined the effects of elevated CO_2 on perennial grasses (Lee et al., 2001; Suter et al., 2002), particularly on their growth and biochemical characteristics. However, some of the recent studies examined the changes in physiology, metabolism, and growth in response to elevated CO_2 combined with heat and drought stresses. The majority of previous studies regarding plant response to elevated CO_2 have been focused on crops (Jahnke, 2001; Tan et al., 2013) or trees (Norby et al., 1999; Asshoff et al., 2006; Lukac et al., 2010; Klein 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. Most of these methods used leaf cuvettes, plant growth chambers, and greenhouses. However, these methods have specific constraints in plot and plant size and require active control of all environmental variables. To overcome some of these limitations, other methodologies were developed, which can perform under heterogeneous conditions. In the present study, cost-effective and easily replicable carbon dioxide-controlled chambers were constructed to perform CO_2 enrichment studies with grass species.

The present study aims to assess the effectiveness of selected grass species in carbon mitigation. The reduction of carbon dioxide in the microclimatic environment owing to the growth and metabolism of specific grass species was compared to determine the species would work best for carbon offset planting and other land management initiatives. Due to their ability to produce higher biomass above and below ground, perennial grasses with high biomass production are selected for the current study. With their high biomass and nutritive values, the chosen grasses are also better choices for fodder and have additional benefits like medicinal uses and soil conservation. The study will offer insightful information about the significance of grass species in mitigating carbon emissions as well as directives regarding the species that are best for carbon offset initiatives.

Objectives of the Present Study

The major aim of the present thesis is to evaluate the carbon dioxide sequestration potential of high biomass yielding tropical grass species under elevated atmospheric CO_2 conditions and to optimize growth conditions for maximum sequestration efficiency**.**

In order to achieve this major aim, the specific objectives of the study are as follows:

- To identify high biomass yielding grass species suitable for tropical climatic conditions.
- To collect and document information on the natural modes of multiplication and growth of selected grass species.
- To propagate selected species in nurseries and maintain them up to the desired stages of growth for experimentation, including standardization of growth conditions and acclimatization of characters.
- To conduct carbon dioxide sequestration studies using Open Top Chambers (OTC)/Free Air Carbon Dioxide Enrichment (FACE)/ Screen Aided Carbon Dioxide Control (SACC) systems under varying concentrations of carbon dioxide and other growth conditions.
- To assess the physiological and biochemical responses of plants exposed to varying levels of carbon dioxide, and to evaluate changes in microclimatic

conditions associated with OTC/FACE/SACC systems resulting from plant growth under elevated CO_2 levels.

- To assess changes in biomass content of plants subjected to elevated concentrations of carbon dioxide.
- To identify and listing out of grass species with higher carbon dioxide sequestration potential and optimize conditions for achieving maximum sequestration efficiency.

The results of the present study are brought out in two chapters for a logical explanation of the aforementioned objectives. In the 1st chapter, the selection of grass species and the strategies for the multiplication of the selected species having sufficient biomass, which enables them to be used as carbon sinks are explained in detail. Chapter I also deals with the design of experimental systems and the standardization of experimental conditions including carbon dioxide supply, which can offer meaningful results. A detailed evaluation of the growth and biochemical responses of the grass species at high CO_2 levels is covered in Chapter II.

CHAPTER 1

Standardization of experimental conditions and studies on the growth responses of selected grass species to elevated levels of CO_2

1.1. INTRODUCTION

The present study was intended to assess the morphological and biochemical responses of selected grass species to elevated levels of CO_2 under controlled experimental conditions. To sustain nearly natural conditions for plant growth in CO_2-enriched environments, previous researchers developed a variety of controlled environmental systems (Kramer, 1978). Several such CO_2-controlled systems are explained in detail in this chapter. It has been known since the early nineteenth century that rising carbon dioxide concentration (CO_2) in the atmosphere can boost plant growth (Kimball, 1983). These studies gave directions on how individual plants, wild plant populations, unmanaged plants, crop plants, and ecosystems react to rising CO_2 levels in the atmosphere. The IPCC (2007) projects that by 2025, the CO_2 concentration will be between 405 and 460 ppm, by 2050, it will be between 445 and 640 ppm, and by 2100, it will be between 720 and 1,020 ppm. Models must effectively capture the responses of entire plants or ecosystems in carbon and water exchange with the atmosphere to have a realistic assessment of the future impacts of climate change and in particular the effects of rising CO_2 concentrations on ecosystems (Smith & Dukes, 2013). The experimental design concerning the present study has been undertaken accordingly. The system helps to regulate the CO_2 concentration and evaluate their fluxes in the microclimatic environment, which is difficult under field conditions.

For the present study, two experimental systems were installed based on a thorough evaluation of the requirements from previous literature. Experiments were conducted with six selected species of grasses. Higher concentrations of CO_2 were supplied in the morning hours, and subsequent changes in the concentration of CO_2 inside the chambers were noted. The experimentation was continued for 15 days. Day and night fluxes of CO_2, attributed by six grass species were compared to determine the species which had higher assimilation potential. A standardization study (without plants) was also undertaken in the same manner to assess the gross and net flux of CO_2 associated with the chambers. The milestones in carbon sequestration research using various systems, along with the present experimental design, process, results, and inferences drawn are detailed in detail in this chapter.

1.2. REVIEW OF LITERATURE

1.2.1 Experimental designs in plant-carbon dioxide research

Various studies have been undertaken to assess the responses of increased atmospheric CO_2 on vegetation (Long et al., 2004). The majority of data regarding the responses of plants to increased CO_2 has come from experimental research conducted in greenhouses, controlled environment chambers with artificial lighting, and transparent enclosures or open-top chambers (OTCs) in the field (Long et al., 2004). A few examples of experimental manipulations include: studies of the effects of nutrient enrichment and soil warming; field studies of the effects of elevated CO_2 by free-air carbon dioxide enrichment (FACE); studies of the effects of elevated CO_2 in open-top chambers; and in situ fertilization studies of marine ecosystems with corresponding measurements of the partial pressure of CO_2 (Prentice et al., 2001).

Important experimental systems for CO_2 enrichment studies during the 1960s and 1999s include leaf chambers and phytotrons. The leaf Chamber is an older technique for calculating gas exchanges between individual leaves. It was a popular method for assessing net gas exchange, which involved measuring CO_2 and H_2O vapour in a flowing air stream and enclosing plant tissue (leaves) in an assimilation chamber or cuvette. This chamber may or may not be able to control the microclimate during measurements. Leaf chambers permit accurate regulation of the leaf environment and ongoing CO_2 and H_2O flux monitoring. It is facilitated with a system for the management of the composition of gas and the atmosphere around the leaf, monitoring a range of physical characteristics such as variations in gas concentrations. There is also the facility for data collection and analysis. Various types of leaf chambers could be found in the works of Musgrave and Moss (1961), Sestak et al. (1971), Sinclair et al. (1980), Valle et al. (1985), Field and Mooney (1990). Syvertsen and Smith (1983) designed a low-cost leaf chamber, where there is a brass plate as a heat exchanger for the control of air temperature (**Figure 1.1**). The fan unit is a 120 VAC motor that can move air at a speed of 28 liters per second with a fan blade that has a diameter of 7.6 cm. To stop air from leaking around the free-spinning shaft, the well-ventilated motor is bolted beneath the insulated base plate and sealed with multiple washers and silicone rubber sealer. The overall size

and fan-blade size of this kind of chamber can be readily adjusted to enclose any leaf.

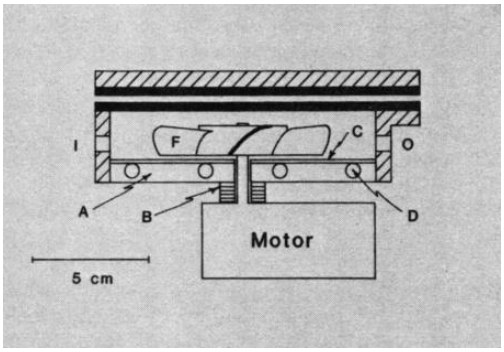


Figure 1.1: Temperature-controlled gas-exchange chamber for single leaves: showing insulation (A), washers (B), brass plate (C), copper tubing (D), fan blade (F), and inlet (I) and outlet (O) ports (Syvertsen & Smith 1983)

The primary shortcomings of leaf chambers were the inability to replicate the natural environment and the inability to evaluate the responses of the entire plant, including plant growth.

Phytotron emerged as a controlled system for CO_2 enrichment studies along with leaf chambers during the 1970s. Among controlled-environment facilities, a phytotron is the most complex type. Various climatic conditions can be provided by using incubators, seed germination chambers, roomettes, photoperiod rooms, mechanically cooled greenhouses, naturally and artificially lighted controlled-environment rooms, and cabinets (Downs,1980). Phytotrons are distinct from greenhouses or growth chambers due to the incorporation of environmental control technologies and a wide range of regulated environmental factors. The researcher can build any environmental gradient in a controlled environment. An advanced phytotron (ESPAS- Experimental Soil Plant Atmosphere System) was created by Gorissen et al. (1996) to measure the dynamics of carbon in the entire plant-soil system (**Figure 1.2**). This facility serves as an instrument for environmental research, estimating the amount of carbon that is transferred from the air to the roots, soil, and microbiological biomass, as well as the breakdown of the plant leftovers and organic matter in the soil. It is possible to quantify how the allocation and yield of carbon between plants and soil are impacted by atmospheric or soil environmental conditions. They studied carbon flow from plant to soil by experimenting with high atmospheric CO_2 using *Lolium perenne* and *Festuca arundinacea*.

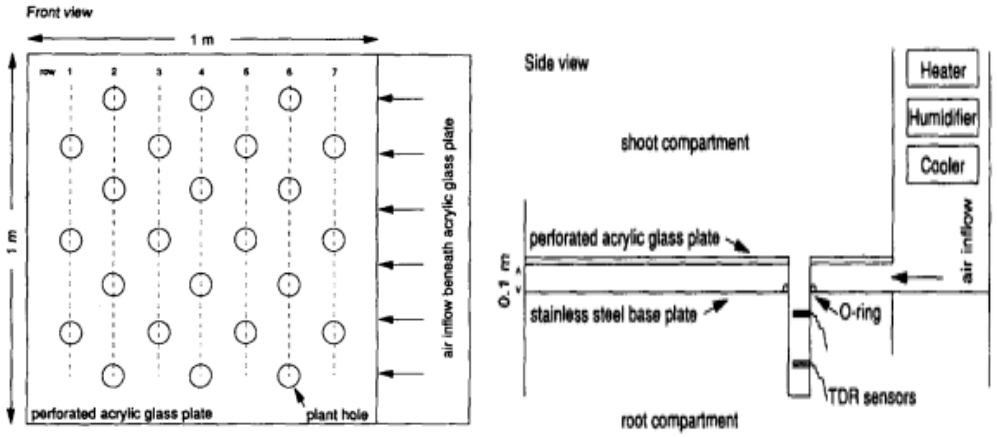


Figure 1.2: Diagrammatic representation of ESPAS Phytotron (Gorrissen et al., 1996)

The experimental activities conducted in phytotrons provide a means of operational and cognitive tools that farmers, cooperative organizations, governmental agencies, and researchers may find very useful. For instance, a range of environmental scenarios can be created and then simulated to measure physiological parameters and look into how the plants develop inside the chambers under various environmental conditions. Extrapolating it to natural settings has drawbacks, including environmental elements that are often consistent, plant size restrictions, and a lack of sunlight.

Greenhouses are the most used experimental system for the assessment of plant responses to elevated CO_2. Glass, fiberglass, polyvinyl chloride, or polyethylene skins are used to cover the structural frames of greenhouses. Greenhouses typically have higher humidity and lower wind speed than the outer environment (Strain & Cure, 1985). Therefore, the crops in the greenhouse are characterized by their development in a more ideal water-relationship environment and by seeming lusher than their short and thick-leaved counterparts that are grown in the field. Its primary drawbacks were the challenges of keeping CO_2 levels stable in specific situations and the difficulty of generalizing findings to the outdoors.

Researchers designed portable chambers to enable air pollution and serve as a low-cost replication of greenhouses. To create a flux density that resembled natural

sunshine, this took advantage of the sunlight that was present within a greenhouse or combined artificial light with sunlight (Carlson & Bazzaz, 1980). To measure actual evapotranspiration (ET) from crops and pastures, a portable, enclosed chamber was built and calibrated by McLeod et al. (2004) (**Figure 1.3**). It was then compared to accepted techniques for calculating ET. Electric fans with variable speeds were installed in the chamber for mixing the air throughout each ET measurement. It has the same drawbacks as other regulated environments.

Figure 1.3: The portable, enclosed chamber used to measure ET (McLeod et al., 2004)

Paradise and Cyr (1995) developed a computer-aided experimental system to measure the uptake of CO_2 by terrestrial plants (**Figure 1.4**). *Helianthus annuus* seedlings were utilised as the experimental plant. A gas environmental chamber, a gas analyzer, high pressure sodium lamps, a light metre, IBM-compatible PCs with A/D boards, and data collection software are all included in the setup.

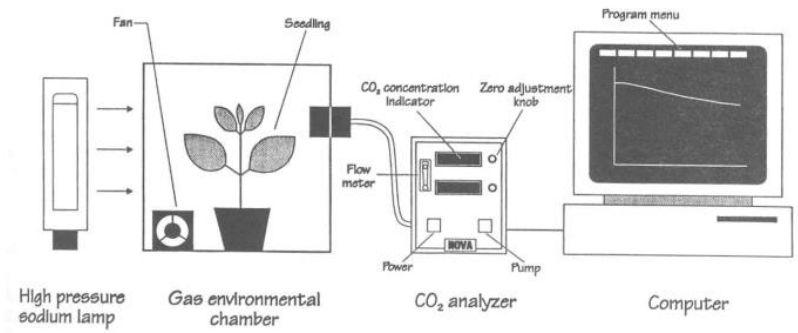


Figure 1.4: System for quantifying CO_2 uptake in terrestrial plants (Paradise & Cyr, 1995)

For studying CO_2 enrichment in the field, sunlit controlled environment chambers (e.g., SPAR, Soil-plant-atmosphere-research) were established by early researchers. Its foundation was a "closed loop" environment that was created in the late 1950s with walls made of Mylar (a registered trademark) polyester film. With

the use of metered CO_2, non-dispersive IRGAs allowed for the rapid monitoring of CO_2 levels and the direct, constant tracking of photosynthesis rates. In these chambers, transpiration and photosynthesis were monitored over time in relation to temperature, light, CO_2 concentration, and soil moisture level (Musgrave & Moss, 1961; Baker & Musgrave, 1964; Egli et al., 1970). With the same quantity of near-natural sunlight reaching each treatment, SPAR facilities can be used to assess physiological, growth, and developmental processes at the plant and canopy levels in carefully controlled environmental settings (Allen et al., 2020). Upon evaluating the distinct features of several systems for studying the consequences of increasing CO_2 levels in the atmosphere, the authors deduced that the SPAR systems possess the benefit of accurate regulation of CO_2 concentration, air temperature, and air humidity within a moderate range of surrounding air conditions.

Its constraints were complex control of the system. The characterization and measurement of C3 and C4 species' responses to CO_2, temperature, and other abiotic variables are best accomplished using SPAR chambers (Fleisher et al., 2010). The Mississippi State University's SPAR unit and related facilities are shown in **Figure 1.5**. Nevertheless, the limited area of each chamber in the SPAR is a disadvantage for unit sizes of plant samples.

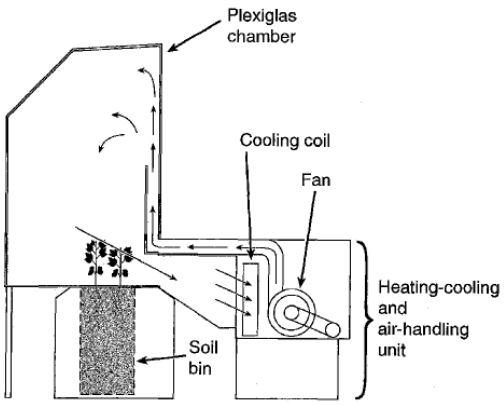


Figure 1.5: An illustration of a Mississippi State University Soil, Plant, Atmosphere, and Research (SPAR) unit with above ground plexiglas compartment, lysimeter, air-handling unit and heaters, fan for air circulation, a dew point sensor and pressure pump (Reddy et al., 2001).

The attempt to evaluate the effects of increased CO_2 on plants over several growing seasons and, in the case of crops, over their whole life cycle has resulted in the development of Free-Air CO_2 Enrichment (FACE) systems (Leaky et al., 2009). For almost twenty years, FACE has been used to expose vegetation to high atmospheric CO_2 concentrations in fully open-air environments, other than

controlled or chambered environments. The FACE method of CO_2 enrichment involves raising the CO_2 in the surrounding air of the plants by employing a system of pipes or plenums located close to the ground. The intention is to prevent the need for a chamber or enclosure surrounding the plants. With the aid of this technology, it is possible to alter the environment around growing plants to accurately replicate future carbon dioxide concentrations in the atmosphere (CO_2).

The FACE method eliminates the need for confinement structures by using a variety of vertical or horizontal vent pipes to discharge jets of pure CO_2 gas or air with higher concentrations of CO_2 at the edges of vegetation plots. FACE disperses the CO_2 throughout the test area via diffusion and natural wind. The original FACE method used blowers or fans to introduce CO_2-enriched air into the experimental area (Hendrey et al., 1993; Lewin et al., 1994). Some field investigations used a FACE approach (Miglietta et al., 2001 ; Okada et al., 2001) to emit pure CO_2 gas as high-velocity jets from emission tubes placed horizontally at the edge of a FACE octagon (through multiple tiny perforations). The main distinctions between FACE and the nearest alternatives, outdoor controlled environment chambers or open-top chambers, are that FACE eliminates chamber impacts such as lowered levels of solar radiation, turbulence, artificial wind flow, and micrometeorological patterns.

The CSIR-National Botanical Research Institute (NBRI), Lucknow established a FACE facility in 2013 to tackle the chamber effect (Hendrey et al., 1993). The facility has been operational since then (Pandey et al., 2017). **Figure 1.6** depicts the workflow of the NBRI-FACE facility. The main components of NBRI-FACE are as follows.

- Hexagonal rings (six), each of which has six arms that are 15 feet long (G.I. pipes) that are anchored in the ground.
- Air combined with CO_2 was pumped into raised rings using air compressors.
- A solenoid valve regulated the CO_2 release within the ring.
- An infrared gas analyzer (IRGA) that monitors the amount of CO_2 within the ring
- A center sensor in each ring measures the following: light intensity, temperature, humidity, wind direction, and speed of the wind.

- All sensors and data gathering is controlled by a Supervisory Control and Data Acquisition (SCADA) system.

The disadvantage of FACE experiments is that the range of CO_2 swings is more than ten times larger than what plants experience in their natural environments, and it is difficult to manage high CO_2 concentrations in turbulent air. According to Allen et al. (2020), responses of plants in FACE are prone to undervalue the advantages of increased CO_2 because CO_2 changes in FACE and new research reveal lower photosynthesis and growth under changing CO_2. Technical feasibility and high cost are other shortcomings associated with the FACE technique.

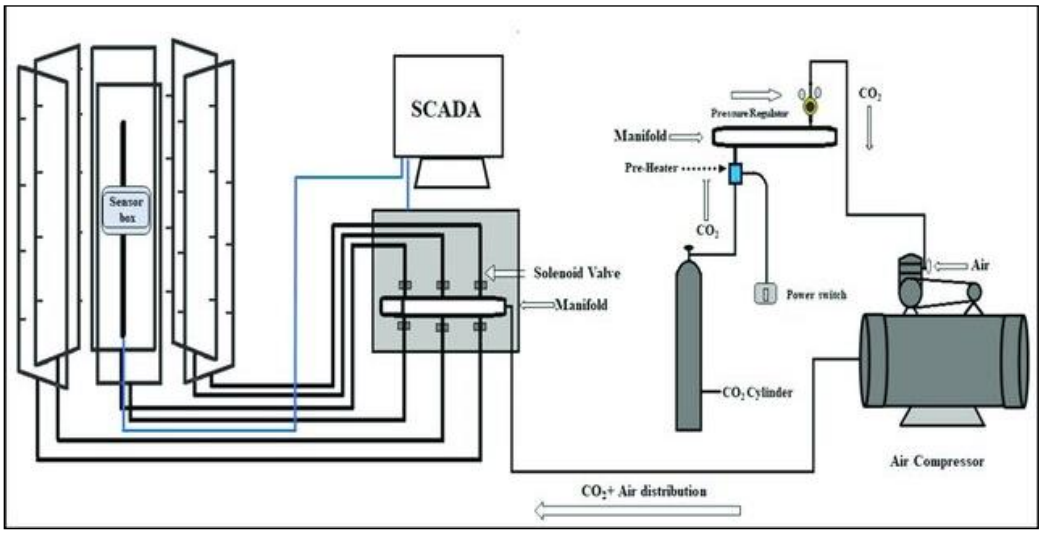


Figure 1.6: Workflow diagram for the NBRI-Free Air Carbon Dioxide Enrichment Facility

Another system for individual plant and field-level responses was established by the Open Top Chamber (OTC). Compared to the FACE experiments, the OTCs are low-cost structures that enable the study of how plants respond to rising CO_2 levels in the field (Ashenden et al., 1992). According to Allen et al. (1992) and Drake et al. (1989), OTCs are divided into three parts: the bottom half, the top half, and the top opening. According to Bhattacharya et al. (1990), building a door on the opposite side of the bottom panel allows for easy access to the chamber, inside. According to Ham et al. (1993), chambers feature a big opening at the top that covers 50-100 percent of their base area. The OTCs can also be readily adjusted to any environment that is being studied. The open-top chamber method worked effectively in the field

for creating and sustaining substantial test environments, and it provided no major challenges once in situ. Electrical power requirements and provisions for large volumes of liquid CO_2 delivery were manageable concerns. Systems for heating and cooling were added to the OTC system in certain earlier experiments (Norby et al., 1997). The following are the effects of OTCs on the microclimate; relative humidity rises, light intensity is generally lowered by up to 20%, wind velocity is lessened and steadier, and temperatures may rise by as much as 3 °C, relying on the chamber design and test site (Leadley & Drake, 1993).

Earlier, open-top chamber experiments by Rogers et al. (1983) used an open-ended cylinder measuring 3 m in diameter and 2.4 m in height (**Figure 1.7**). To maintain the internal temperature and humidity comparable to that of the environment outside, elevated ventilation was provided. To maintain the required CO_2 concentration over the top of the chamber, a "frustum" was added, and it caused the upper entrance's size to be limited to half of the chamber's ground surface (Rogers et al., 1984). The OTCs were made of a structural frame of aluminum covered by translucent polyvinyl chloride plastic film panels. The lower panel included two walls and was equipped with 2.5 cm holes perforated into the inner wall, which served as a duct to evenly distribute the CO_2-air mixture throughout the chamber. To ensure proper mixing, air was supplied to this duct by an axial fan positioned in a sheet metal plenum box ahead of the fan.

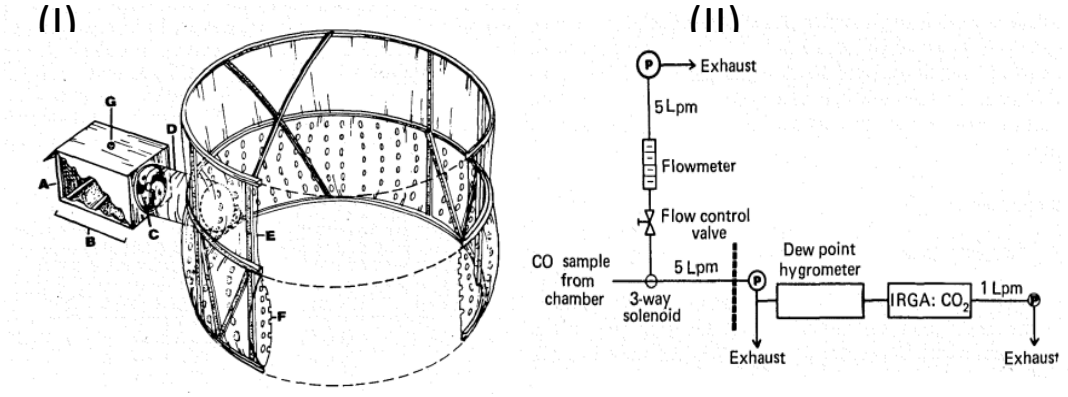


Figure 1.7: (I). The earliest cylindrical OTC was with a transparent PVC plastic film covering the aluminum frame. A. Particulate filter made of fiberglass; B. Sheet metal box; C. Axial fan D. Connecting duct, F. Upper panel G. CO_2 injection port. (II) Diagram showing the CO_2 monitoring system. (Rogers et al., 1983)

Open-top chambers with various modifications have been used widely in CO_2 research to date. Using a slightly modified version of Roger et al. (1983), Kant et al. (2007) studied the active carbon pools in the rhizosphere of wheat growing under elevated CO_2. Each OTC was 1.8 meters high and had a diameter of 1.5 meters. Within the chambers, polyvinyl chloride (PVC) sheets transferred over 85% of the incident solar energy. To allow the CO_2 and air mixture to enter the chamber, a hollow circular PVC ring with holes spaced 30 cm apart was employed as the chamber foundation. Barton et al. (2010) made use of OTCs big enough to hold entire trees. Drake (2014) published findings from a 28-year study in which portable OTCs were used in a marsh that experienced erratic flood tides. In the most recent study by Vijayalakshmi et al. (2024), plants were grown inside an OTC made of polycarbonate sheet that measured 4 m x 4 m × 4 m. The chambers were outfitted with wireless signal transmission features, SCADA integration technologies, and CO_2, temperature, and humidity monitoring controls.

Though OTCs are quite popular, their impact on microclimate poses a major limitation due to potential interactions between changed microclimate and elevated CO_2 (Leadley et al., 1997). OTCs are facilities that are particularly helpful for conducting studies on the impact of CO_2 on crop growth and yield in field crops with relatively short cycles (Andrea & Rinaldi, 2010). A significant disadvantage is the chamber effects caused by humidity and wind gradients. Another shortcoming associated with OTC is that the plant growth varies inside and outside and that multiple sample chambers are required to deal with the natural variety of ecosystems.

To overcome the disadvantages of OTC and operational expenses associated with FACE, Screen Aided CO_2 Control (SACC) was developed in the late 1990's. SACC has substantially lower operating expenses per experiment than FACE and helps with the microclimate issues related to OTCs (Leadley et al., 1997). According to Leadley et al. (1997), FACE requires huge quantities of CO_2 and large plots; hence the exposure to CO_2 is fully unconfined. Thus the screens are employed in SACC to stop the wind, and in addition, air jets enhanced with CO_2 produce turbulent mixing inside the SACC unit. The SACC system developed by Leadley et al, (1997) is illustrated below (**Figure 1.8**)

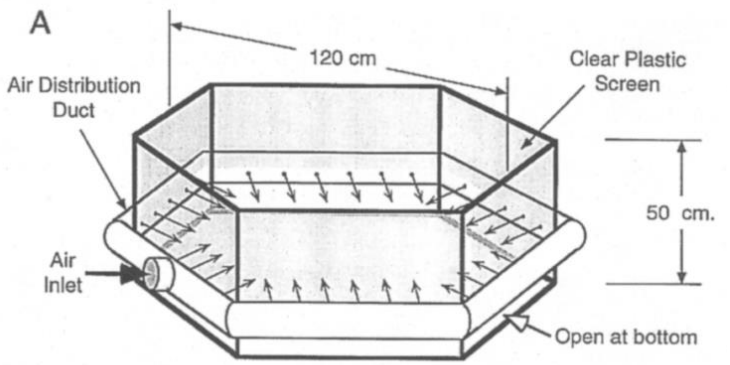


Figure 1.8: Schematic representation of Screen Aided CO_2 control (SACC) developed by Leadley et al, (1997).

1.2.2 Sidewall materials under use

The experimental chambers employed in previous studies were constructed specifically to fulfill the objectives. Consequently, different investigations started using various facilities. One common type of experimental setup is the open-top chamber. The chambers come in a variety of volumes and shapes, such as hexagonal, hexahedron, octagon, and cylindrical. On a review of 3 decades of studies on the elevated CO_2 responses of grass species, Sashna et al. (2022) revealed that the volumes of earlier chambers varied from 0.3 m^3 to 64 m^3. The shape of a chamber affects air circulation and CO_2 distribution. Most OTCs had a frustum maintained at the top to reduce the impact of air current dilution inside the chamber (Vanaja et al., 2006). In general, OTC's shape and dimensions are determined by the nature of studies carried out and the objectives set forth. The materials of the side walls have a greater influence on the overall results of the experiments and the pattern of irradiance. Commonly utilized side wall materials for chamber construction are polyethylene film, polycarbonate sheets, and PVC film. Researchers also employed different kinds of other materials depending on the objectives of the experiment.

Polyethylene is a polymer composed of long chains of ethylene (C_2H_4) monomer units. The chemical structure is a long-chain hydrocarbon made up of only carbon and hydrogen atoms. Polyethylene was used to wrap chambers in early OTCs. Knapp et al. (1993) used a 1.5mm (6 mil) thick UV-resistant polyethylene film to construct a large cylindrical OTC with a volume of 63.578 m^3. It was a field experiment carried out in tall grass prairie. Even though the top opening is there, the effect of the wrapping material resulted in a reduction of photosynthetically active

radiation to 11%. Johnson et al. (2000) found that the polyethylene covering transmits 85-95% of incident PPFD under most day conditions. Hagopian et al. (2015) constructed a low-cost growth chamber using 6 mil UV-resistant dura-film super 4 polyethylene greenhouse film. A 91% light transmission rating was provided by the material. UV-resistant polyethylene film is a common choice for CO_2 enrichment studies in earlier works (Knapp et al., 1993; Bremer et al., 1996).

Polycarbonates are engineered thermoplastics that are strong, stiff, hard, tough, and clear. This material can maintain rigidity at 140°C and toughness at -20°C, or even lower in certain grades. Several locations in Europe employ polycarbonate material year-round. Schapendonk et al. (2000) used chambers without chamber supports that were composed of 3-mm polycarbonate. Because polycarbonate is virtually unbreakable and can be cold bent, simpler designs can be used. Compared to other materials, this one is more costly. For the construction of OTC, Oijen et al. (1999) utilized 3mm polycarbonate lexan. Although it absorbs UV-b, it is 88% transparent to light used in photosynthetic reactions. The material is amorphous, which results in excellent mechanical properties and dimensional stability. Morgan et al. (2001) used clear lexan panels, a type of polycarbonate material, to build OTC for their CO_2 enrichment study in a shortgrass steppe. More than 95% of incident photosynthetically active radiation (PAR) was passed through the Lexan chamber walls, based on the data from quantum sensors positioned inside the chambers. On the other hand, the daily photon flux inside the chambers was 28% lower than outside due to shading caused by the chamber framework. Roy et al. (2012) used transparent multilayered polycarbonate sheets to construct circular, UV-shielded open-top chambers for a three-year study on the combined effects of CO_2 enrichment and temperature on rice and the chamber transmitted 84-68% of the incoming light.

When one of the hydrogens on an ethylene molecule is replaced with chlorine, the molecule becomes vinyl chloride, which serves as the basis for polyvinyl chloride (PVC). PVC is divided into two main groups: rigid PVC (rPVC) and flexible PVC (fPVC). The density of flexible PVC, also known as plasticized PVC, typically ranges from 1.1 to 1.35 g/cm^3 (Pyeon et al., 2017). PVC does not crack when temperatures are raised to 140°F and then lowered to 0°F again. To

create it, pure PVC is mixed with suitable plasticizers to reduce its crystallinity. These plasticizers give fPVC a lubricating effect, which makes PVC plastics more flexible and clear (Mnyango & Hlangothi, 2024). In the current study, flexible PVC is used as the side wall material. Previously many researchers developed OTCs with PVC sheets as covering material. In the earlier construction by Rogers et al. (1983), which forms the basic model for all subsequent OTCs, they used PVC film as covering material. Leadley and Drake (1993) used an OTC where PVC film was used as covering material, to measure net gas exchange in plant canopies exposed to higher CO_2 levels. Here the chamber cover did not affect the radiation quality in the visible and far-red light, but it significantly decreased UV-B penetration (Drake et al. 1989). An OTC for continuously checking and sustaining the appropriate level of CO_2, temperature, and relative humidity was developed by Vanaja et al. (2006). To achieve over 90% light transmittance, PVC sheets measuring a thickness of 120 microns were used to cover these OTCs. The growth, yield, and quality of maize under high temperatures and atmospheric carbon dioxide were investigated by Abebe et al. (2016) using OTCs made up of PVC sheets that were nearly 90% transparent.

Apart from the aforementioned materials, fiberglass and Plexiglas are also often used in OTCs (Andrea & Rinaldi, 2010). These materials are used in the construction of OTCs for horticultural purposes. Davey et al. (1999) developed a cylindrical OTC with UV-stabilized plastic sheets as side walls and lightweight steel frames. UV stabilizers are frequently used in plastic products to increase their stability and reduce UV radiation-induced deterioration (Yuan & Xu, 2023). Here the chamber has a volume of $1.13m^3$. Using this chamber the photosynthetic response to high CO_2 and nutrient stress was studied for *Agrostis capillaris*, *Lolium perenne*, and *Trifolium repens.* A CO_2 growth chamber was created by Nackley et al. (2014) using PVC-framed units covered in Mylar polyester sheeting, and this model was constructed following Kinmonth-Schultz and Kim (2011). Mylar is a polyester film noted for its strength, resilience, and adaptability. Since Mylar film is usually transparent, light can travel through it (Borra et al., 1992). Acrylic sheets are another material used in certain chambers. Outstanding optical qualities, weather ability, and a wide spectrum of transparent, translucent, and opaque colors are characteristics of acrylic polymers, which are also known as Polymethyl Methacrylate (PMMA). Methyl methacrylate is used for making hard, transparent sheets of acrylic (Campo,

2008). In 1998, Jongen and Jones built an OTC using a framework of galvanized steel, and clear, UV-stabilized 2mm thick acrylic sheets affixed to the exterior of the framework. Similarly, Bunce (2016) built an OTC covering 1.2 m × 2.3 m of ground, with walls made of transparent acrylic sheets, but the framework here was wooden corner posts.

Most of the technical specifications of OTCs are especially useful for research on how CO_2 affects crop growth and yield in field crops. Since the goal of this study is to measure the performance of individual plants at elevated CO_2, a varied construct was used. The chamber materials are selected based on the specific requirements and cost-effectiveness. The frames are constructed with PVC pipes and the side wall material used was clear PVC film (1mm thick). The whole experimental procedure including the installation of the experimental system is detailed in the next section.

1.3. MATERIALS AND METHODS

The primary aim of the study was to assess the changes in microclimate brought about by the growth and metabolism of selected grass species subjected to elevated levels of carbon dioxide. Furthermore, the study focused on the relative changes in morphology and biochemistry of selected grass species grown at an elevated condition of CO_2. These requirements are followed in the construction of the chambers and setting-related facilities. A detailed explanation is provided for the chamber installation, the system specification and operation, and the standardization.

1.3.1 Installation of CO_2-controlled chambers

Two chambers, each with a size of 6.32 m^3 were installed using the materials and facilities mentioned in **Table 1.1**. 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 the 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. The CO_2-controlled chamber and associated facilities are depicted in **Plate 1.1**

Table 1.1: Materials used and facilities provided with CO_2-controlled experimental systems

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

* Unplasticiized PVC, **Non-dispersive infrared

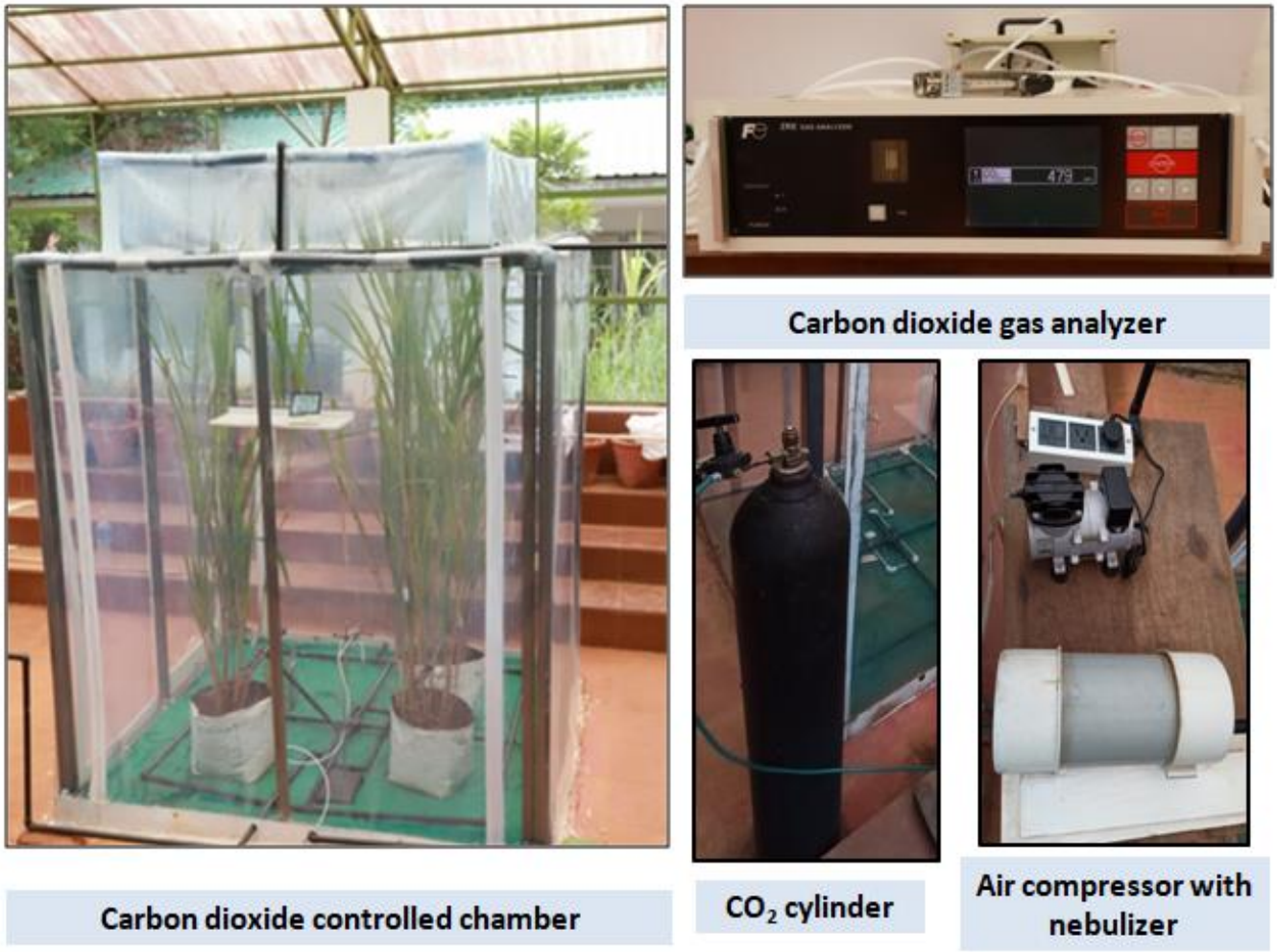


Plate 1.1: Experimental chambers with associated facilities

1.3.2 Standardization study in the experimental system

The installation and standardization of chambers were for experimentation using selected tree and grass species by two contemporary researchers. The standardization results of empty chambers with the respective gas composition were already reported by Sreekumar (2024), for .tree species and this being simultaneous research, the outcomes of the earlier finding have also been used in this study. The justification for standardization studies and the methods undertaken for the same are listed below:

Standardization studies were undertaken in both chambers (empty) for 15 days to assess the flux (day and night) of CO_2 associated with the chambers as a result of dissipation and subsequent retention at specific conditions of temperature and humidity. At the beginning of the standardization study chambers were properly sealed. Afterward, one chamber (CO_2 treatment chamber) was supplied with CO_2 - ambient air mixture at 9.00 a.m. A concentration of 900-1000 ppm CO_2 was ensured inside the chamber by monitoring through the CO_2 gas analyzer. This range of CO_2 was attained inside the chamber in about 15 minutes. The other chamber (control) was provided with ambient air for 15 minutes. The CO_2 composition of the control chamber was also assessed. Using a Thermo-Hygrothermometer, initial temperature and humidity within the chambers were noted along with CO_2 concentration. In the evening, CO_2 concentration, temperature, and humidity were again recorded. Day flux of CO_2 and day variations in temperature and humidity were calculated from their respective morning and evening values. Supply of CO_2 and monitoring of CO_2, temperature, and humidity were repeated the next day morning, after the initial analysis. The Night flux of CO_2 and night variations in temperature and humidity concerning both chambers were obtained from morning records, concerning the evening records of the previous day. All these procedures were repeated for 15 days. The retention value of CO_2 in the daytime and the night-time were retained as day flux and night flux associated with the chambers.

The data obtained in the standardization study determines the pattern of CO_2 flux, temperature, and humidity with changing CO_2 concentrations in the chamber at ideal conditions without plants and is referred to as the chamber effect. While

analysing the actual flux/actual intake of CO_2 by the individual grass species the chamber effect, as obtained in the standardization study, has been taken into account. **Figures 1.9** and **1.10** display the experimental setup concerning the standardization study.

Figure 1.9: Control chamber set up for Standardization study

Figure 1.10: Treatment chamber set up for Standardization study

1.3.3 Identification and selection of high biomass-yielding grass species suitable for tropical climatic conditions

To determine which species of grass would be best for the study, a comprehensive review of the literature was conducted. Analysis was done on 52 grass genera. According to reports by Van Dyne et al. (1977) and Singh and Gupta (1993) on a variety of grasslands around the world, the main species of grass are identified as belonging to the genera listed below.

Dominant grass genera of the world grasslands

1. *Aeluropus*
2. *Agropyron*
3. *Andropogon*
4. *Aristida*
5. *Arundinela*
6. ***Arundo***
7. *Astrebla*
8. *Avena*
9. *Bambusa*
10. *Bothriochloa*
11. *Bromus*
12. *Calamovilfa*
13. *Cenchrus*
14. *Chloris*
15. ***Chrysopogon***
16. ***Cymbopogon***
17. *Cynodon*
18. *Dactyloctenium,*
19. *Danthovia*
20. *Dendrocalamus*
21. *Dichanthium*
22. *Digitaria*
23. *Dimeria*
24. *Ehrharta*
25. *Eleusine*
26. *Elymus*
27. *Erianthus*
28. *Festuca*
29. *Eragrostis*
30. *Heteropogon*
31. *Hordeum*
32. *Hyparrhenia*
33. *Imperata*
34. *Ischaemum*
35. *Koeleria*
36. *Lesiurus*
37. *Melocanna*
38. *Oryzopis*
39. ***Megathyrsus***
40. *Paspalum*
41. ***Pennisetum***
42. *Phragmites*
43. *Poa*
44. ***Saccharum***
45. *Sehima*
46. *Setaria*
47. *Sorghastrum*
48. *Sporobolus*
49. *Stipa*
50. *Tetrachne*
51. *Themeda*
52. *Vulpia*

The selection of six grass species belonging to the six genera mentioned above was based on several factors, such as their greater biomass, suitability for tropical climatic conditions, and the dearth of studies on CO_2 sequestration of these species with the specific experimental conditions and procedures outlined in the present study. Type specimens of the selected species were handed over to the Calicut University Herbarium (CALI). A general description of the species of grass chosen for the present study is illustrated. **Plate 1.2** shows the nursery-grown grass species ready for experimentation.

1) *Megathyrsus maximus (*Jacq.) B.K.Simon & S.W.L.Jacobs (Guinea grass) (CALI NO: 7188)

Family : Poaceae

Genus : Megathyrsus

Species : *Megathyrsus maximus (*Jacq.) B.K.Simon & S.W.L.Jacobs

Megathyrsus maximus is a robust, tillered, upright perennial plant that grows to a height of 1-2 meters (**Pate 1.2**). Typically, it grows in large bunches from short, stout rhizomes that reach 1-3 meters. It spreads by rhizomes and seeds (Lorenzi, 2000). *M. maximus*, is a type of forage grass indigenous to South Africa (Soti & Thomas, 2022). The species is an excellent candidate for biomass production under tropical environmental conditions. The species can thrive in a range of well-drained soil types and is resistant to drought. According to Bogdan (1977), the plant has a preference for light-textured soils, especially sandy loams, and avoids heavy clays. On heavy soils, the initial growth is slow and leaf area is modest (Alves & Xavier, 1986). It doesn't do well in areas that experience ongoing flooding or waterlogging, nor does it do well on saline soils. In conditions with high temperatures and bright lights, the plant grows more quickly. The species possesses numerous advantageous properties, such as excellent quality animal feed, high digestibility and palatability, highly viable seeds, resilience to diseases and insects, tolerance to poor fertility soils and drought, and creeping traits with stoloniferous and rhizotomous reproduction (Rhodes et al., 2021). A positive increase in photosynthesis and biomass accumulation by the species under elevated CO_2 levels was observed by Faria et al. (2018). Similarly higher growth and biomass production of *M. maximus* at increasing CO_2 levels were observed in an OTC study by Bhatt et al. (2010). However, previous studies did not provide information on its CO_2 intake potential (mitigation potential) at elevated CO_2 levels or on CO_2 uptake per biomass in comparison to other species.

2) *Saccharum arundinaceum* Retz. (Hardy sugarcane) (CALI NO: 7189)

Family : Poaceae

Genus : *Saccharum*

Species : *arundinaceum* Retz.

According to *Royal Botanic Gardens, Kew* (n.d.) the species is first published in Observ. Bot. 4: 14 (1786). This name is a synonym of *Tripidium arundinaceum* (Retz.) Welker, Voronts. & E.A. Kellogg. The species is indigenous to tropical and subtropical regions of Asia, ranging from India to Korea and New Guinea. The plant is distributed throughout Kerala (https://indiabiodiversity.org/ species/ show/243193). It is known as wild sugarcane or hardy sugarcane in general. The plant is a wild species of *S. officinarum* (sugarcane) that has good resistance to infertile soils, cold, drought, and pests (Lu et al., 2013). It is a perennial grass and gathers in big clumps (**Pate 1.2)**. Culms are glabrous, robust, and grow up to a height of 6 meters and 1-2 cm in diameter. Leaf-sheaths have ciliate edges and mouths and can be pubescent or glabrous. The inflorescence has a glabrous axis and is a branched panicle that is between 30 and 80 cm long (https://www.worldfloraonline.org / taxon/ wfo- 0000896536). The species exhibits a C4 photosynthetic pathway. The plant grows well along streams and field borders. Flowering and fruiting time is December to April. The higher biomass yield of the species is previously reported. According to Mislevy et al. (1997), the species yielded 5.2 to 51.5 t DM/ha/yr biomass on average over four years in the USA. Second-year yields were 4.6-23.3 t DM/ha/yr, according to Deren et al. (1991). Mirshad et al. (2014) reported the tolerance of this species in saline soils, and it is reported to be a species with sustained biomass yield (Feng et al., 2015). The sustainable lignocellulosic biomass residues of *S. arundinaceum* have been reported by Muthuvelu et al. (2019) to be promising feedstocks for the synthesis of bioethanol and other value-added products.

3) *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson (Cochin lemongrass) (CALI NO: 7183)

Family : Poaceae

Genus : *Cymbopogon*

Species : *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson

Cymbopogon flexuosus or lemongrass is native to India and cultivated in Kerala, Assam, Maharashtra, and Uttar Pradesh. Lemongrass is widely grown in several countries, including Brazil, Mexico, Dominica, Haiti, Madagascar, Indonesia, and China (Ganjewala & Gupta, 2013). It is a tufted robust perennial grass that reaches a height of about 3 meters **(Plate 1.2).** The plant utilizes the C4 photosynthetic pathway. It is also known as Malabar grass. The leaves can grow up to one meter in length (Skaria et al., 2006). Crushed leaves have a distinct citrus scent due to lemongrass oil of commercial importance. As 90% of East Indian lemongrass oil was previously shipped from Cochin Port, it is commonly referred to as "Cochin oil" in international trade (Skaria et al., 2006). It freely blooms and every flowering unit consists of two yellow-brown structures known as glumes at the base of a spikelet. Pairs of spikelets are present (https://www.worldfloraonline.org/taxon/wfo-0000860994). The colour of the stems distinguishes two varieties of this species: red grass and white grass (Skaria et al., 2006). Lemongrass can thrive in a variety of soil and climate conditions. However, well-drained sandy loam soil that is highly fertile and exposed to sunlight yields vigorous growth (Sugumaran et al., 2005). Khan and Verma (2020) studied the carbon sequestration potential of aromatic plants, including *C. flexuosus,* in marginal soils in India, and according to them, in marginal soils, efficient recycling of the plant's distillation waste will improve biomass yield and sequester carbon in the soil. While there are no previous studies on the CO_2 sequestration capability of the species using controlled systems with elevated CO_2 environments.

4) *Chrysopogon zizanioides* (L.) Roberty (Vetiver grass) (CALI NO: 7191)

Family : Poaceae

Genus : *Chrysopogon*

Species : *Chrysopogon zizanioides* (L.) Roberty

Chrysopogon zizanioides is a perennial tussocky grass, that reaches up to 200cm high or more (**Pate 1.2)**. The leaves are linear and pale green, with glabrous sheaths that are lower sharply keeled and imbricate in fan-like clusters. Inflorescence is a panicle with an oblong outline, 20-30 cm, usually thin and purplish with many branches (https://www.worldfloraonline.org/taxon/wfo-0000859932). In favorable conditions, its roots grow quickly and can reach a depth of 3-5 meters in the first year of cultivation. This allows them to withstand harsh weather conditions like drought and floods (De Guzmam & Oyen 1999). *C. zizanioides* has been grown for the extraction of essential oils with economic and medicinal use (Chou et al., 2016). The root portion of the plant is the most valuable for oil extraction, which is used in food and drink, spa treatments, household appliances, and medicinal applications. The global market for vetiver oil was 408.8 tonnes in 2019, with a 7.8% increase expected between 2020 and 2027 (Grand View Research, 2020). The species has numerous potential uses because of its distinct physiological and ecological traits. It can be found in many different environments with different soil and environmental conditions. Currently, more than 120 countries use this grass species. *C. zizanioides* is traditionally used as a boundary fence in India (Chou et al., 2016). Ecological applications of *C. zizanioides* include soil and water conservation systems in agricultural settings, stabilization of mountain slopes, reclamation of mines, polluted soil, and saline land, as well as wastewater treatment (Mickovski et al., 2005). The ability of *C. zizanioides* to withstand high levels of several heavy metals without compromising its growth and development has been revealed. The carbon sequestration potential of *C. zizanioides* is previously studied by Lavania and Seshu (2009). According to them with its rapidly spreading tufted root structure that can reach 3 m in just a single year, *C. zizanioides* has the potential to be a perfect global candidate for sequestering 1 kilogram of atmospheric carbon annually from a surface area of one square meter deep into the soil pool. The researchers mainly focused on the belowground growth and soil carbon sequestration, while the present study addressed the CO_2 uptake capability of aboveground and CO_2 uptake per biomass of the plant under CO_2 enriched conditions of a controlled chamber.

5) *Arundo donax* L. (Giant reed) (CALI NO: 7182)

Family : Poaceae

Genus : *Arundo*

Species : *Arundo donax* L.

Arundo donax is a perennial, rhizomatous grass species that is not tufted (**Pate 1.2)**. The strong, upright stalks reach up to 6 meters, 1-3 cm in diameter, and are either unbranched or have clusters of slender branches that resemble bamboo growing from nodes. Inflorescence is a dense purplish-coloured panicle. The plant is also known as "giant reed" or "giant cane". *A. donax* grows naturally in a variety of habitats and is found throughout temperate and hot climates worldwide. The species is a C3 plant with high photosynthetic CO_2 exchange rates (Rossa et al., 1998). According to Else (1996), the plant is sterile, so new plants can be produced (annually) directly from rhizomes. The species has many benefits because of its high biomass production, low input needs, and adaptability to different environments, soil types, and growing conditions (Corno et al., 2014). *A. donax* is used in the biological fermentation process for the synthesis of biofuels and bioenergy, as well as direct biomass combustion (Dahl & Obernberger, 2004). Ragaglini et al. (2014) provide information on the plant's biogas production potential. Bio-ethanol production from the species was reported by Jaradat (2010). According to Tracy and DeLoach (1998), a lot of industrial applications and chemical compound extraction are already recognized. Furthermore, the plant has a great deal of potential for phytoremediation of contaminated soils (Jambor & Torok, 2019). In a closed-topped CO_2 chamber experiment using *A.donax*, Nackley et al. (2014) observed a 100% drop in transpiration rates at CO_2 enrichment. The authors reported that at extreme drought, reduced transpiration could not stop desiccation and photosynthetic decrease, but it could postpone drought responses and assimilation periods in this species. Water usage efficiencies (WUE) were also enhanced by decreased transpiration. The objective of the current study is to quantify CO_2 uptake capability, growth response, and CO_2 uptake per plant biomass of the species.

6) *Pennisetum pedicellatum* Trin. (Deenanth grass) (CALI NO: 7184)

Family : Poaceae

Genus : *Pennisetum*

Species : *Pennisetum pedicellatum* Trin.

The name *Pennisetum pedicellatum* Trin. is a synonym of *Cenchrus pedicellatus* (Trin.) Morrone. It is native to West Africa and was brought to India, from where it subsequently expanded to Northern Australia and Southeast Asia (Schmelzer, 1997). It is a C4 perennial forage grass, and the height of the culm ranges from 30cm to 150 cm (**Pate 1.2)**. The inflorescence is panicle and its length ranges from 5 to 15 cm (https://www.worldfloraonline.org/taxon/wfo-0000889043). The species has a massive root system, and due to its quick growth and higher biomass production, it is ideal for remediation studies to improve land degradation as a restoration grass (Kumar & Fulekar, 2022). It grows on soils with a wide pH range, 500–1500 mm of rainfall, and periods of severe drought lasting 4-6 months. It is found near roadsides, disturbed areas, and abandoned land (Schmelzer, 1997; FAO, 2010). Varshney and Baijal (1977) reported that the plant can withstand salinity, while increased salinity will hinder its growth. The high nutritional value of *P. pedicellatum* makes it mostly useful as cattle feed, especially in the early stages of feeding (90 to 120 days after planting).

According to Asmare (2016), the plant performs well at both mid- and high altitudes. The impact of elevated CO_2 on biomass and nutritional value of *P. pedicellatum* was examined by Tom-Dery et al. (2018). They found that while biomass allocation reduced the root-to-shoot ratio, elevated CO_2 increased the leaf area. There are no previous studies on the plant's capacity to absorb CO_2 under conditions of elevated CO_2 in controlled chambers or CO_2 uptake data based on biomass. Therefore, the current study investigates the plant's capacity to sequester carbon in this regard.

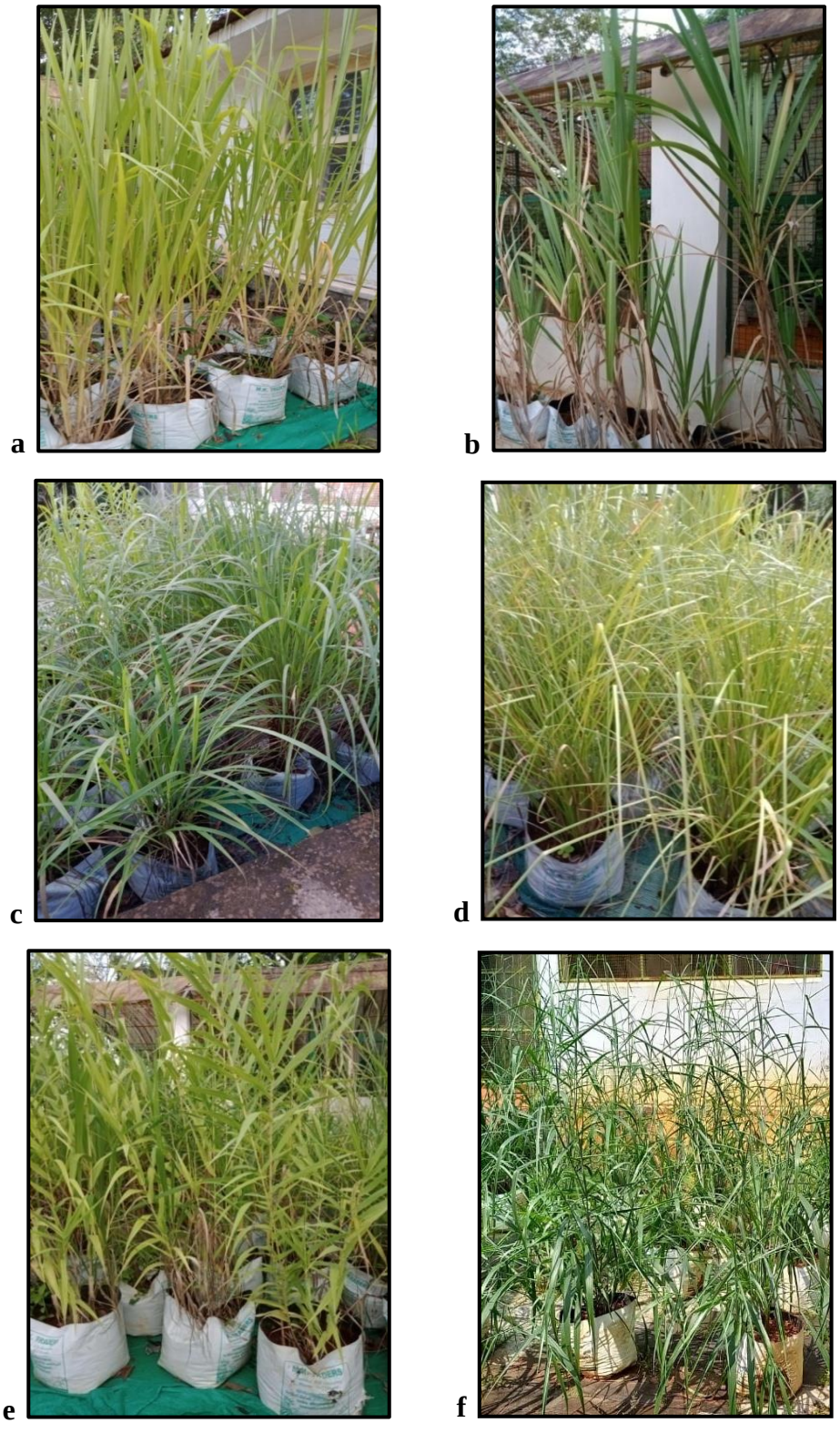


Plate 1.2: Plants grown for experimentation. a) ***Megathyrsus maximus*** **b)** ***Saccharum arundinaceum*** **c)** ***Cymbopogon fexuosus*** **d)** ***Chrysopogon zizanioides*** **e)** ***Arundo donax*** **f)** ***Pennisetum pedicellatum***

1.3.4 Collection, Multiplication, and growth of grass species for experimentation

Plantlets were collected from the Kannur and Malappuram districts of Kerala, India. 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 rearing process and were then grown for 6-7 months to attain sizable biomass for experimentation.

1.3.5 Experimentation on plants using elevated CO_2 and monitoring of microclimatic conditions associated with the growth chambers

Mature plants of *Megathyrsus maximus*, *Saccharum arundinaceum*, *Cymbopogon flexuosus*, *Chryzopogon zizanioides*, *Arundo donax,* and *Pennicetum pedicellatum* were subjected to experimentation at specific intervals. For each study, 2 sets of plants (3 each) were selected and maintained in the Control Chamber (CC) and CO_2 treatment Chamber (TC) respectively. Before the introduction of the plants in the chamber, morphological parameters including plant height, number of leaves, leaf length and breadth, culm diameter, and number of tillers and tiller height were measured. For initial soil quality analysis, samples were taken from each grow bag before experimentation. The functionality of the irrigation system, CO_2 supply system, and hygro-thermometer were confirmed before sealing the chamber. As per the procedure explained in the standardization study, ambient air was supplied to CC for 15 minutes in the morning (9 a.m.). Similarly, through the mixing tube/nebulizer, an elevated amount of CO_2 was supplied to the TC for 15 minutes (9 a.m.). After the supply of air and 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. **Figure 1.11** is the schematic representation of the entire experimental setup with chambers and associated facilities.

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 was calculated by subtracting evening measurements from morning measurements

(after the 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 morning gives the day CO_2 flux, ie. the amount of CO_2 assimilated by the plants in the chamber. Monitoring is repeated in the same way in the next day's morning (before the supply of CO_2) and the night CO_2 flux and night variations in the temperature and humidity were calculated by subtracting morning measurements from the measurements (evening) of the previous day. The monitoring was continued for fifteen days and the results of standardization studies were taken for the validation of results. Every evening, plants were irrigated using a semi-automated system from outside the chamber. Plants were monitored for the growth measurements in the initial and final days of the study. Similarly biochemical attributes were monitored on the 1^{st}, 5^{th}, 10^{th} and 15^{th} days of the study. On the fifth and tenth day of experimentation, the sealing of the chambers was removed to collect leaf samples for biochemical analysis. Accordingly, the changes in morphological and biochemical attributes of the plants associated with experimental conditions and soil characteristics were estimated and are dealt with in Chapter II. The experiments carried out with *M. maximus* (**Figure 1.12 a,b**), *S. arundinaceum* (**Figure 1.12 c,d**), *C. flexuosus* (**Figure 1.12 e,f**), *C. zizanioides* (**Figure 1.12 g,h**), *A. donax* (**Figure 1.12 i,j**), *P. pedicellatum* (**Figure 1.12 k,l**) are represented below.

1.3.6 Statistical analysis

To validate the significance of the outcomes obtained, all statistical analyses of the climatological parameters were performed using *SPSS version 26*. The statistical tools carried out in the present chapter were the T-test, One-way ANOVA, ANOVA post-hoc test, and Pearson correlation. The 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. Furthermore, Pearson correlation analyses were conducted to estimate the relationship between microclimatic variables such as CO_2 concentration, temperature, and humidity during the day and nighttime.

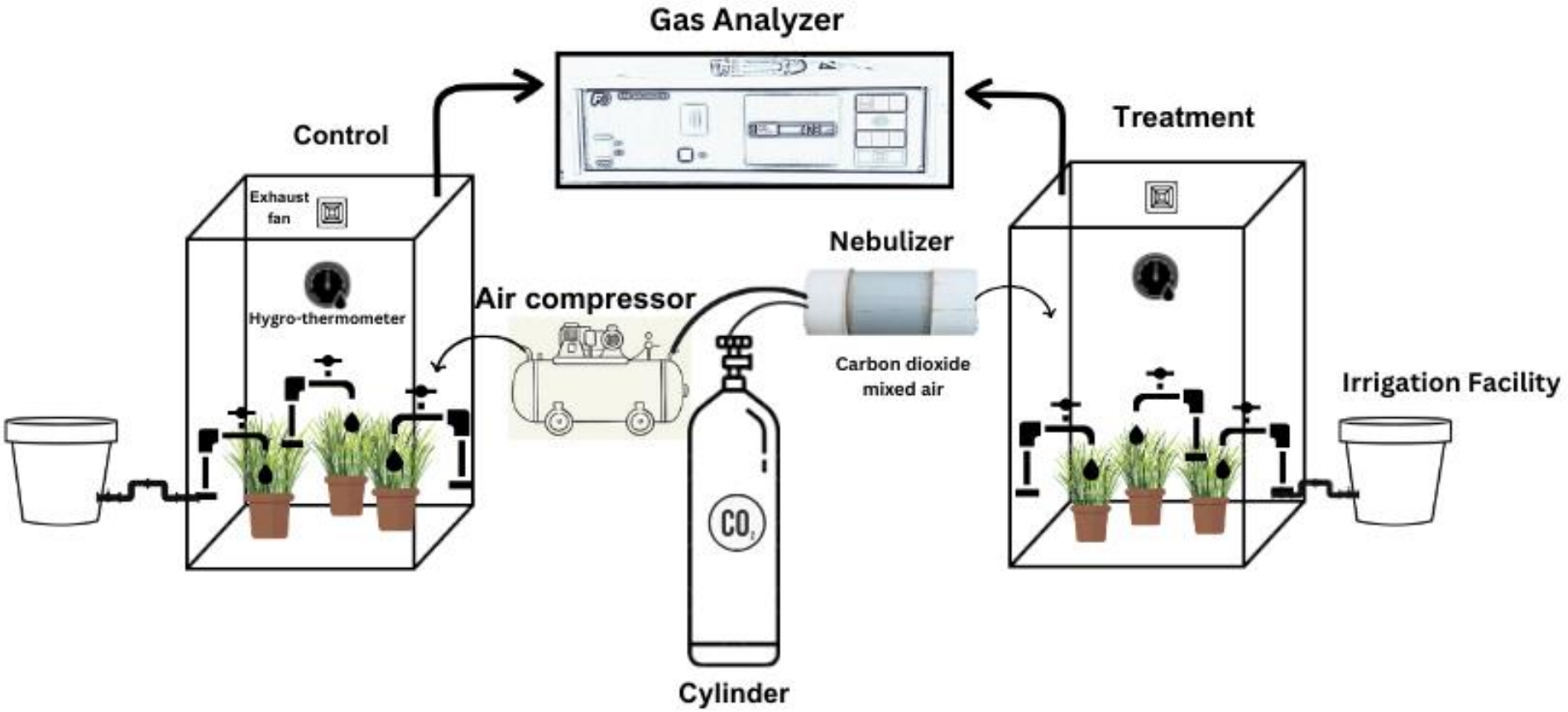


Figure 1.11: Schematic representation of the experiment

Figure 1.12 a: *M. maximus* (Control)

Figure 1.12 b: *M. maximus* (Treatment)

Figure 1.12 c: *S. arundinaceum* (Control)

Figure 1.12 d: *S. arundinaceum* (Treatment)

Figure 1.12 e: *C. flexuosus* (Control)

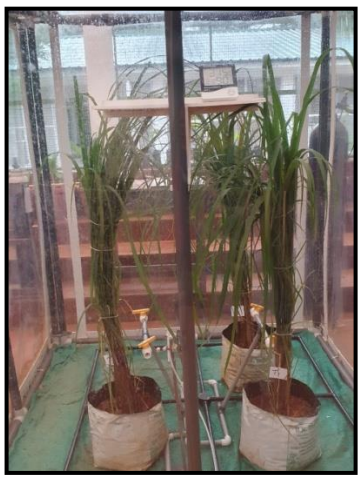


Figure 1.12 f: *C. flexuosus* (Treatment)

Figure 1.12 e: *C. flexuosus* (Control)

Figure 1.12 f: *C. flexuosus* (Treatment)

Figure 1.12 g: *C. zizanioides* (Control)

Figure 1.12 h: *C. zizanioides* (Treatment)

Figure 1.12 i: *A .donax* (Control)

Figure 1.12 j: *A. donax* (Treatment)

Figure 1.12 k: *P. pedicellatum* (Control)

Figure 1.12 l: *P. pedicellatum* (Treatment)

1.4. RESULTS

The outcomes of the experiments carried out are depicted in two sessions.

Session I, deals with the results of the standardization studies conducted in empty chambers (without plants). These results include variations in temperature, humidity, and CO_2 as a result of the supply of air (CC) and air-CO_2 mixture (TC).

Session II focuses on the changes in temperature, humidity, and CO_2 that occurred as a result of the supply of air (CC) and air-CO_2 mixture (TC) during the experimentation with grass species. These changes are related to the growth and metabolism of six different species of grasses.

In both experiments, i.e., the standardization studies and the experiment with plant species, the variations in microclimatic parameters such as day CO_2 flux, and the day variation in temperature, and humidity were calculated by subtracting the measurements obtained during the evening with those from the morning (after air/air+CO_2 supply in CC and TC, respectively). Night CO_2 flux and night variations of temperature and humidity were obtained by subtracting the measurements obtained in the next day's morning hours (before air/air+CO_2 supply in CC and TC, respectively) from those of the previous day's evening. CO_2 flux values with a negative sign indicate a reduction in the concentration of CO_2 in the experimental chambers and a positive value indicates attribution. The percentage of day and night fluxes of CO_2 and the percentage variation in temperature and humidity were calculated from the respective individual values. Statistical validation of the results was done with one-way ANOVA univariate analysis and Pearson correlation analysis.

1.4.1 Carbon dioxide flux associated with the experimental chambers

Session I - Outcomes of standardization studies

The results of the standardization experiments regarding CO_2 concentration in CC and TC are depicted in **Table 1.2**. In the standardization experiment, the concentration of CO_2 in CC and TC after the supply of ambient air/CO_2+ ambient air ranges from 602–694 ppm and 996–1045 ppm, respectively. Whereas the average concentration of CO_2 retained in the evening hours is 634.29±11.56ppm and

941.36±9.52ppm, respectively, in CC and TC. Day flux calculated from this morning (after ambient air/CO_2+ ambient air supply) and evening concentrations are -29.14±16.41ppm in CC and -77.07±16.83ppm in TC. The average values of CO_2 retained in the morning are 671.21±13.64ppm and 964.57±14.60ppm in CC and TC, respectively. Accordingly, the night flux values, which are estimated by subtracting CO_2 retained in the next morning from that of the evening hours, are 36.93±12.50ppm for CC and 23.21±16.16ppm for TC, respectively. The outcomes specify the day and night fluxes in TC as -77.07±16.83 ppm and 23.21±16.16 ppm, respectively, and the flux values are significant ($p<0.001$) as per the T-test done for CC and TC. These differences in CC and TC are expected to be due to specific physico-chemical properties of CO_2 gas, materials used for the installation of the chamber, and other specific conditions brought about by the CO_2 enrichment inside the chamber as explained in detail in the discussion section.

Session II - Outcomes associated with grass species under study

As mentioned in previous sections, six species of grasses are analyzed for efficiency at elevated CO_2 and metabolic responses at the anticipated microclimatic conditions. The outcomes of the study regarding CO_2 concentration in CC and TC associated with the species *Megathyrsus maximus* (**Table 1.3**), *Saccharum arundinaceum* (**Table 1.4**), *Cymbopogon flexuosus* (**Table 1.5**), *Chryzopogon zizanioides* (**Table 1.6**), *Arundo donax* (**Table 1.7**) and *Pennicetum pedicellatum* (**Table 1.8**) are represented below.

In the experiments with *M. maximus*, the concentration of CO_2 in CC and TC after the supply of ambient air/CO_2+ambient air ranges from 410ppm to 530ppm and 994ppm to 1070ppm respectively. The average CO_2 retained in the evening hours is 464.07±57.00ppm and 627.79±81.56ppm respectively in CC and TC. Consequently, the growth and metabolism of *M. maximus* under experimentation show an average day flux of -10.64±53.37ppm in CC and -398.14±85.05ppm in TC. Similarly, the average amount of CO_2 retained in the morning in CC, 574.36±127.47ppm and TC 677.50±143.24ppm gives the night flux attributed by *M. maximus* as 110.29±112.25ppm and 49.71±94.33ppm in CC and TC respectively. Pearson correlation studies signify a strong negative correlation between night temperature

variation and night flux of CO_2 in TC at 0.01 levels (r-value = -0.883**, p-value = 0.000).

The ranges of CO_2 in CC and TC after the supply of ambient air/CO_2+ambient air in CC and TC relating to the species *S. arundinaceum* are 439-517ppm and 1040-1100ppm respectively. CO_2 retention (average) in the experiment with this species at evening hours is 517.07±29.03ppm and 643.50±63.39ppm respectively in CC and TC. Accordingly, the day flux is 28.00±27.81ppm in CC and -415.71±63.92ppm in TC. The mean retention of CO_2 in CC and TC in the morning is 551.07±44.78ppm and 721.79±44.22ppm respectively. Consequently, the night flux is 34.00±31.00ppm in CC and 78.29±46.58ppm in TC. Here night flux shows a correlation (0.05 levels) with daytime humidity variation (r-value = -0.558*, p-value = 0.038). As well there is a negative correlation between day flux and night flux noticed (r-value = -0.681**, p-value = 0.007).

C. flexuosus gives a CO_2 retention average of 520.00±32.81ppm in CC and 698.86±114.79ppm in TC at evening hours. This retention happened from an average CO_2 concentration of 546.21±47.41ppm and 1016.79±35.24ppm in CC and TC respectively after the supply of ambient air/CO_2+ambient air in the morning. Thus the day CO_2 flux is -26.21±38.72ppm in CC and -317.93±93.55ppm in TC. Here the average morning retention values of CO_2 in CC and TC respectively are 569.21±50.72ppm and 760.50±121.79ppm. Therefore there is a flux of 49.21±53.01ppm CO_2 in CC and 61.64±145.83ppm in TC occurred at night. The night flux is strongly correlated (0.01 level) with temperature (r-value = -0.702**, p-value = 0.005) and humidity (r-value = -0.679**, p-value = 0.008) variations of night time.

The ranges of ambient air/ CO_2+ambient air supplied in CC and TC, where the species *C. zizanioides* was experimented is 473-621ppm and 964-110ppm respectively. The average retention of CO_2 during the evening is 568.29±25.56ppm in CC and 709.29±88.14ppm in TC, thus the respective day flux is 40.50±35.23ppm and -300.00±86.91ppm. Similarly, the average retention of CO_2 in morning hours from evening CO_2 concentrations is 553.79±43.09ppm in CC and 693.64±94.38ppm in TC.

Accordingly, the night flux in CC is -14.50±39.25ppm and in TC -15.64±68.09ppm. Here no significant correlations are noticed between the microclimatic variables.

The average retention of CO_2 associated with the species *A. donax* during the evening hours is 586.71±26.92ppm and 687.21±105.09ppm in CC and TC respectively. Here the morning averages of CO_2 after the supply of ambient air/ CO_2+ambient air in CC and TC respectively are 595.29±21.58ppm and 1022.14±47.43ppm. Thereby the day flux of CO_2 in CC is -8.57±20.19ppm and in TC it is -334.93±123.16ppm. The average retention of CO_2 in the morning from the evening concentrations of CO_2 is 590.36±21.65ppm and 682.36±90.40ppm in CC and TC respectively. Thus the night flux in CC is 3.64±29.55ppm and in TC -4.86±74.73ppm. No significant correlation is noticed between CO_2 fluxes and other microclimatic variables under study.

In the experiments with *P. pedicellatum*, the concentration of CO_2 in CC and TC after the supply of ambient air/CO_2+ ambient air ranges from 564ppm to 710ppm and 936ppm to 1074ppm respectively. The average retention of CO_2 in the evening is 628.21±25.99ppm in CC and 695.14±40.25ppm in TC. Accordingly the respective day fluxes are -31.50±54.86ppm and -322.57±61.54ppm. In the morning an average concentration of 622.57±60.44ppm and 716.07±66.55ppm CO_2 was retained in CC and TC respectively. Thus the night flux in CC is -5.64±62.64ppm and 20.93±70.13ppm in TC. A strong negative correlation is noticed between night temperature variation and night CO_2 flux in TC at 0.01 levels (r-value = -0.852**, p-value = 0.000).

In comparison to the standardization study, the results validate that grass plants have a higher day flux. Furthermore, T-test results show that, in all plants, day flux differs significantly between treatment and control groups ($p<0.05$). The increased day flux of CO_2 in TCs associated with grass species is also indicated ($P<0.001$) by the one-way ANOVA univariate analysis (**Table 1.9**). The results of the one-way ANOVA univariate analysis, where night flux as the dependent variable showed no significance between control and treatment (**Table 1.10**). Multiple comparisons of the mean difference of the day flux of all plants with the standardization study show significant day flux for all species under study (**Table 1.11**). Thus as mentioned, the noteworthy

result of the present study is the significant ($p<0.001$) day flux associated with selected grass species in TC, where *S. arundinaceum* (-415.71±63.92ppm) shows higher flux followed by *M. maximus* (-398.14±85.05ppm), *A. donax* (-334.93±123.16ppm, *P. pedicellatum* (-322.57±61.54ppm), *C. flexuosus* (-317.93±93.55ppm) and *C. zizanioides* (-300.00±86.91ppm). The day flux for each grass species mentioned above represents the concentrations of CO_2 without deducting the dissipation rates at ideal conditions that are obtained in the standardization study. The actual day/night fluxes associated with the plant assimilation (net flux) are obtained by deducting the day/night fluxes of the standardization study. Accordingly, the day CO_2 flux associated with each grass species in TC are as follows, *M. maximus* (-321.07±78.16ppm), *S. arundinaceum* (-338.64 ± 65.01ppm), *C. flexuosus* (-240.86 ± 93.26ppm), *C. zizanioides* (-222.93 ± 89.46ppm), *A. donax* (-257.86±115.64ppm), and *P. pedicellatum* (-245.50±67.13ppm).

The day flux as mentioned, is the ppm of CO_2 reduced from the chamber in the anticipated time and is an index of the respective assimilation of CO_2 by plants. According to the outcomes of day flux in ppm and that of percentage change in day flux, *S. arundinaceum* (-39.24±5.96%) is found to have higher CO_2 intake potential followed by *M. maximus* (-38.78±7.95%), *A. donax* (-32.56±11.49%), *P. pedicellatum* (-31.57±5.23%), *C. flexuosus* (-31.44±9.57) and *C. zizanioides* (-29.70±8.36%). **Figure 1.13** illustrates the day CO_2 fluxes in experimental chambers regarding each species during the entire experiment. In addition, Pearson correlation analysis signifies strong correlation between night flux and temperature variations regarding *M. maximus* (r-value = -0.883**, p-value = 0.000), *C. flexuosus* (r-value = -0.702**, p-value = 0.005), and *P. pedicellatum* (r-value = -0.852**, p-value = 0.000).

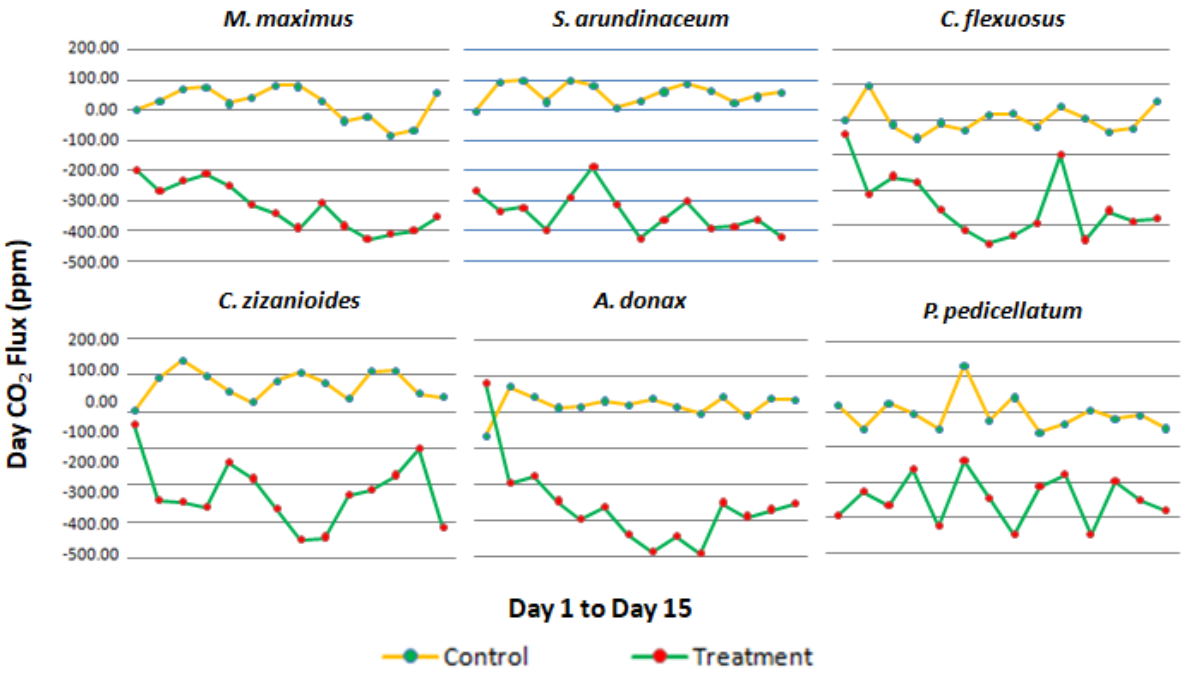


Figure 1.13: Day CO_2 flux in experimental chambers

Table 1.2: Estimate of the fluxes of CO_2 in experimental chambers (standardization studies)

Control chamber									CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change
1	583	602	617	656	15	2.49	39	6.32	615	1025	955	973	-70	-6.83	18	1.88
2	656	660	622	685	-38	-5.76	63	10.13	973	996	938	999	-58	-5.82	61	6.50
3	685	675	622	649	-53	-7.85	27	4.34	999	999	942	978	-57	-5.71	36	3.82
4	649	650	622	660	-28	-4.31	38	6.11	978	1020	947	949	-73	-7.16	2	0.21
5	660	653	627	649	-26	-3.98	22	3.51	949	1026	941	960	-85	-8.28	19	2.02
6	649	647	625	671	-22	-3.40	46	7.36	960	1005	929	940	-76	-7.56	11	1.18
7	671	658	641	670	-17	-2.58	29	4.52	940	1030	949	962	-81	-7.86	13	1.37
8	670	670	637	676	-33	-4.93	39	6.12	962	1001	945	973	-56	-5.59	28	2.96
9	676	671	639	687	-32	-4.77	48	7.51	973	1025	929	959	-96	-9.37	30	3.23
10	687	675	646	687	-29	-4.30	41	6.35	959	1045	936	955	-109	-10.43	19	2.03
11	687	680	644	672	-36	-5.29	28	4.35	955	1009	941	963	-68	-6.74	22	2.34
12	672	673	654	674	-19	-2.82	20	3.06	963	1030	936	953	-94	-9.13	17	1.82
13	674	680	638	690	-42	-6.18	52	8.15	953	1025	930	976	-95	-9.27	46	4.95
14	690	694	646	671	-48	-6.92	25	3.87	976	1022	961	964	-61	-5.97	3	0.31
Mean	664.93	663.43	634.29	671.21	-29.14	-4.33	36.93	5.84	939.64	1018.43	941.36	964.57	-77.07	-7.55	23.21	2.47
STDEV	27.24	22.04	11.56	13.64	16.41	2.46	12.50	2.01	94.57	14.22	9.52	14.60	16.83	1.56	16.16	1.73

Table 1.3: Estimate of the fluxes of CO_2 in experimental chambers (*Megathyrsus maximus*)

Control chamber									CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change
1	454	410	410	704	0	0	294	71.71	484	1070	800	879	-270	-25.23	79	9.88
2	704	494	485	692	-9	-1.82	207	42.68	879	1030	705	801	-325	-31.55	96	13.62
3	692	530	544	803	14	2.64	259	47.61	801	1015	725	910	-290	-28.57	185	25.52
4	803	500	545	740	45	9.00	195	35.78	910	1010	728	899	-282	-27.92	171	23.49
5	740	493	488	482	-5	-1.01	-6	-1.23	899	994	658	623	-336	-33.80	-35	-5.32
6	482	472	492	478	20	4.24	-14	-2.85	623	1016	627	545	-389	-38.29	-82	-13.08
7	478	447	510	471	63	14.09	-39	-7.65	545	1002	580	534	-422	-42.12	-46	-7.93
8	471	442	487	715	45	10.18	228	46.82	534	1000	552	734	-448	-44.80	182	32.97
9	715	501	500	558	-1	-0.20	58	11.60	734	994	587	577	-407	-40.95	-10	-1.70
10	558	490	425	465	-65	-13.27	40	9.41	577	1058	563	506	-495	-46.79	-57	-10.12
11	465	465	408	478	-57	-12.26	70	17.16	506	1061	564	586	-497	-46.84	22	3.90
12	478	478	375	553	-103	-21.55	178	47.47	586	1057	553	706	-504	-47.68	153	27.67
13	553	483	375	441	-108	-22.36	66	17.60	706	1046	551	580	-495	-47.32	29	5.26
14	441	441	453	461	12	2.72	8	1.77	580	1010	596	605	-414	-40.99	9	1.51
Mean	573.86	474.71	464.07	574.36	-10.64	-2.11	110.29	24.13	668.86	1025.93	627.79	677.50	-398.14	-38.78	49.71	7.55
STDEV	127.96	31.22	57.00	127.47	53.37	11.26	112.25	24.34	151.37	27.17	81.56	143.24	85.05	7.95	94.33	15.07

Table 1.4: Estimate of the fluxes of CO_2 in experimental chambers (*Saccharum arundinaceum*)

Control chamber									CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change
1	491	478	478	566	0	0	88	18.41	512	1075	737	725	-338	-31.44	-12	-1.63
2	566	507	563	593	56	11.05	30	5.33	725	1052	660	751	-392	-37.26	91	13.79
3	593	510	557	583	47	9.22	26	4.67	751	1054	676	795	-378	-35.86	119	17.60
4	583	517	520	552	3	0.58	32	6.15	795	1061	593	726	-468	-44.11	133	22.43
5	552	474	549	606	75	15.82	57	10.38	726	1045	672	745	-373	-35.69	73	10.86
6	606	505	566	632	61	12.08	66	11.66	745	1061	797	803	-264	-24.88	6	0.75
7	632	508	503	556	-5	-0.98	53	10.54	803	1047	656	735	-391	-37.34	79	12.04
8	556	497	496	482	-1	-0.20	-14	-2.82	735	1066	587	673	-479	-44.93	86	14.65
9	482	473	506	557	33	6.98	51	10.08	673	1054	598	674	-456	-43.26	76	12.71
10	557	439	501	484	62	14.12	-17	-3.39	674	1040	630	656	-410	-39.42	26	4.13
11	484	469	502	548	33	7.04	46	9.16	656	1100	642	709	-458	-41.64	67	10.44
12	548	489	499	538	10	2.04	39	7.82	709	1059	581	739	-478	-45.14	158	27.19
13	538	501	507	494	6	1.20	-13	-2.56	739	1049	592	688	-457	-43.57	96	16.22
14	494	480	492	524	12	2.50	32	6.50	688	1066	588	686	-478	-44.84	98	16.67
Mean	548.71	489.07	517.07	551.07	28.00	5.82	34.00	6.57	709.36	1059.21	643.50	721.79	-415.71	-39.24	78.29	12.70
STDEV	47.12	21.41	29.03	44.78	27.81	5.82	31.00	6.16	71.25	15.04	63.39	44.22	63.92	5.96	46.58	7.81

Table 1.5: Estimate of the fluxes of CO_2 in experimental chambers (*Cymbopogon flexuosus*)

Control chamber									CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change
1	485	583	583	505	0	0	-78	-13.38	588	1089	980	648	-109	-10.01	-332	-33.88
2	505	480	541	674	61	12.71	133	24.58	648	1024	758	998	-266	-25.98	240	31.66
3	674	635	566	658	-69	-10.87	92	16.25	998	1011	792	980	-219	-21.66	188	23.74
4	658	644	561	622	-83	-12.89	61	10.87	980	1034	787	945	-247	-23.89	158	20.08
5	622	555	518	577	-37	-6.67	59	11.39	945	1002	664	777	-338	-33.73	113	17.02
6	577	548	500	534	-48	-8.76	34	6.80	777	993	608	714	-385	-38.77	106	17.43
7	534	498	497	562	-1	-0.20	65	13.08	714	1028	598	656	-430	-41.83	58	9.70
8	562	510	497	553	-13	-2.55	56	11.27	656	957	574	692	-383	-40.02	118	20.56
9	553	534	485	563	-49	-9.18	78	16.08	692	980	594	717	-386	-39.39	123	20.71
10	563	523	533	511	10	1.91	-22	-4.13	717	1003	796	687	-207	-20.64	-109	-13.69
11	511	513	483	557	-30	-5.85	74	15.32	687	1016	606	705	-410	-40.35	99	16.34
12	557	544	494	580	-50	-9.19	86	17.41	705	993	640	767	-353	-35.55	127	19.84
13	580	555	492	547	-63	-11.35	55	11.18	767	1026	646	692	-380	-37.04	46	7.12
14	547	525	530	526	5	0.95	-4	-0.75	692	1079	741	669	-338	-31.33	-72	-9.72
Mean	566.29	546.21	520.00	569.21	-26.21	-4.42	49.21	9.71	754.71	1016.79	698.86	760.50	-317.93	-31.44	61.64	10.49
STDEV	54.45	47.41	32.81	50.72	38.72	6.99	53.01	9.84	128.22	35.24	114.79	121.79	93.55	9.57	145.83	17.78

Table 1.6: Estimate of the fluxes of CO_2 in experimental chambers (*Chrysopogon zizanioides*)

Control chamber									CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change
1	558	621	621	599	0	0	-22	-3.54	568	993	885	845	-108	-10.88	-40	-4.52
2	599	566	616	537	50	8.83	-79	-12.82	845	1042	741	656	-301	-28.89	-85	-11.47
3	537	496	580	550	84	16.94	-30	-5.17	656	965	661	623	-304	-31.50	-38	-5.75
4	550	511	577	601	66	12.92	24	4.16	623	964	628	657	-336	-34.85	29	4.62
5	601	560	587	562	27	4.82	-25	-4.26	657	1100	875	822	-225	-20.45	-53	-6.06
6	562	547	550	550	3	0.55	0	0.00	822	1007	746	743	-261	-25.92	-3	-0.40
7	550	489	553	500	64	13.09	-53	-9.58	743	982	634	639	-348	-35.44	5	0.79
8	500	481	552	522	71	14.76	-30	-5.43	639	1033	626	640	-407	-39.40	14	2.24
9	522	494	539	511	45	9.11	-28	-5.19	640	1066	626	639	-440	-41.28	13	2.08
10	511	544	546	525	2	0.37	-21	-3.85	639	1046	707	571	-339	-32.41	-136	-19.24
11	525	473	544	511	71	15.01	-33	-6.07	571	970	687	732	-283	-29.18	45	6.55
12	511	476	566	640	90	18.91	74	13.07	732	974	704	852	-270	-27.72	148	21.02
13	640	561	566	611	5	0.89	45	7.95	852	978	780	721	-198	-20.25	-59	-7.56
14	611	570	559	534	-11	-1.93	-25	-4.47	721	1010	630	571	-380	-37.62	-59	-9.37
Mean	555.50	527.79	568.29	553.79	40.50	8.16	-14.50	-2.52	693.43	1009.29	709.29	693.64	-300.00	-29.70	-15.64	-1.93
STDEV	42.72	45.16	25.56	43.09	35.23	7.24	39.25	6.82	94.68	42.26	88.14	94.38	86.91	8.36	68.09	9.61

Table 1.7: Estimate of the fluxes of CO_2 in experimental chambers (*Arundo donax*)

Control chamber									CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change
1	643	637	588	627	-49	-7.69	39	6.63	657	985	994	810	9	0.91	-184	-18.51
2	627	626	659	609	33	5.27	-50	-7.59	810	1017	764	733	-253	-24.88	-31	-4.06
3	609	620	610	598	-10	-1.61	-12	-1.97	733	972	739	693	-233	-23.97	-46	-6.22
4	598	609	594	619	-15	-2.46	25	4.21	693	973	655	688	-318	-32.68	33	5.04
5	619	582	572	588	-10	-1.72	16	2.80	688	980	600	596	-380	-38.78	-4	-0.67
6	588	593	603	588	10	1.69	-15	-2.49	596	950	612	602	-338	-35.58	-10	-1.63
7	588	576	580	600	4	0.69	20	3.45	602	1026	607	629	-419	-40.84	22	3.62
8	600	602	606	558	4	0.66	-48	-7.92	629	1100	659	587	-441	-40.09	-72	-10.93
9	558	574	558	590	-16	-2.79	32	5.73	587	1024	585	609	-439	-42.87	24	4.10
10	590	593	562	560	-31	-5.23	-2	-0.36	609	1100	602	527	-498	-45.27	-75	-12.46
11	560	560	566	604	6	1.07	38	6.71	527	1017	699	775	-318	-31.27	76	10.87
12	604	590	561	583	-29	-4.92	22	3.92	775	1063	681	781	-382	-35.94	100	14.68
13	583	580	576	585	-4	-0.69	9	1.56	781	1065	701	784	-364	-34.18	83	11.84
14	585	592	579	556	-13	-2.20	-23	-3.97	784	1038	723	739	-315	-30.35	16	2.21
Mean	596.57	595.29	586.71	590.36	-8.57	-1.42	3.64	0.77	676.50	1022.14	687.21	682.36	-334.93	-32.56	-4.86	-0.15
STDEV	23.44	21.58	26.92	21.65	20.19	3.28	29.55	4.92	89.09	47.43	105.09	90.40	123.16	11.49	74.73	9.65

Table 1.8: Estimate of the fluxes of CO_2 in experimental chambers (*Pennisetum pedicellatum*)

Control chamber									CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day flux (ppm)	Day flux percentage change	Night Flux (ppm)	Night flux percentage change
1	601	619	657	724	38	6.14	67	10.20	684	1040	676	720	-364	-35.00	44	6.51
2	724	710	626	689	-84	-11.83	63	10.06	720	1001	721	758	-280	-27.97	37	5.13
3	689	680	655	618	-25	-3.68	-37	-5.65	758	1004	684	656	-320	-31.87	-28	-4.09
4	618	680	649	694	-31	-4.56	45	6.93	656	936	703	772	-233	-24.89	69	9.82
5	694	698	626	537	-72	-10.32	-89	-14.22	772	1045	638	590	-407	-38.95	-48	-7.52
6	537	564	675	588	111	19.68	-87	-12.89	590	977	765	714	-212	-21.70	-51	-6.67
7	588	670	633	552	-37	-5.52	-81	-12.80	714	1074	749	648	-325	-30.26	-101	-13.48
8	552	582	593	609	11	1.89	16	2.70	648	1026	624	721	-402	-39.18	97	15.54
9	609	693	606	528	-87	-12.55	-78	-12.87	721	992	685	621	-307	-30.95	-64	-9.34
10	528	680	621	589	-59	-8.68	-32	-5.15	621	982	699	705	-283	-28.82	6	0.86
11	589	623	594	657	-29	-4.65	63	10.61	705	1069	655	757	-414	-38.73	102	15.57
12	657	687	650	620	-37	-5.39	-30	-4.62	757	1024	736	759	-288	-28.13	23	3.13
13	620	660	611	672	-49	-7.42	61	9.98	759	1053	711	825	-342	-32.48	114	16.03
14	672	690	599	639	-91	-13.19	40	6.68	825	1025	686	779	-339	-33.07	93	13.56
Mean	619.86	659.71	628.21	622.57	-31.50	-4.29	-5.64	-0.79	709.29	1017.71	695.14	716.07	-322.57	-31.57	20.93	3.22
STDEV	60.49	44.99	25.99	60.44	54.86	8.75	62.64	9.95	64.45	38.42	40.25	66.55	61.54	5.23	70.13	10.16

Table 1.9: Results of one-way ANOVA Univariate analysis (Dependent variable - Day flux in ppm)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5541112.342a	13	426239.411	103.031	0.000
Intercept	4742350.573	1	4742350.573	1146.323	0.000
Species	445030.860	6	74171.810	17.929	0.000
Groups	4444418.230	1	4444418.230	1074.307	0.000
Species * Group	657346.400	6	109557.733	26.482	0.000
Error	736387.736	178	4137.010		
Total	11235995.000	192			
Corrected Total	6277500.078	191			

Table 1.10: Results of one-way ANOVA Univariate analysis (Dependent variable - Night flux in ppm)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	250852.204a	13	19296.323	3.806	0.000
Intercept	180568.141	1	180568.141	35.618	0.000
sp1	203514.762	6	33919.127	6.691	0.000
gp1	0.222	1	0.222	0.000	0.995
sp1 * gp1	47256.530	6	7876.088	1.554	0.163
Error	917592.791	181	5069.573		
Total	1351893.000	195			
Corrected Total	1168444.995	194			

Table 1.11: Multiple comparisons of mean difference of day flux of all plants with standardization study (Treatment)

(I)	(J) Species	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Standardization study	*Megathyrsus maximus*	321.07143	30.92567	0.000*	259.6414	382.5015
	Saccharum arundinaceum	338.64286	30.92567	0.000*	277.2128	400.0729
	Cymbopogon flexuosus	240.85714	30.92567	0.000*	179.4271	302.2872
	Chrysopogon zizanioides	222.92857	30.92567	0.000*	161.4985	284.3586
	Arundo donax	257.85714	30.92567	0.000*	196.4271	319.2872
	Pennisetum pedicellatum	245.50000	30.92567	0.000*	184.0700	306.9300

1.4.2 Temperature variations in the experimental chambers

Session I - Outcomes of standardization studies

The results of the temperature variations in chambers associated with standardization studies are represented in **Table 1.12**. The temperature in CC after the supply of air ranges from 37.70°C to 45.00°C, whereas in TC it ranges from 37.90°C to 44.20°C. Evening temperature ranges are 32.30°C to 37.30°C in CC and 32.50°C to 37.10°C in TC. Thus the average variation of day temperature in CC and TC respectively is -5.86±2.44°C and -6.04±2.15°C. Similarly, the mean variations in night temperature, obtained by subtracting evening temperature from morning temperature in CC is 4.78±2.53°C and in TC it is 5.39±2.30°C. Pearson correlation analysis signifies strong negative correlations between humidity and temperature variations of the day (r-value = -0.723**, p-value= 0.003) and that of the night (r-value = -0.879**, p-value =0.000).

Session II - Outcomes associated with grass species under study

The temperature ranges (14-day average) associated with the species *M. maximus* in morning hours after the supply of ambient air in CC is 34.90°C to 50.40°C, while in TC after the supply of CO_2+ambient air mixture is 30.20°C to 46.50°C (**Table 1.13**). Moreover, the average evening ranges are 30.20°C to 34.20°C and 30.10°C to 33.30°C in CC and TC respectively. Hence the average day variation in temperature is -7.87±3.84°C in CC and -6.21±4.81°C in TC. Similarly, the respective variations in the night temperature in CC and TC are 6.09±3.92°C and 5.31±3.92°C. Pearson correlation studies signify a strong negative correlation between night temperature variation and night CO_2 flux in TC at 0.01 levels (r-value = -0.883**, p-value = 0.000).

The species, S. *arundinaceum* in the CC maintained the temperature ranges from 31.70°C to 45.10°C (in the morning after ambient air supply), and in TC from 30.90°C to 43.70°C in the morning after the supply of CO_2+ambient air (**Table 1.14**). Evening ranges of temperature associated with this species are 29.30°C to 32.30°C in CC and 29.70°C to 32.40°C in TC. Accordingly, the average temperature variation in the time is -6.99±2.88°C and -5.06±2.63°C in CC and TC respectively. Similarly, night temperature variations in CC is 5.34±2.12°C and in TC 1.85±1.00°C. Here Pearson correlation analysis signifies a negative correlation between variations of day temperature and humidity in TC at 0.01 levels (r-value = -0.682**, p value = 0.007).

Morning ranges of temperature in CC and TC respectively after the supply of ambient air/CO_2+ambient air associated with *C. flexuous* is 29.50°C - 43.70°C and 29.90°C - 43.00°C. Whereas the evening temperature ranges are 26.80°C – 35.60°C in CC and 26.90°C to 35.70°C in TC (**Table 1.15**). Hence the day temperature variation gives an average value of -2.75±2.95°C and -2.68±2.88°C in CC and TC respectively. Night temperature variations during the experimental period are 2.24±3.66°C in CC and 2.74±3.78°C in TC. Regarding *C. flexuous* there is a negative correlation between temperature variations of the daytime and humidity variations of the nighttime at TC and it is significant at 0.05 levels (r-value = -0.601*, p-value = 0.023). Similarly, a negative correlation between the night temperature variations and night CO_2 flux at a 0.01 significance level (r-value = -0.702**, p-value = 0.005) is noticed.

The CC associated with *C. zizanioides* shows a morning temperature range of 32.30°C to 47.70°C. Similarly in TC a temperature range of 31.10°C to 40.40°C at morning after the supply of CO_2+ambient air is noticed (**Table 1.16**). Evening ranges of temperature in CC is 28.60°C -32.50°C and in TC 26.90°C – 35.70°C. Thus the day variations of temperature calculated from this data provide a mean value of -8.07±3.81 in CC and -5.66±2.89 in TC. As well the night temperature variations in both chambers during the study period associated with *C. zizanioides* are 5.24±2.39°C (CC) and 3.86±3.37°C (TC). Significant correlations are not noticed in microclimatic variables associated with this species.

Experiment with *A. donax* shows a morning temperature range of 37.00°C to 43.60°C and 35.60°C to 43.90°C in CC and TC respectively (**Table 1.17**). Also a range of 28.10°C to 35.90°C in CC and 28.40°C to 35.50°C is noticed in TC during evening hours. Accordingly, the daytime temperature varies at an average rate of -6.51±2.69°C in CC and -6.21±3.04°C in TC. Similarly, the mean nighttime temperature variations during the study period in CC is 4.24±2.51°C and in TC 5.26±2.76°C. Here, Pearson correlation analysis shows that there is a negative correlation between temperature and humidity variations during the day at the 0.01 level (r-value = -0.713**, p-value=0.004) and at night at the 0.05 level (r-value = 0.623*, p-value = 0.017).

The temperature ranges associated with *P. pedicellatum* in the morning after the supply of ambient air/CO_2+ambient in CC and TC respectively are 32.50°C to 49.70°C and 32.70°C to 42.90°C and at evening hours 27.50°C to 33.60°C in CC and 27.60°C to 33.30°C in TC during the study period (**Table 1.18**). Thus the average daytime temperature variation in CC is -7.34±4.48°C and in TC -6.60±3.55°C. Night temperature variations during the study period are 4.84±3.70°C and 5.06±3.67°C in CC and TC respectively. The Pearson correlation analysis signifies a negative correlation between daytime temperature variations and humidity variations at 0.05 levels (r-value = -0.646*, p-value = 0.013) and a strong negative correlation between night temperature variation and night CO_2 flux in TC at 0.01 levels (r-value = -0.852**, p-value = 0.000).

Table 1.12: Standardization studies on experimental chambers (Temperature in °C)

Control chamber									CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation(% change)	Night variation in the temperature	Night variation(% change)	Temperature morning hours (before CO_2supplementation)	Temperature morning hours (after CO_2supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation(% change)	Night variation in temperature	Night variation(% change)
1	38.80	45.00	37.30	39.10	-7.70	-17.11	1.80	4.83	40.10	44.20	37.10	40.10	-7.10	-16.06	3.00	8.09
2	39.10	40.40	32.90	36.70	-7.50	-18.56	3.80	11.55	40.10	40.60	33.10	37.00	-7.50	-18.47	3.90	11.78
3	36.70	41.14	34.30	39.60	-6.84	-16.63	5.30	15.45	37.00	41.00	34.30	39.70	-6.70	-16.34	5.40	15.74
4	39.60	41.30	36.50	37.50	-4.80	-11.62	1.00	2.74	39.70	40.30	35.90	37.90	-4.40	-10.92	2.00	5.57
5	37.50	37.70	34.03	43.80	-3.67	-9.73	9.77	28.71	37.90	37.90	34.10	44.40	-3.80	-10.03	10.30	30.21
6	43.80	43.60	36.80	42.80	-6.80	-15.60	6.00	16.30	44.40	44.00	36.30	43.50	-7.70	-17.50	7.20	19.83
7	42.80	44.00	33.70	39.60	-10.30	-23.41	5.90	17.51	43.50	43.90	33.70	39.90	-10.20	-23.23	6.20	18.40
8	39.60	40.00	36.30	44.00	-3.70	-9.25	7.70	21.21	39.90	40.00	35.90	44.00	-4.10	-10.25	8.10	22.56
9	44.00	42.10	37.10	40.20	-5.00	-11.88	3.10	8.36	44.00	43.20	36.80	40.50	-6.40	-14.81	3.70	10.05
10	40.20	39.80	35.90	39.30	-3.90	-9.80	3.40	9.47	40.50	40.10	36.10	40.10	-4.00	-9.98	4.00	11.08
11	39.30	41.40	32.30	39.90	-9.10	-21.98	7.60	23.53	40.10	41.00	32.50	40.10	-8.50	-20.73	7.60	23.38
12	39.90	40.70	33.20	38.50	-7.50	-18.43	5.30	15.96	40.10	40.50	33.30	39.30	-7.20	-17.78	6.00	18.02
13	38.50	39.30	36.40	40.90	-2.90	-7.38	4.50	12.36	39.30	39.50	35.90	40.50	-3.60	-9.11	4.60	12.81
14	40.90	39.60	37.30	39.00	-2.30	-5.81	1.70	4.56	40.50	40.00	36.70	40.20	-3.30	-8.25	3.50	9.54
Mean	40.05	41.15	35.29	40.06	-5.86	-14.08	4.78	13.75	40.51	41.16	35.12	40.51	-6.04	-14.53	5.39	15.51
STDEV	2.18	1.99	1.79	2.17	2.44	5.49	2.53	7.56	2.12	1.92	1.55	2.12	2.15	4.77	2.30	6.91

Table 1.13: Temperature studies (°C) on experimental chambers containing *Megathyrsus maximus*

Control chamber										CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in the temperature	Night variation(% change)		Temperature morning hours (before CO_2supplementation)	Temperature morning hours (after CO_2supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation % change)	Night variation in temperature	Night variation (% change)
1	49.10	50.40	32.20	40.50	-18.20	-36.11	8.30	25.78		42.90	46.50	31.70	38.90	-14.80	-31.83	7.20	22.71
2	40.50	37.80	32.50	37.90	-5.30	-14.02	5.40	16.62		38.90	38.90	32.10	35.20	-6.80	-17.48	3.10	9.66
3	37.90	38.50	30.20	30.80	-8.30	-21.56	0.60	1.99		35.20	37.10	30.10	30.10	-7.00	-18.87	0.00	0.00
4	30.80	34.90	30.20	31.40	-4.70	-13.47	1.20	3.97		30.10	30.20	30.00	28.40	-0.20	-0.66	-1.60	-5.33
5	31.40	37.40	32.00	43.90	-5.40	-14.44	11.90	37.19		28.40	30.40	31.20	39.50	0.80	2.63	8.30	26.60
6	43.90	41.50	32.80	43.10	-8.70	-20.96	10.30	31.40		39.50	43.80	32.20	40.80	-11.60	-26.48	8.60	26.71
7	43.10	43.90	32.90	42.40	-11.00	-25.06	9.50	28.88		40.80	42.20	32.30	41.40	-9.90	-23.46	9.10	28.17
8	42.40	43.90	32.70	33.20	-11.20	-25.51	0.50	1.53		41.40	41.10	32.40	30.90	-8.70	-21.17	-1.50	-4.63
9	33.20	35.60	31.40	34.10	-4.20	-11.80	2.70	8.60		30.90	32.30	31.10	37.70	-1.20	-3.72	6.60	21.22
10	34.10	39.40	32.00	39.50	-7.40	-18.78	7.50	23.44		37.70	33.10	31.30	41.80	-1.80	-5.44	10.50	33.55
11	39.50	40.00	33.00	39.40	-7.00	-17.50	6.40	19.39		41.80	39.00	32.40	37.90	-6.60	-16.92	5.50	16.98
12	39.40	40.07	34.00	36.50	-6.07	-15.15	2.50	7.35		37.90	38.20	33.20	38.50	-5.00	-13.09	5.30	15.96
13	36.50	37.50	34.20	43.50	-3.30	-8.80	9.30	27.19		38.50	35.70	33.30	41.20	-2.40	-6.72	7.90	23.72
14	43.50	40.07	30.70	39.90	-9.37	-23.38	9.20	29.97		41.20	42.30	30.50	35.90	-11.80	-27.90	5.40	17.70
Mean	38.95	40.07	32.20	38.29	-7.87	-19.04	6.09	18.81		37.51	37.91	31.70	37.01	-6.21	-15.08	5.31	16.64
STDEV	5.32	3.99	1.24	4.47	3.84	7.08	3.92	12.10		4.65	5.07	1.05	4.40	4.81	10.82	3.92	12.37

Table 1.14: Temperature studies (°C) on experimental chambers containing *Saccharum arundinaceum*

Control chamber										CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in the temperature	Night variation (% change)		Temperature morning hours (before CO_2supplementation)	Temperature morning hours (after CO_2supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation % change)	Night variation in temperature	Night variation (% change)
1	44.80	45.10	32.30	35.50	-12.80	-28.38	3.20	9.91		41.90	43.70	32.40	33.00	-11.30	-25.86	0.60	1.85
2	35.50	36.10	31.10	34.10	-5.00	-13.85	3.00	9.65		33.00	34.60	31.30	33.20	-3.30	-9.54	1.90	6.07
3	34.10	33.90	30.90	38.20	-3.00	-8.85	7.30	23.62		33.20	33.90	31.10	33.90	-2.80	-8.26	2.80	9.00
4	38.20	38.70	30.30	36.60	-8.40	-21.71	6.30	20.79		33.90	37.10	30.70	32.40	-6.40	-17.25	1.70	5.54
5	36.60	38.80	31.20	35.40	-7.60	-19.59	4.20	13.46		32.40	35.10	31.50	33.70	-3.60	-10.26	2.20	6.98
6	35.40	35.20	29.30	31.10	-5.90	-16.76	1.80	6.14		33.70	35.00	29.70	32.30	-5.30	-15.14	2.60	8.75
7	31.10	31.70	30.70	37.50	-1.00	-3.15	6.80	22.15		32.30	30.90	30.90	33.50	0.00	0.00	2.60	8.41
8	37.50	38.50	31.70	39.90	-6.80	-17.66	8.20	25.87		33.50	36.10	32.00	35.20	-4.10	-11.36	3.20	10.00
9	39.90	41.00	31.90	34.70	-9.10	-22.20	2.80	8.78		35.20	38.90	32.00	33.10	-6.90	-17.74	1.10	3.44
10	34.70	40.10	30.50	38.90	-9.60	-23.94	8.40	27.54		33.10	37.90	30.90	34.30	-7.00	-18.47	3.40	11.00
11	38.90	39.50	30.90	37.10	-8.60	-21.77	6.20	20.06		34.30	37.90	31.10	33.00	-6.80	-17.94	1.90	6.11
12	37.10	38.10	31.30	37.10	-6.80	-17.85	5.80	18.53		33.00	36.40	31.70	32.20	-4.70	-12.91	0.50	1.58
13	37.10	38.10	32.00	38.50	-6.10	-16.01	6.50	20.31		32.20	35.70	32.10	32.70	-3.60	-10.08	0.60	1.87
14	38.50	39.30	32.10	36.30	-7.20	-18.32	4.20	13.08		32.70	37.30	32.20	33.00	-5.10	-13.67	0.80	2.48
Mean	37.10	38.15	31.16	36.49	-6.99	-17.86	5.34	17.14		33.89	36.46	31.40	33.25	-5.06	-13.46	1.85	5.94
STDEV	3.16	3.24	0.82	2.26	2.88	6.31	2.12	6.88		2.45	2.90	0.73	0.82	2.63	6.10	1.00	3.25

Table 1.15: Temperature studies (°C) on experimental chambers containing *Cymbopogon flexuosus*

Control chamber										CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in the temperature	Night variation (% change)		Temperature morning hours (before CO_2supplementation)	Temperature morning hours (after CO_2supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation % change)	Night variation in temperature	Night variation (% change)
1	31.50	29.30	31.70	37.50	2.40	8.19	5.80	18.30		32.00	29.90	31.60	38.80	1.70	5.69	7.20	22.78
2	37.50	43.70	35.60	36.50	-8.10	-18.54	0.90	2.53		38.80	43.00	35.70	37.20	-7.30	-16.98	1.50	4.20
3	36.50	38.20	33.00	35.80	-5.20	-13.61	2.80	8.48		37.20	37.50	33.10	36.70	-4.40	-11.73	3.60	10.88
4	35.80	36.50	32.30	31.50	-4.20	-11.51	-0.80	-2.48		36.70	36.50	32.40	31.70	-4.10	-11.23	-0.70	-2.16
5	31.50	34.30	32.30	32.00	-2.00	-5.83	-0.30	-0.93		31.70	32.90	32.20	32.20	-0.70	-2.13	0.00	0.00
6	32.00	34.00	32.00	32.90	-2.00	-5.88	0.90	2.81		32.20	32.70	31.90	33.00	-0.80	-2.45	1.10	3.45
7	32.90	36.10	32.90	37.60	-3.20	-8.86	4.70	14.29		33.00	34.10	32.70	39.30	-1.40	-4.11	6.60	20.18
8	37.60	38.90	33.50	33.30	-5.40	-13.88	-0.20	-0.60		39.30	41.00	33.30	33.70	-7.70	-18.78	0.40	1.20
9	33.30	36.10	31.80	33.00	-4.30	-11.91	1.20	3.77		33.70	35.00	31.80	33.00	-3.20	-9.14	1.20	3.77
10	33.00	29.50	26.80	36.30	-2.70	-9.15	9.50	35.45		33.00	31.00	26.90	36.70	-4.10	-13.23	9.80	36.43
11	36.30	32.20	32.50	31.10	0.30	0.93	-1.40	-4.31		36.70	34.00	32.10	31.10	-1.90	-5.59	-1.00	-3.12
12	31.10	30.20	31.30	29.60	1.10	3.64	-1.70	-5.43		31.10	30.70	31.00	29.80	0.30	0.98	-1.20	-3.87
13	29.60	31.20	31.30	32.30	0.10	0.32	1.00	3.19		29.80	30.20	31.00	32.00	0.80	2.65	1.00	3.23
14	32.30	33.00	27.70	36.70	-5.30	-16.06	9.00	32.49		32.00	32.50	27.80	36.60	-4.70	-14.46	8.80	31.65
Mean	33.64	34.51	31.76	34.01	-2.75	-7.30	2.24	7.68		34.09	34.36	31.68	34.41	-2.68	-7.18	2.74	9.19
STDEV	2.60	4.08	2.21	2.64	2.95	7.95	3.66	12.98		3.04	3.96	2.18	3.05	2.88	7.59	3.78	13.23

Table 1.16: Temperature studies (°C) on experimental chambers containing *Chrysopogon zizanioides*

Control chamber										CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in the temperature	Night variation (% change)		Temperature morning hours (before CO_2supplementation)	Temperature morning hours (after CO_2supplementation)	Temperature evening hours	Temperature in the next day morning	Day variation in temperature	Day variation % change)	Night variation in temperature	Night variation (% change)
1	40.60	33.00	31.10	38.00	-1.90	-5.76	6.90	22.19		39.00	35.90	31.60	36.00	-4.30	-11.98	4.40	13.92
2	38.00	41.10	31.00	38.50	-10.10	-24.57	7.50	24.19		36.00	37.90	35.70	36.90	-2.20	-5.80	1.20	3.36
3	38.50	42.70	31.50	35.90	-11.20	-26.23	4.40	13.97		36.90	40.10	33.10	35.30	-7.00	-17.46	2.20	6.65
4	35.90	41.30	31.10	33.80	-10.20	-24.70	2.70	8.68		35.30	38.70	32.40	33.20	-6.30	-16.28	0.80	2.47
5	33.80	42.30	31.70	34.90	-10.60	-25.06	3.20	10.09		33.20	40.40	32.20	34.30	-8.20	-20.30	2.10	6.52
6	34.90	35.00	31.00	37.60	-4.00	-11.43	6.60	21.29		34.30	34.10	31.90	36.40	-2.20	-6.45	4.50	14.11
7	37.60	47.70	32.10	37.00	-15.60	-32.70	4.90	15.26		36.40	39.50	32.70	35.60	-6.80	-17.22	2.90	8.87
8	37.00	39.70	32.10	37.50	-7.60	-19.14	5.40	16.82		35.60	37.50	33.30	36.60	-4.20	-11.20	3.30	9.91
9	37.50	39.70	32.90	38.90	-6.80	-17.13	6.00	18.24		36.60	37.90	31.80	38.00	-6.10	-16.09	6.20	19.50
10	38.90	39.10	31.90	38.30	-7.20	-18.41	6.40	20.06		38.00	38.40	26.90	37.00	-11.50	-29.95	10.10	37.55
11	38.30	41.80	32.50	37.30	-9.30	-22.25	4.80	14.77		37.00	39.30	32.10	36.80	-7.20	-18.32	4.70	14.64
12	37.30	41.30	30.10	31.50	-11.20	-27.12	1.40	4.65		36.80	39.70	31.00	31.60	-8.70	-21.91	0.60	1.94
13	31.50	32.30	28.60	31.10	-3.70	-11.46	2.50	8.74		31.60	32.30	31.00	31.00	-1.30	-4.02	0.00	0.00
14	31.10	32.30	28.70	39.30	-3.60	-11.15	10.60	36.93		31.00	31.10	27.80	38.90	-3.30	-10.61	11.10	39.93
Mean	36.49	39.24	31.16	36.40	-8.07	-19.79	5.24	16.85		35.55	37.34	31.68	35.54	-5.66	-14.83	3.86	12.81
STDEV	2.77	4.52	1.28	2.64	3.81	7.66	2.39	8.13		2.30	2.93	2.18	2.29	2.89	7.04	3.37	12.34

Table 1.17: Temperature studies (°C) on experimental chambers containing *Arundo donax*

Control chamber										CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in the temperature	Night variation (% change)		Temperature morning hours (before CO_2supplementation)	Temperature morning hours (after CO_2supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation % change)	Night variation in temperature	Night variation (% change)
1	37.00	37.00	34.50	35.80	-2.50	-6.76	1.30	3.77		36.70	36.60	34.20	36.30	-2.40	-6.56	2.10	6.14
2	35.80	40.90	34.40	40.30	-6.50	-15.89	5.90	17.15		36.30	39.80	34.10	42.00	-5.70	-14.32	7.90	23.17
3	40.30	41.20	28.10	35.40	-13.10	-31.80	7.30	25.98		42.00	42.50	28.40	35.70	-14.10	-33.18	7.30	25.70
4	35.40	37.30	32.20	34.10	-5.10	-13.67	1.90	5.90		35.70	37.00	32.20	34.30	-4.80	-12.97	2.10	6.52
5	34.10	37.50	33.00	37.90	-4.50	-12.00	4.90	14.85		34.30	35.60	32.90	38.70	-2.70	-7.58	5.80	17.63
6	37.90	39.50	34.10	39.90	-5.40	-13.67	5.80	17.01		38.70	39.40	34.00	40.50	-5.40	-13.71	6.50	19.12
7	39.90	43.10	35.90	34.70	-7.20	-16.71	-1.20	-3.34		40.50	42.20	35.50	34.90	-6.70	-15.88	-0.60	-1.69
8	34.70	38.60	31.10	36.00	-7.50	-19.43	4.90	15.76		34.90	37.50	31.40	36.80	-6.10	-16.27	5.40	17.20
9	36.00	37.50	34.30	37.80	-3.20	-8.53	3.50	10.20		36.80	37.30	33.90	38.50	-3.40	-9.12	4.60	13.57
10	37.80	38.20	32.90	40.30	-5.30	-13.87	7.40	22.49		38.50	37.90	32.70	41.70	-5.20	-13.72	9.00	27.52
11	40.30	43.60	33.50	37.30	-10.10	-23.17	3.80	11.34		41.70	43.90	33.50	39.00	-10.40	-23.69	5.50	16.42
12	37.30	40.00	33.30	36.10	-6.70	-16.75	2.80	8.41		39.00	39.90	32.90	36.70	-7.00	-17.54	3.80	11.55
13	36.10	40.30	33.00	36.70	-7.30	-18.11	3.70	11.21		36.70	40.00	32.90	38.00	-7.10	-17.75	5.10	15.50
14	36.70	39.90	33.20	40.50	-6.70	-16.79	7.30	21.99		38.00	39.10	33.20	42.30	-5.90	-15.09	9.10	27.41
Mean	37.09	39.61	33.11	37.34	-6.51	-16.23	4.24	13.05		37.84	39.19	32.99	38.24	-6.21	-15.53	5.26	16.13
STDEV	1.98	2.10	1.84	2.18	2.69	6.14	2.51	7.95		2.38	2.42	1.65	2.63	3.04	6.72	2.76	8.54

Table 1.18: Temperature studies (°C) on experimental chambers containing *Pennisetum pedicellatum*

Control chamber										CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in the temperature	Night variation (% change)		Temperature morning hours (before CO_2supplementation)	Temperature morning hours (after CO_2supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation % change)	Night variation in temperature	Night variation (% change)
1	39.90	49.70	30.40	29.50	-19.30	-38.83	-0.90	-2.96		40.00	42.90	30.40	29.80	-12.50	-29.14	-0.60	-1.97
2	29.50	35.30	30.10	33.90	-5.20	-14.73	3.80	12.62		29.80	31.60	30.50	34.00	-1.10	-3.48	3.50	11.48
3	33.90	34.30	29.90	35.30	-4.40	-12.83	5.40	18.06		34.00	34.60	29.70	35.20	-4.90	-14.16	5.50	18.52
4	35.30	35.70	29.90	30.60	-5.80	-16.25	0.70	2.34		35.20	35.00	29.80	30.80	-5.20	-14.86	1.00	3.36
5	30.60	35.70	31.00	41.00	-4.70	-13.17	10.00	32.26		30.80	35.30	30.50	40.90	-4.80	-13.60	10.40	34.10
6	41.00	42.80	28.50	38.00	-14.30	-33.41	9.50	33.33		40.90	42.90	28.60	38.20	-14.30	-33.33	9.60	33.57
7	38.00	37.00	28.50	38.60	-8.50	-22.97	10.10	35.44		38.20	36.30	28.50	38.80	-7.80	-21.49	10.30	36.14
8	38.60	40.20	33.60	34.50	-6.60	-16.42	0.90	2.68		38.80	40.20	33.30	34.30	-6.90	-17.16	1.00	3.00
9	34.50	33.10	30.10	39.30	-3.00	-9.06	9.20	30.56		34.30	34.00	30.10	39.30	-3.90	-11.47	9.20	30.56
10	39.30	38.10	31.10	37.00	-7.00	-18.37	5.90	18.97		39.30	37.60	31.00	37.60	-6.60	-17.55	6.60	21.29
11	37.00	38.30	32.90	34.60	-5.40	-14.10	1.70	5.17		37.60	38.10	32.50	34.90	-5.60	-14.70	2.40	7.38
12	34.60	36.80	27.50	32.30	-9.30	-25.27	4.80	17.45		34.90	37.40	27.60	32.40	-9.80	-26.20	4.80	17.39
13	32.30	32.50	29.20	32.00	-3.30	-10.15	2.80	9.59		32.40	32.70	29.30	32.50	-3.40	-10.40	3.20	10.92
14	32.00	35.90	30.00	33.80	-5.90	-16.43	3.80	12.67		32.50	35.70	30.10	34.10	-5.60	-15.69	4.00	13.29
Mean	35.46	37.53	30.19	35.03	-7.34	-18.71	4.84	16.30		35.62	36.74	30.14	35.20	-6.60	-17.37	5.06	17.07
STDEV	3.61	4.42	1.63	3.39	4.48	8.63	3.70	12.63		3.55	3.42	1.50	3.33	3.55	7.87	3.67	12.57

1.4.3 Humidity variations associated with the experimental chambers

Session I - Outcomes of standardization studies

Percentage humidity decreased in both chambers after the supply of ambient air/CO_2+ ambient air in the morning (**Table 1.19**). The day variation in the percentage of humidity is 9.29±6.24% in CC and 15.86±6.09% in TC, i.e. an average increase of 9.73±13.56% and 30.12±12.66% was observed in CC and TC respectively during 15 days of standardization study. Similarly, night variations of percentage humidity in CC and TC are, -6.29±5.88% and -14.75±5.49%. Contrary to the day humidity in chambers, night humidity shows a decreasing trend; a decrease of -10.29±9.18% in CC and -20.97±7.00% in TC. Pearson correlation signifies a strong negative correlation in TC between day temperature variations and day humidity variations (r-value = -0.723**, p-value = 0.003) and between night temperature variations and night humidity variations (r-value = -0.879**, p-value =0.000) at 0.01 levels. A significant correlation (negative) is also seen between humidity variations of day and night (r-value = -0.558*, p-value = 0.038). Nevertheless, CO_2 flux during the day and night shows only a weak correlation with temperature and humidity and is not significant.

Session II - Outcomes associated with grass species under study

The outcomes of humidity variations in chambers associated with plants under study are depicted in the tables listed below (*M. maximus*; **Table 1.20**, *S. arundinaceum*; **Table 1.21,** *C. flexuous*; **Table 1.22**, *C. zizanioides*; **Table 1.23**, *A. donax*; **Table 1.24**, *P. pedicellatum*; **Table 1.25.).** The experiment using *M. maximus* shows an average daytime increase of 6.46±14.33% humidity in CC and 4.08±15.27 in TC. At night time the changes were not noticed in TC regarding the humidity. This is because the Hygrothermometer measurements showed 99% in the evening and in the morning hours before ambient air/CO_2+ ambient air supply. The fact is that the instrument is calibrated to a maximum value of 99% and, a humidity hike over it will not be displayed in the instrument. In the TC of *M. maximus*, a moderate, but negative correlation is evident between the variations of the temperature and humidity during the daytime, and is not significant. In the CC and TC associated with the study of *S. arundinaceum*, day variations of humidity (%) are 3.29±8.89% and 2.64±9.89% respectively with a meager increase of 4.58±13.34% and 4.26±15.95%. Whereas at

night time humidity measurements are noticed constant (99%) throughout the study period. Here humidity variations of the day are negatively correlated with both temperature variations of the day (r-value = -0.682**, p-value = 0.007) and night CO_2 flux (r-value = -0.558*, p-value = 0.038).

The percentage change in the humidity variations in CC and TC associated with *C. flexuous* at daytime is 2.73±8.32% and 0.59±2.52%, respectively. Similarly, at night, a meager decrease of 1.03±8.10% and an increase of 0.16±1.69% are observed in CC and TC, respectively. Humidity variations at night are strongly correlated with daytime humidity (r-value = -0.922**, p-value = 0.000) variations and also with nighttime CO_2 flux (r-value = -0.679**, p-value= 0.008) at the 0.01 significance level. *C. zizanioides* at elevated CO_2 attributed a humidity increase of 7.17± 6.78% in CC and 6.07±5.54% in TC. Similar to *M. maximus* and *S. arundinaceum*, both CC and TC associated with *C. zizanioides* throughout the experiment had consistent nighttime humidity readings (99%). Significant correlations are not found in the microclimatic variables related to this species, as was discussed in the previous session regarding temperature.

A. donax shows an average humidity rise of 7.15±8.95% and 9.43±6.66% in CC and TC respectively in the daytime. Similarly average increases of 2.77±6.83% in CC while a decrease of 5.04±5.58% in TC at nighttime was noticed. A negative correlation in TC related to *A. donax* is shown by Pearson correlation analysis between temperature and humidity variations during the day at the 0.01 level (r-value = -0.713**, p-value =0.004) and at night at the 0.05 level (r-value = -0.623*, p-value =0.017). Regarding the species *P. pedicellatum* percentage increase in the day variations of humidity in CC and TC respectively are 4.50±14.97% and 5.08±11.76%. Here during the entire study, the average nighttime variation of humidity in CC is 0.63±2.35. Here also throughout the experiment had consistent nighttime humidity readings (99%) like that of the situation mentioned above. The Pearson correlation analysis signifies a negative correlation between daytime temperature variations and humidity variations at 0.05 levels (r-value = -0.646*, p-value = 0.013) in TC associated with this species.

Table 1.19: Standardization studies on experimental chambers (Humidity in %)

Control chamber										CO_2 treated chamber						
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity in morning hours (after air	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (%change)	Night variation in the humidity	Night variation (% change)	Humidity in morning hours (before CO2supplementation)	Humidity in morning hours (after CO2supplementation)	Humidity in evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
1	56.00	40.00	51.00	53.00	11.00	27.50	2.00	3.92	53.00	54.00	59.00	54.00	5.00	9.26	-5.00	-8.47
2	53.00	50.00	62.00	59.00	12.00	24.00	-3.00	-4.84	54.00	52.00	73.00	61.00	21.00	40.38	-12.00	-16.44
3	59.00	48.00	60.00	54.00	12.00	25.00	-6.00	-10.00	61.00	53.00	70.00	56.00	17.00	32.08	-14.00	-20.00
4	54.00	50.00	54.00	53.00	4.00	8.00	-1.00	-1.85	56.00	55.00	67.00	58.00	12.00	21.82	-9.00	-13.43
5	53.00	52.00	59.00	45.00	7.00	13.46	-14.00	-23.73	58.00	58.00	71.00	47.00	13.00	22.41	-24.00	-33.80
6	45.00	43.00	51.00	46.00	8.00	18.60	-5.00	-9.80	47.00	46.00	64.00	49.00	18.00	39.13	-15.00	-23.44
7	46.00	43.00	62.00	51.00	19.00	44.19	-11.00	-17.74	49.00	48.00	74.00	55.00	26.00	54.17	-19.00	-25.68
8	51.00	50.00	54.00	44.00	4.00	8.00	-10.00	-18.52	55.00	55.00	66.00	47.00	11.00	20.00	-19.00	-28.79
9	44.00	45.00	53.00	52.00	8.00	17.78	-1.00	-1.89	47.00	49.00	64.00	55.00	15.00	30.61	-9.00	-14.06
10	52.00	52.00	59.00	54.00	7.00	13.46	-5.00	-8.47	55.00	55.00	68.00	56.00	13.00	23.64	-12.00	-17.65
11	54.00	49.00	70.00	52.00	21.00	42.86	-18.00	-25.71	56.00	54.00	79.00	56.00	25.00	46.30	-23.00	-29.11
12	52.00	51.00	66.00	54.00	15.00	29.41	-12.00	-18.18	56.00	55.00	78.00	59.00	23.00	41.82	-19.00	-24.36
13	54.00	52.00	50.00	50.00	-2.00	-3.85	0.00	0.00	59.00	58.00	69.00	57.00	11.00	18.97	-12.00	-17.39
14	50.00	51.00	55.00	51.00	4.00	7.84	-4.00	-7.27	57.00	57.00	69.00	54.50	12.00	21.05	-14.50	-21.01
Mean	51.64	48.29	57.57	51.29	9.29	19.73	-6.29	-10.29	54.50	53.50	69.36	54.61	15.86	30.12	-14.75	-20.97
STDEV	4.22	3.93	6.02	4.03	6.24	13.56	5.88	9.18	4.24	3.63	5.47	4.22	6.09	12.66	5.49	7.00

Table 1.20: Humidity studies (%) on experimental chambers containing *Megathyrsus maximus*

Control chamber									CO_2 treated chamber							
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity in morning hours (after air supplementation)	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (%change)	Night variation in the humidity	Night variation (% change)	Humidity in morning hours (before CO2supplementation)	Humidity in morning hours (after CO2supplementation)	Humidity in evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
1	61.00	64.00	99.00	99.00	35.00	54.69	0.00	0.00	60.00	63.00	99.00	99.00	36.00	57.14	0.00	0.00
2	99.00	91.00	99.00	99.00	8.00	8.79	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
3	99.00	90.00	99.00	99.00	9.00	10.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
4	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
5	99.00	99.00	99.00	92.00	0.00	0.00	-7.00	-7.07	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
6	92.00	93.00	99.00	97.00	6.00	6.45	-2.00	-2.02	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
7	97.00	94.00	99.00	99.00	5.00	5.32	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
8	99.00	97.00	99.00	99.00	2.00	2.06	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
9	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
10	99.00	96.00	99.00	99.00	3.00	3.13	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
11	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
12	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
13	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
14	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
Mean	95.64	94.14	99.00	98.36	4.86	6.46	-0.64	-0.65	96.21	96.43	99.00	99.00	2.57	4.08	0.00	0.00
STDEV	10.15	9.26	0.00	1.91	9.26	14.33	1.91	1.92	10.42	9.62	0.00	0.00	9.62	15.27	0.00	0.00

Table 1.21: Humidity studies (%) on experimental chambers containing *Saccharum arundinaceum*

Control chamber										CO_2 treated chamber							
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity in morning hours (after air supplementation)	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (%change)	Night variation in the humidity	Night variation(% change)		Humidity in morning hours (before CO_2supplementation)	Humidity in morning hours (after CO_2supplementation)	Humidity in evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
1	65.00	66.00	99.00	99.00	33.00	50.00	0.00	0.00		59.00	62.00	99.00	99.00	37.00	59.68	0.00	0.00
2	99.00	91.00	99.00	99.00	8.00	8.79	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
3	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
4	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
5	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
6	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
7	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
8	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
9	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
10	99.00	94.00	99.00	99.00	5.00	5.32	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
11	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
12	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
13	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
14	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
Mean	96.57	95.71	99.00	99.00	3.29	4.58	0.00	0.00		96.14	96.36	99.00	99.00	2.64	4.26	0.00	0.00
STDEV	9.09	8.89	0.00	0.00	8.89	13.34	0.00	0.00		10.69	9.89	0.00	0.00	9.89	15.95	0.00	0.00

Table 1.22: Humidity studies (%) on experimental chambers containing *Cymbopogon flexuosus*

Control chamber									CO_2 treated chamber							
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity in morning hours (after air supplementation)	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (%change)	Night variation in the humidity	Night variation(% change	Humidity in morning hours (before CO_2supplementation)	Humidity in morning hours (after CO_2supplementation)	Humidity in evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
1	78.00	95.00	98.00	71.00	3.00	3.16	-27.00	-27.55	86.00	99.00	98.00	95.00	-1.00	-1.01	-3.00	-3.06
2	71.00	69.00	90.00	99.00	21.00	30.43	9.00	10.00	95.00	86.00	94.00	99.00	8.00	9.30	5.00	5.32
3	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
4	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
5	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
6	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
7	99.00	99.00	96.00	99.00	-3.00	-3.03	3.00	3.13	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
8	99.00	92.00	99.00	99.00	7.00	7.61	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
9	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
10	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
11	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
12	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
13	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
14	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
Mean	95.50	96.07	98.07	97.00	2.00	2.73	-1.07	-1.03	97.79	98.07	98.57	98.71	0.50	0.59	0.14	0.16
STDEV	9.00	8.06	2.46	7.48	5.90	8.32	7.86	8.10	3.56	3.47	1.34	1.07	2.18	2.52	1.61	1.69

Table 1.23: Humidity studies (%) on experimental chambers containing *Chrysopogon zizanioides*

Control chamber									CO_2 treated chamber							
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity in morning hours (after air supplementation)	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (%change)	Night variation in the humidity	Night variation(% change)	Humidity in morning hours (before CO2supplementation)	Humidity in morning hours (after CO2supplementation)	Humidity in evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
1	66.00	92.00	99.00	99.00	7.00	7.61	0.00	0.00	70.00	85.00	99.00	99.00	14.00	16.47	0.00	0.00
2	99.00	84.00	99.00	99.00	15.00	17.86	0.00	0.00	99.00	91.00	99.00	99.00	8.00	8.79	0.00	0.00
3	99.00	82.00	99.00	99.00	17.00	20.73	0.00	0.00	99.00	87.00	99.00	99.00	12.00	13.79	0.00	0.00
4	99.00	90.00	99.00	99.00	9.00	10.00	0.00	0.00	99.00	94.00	99.00	99.00	5.00	5.32	0.00	0.00
5	99.00	90.00	99.00	99.00	9.00	10.00	0.00	0.00	99.00	92.00	99.00	99.00	7.00	7.61	0.00	0.00
6	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
7	99.00	93.00	99.00	99.00	6.00	6.45	0.00	0.00	99.00	94.00	99.00	99.00	5.00	5.32	0.00	0.00
8	99.00	93.00	99.00	99.00	6.00	6.45	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
9	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
10	99.00	90.00	99.00	99.00	9.00	10.00	0.00	0.00	99.00	89.00	99.00	99.00	10.00	11.24	0.00	0.00
11	99.00	89.00	99.00	99.00	10.00	11.24	0.00	0.00	99.00	91.00	99.00	99.00	8.00	8.79	0.00	0.00
12	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	92.00	99.00	99.00	7.00	7.61	0.00	0.00
13	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
14	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
Mean	96.64	92.71	99.00	99.00	6.29	7.17	0.00	0.00	96.93	93.57	99.00	99.00	5.43	6.07	0.00	0.00
STDEV	8.82	5.72	0.00	0.00	5.72	6.78	0.00	0.00	7.75	4.83	0.00	0.00	4.83	5.54	0.00	0.00

Table 1.24: Humidity studies (%) on experimental chambers containing *Arundo donax*

Control chamber									CO_2 treated chamber							
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity in morning hours (after air supplementation)	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (%change)	Night variation in the humidity	Night variation(% change)	Humidity in morning hours (before CO2supplementation)	Humidity in morning hours (after CO2supplementation)	Humidity in evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
1	71.00	74.00	81.00	99.00	7.00	9.46	18.00	22.22	75.00	79.00	90.00	92.00	11.00	13.92	2.00	2.22
2	99.00	87.00	93.00	94.00	6.00	6.90	1.00	1.08	92.00	90.00	99.00	86.00	9.00	10.00	-13.00	-13.13
3	94.00	80.00	99.00	99.00	19.00	23.75	0.00	0.00	86.00	81.00	99.00	99.00	18.00	22.22	0.00	0.00
4	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	93.00	99.00	92.00	6.00	6.45	-7.00	-7.07
5	99.00	99.00	93.00	99.00	-6.00	-6.06	6.00	6.45	92.00	99.00	99.00	93.00	0.00	0.00	-6.00	-6.06
6	99.00	92.00	99.00	91.00	7.00	7.61	-8.00	-8.08	93.00	90.00	99.00	86.00	9.00	10.00	-13.00	-13.13
7	91.00	84.00	94.00	99.00	10.00	11.90	5.00	5.32	86.00	87.00	99.00	99.00	12.00	13.79	0.00	0.00
8	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
9	99.00	96.00	93.00	99.00	-3.00	-3.13	6.00	6.45	99.00	94.00	94.00	94.00	0.00	0.00	0.00	0.00
10	99.00	89.00	93.00	92.00	4.00	4.49	-1.00	-1.08	94.00	91.00	99.00	87.00	8.00	8.79	-12.00	-12.12
11	92.00	79.00	99.00	99.00	20.00	25.32	0.00	0.00	87.00	84.00	99.00	91.00	15.00	17.86	-8.00	-8.08
12	99.00	89.00	93.00	99.00	4.00	4.49	6.00	6.45	91.00	87.00	99.00	99.00	12.00	13.79	0.00	0.00
13	99.00	94.00	99.00	99.00	5.00	5.32	0.00	0.00	99.00	92.00	99.00	96.00	7.00	7.61	-3.00	-3.03
14	99.00	90.00	99.00	99.00	9.00	10.00	0.00	0.00	96.00	92.00	99.00	89.00	7.00	7.61	-10.00	-10.10
Mean	95.57	89.36	95.21	97.57	5.86	7.15	2.36	2.77	92.00	89.86	98.00	93.00	8.14	9.43	-5.00	-5.04
STDEV	7.63	7.93	5.03	2.90	7.33	8.95	5.85	6.83	6.82	5.89	2.66	4.88	5.48	6.66	5.51	5.58

Table 1.25: Humidity studies (%) on experimental chambers containing *Pennisetum pedicellatum*

Control chamber										CO_2 treated chamber							
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity in morning hours (after air supplementation)	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (%change)	Night variation in the humidity	Night variation(% change)		Humidity in morning hours (before CO_2supplementation)	Humidity in morning hours (after CO_2supplementation)	Humidity in evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
1	71.00	64.00	99.00	99.00	35.00	54.69	0.00	0.00		76.00	69.00	99.00	99.00	30.00	43.48	0.00	0.00
2	99.00	99.00	91.00	99.00	-8.00	-8.08	8.00	8.79		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
3	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
4	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
5	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
6	99.00	93.00	99.00	99.00	6.00	6.45	0.00	0.00		99.00	90.00	99.00	99.00	9.00	10.00	0.00	0.00
7	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
8	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	93.00	99.00	99.00	6.00	6.45	0.00	0.00
9	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
10	99.00	90.00	99.00	99.00	9.00	10.00	0.00	0.00		99.00	89.00	99.00	99.00	10.00	11.24	0.00	0.00
11	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
12	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
13	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
14	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
Mean	97.00	95.43	98.43	99.00	3.00	4.50	0.57	0.63		97.36	95.07	99.00	99.00	3.93	5.08	0.00	0.00
STDEV	7.48	9.46	2.14	0.00	9.92	14.97	2.14	2.35		6.15	8.32	0.00	0.00	8.32	11.76	0.00	0.00

The tables depicted below (**Table 1.26** to **Table 1.32**) are the correlation tables for standardization study and studies with grass species regarding microclimatic variables as mentioned in previous sessions. The tables represent Pearson correlation between day and night CO_2 fluxes, day and night temperature variations, and day and night humidity variations during the experimental period.

Table 1.26: Correlation between microclimatic variables (Standardization study)

	Temperature variation Day	Temperature variation Night	Humidity variation Day	Humidity variation Night	Day flux (ppm)	Night Flux (ppm)
Temperature variation Day	1	-0.100	**-.723****	0.129	-0.138	-0.068
Temperature variation Night		1	0.315	**-.879****	0.055	-0.004
Humidity variation Day			1	**-.558***	0.004	0.097
Humidity variation Night				**1**	-0.071	0.110
Day flux (ppm)					1	0.108
Night Flux (ppm)						1

Table 1.27: Correlation between microclimatic variables (*M. Maximus*)

	Temperature variation Day	Temperature variation Night	Humidity variation Day	Humidity variation Night	Day flux (ppm)	Night Flux (ppm)
Temperature variation Day	1	-0.030	-0.514	.a	-0.146	0.000
Temperature variation Night		1	0.138	.a	-0.372	**-.883****
Humidity variation Day			1	.a	0.434	0.089
Humidity variation Night					.a	.a
Day flux (ppm)						0.291
Night Flux (ppm)						1

Table 1.28: Correlation between microclimatic variables (*S. arundinaceum*)

	Temperature variation Day	Temperature variation Night	Humidity variation Day	Humidity variation Night	Day flux (ppm)	Night Flux (ppm)
Temperature variation Day	1	0.341	**-.682****	.b	-0.065	0.505
Temperature variation Night		1	-0.360	.b	0.298	-0.217
Humidity variation Day			1	.b	0.350	-.**558***
Humidity variation Night				.b	.b	.b
Day flux (ppm)					1	-.**681****
Night Flux (ppm)						1

Table 1.29: Correlation between microclimatic variables (*C. flexuosus*)

	Temperature variation Day	Temperature variation Night	Humidity variation Day	Humidity variation Night	Day flux (ppm)	Night Flux (ppm)
Temperature variation Day	1	-0.038	-0.508	-.**601***	-0.015	-0.431
Temperature variation Night		1	-0.134	-0.247	0.424	-.**702****
Humidity variation Day			1	.**922****	0.078	0.442
Humidity variation Night				1	-0.187	.**679****
Day flux (ppm)					1	-0.485
Night Flux (ppm)						1

Table 1.30: Correlation between microclimatic variables (*C. zizanioides*)

	Temperature variation Day	Temperature variation Night	Humidity variation Day	Humidity variation Night	Day flux (ppm)	Night Flux (ppm)
Temperature variation Day	1	-0.205	-0.484	.a	0.171	-0.119
Temperature variation Night		1	-0.095	.a	-0.370	-0.404
Humidity variation Day			1	.a	0.493	-0.157
Humidity variation Night				.a	.a	.a
Day flux (ppm)					1	-0.090
Night Flux (ppm)						1

Table 1.31: Correlation between microclimatic variables (*A. donax*)

	Temperature variation Day	Temperature variation Night	Humidity variation Day	Humidity variation Night	Day flux (ppm)	Night Flux (ppm)
Temperature variation Day	1	-0.202	-.**713****	-0.089	-0.004	-0.278
Temperature variation Night		1	-0.053	-.**623***	-0.148	-0.085
Humidity variation Day			1	0.023	0.427	0.043
Humidity variation Night				1	0.173	-0.093
Day flux (ppm)					1	-0.499
Night Flux (ppm)						1

Table 1.32: Correlation between microclimatic variables (*P. pedicellatum*)

	Temperature variation Day	Temperature variation Night	Humidity variation Day	Humidity variation Night	Day flux (ppm)	Night Flux (ppm)
Temperature variation Day	1	-0.048	-.**646***	.b	-0.214	0.218
Temperature variation Night		1	-0.347	.b	0.197	-.**852****
Humidity variation Day			1	.b	-0.049	0.046
Humidity variation Night				.b	.b	.b
Day flux (ppm)					1	-0.289
Night Flux (ppm)						1

1.5. DISCUSSION

The entire experiment was carried out to assess the relative ability of grass species to assimilate CO_2. A comprehensive review of the literature was carried out to list the grass species that can be included in this study. As mentioned in the materials and methods, 52 grass genera were identified as the most common grass genera found in grasslands across various habitats, worldwide (Van Dyne et al.1978; Singh & Gupta, 1993). Based on the predefined criteria, six species, representing six genera were selected for the current study. The plants were collected from their natural habitats, multiplied, and grown to a suitable size under nursery conditions. For this study, mature plants that are grown for six to eight months and have adequate biomass have been employed.

In addition to the rearing of plants, a detailed analysis of the literature was conducted to design a controlled system for the study that would meet the expected needs. Accordingly, two identical chambers of size 6.32 m^3 are installed, and all prerequisites are fulfilled, as detailed in the materials and methods. After analysis, conclusions were drawn on the climatological, growth, and biochemical responses of the selected species under elevated levels of CO_2 supply. The present discussion will deal with the salient outcomes that have resulted from the growth and metabolism of each grass species at elevated levels of CO_2.

1.5.1 Changes in microclimatic variables associated with standardization studies

The study was carried out in two phases and in the first phase, standardization studies are carried out in the growth chambers to assess the retention pattern of CO_2 under ideal conditions. For this, a 15-day experiment was conducted simultaneously in both the growth chambers in the absence of plants, where CC (Control Chamber) and TC (Treatment Chamber) were supplied with ambient air and CO_2+ ambient air combination respectively for 15 minutes during morning hours. The extent of CO_2 in both chambers was recorded, along with an assessment of the extent of temperature and humidity. The amount of CO_2 retained was measured during evening hours to estimate the day flux and those of the next day morning to evaluate the night flux. All these measurements are carried out to assess the natural dissipation/assimilation rate and resultant retention of CO_2 on a day and night time scale. The temperature and humidity

associated with the chambers were also measured along with CO_2 concentration. Elevated CO_2 experiments in enclosed systems may experience dissipation or leakage and this will also affect the measurement of microclimatic variables. Anyhow, in the present study, the standardization experiment has been undertaken to eliminate the errors, which are likely to appear during the estimation of the CO_2 assimilation potentials of selected grass species. The outcomes obtained in the standardization study are used to eliminate the 'chamber effect' from the results associated with microclimatic conditions obtained during the experimentation with grass species. The day and night fluxes of CO_2 associated with the standardization study have also been incorporated into the results of the grass species to get a realistic estimation of the growth and metabolic performances. Thus the CO_2 flux (day and night) calculated with each grass species is the respective gross flux and the values of the day and night fluxes obtained during standardization studies have been subtracted from the individual values of grass species to get the net flux or net assimilation. Changes associated with the chambers regarding CO_2, temperature, and humidity are discussed in this chapter and those changes associated with the growth, and biochemistry of grass species, along with their soil parameters are dealt with in Chapter II.

Ahead of experimentation, the chambers (CC and TC) and associated facilities were checked to ensure their proper functioning and were identical before the first supply of ambient air / CO_2+ ambient air mixture. The percentage change in day flux associated with the standardization study is -4.33±2.46 % in CC and -7.55±1.56 % in TC. Several factors could contribute to the reduction in the amount of CO_2 in the standardization study. It is assumed to be associated with the physical and chemical properties of CO_2 gas and the adsorption/permeability properties of the materials that are used for the construction of chambers.

The primary cause of carbon dioxide dissipation in chambers associated with standardization study, especially TC following the supply of CO_2 + ambient air is thought to be due to the properties of the CO_2 gas. In the atmosphere, CO_2 gas can diffuse and spread out to occupy free space. The gradient in CO_2 concentration between two points, temperature, and pressure are some of the variables that affect the diffusion rate (Liu et al., 2023). Increased molecular motion at higher temperatures can speed up the diffusion and dissipation of CO_2 (Fortunato et al., 2023). The dissipation of CO_2

during the day may be caused by higher morning temperatures (average) in the CC (41.15°C) and TC (41.16°C). Furthermore, as a greenhouse gas, carbon dioxide increases the temperature inside the chamber by absorbing more reflected sunlight, thereby enhancing the greenhouse effect (Zhong & Haigh, 2013). According to Long and Hallgren (1985), an increase in the number of moles of gas will increase pressure inside the chamber; however, if the system is not entirely airtight, this rise in pressure will cause a loss of CO_2. Similar processes might have occurred in the TC associated with the standardization study. Further, diffusion of CO_2 occurs more quickly in environments with high CO_2 concentrations (Fortunato et al., 2023), and this also explains the higher day flux rates in TC (7.55±1.56 %) compared to CC (-4.33±2.46 %). The dissipation characteristics of CO_2 will also be influenced by how long it stays inside the chamber. If CO_2 is not continuously supplied, it will eventually disappear and come into balance with the surroundings. The water solubility property of CO_2 gas may also contribute to the reduction of CO_2 or negative day flux. Carbon dioxide can dissolve in water vapour to produce carbonic acid (Topham, 2000). In the daytime, there was a noticeable rise in humidity in both chambers, which probably contributed to the atmospheric water vapour. In comparison to CC (19.73±13.56%), TC experienced an average humidity rise of 30.12±12.66%, and both chambers had a chance of carbonic acid generation during the day, with TC experiencing this more frequently, and accordingly, there is a negative daytime flux of CO_2. With a rise in pressure (due to the supply of air/CO_2), CO_2 gas becomes more soluble in water (He et al., 2021), and this may also contribute to carbonic acid formation. In addition, a negative correlation is noticed in TC between temperature and humidity during day and night hours. Day and nighttime humidity variations are also negatively correlated.

The permeability characteristics of the PVC sheet and pipes used as chamber materials are thought to be another factor contributing to the decrease in CO_2 concentrations associated with the standardization study. Small molecules are moved through polymeric media by successive unit diffusion jumps under the effect of a concentration gradient. The availability of free volume, which is based on the relative mobility of the penetrant molecules and polymer chain segments, is connected to the amount of transport (Kumins & Kwei, 1968). However, polyvinyl chloride (PVC) is an example of a non-permeable and inert material that has to be used in the construction of chambers to measure CO_2 fluxes from terrestrial ecosystems (Pavelka et al., 2018).

Previous studies have also confirmed that PVC membranes have low gas permeability (Tiemblo et al., 2001, 2002; Bierbrauer et al., 2010). According to the literature, polyvinyl chloride membranes are therefore impermeable, and the decrease in CO_2 in the standardization study might be due to other reasons. However, previous researchers claim that the permeability of PVC membranes decreases when plasticizers are used to reduce their rigidity. Phthalate-based plasticizers are commonly used, and Shailaja and Yaseen (1993) found that the higher phthalate concentration of the membranes accelerated the permeability process. The phenomenon is affected by variations in component concentration, size, form, and interaction. As a result, a 1 mm-thick PVC membrane, which is made flexible by plasticizers, will undoubtedly help lower the concentrations of CO_2 in both CC and TC. Furthermore, a slighter decrease in CC is brought about by a lower CO_2 concentration in the supplied air, as mentioned above.

During the 15 days of the standardization study, the night flux in CO_2 exhibits a reversal from the day flux, with concentrations showing a slight rising trend. The average increase in night flux is 2.47±1.73 in CC and 5.84±2.01 in TC. There is no ambient air/CO_2+ ambient air supply in chambers during the evening hours. The materials in the chamber that adsorbed CO_2 during the day may desorbed it as the temperature drops at night (Rayment, 2000) which could account for the observed rise in night flux (in comparison to day flux). The average temperature (35.29±1.79°C I CC and 35.12±1.55°C I TC) during evening hours is less compared to morning hours and is about a 5°C increase in both chambers in the next day morning. Thus the positive night flux values could also be attributed to the expansion of CO_2 gas at a temperature rise. The concentration of CO_2 in the chamber will rise if a specific volume of gas, at a given pressure and temperature, expands when the temperature rises (Duan et al., 2023). Consequently, a greater volume of CO_2 is diffused, increasing the concentration of CO_2 in the chamber.

Materials used in the chamber might have an impact on the CO_2 flux in the standardization experiment. In this case, pressure-mediated gas diffusion and the characteristics of the chamber material upon which it is made (PVC sheet and pipes) are the only aspects influencing the pattern of rise and fall in CO_2 concentrations. Thus, an FTIR analysis is carried out to confirm the composition of the PVC sheet (**Figure 1.14**).

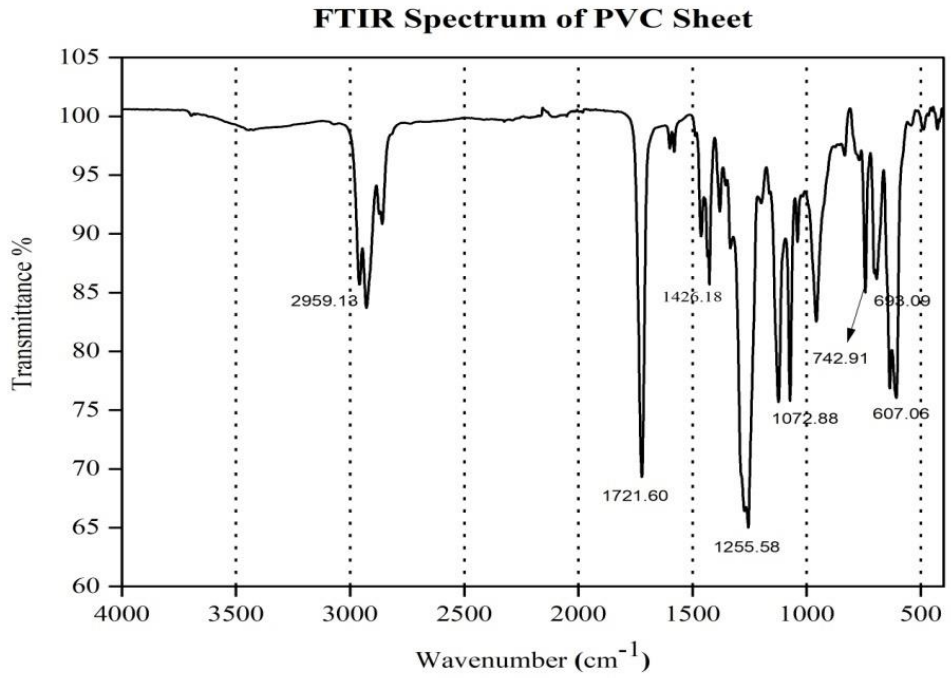


Figure 1.14: FTIR spectrum of PVC sheet

There are a few prominent peaks in the PVC films: C-H out-of-plane trans deformation at 960 cm-1, CH_2-Cl angular deformation at 1426 cm-1, and C-Cl bond stretching at 831, 691, and 615 cm-1 (Chen et al., 2018). Likewise, in the present spectrum, a prominent peak is obtained at 1426.18 cm1. Peaks nearer to the mentioned peaks were noticed in the present spectrum at 742.91 cm1, 693.09 cm1, and 607.06 cm1. Methylene (CH_2) groups inside the PVC polymer chains may be represented by the peak at 1426.18 cm1. Repeating vinyl chloride monomers makeup PVC, and the polymer backbone frequently contains CH_2 groups. The presence of C-Cl bonds is distinctive of the PVC structure; the peaks at 693.09 cm1 and 607.06 cm1 may indicate the bending vibration of C-Cl bonds inside the PVC polymer chains. Manufacturers are copolymerizing PVC with additional monomers and adding additives like plasticizers, heat stabilizers, lubricants, fillers, and other polymers to enhance its qualities (Liu & Zhang, 2007). This explains why the current spectrum differs from the FTIR spectrum of pure PVC. Chen et al. (2018) reported a change in the FTIR spectrum of PVC films due to the addition of plasticizers. According to them, with the addition of plasticizers to the formulations, the band representing C=O groups in commercial plasticizer (dioctyl phthalate (DOP) at 1724 cm-1 gets shifted by roughly 4-5 cm-1 towards the lower frequency, and hence its width increases. In the current spectra, a peak at 1721.60

cm-1, which is closer to the mentioned band is observed, which denotes the apparent presence of plasticizer and the presence of C=O groups. Moreover, the glycidyl ether group in the natural plasticizer - cardanol derivatives of glycidyl ether (CGE) changes to 908, 858, 834 cm^{-1} from 910, 859, 847 cm^{-1}. The inclusion of plasticizers is thought to have caused the peaks in the current spectrum to differ from the FTIR spectrum of pure PVC and other commercial PVC films. Accordingly, the structural composition of PVC sheets might influence the CO_2 fluxes relating to the day and night.

1.5.2 Changes in microclimatic variables associated with grass species under study

The measurement of the variation in CO_2 concentration of the air within the chamber yields the rate of CO_2 assimilation by the enclosed material (Long & Hallgren, 1985). Accordingly, in the present study the day flux, which is determined by calculating the difference in CO_2 levels in the experimental chamber between morning and evening hours, is thought to represent the amount of CO_2 that the plant assimilates. Similarly, the night flux of CO_2 is the respiratory contribution. 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 flux data concerning the standardization studies were used. Accordingly, the net flux in CO_2 is obtained by subtracting the CO_2 flux values of the standardization study from the respective gross flux of each species under study. The table below depicts the respective gross and net fluxes of each plant. Negative signs correspond to the reduction of CO_2 within the chamber atmosphere.

CO_2 fluxes (ppm)	CC (DF)	TC (DF)	CC (NF)	TC (NF)
Standardization (Control)	-29.14±16.41	--	36.93±12.50	--
Standardization (Treated)	--	-77.07±16.83	0	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.zizanioides (G)	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, CC is the control chamber; TC is the treatment chamber; DF is the day flux; NF is the night flux; G is the gross assimilation and N is the net assimilation

The discrepancy between the gross and net assimilation values of CO_2 flux is due to the elimination of CO_2 reduction by various other factors in the net assimilation value. The net assimilation represents the actual assimilation of CO_2 or the actual amount of CO_2 absorption or could be considered as stomatal intake. The nighttime CO_2 flux inside the chamber includes respiratory release. Thus the night fluxes are positive values concerning certain species of the present study. Gross day and night fluxes of CO_2 regarding *M. maximus* in TC are -398.14±85.05 ppm and 49.71±94.33ppm respectively, and the day flux in TC regarding standardization study is -77.07±16.83ppm and night flux is 23.21±16.16ppm. Consequently, the net flux associated with *M. maximus* in TC is -321.07±78.16ppm and 26.50±92.34ppm respectively at day and night. Likewise in TC concerning *S. arundinaceum*, respective net day and night fluxes are -338.64±65.01ppm and 55.07±47.75ppm. The net day flux noticed in TC of the species *C. flexuosus* is -240.86±93.26ppm and here the net night flux is 38.43±140.87. In *C. zizanioides*, the 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 day flux in TC is -257.86±115.64 and the night flux is -28.07±77.05. The net day flux attributed by *P. pedicellatum* is -245.50±67.13 and the associated night flux is -2.29±70.17.

Percentage change in CO_2 flux during the experiment is also worked out to understand the relative efficiency of plants in carbon assimilation. Percentage change in both CC and TC at day and night regarding all species is represented in the following

table. The net percentage change in CO_2 flux is obtained by subtracting percentage changes in CO_2 flux regarding standardization study from that associated with each plant species.

% Change in CO_2 Flux	CC (DF)	TC (DF)	CC (NF)	TC (NF)
Standardization (Control)	-4.33±2.46	--	5.84±2.01	--
Standardization (Treated)	--	-7.55±1.56	0	2.47±1.73
M.maximus (G)	-2.11±11.26	-38.78±7.95	24.13±24.34	7.55±15.07
M.maximus (N)	2.21±11.33	-31.22±7.34	18.30±24.16	5.07±14.82
S.arundinaceum (G)	5.82±5.82	-39.24±5.96	6.57±6.16	12.70±7.81
S.arundinaceum (N)	10.14±6.88	-31.69±6.00	0.73±6.87	10.23±7.90
C.flexuosus (G)	-4.42±6.99	-31.44±9.57	9.71±9.84	10.49±17.78
C.flexuosus (N)	-0.10±7.15	-23.89±9.48	3.88±9.82	8.02±17.21
C.zizanioides (G)	8.16±7.24	-29.70±8.36	-2.52±6.82	-1.93±9.61
C.zizanioides (N)	12.49±8.07	-22.15±8.66	-8.35±7.51	-4.41±10.24
A.donax (G)	-1.42±3.28	-32.56±11.49	0.77±4.92	-0.15±9.65
A.donax (N)	2.91±5.00	-25.00±10.81	-5.07±5.90	-2.62±9.88
P.pedicellatum (G)	-4.29±8.75	-31.57±5.23	-0.79±9.95	3.22±10.16
P.pedicellatum (N)	0.04±7.96	-24.02±5.80	-6.62±9.58	0.74±10.14

Where, CC is the control chamber; TC is the treatment chamber; DF is day flux; NF is night flux; G is gross assimilation, and N is net assimilation

Changes in CO_2 fluxes, both net and gross, are shown in this table. Where an increase in CO_2 flux would be regarded as respiratory release by the plant (night) and a decrease as absorption (day). Compared to CC, the day flux in TC is significantly higher. In the TC associated with *M. maximus*, net CO_2 fluxes are -31.22±7.34% and 5.07±14.82% at day and night, respectively, whereas with *S. arundinaceum,* the respective net changes are -31.69±6.00% at day and 10.23±7.90% at night in TC. The *C. flexuosus* attributed a day flux of -23.89±9.48 and a night flux of 8.02±17.21 at elevated CO_2 treatment. *C. zizanioides* shows a day flux of -22.15±8.66 and a night flux of -4.41±10.24%. Similarly, the respective day and night fluxes associated with CO_2

treatment in *A. donax* are -25.00±10.81% and -2.62±9.88%, respectively and the net day flux of *P. pedicellatum* is -24.02±5.80%, and the net night flux is 0.74±10.14%.

It is evident from the tables above that the net values of the day and night fluxes differ from their gross values, where the removal of CO_2 by other factors is eliminated. 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 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, which is depicted in the following table.

Species	Net CO_2 exchange (%)	
	Control	Treatment
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 species 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 assimilation of *S. arundinaceum* (-31.69±6.00%) and *M. maximus* (-31.22±7.34%) is higher during the day than *A. donax* (-25.00±10.81%) and *C. zizanioides* (-22.15±8.66). Despite having higher 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.

Physiological mechanisms and genetic traits determine how different species absorb, assimilate, and release CO_2. Also, the amount to which different plant species can assimilate carbon varies. Distinct physiological techniques used to fix carbon (C3/C4/CAM photosynthesis) or varying capacities to metabolize carbohydrates by actively growing plant parts might lead to limitations (Poorter, 1993). Among the selected species, only *A. donax* exhibits a C3 photosynthetic pathway and the other five species show a C4 pathway. 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 experimented 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; 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 on each day of the present study. According to Morison and Gifford (1983), a ratio between intercellular and ambient CO_2 concentration (Ci/Ca) has a role in stomatal sensitivity. Ci/Ca has been reported to be greater in C3 species compared to C4 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 (LA) of each species measured in the initial (D1) and final (D15) 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)			
	Control		Treatment	
	D1	D15	D1	D15
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

In the present study, all the above-mentioned factors may contribute to CO_2 exchange. In the present study, *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 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. pedicelllatum (*-0.74±10.14%).

Night flux is an index of leaf respiration and it is influenced by multiple factors such as temperature, humidity, and carbohydrate availability. While conditions like water stress or limited carbohydrate availability may cause respiration rates to decrease, warmer temperatures have the potential to increase it (Rashid et al., 2020). Specific metabolic activity and physiological processes also influence nighttime respiration. In a previous study by Tan et al. (2015), the night respiration of winter wheat (C3) decreased by 11% at 560 ppm of CO_2. Similarly in the present study, the C3 species *A. donax* shows a marginal decrease of CO_2 during nighttime (indicative of a decrease in respiration) in the TC and CC, where average CO2 concentration during nighttime is 590.36±21.65 and 682.36±90.40 respectively. As mentioned above *C. zizanioides* (C4) also shows negative night flux, respiratory release is not obvious in this case also. The plant has a larger leaf area than other species, but its accessible carbohydrate content is

comparable with other species, therefore this lower nighttime respiration may be explained by the plant's insufficient carbohydrates.

1.5.3 Changes in Temperature and Humidity and its Influence on CO_2 flux

Temperature and humidity associated with each plant species change according to the flux of CO_2 and vice versa. This will also affect the metabolism and growth of plants. Also, plant metabolism affects the temperature and humidity associated with the system under study. Stomatal response to CO_2 is discussed in the previous session; in addition, guard cells can detect variations in temperature (Srivastava et al., 1995) and humidity (Sheriff, 1979; Assmann et al., 2000). Growth chambers are reported to have a higher relative humidity (Talbott et al., 2003), likewise in the present study average humidity in TC associated with all experimental plants exceeds 99%. Talbott et al. (2003) reported that higher relative humidity increases stomatal sensitivity. The inference drawn in the present study is contrary to it and the higher CO_2 intake exhibited by the grass species in the present study determines its higher conductance rate. Likewise in previous research, Kawamitsu et al. (1993) observed higher conductance rates and maximal assimilation rates in *Oryza sativa* (C3) plants grown at 85% relative humidity compared to plants grown at 35% relative humidity. As well the CO_2 conductance of *A. donax* at higher humidity conditions is maximal. In addition, when *Panicum maximum* (*Megathyrsus maximus*), a C4 species, was grown in similar varying relative humidity conditions, no differences were observed in any of these parameters. Similarly in the present study, the majority of the grass species is C4 and at higher humidity conditions these species show higher conductance, which is evident from the day flux under elevated CO_2 treatment. According to Bencloski (1982), relative humidity will rise with rising air temperature and fall with falling air temperature. Similarly, day temperature is negatively correlated with day humidity in *S. arundinaceum, A.donax,* and *P. pedicellatum*. The Hygrothermometer attached to the experimental system is calibrated to a maximum value of 99% and, a humidity hike over it will not be displayed in the instrument. As mentioned, the average humidity in TC associated with all experimental plants exceeded 99% and this hike in no way affected the conductance of grass species as reported by Kawamitsu et al. (1993). The response of plants with C3 metabolism to increased CO_2 is predicted by biochemical photosynthesis models to be largest at high temperatures and smallest at low

temperatures (Long, 1992; Bowes, 1996; Gifford, 2003). As well the C3 species *A. donax* in the present study shows maximum uptake of CO_2 than other C4 grasses under study. It is widely accepted that C4 plants, due to their CO_2 concentrating mechanism, will exhibit minimal CO_2 stimulation regardless of temperature (Bowes, 1996; Gifford, 2003). Nonetheless, recent research has demonstrated a significant stimulation of biomass in C4 species (Ghannoum et al., 1997, reported a 28% rise in *Panicum antidotale*). In the present study, CO_2 uptake responses of C4 grass species also show a higher stimulation at elevated CO_2 and increased temperature environments. The air temperature in the chamber is typically 0-2°C higher than the air temperature outside the chamber as in most studies. Since the C4 and C3 plants show a similar pattern of temperature response (although with very species-specific quantitative characteristics), it is probable that the complex responses of the C4 plants to the Temperature × CO_2 interactions will resemble those of the C3 plants (Morison & Lawlor, 1999). Present research on the six grass species is consistent with this conclusion.

1.6 SUMMARY AND CONCLUSION

The current study aims to assess the effectiveness of selected grass species in carbon mitigation. 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.

The specific objectives of the study include, 1) Identification of high biomass yielding grass species suitable for tropical climatic conditions, 2) Collection and multiplication in nurseries and maintenance up to desired stages of growth for experimentation; standardization of growth conditions and acclimatization of characters, 4) Conduct of carbon dioxide sequestration studies in plants using carbon dioxide controlled systems under varying concentrations of carbon dioxide and other growth conditions, 5) Assessment of physiological and biochemical responses of plants under varying levels of carbon dioxide supply; assessment of the changes in micro climatic conditions associated with controlled environmental systems brought about by the growth of plants under varying levels of carbon dioxide supply, 6) Assessment of the changes in biomass content associated with the plants subjected to varying concentrations of carbon dioxide, 7) Listing of grass species having higher carbon dioxide sequestration potential and optimization of conditions of highest sequestration efficiency.

To determine which species of grass would be best for the study, a comprehensive review of the literature was conducted, 52 genera were analyzed and six species such as *Megathyrsus maximus*, *Saccharum arundinaceum*, *Cymbopogon flexuosus*, *Chryzopogon zizanioides*, *Arundo donax* and *Pennicetum pedicellatum* were selected for the study. The selection of six grass species was based on several factors, such as their greater biomass, suitability for tropical climatic conditions, and the dearth of studies on CO_2 sequestration of these species with the specific experimental conditions and procedures as outlined in the present study.

The experimental setup for the study consisted of two chambers (6.32 m^3 each) made with PVC frames (1.0 inch diameter) and clear transparent PVC walls of 1.0 mm thick. The control chamber was installed with the facility for the supply of air and the

treatment chamber with CO_2 - air mixture using an air compressor and a nebulizer. Both the chambers were equipped with an exhaust facility to control the gasses when required. During experimentation, monitoring of CO_2 concentration (ppm) within the chambers was made through an automated CO_2 analyzer, and the extent of temperature (°C) and humidity (%) using a hygro-thermometer. An irrigation facility was also attached to both chambers. For each study, two sets of plants were taken, in which one set was maintained in the control chamber, and the other in the treatment chamber. Both the chambers were sealed from the outside. Ambient air was supplied to the control chamber for about 15 minutes in the morning (9 am). Similarly, a CO_2-air mixture was supplied to the treatment chamber for the same period, ensuring an elevated CO_2 concentration within the chamber (900 - 1000 ppm). The experiment was repeated at 6.00 pm with constant monitoring of the CO_2 (ppm), temperature (°C), and humidity (%) within the chambers. These measurements, which were procured for 15 days, were used to estimate the relative day and night fluxes of CO_2 brought about by the growth of plants in the respective chambers. Similarly, a standardization study (without plants) was also undertaken for fifteen days in the same manner to assess the gross and net flux in CO_2 associated with the chambers.

The present chapter (Chapter I) deals with an evaluation of the microclimatic conditions attributed by grass species to varying levels of CO_2 to assess the relative efficiencies of plants in carbon assimilation. 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 grasses, 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. 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 taken as the net CO_2 exchange. The net exchange reveals that the actual CO_2 concentration decreased over the experimental period in the enclosed chamber associated with each grass species. Accordingly, all species were noted to be potential species in terms of carbon sequestration with higher net CO_2 exchange at elevated CO_2. However, *A.*

donax is identified as having higher potential, followed by *C. zizanioides*, *M. maximus*, *P. pedicellatum*, *S. arundinaceum*, and *C. flexuosus.* The net day flux of *S. arundinaceum* and *M. maximus* was higher than *A. donax* and *C. zizanioides*. Despite having superior net day flux, these species cannot be regarded as the most efficient species for sequestering carbon due to the higher net night flux associated with them. Although *C. zizanioides* has a lower net day flux, its CO_2 release during the night is minimal and even lowers the CO_2 levels at night. Consequently with higher net CO_2 exchange *A. donax and C. zizanioides* become the most potential species.

Temperature and Humidity associated with each plant species change according to the flux of CO_2 and vice versa. The average humidity in the treatment chamber associated with all experimental plants exceeded 99%. Higher conductance rates and maximal assimilation rates at higher humidity conditions were shown by the C3 grass *A. donax* as previously reported for other C3 species. In the present study, other grass species were C4 which shows similar phenomena were higher conductance of CO_2 under high humidity conditions, which is evident from the day flux under elevated CO_2 treatment.

The present chapter explains the CO_2 assimilation potential of the grass species through experiments in CO_2-controlled chambers using various aspects such as day and night flux of CO_2; net day and night flux and net CO_2 exchange rate. According to a comprehensive analysis of these aspects, *A. donax* was identified as superior in carbon assimilation efficiency followed by *C. zizanioides*, *M. maximus*, *P. pedicellatum*, *S. arundinaceum* and *C. flexuosus.*

CHAPTER II

Carbon sequestration studies on selected grass species

2.1 INTRODUCTION

Carbon sequestration using biomass is the most significant, sustainable, and renewable method being developed to address the challenges related to global warming and climate change (Jansson et al., 2010). Plants capture atmospheric carbon dioxide (CO_2) through photosynthesis and store enormous quantities of organic carbon in above and below-ground biomass (Eloka-Eboka et al., 2019). The grass family, Poaceae, is one of the most ecologically adapted families of angiosperms among terrestrial plants, and it may be found in any type of habitat (Tzvelev, 1989). According to Frank et al. (2004), the subsurface biomass of grasslands acts as a sink for carbon storage. A large number of these grasses have vast root systems, which aid in the annual transfer and storage of carbon into the soil through the shedding of extensive roots (Anderson et al., 2008; Odiwe et al., 2016). Because of rapid development and turnover, grasses can accumulate and turn over biomass rapidly. Grasses have the highest average root-to-shoot carbon stock ratios, with the roots holding up to 45% of the plant's total carbon stock (Bhattacharya et al., 2023).

Specific plant functional traits could react to new environmental conditions to balance with 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 subsequent transport of it to 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 growth, physiology, and chemical composition of plants (IPCC, 2013; Myers et al., 2014).

Perennial crops including grasses are more energy-producing, require less input, and help reduce greenhouse gas emissions more than other crops (Jansson et al., 2010). Unlike grasslands, owing to the growth or life cycle patterns, many grass species are less studied as sinks of carbon storage. Annual grasses are short-lived and thus need to be managed each year, and with perennial grasses, climatic limitations may hinder their cultivation (Scordia & Cosentino, 2019). Thus research on elevated CO_2 responses of individual perennial grass species becomes limited. In this regard, the

present initiative assesses the responses of selected perennial grass species to elevated CO_2. Morphological traits, pigments, metabolites, nutrients, and soil characteristics of the species such as *Megathyrsus maximus*, *Saccharum arundinaceum*, *Cymbopogon flexuosus*, *Chrysopogon zizanioides*, *Arundo donax,* and *Pennisetum pedicellatum* were analyzed at experimental conditions and are detailed in Chapter II. The outcomes will assist in determining which grass species are most suitable for carbon offsets and agroforestry. The objectives of this chapter are summarized as follows:

- Evaluation of the growth/development and changes in biomass of the selected grass species at elevated levels of carbon dioxide supply.
- Evaluation of the biochemical responses of selected grass species to elevated levels of carbon dioxide supply.
- Listing of plants with the greatest potential for sequestering carbon dioxide and optimizing conditions for better efficiency.

Different plants respond uniquely to varying carbon dioxide concentrations. Several studies with different objectives have been undertaken in the past on the effects of higher CO_2 on grass species. In light of this, the following is a review of the morphological/growth, biochemical, mineralogical, and soil properties of grasses under elevated CO_2 conditions. An overview of previous research in this area is detailed.

2.2 REVIEW OF LITERATURE

Grass species are essential to maintaining the energy, health, and harmony of our ecosystems. Grass plants ensure the survival of a wide range of organisms, from giant herbivores to tiny insects, thereby fostering biodiversity. They also play a critical role in keeping the land fertile for agriculture, avoiding erosion, and preserving the health of the soil. However, these advantages go beyond that. In the struggle against climate change, grasses are also vital participants. With the removal of carbon dioxide from the atmosphere, they contribute to lowering greenhouse gas emissions and slowing down global warming. Studies on the responses of grass species (Poaceae) to enriched CO_2 environments gained traction in the early 1990s. Poaceae, with about 11,000 species, is the seventh most species-rich family of angiosperms (Clayton et al., 2006). Of this, the C3 and C4 grasses are the predominant species in the world's savannas and grasslands. These ecosystems cover almost 20% of the land area on Earth and account for 30% of net primary productivity worldwide (Mishra & Young, 2020). Grassland area accounts for around 24% of the total land area in India (Dey et al., 2024).

Higher atmospheric carbon dioxide concentrations enhance photosynthesis, which in turn may promote more plant growth. The process of photosynthesis requires CO_2 and photosynthesis rises when CO_2 levels increase, but only in the absence of any other limiting factors (Boretti & Florentine, 2019). Some studies reported that elevated CO_2 may increase transpiration rate, stomatal conductance, water use efficiency (WUE), carbon content, shoot and root biomass, stem diameter, wood density, and root growth (Bu et al., 2019; Srinivasan et al., 2017; Ali et al., 2016; Benlloch-Gonzalez et al., 2014). Increased photosynthesis results in an increase in the production of photoassimilate. In this regard, plant resource allocation patterns, or carbon partitioning, are crucial (Thompson et al., 2017), as carbon regulates a variety of morpho-physio-anatomical traits. Plant growth is often influenced by four main factors: sunlight, water, temperature, and nutrients such as CO_2, nitrogen, and phosphorus (Epstein, 1977). Thus the effect of elevated CO_2 on plants is always coupled with these factors. For example, the opposite effect may be seen when elevated CO_2 and temperature rise together, greater temperatures may inhibit root growth while elevated

CO_2 may promote it (Benlloch-Gonzalez et al., 2014). Here are the effects of elevated CO_2 on different plant traits, with a focus on the effects on grasses.

2.2.1 Modifications to plant morphological characteristics and biomass in environments with higher levels of CO_2: Facts from studies on grasses

The growth and biomass production in grasses vary greatly with elevated levels of CO_2. Under high CO_2, growth and biomass production are almost always enhanced (Leakey et al., 2009). The formation of more fertile tillers in wheat is influenced by increased tillering under elevated CO_2 (Tausz-Posch et al., 2015), and in a biofuel crop, increased growth and branching under elevated CO_2 has been noticed, indicating enhanced sink strength (Kumar et al., 2014). In a FACE experiment using perennial Ryegrass (*Lolium perenne*) under elevated CO_2, significant changes were seen in the size of individual plants, indicated by culm elongation, and leaf characteristics. These morphological modifications were observed across a broad range of water availability. Additionally, these variations persisted for quite a while of the year, indicating that the effects were not greatly affected by temperature. There is evidence that major morphological features can be changed by exposure to increased CO_2. For instance, increased CO_2 improves plant height by 14% and leaf number by 8% on average, according to a meta-analysis of FACE studies (Ainsworth & Long, 2005). Specific leaf area (SLA - the ratio of leaf area to leaf dry mass) sometimes decreases under elevated CO_2 conditions, yet total leaf area and leaf size are often greater than control conditions (Pritchard et al., 1999; Ainsworth & Long, 2005). Temme et al. (2015) provided support for these results. In a study conducted by Tipping and Murray (2000), three species of *Panicum* with three distinct photosynthetic modes (C3, C4, and C3/C4 intermediate) showed no qualitative changes in their leaf architecture when exposed to elevated CO_2. There were noticeable variations in leaf thickness, though: the C3 species showed a 10% rise, whereas the C4 and C3/C4 intermediate species showed declines. The mean SLA decreased by 12%, 22%, and 34% for the C3 species, C4 species, and C3/C4 intermediate, respectively. In the same study, the C3 species had a roughly 22% decrease in stomatal frequency for the abaxial epidermis. On the other hand, for the other two species, the stomatal frequency had increased by nearly 30 percent. As mentioned in Chapter I, percentage gas exchange is determined by stomatal frequency, which in turn influences carbon assimilation. Elevated CO_2 enhanced individual leaf

area of *Cenchrus pedicellatus*, according to Tom Dery et al. (2018), making the grass more appealing as fodder.

Elevated CO_2-induced morphological alterations can have a range of effects on physiological processes (Brinkhoff et al., 2019). The efficiency of essential physiological functions like light interception and gas exchange may be impacted by morphological changes. The potential of certain species to sequester carbon would be significantly impacted by this. According to studies by Honda and Fisher (1978), Falster and Westoby (2003), Kern et al. (2004), and others, leaf size, leaf arrangement, internode length, and branching angle have been demonstrated to have an impact on carbon assimilation by influencing the degree of self-shading and, consequently, the light interception efficiency. For instance, petiole length and angle are substantially responsible for the degree of self-shading, light interception, and carbon uptake of a tropical pioneer species. (Yamada et al., 2000). More nitrogen can be retained in leaf blades when high CO_2 decreases SLA in wheat, (Thilakarathne et al., 2012). This may speed up the photosynthetic rate and delay leaf senescence. In contrast, non-structural carbohydrate accumulation may be the cause of a decrease in SLA under increased CO_2, which might hinder photosynthesis (Pritchard et al., 1999).

There has been a good deal of research on how plants respond biomass-wise to high CO_2 levels. According to published research, biomass has increased in response to high CO_2 levels. Due to internal CO_2 concentrating processes, C4 grasses do not benefit more from increasing CO_2, but they have been shown to exhibit increased biomass compared to C3 grasses. Faster inflorescence development, altered partitioning, delay of leaf senescence, or increased water potential are all effects of these increases (Carter & Petersen, 1983; Potvin & Strain, 1985; Knapp et al., 1993). Some studies indicate that an increase in leaf area is responsible for the growth and biomass increase during CO_2 ascent (Poorter & Remkes, 1990). Additionally, Roumet and Roy (1996) reported that in 11 closely related grasses, there was a positive link between plant growth and SLA. The responses of biomass of several grass species experimented under elevated CO_2, and reported during the last few years are shown in **Table 2.1**.

Table 2.1: Changes in biomass of various grass species at elevated CO_2

Sl. No.	Plant species	Duration of elevated CO_2 & supplementary treatments	Biomass	Reference
1	*Bouteloua gracilis* (C_4)	7 weeks	35% increase	Riechers & strain, 1988
2	*Lolium perenne* (C_3)	49 days	Increased	Ryle et al., 1992
3	*Andropogon gerardii* (C_4)	2 growing seasons one with water stress	41% increase	Knapp et al., 1993
4	*Agrostis capillaries* (C_3)	79 days	Increased	Baxter et al., 1994
5	*Poa alpinia* (C_3)	105 days	Increased	Baxter et al., 1994
6	*Festuca vivipara* (C_3)	189 days	48% increase	Baxter et al., 1994
7	*Lolium perenne*-Root (C_3)	One growing season	48% increase	Jongen et al., 1995
8	*Panicum laxum* (C_3)	1.5 months (low light/high light)	Increased by 1.41 fold in low light. Increased by 1.71-fold in high light.	Ghannoum et al., 1997
9	*Panicum antidotale* (C_4)	1.5 months (low light/high light)	Increased by 1.28 fold in high light	Ghannoum et al., 1997
10	*Triticum aestivum* (C_4)	2 growing seasons	Increased	Oijen et al., 1999
11	*Agrostis capillaries* (C_3)	2 years (low & moderate nutrient supply)	Decreased by 23% at low nutrient supply. Decreased by 16% at moderate nutrient supply.	Davey et al., 1999
12	*Lolium perenne* (C_3)	2 years (low & moderate nutrient supply)	Decreased by 29% at low nutrient supply. Decreased by 17% at moderate nutrient supply.	Davey et al., 1999
13	*Bouteloua gracilis* (C_4)	7 months	Increased	Morgan et al., 2001
14	*Pascopyrum smithii* (C_3)	7 months	Increased	Morgan et al., 2001
15	*Agrostis stolonifera* (C_3)	65 days	No significant change	Goverde et al., 2002
16	*Anthoxanthum odoratum* (C_3)	65 days	Increased	Goverde et al., 2002
17	*Festuca rubra* (C_3)	65 days	Increased	Goverde et al., 2002
18	*Poa pratensis* (C_3)	65 days	No significant change	Goverde et al., 2002
19	*Saccharum officinarum* (C_4)	50 weeks	40% increase	De Souza et al., 2008
20	*Panicum maximum* (C_4)	2 growing seasons	Increased	Bhatt et al., 2010
21	*Agrostis stolonifera* (C_3)	84 days	35% increase	Burgess & Huang, 2014
22	*Poa pratensis* (C_3)	2 weeks (temperature)	Increased	Song et al., 2014
23	*Urochloa brizantha* (C_4)	75days (temperature)	54.5% increase	Faria et al., 2018
24	*Megathyrsus maximus* (C_4)	75 days temperature	56.7% increase	Faria et al., 2018
25	*Stipa baicalensis* (C_3)	3 months	Increased	Wang et al., 2019

+, increase; -, decrease; NS, change is not significant

The biomass of grasses is a major sink of atmospheric carbon. Likewise, 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). The long-term sequestration of atmospheric carbon in soil is facilitated by grasses with rapid growth. A square meter of vetiver grass can store one kilogram of atmospheric carbon each year (Pinners, 2014). Fisher et al. (1994) estimated the carbon sequestration potential of deep-rooted grasses in South American savannas and reported sequestration of 100-507 Mt carbon per year. In their study, Khan et al. (2006) examined the biomass potential of perennial grass species in Pakistan's Cholistan desert. They found that each year, *Panicum antidotale* and *Cenchrus ciliaris* sequestered 10.8 and 10 tones per hectare of carbon, respectively. **Table 2.2** shows the potential of various grasslands to sequester carbon that has been previously documented by the several authors (From year 2006 to 2022).

Table 2.2: Carbon sequestration potential of various grasslands

Sl No	Grassland type	Carbon sequestration (tons ha^{-1} $year^{-1}$)*	References
1	Calcareous grassland dominated by *Bromus erectus* Huds.	0.9	Niklaus & Falloon, 2006
2	Bioenergy crops include herbaceous bunch-type grasses like switchgrass (*Panicum virgatum* L.), elephant grass (*Pennissetum purpureum* Schum.), tall fescue (*Fetusca arundinacea* L.), etc.	0.34	Lemus & Lal, 2005
3	Temperate Pastures in central Pennsylvania, dominated by a mixture of cool-season grasses	0.19	Skinner, 2008
4	Grassland	5 ± 0.3	Soussana et al., 2010
5	Turf grass systems	1	Guertal, 2012
6	Mediterranean annual grasslands	1.1×10^{-3}	DeLonge et al., 2013
7	World grasslands	0.2	Lorenz, 2013
8	World grassland soils	2.9×10^{-3} to 8.6×10^{-2}	Ghosh & Mahanta, 2014
9	European arable soils dominated by grasses	0.4 to 0.8	Lugato et al., 2014
10	Pasture systems in arid northwestern India, dominated by ms (*Cenchrus ciliaris* and *Cenchrus seteger*)	2.96	Mangalassery, 2014
11	World grassland soils	2.9×10^{-3} to 8.6×10^{-2}	Nair et al., 2015
12	Pastures in the western Brazilian Amazon, dominated by *Brachiaria humidicola* (Rendle)	0.56	Costa et al., 2016
13	Water Meadows field dominated by wheat and Long	0.5	Goseling, 2017

	Close field dominated by Rye grass (*Lolium* spp.)		
14	Urban turf grasses	2.6×10^{-3}	Shchepeleva et al., 2017
15	World grassland soils	0.5 to 2	Ussiri et al., 2017
16	World grassland soils	1.4×10^{-1}	Lorenz & Lal, 2018
17	Olive orchards dominated by *Brachypodium distachyon*	2.56 [1]	Repullo et al., 2018
18	World grassland soils	2.9×10^{-3} [-1] to 8.6×10^{-2}	Gakaev, 2022

*Tons of carbon sequestration by grassland per hector per year

It is clear from various literature that grasslands have a greater potential for carbon storage. The approximate average carbon sequestration by various grasslands from the above data is 0.9 tons ha^{-1} $year^{-1}$. Moreover up to $8.6x10^{-2}$ tons ha^{-1} $year^{-1}$ carbon sequestration was exhibited by various grasslands represented in the above table. Changes in moisture relationships have a key role in grassland productivity (Ownesby et al., 1993). Previous studies revealed that elevated CO_2 significantly stimulated plant growth even at water-limited conditions (Gifford & Morison, 1985; King & Greer, 1986; Samarakoon & Gifford, 1995; Owensby et al., 1996). According to Pritchard et al. (1999), stimulation of cell wall-related genes at elevated CO_2 upholds enhanced cell production and cell elongation. This view is a possible explanation for biomass increase at elevated CO_2. Temperature is yet another factor, which defines growth at elevated CO_2. Growth and storage of carbohydrates are limiting at low temperatures, however, an increase in temperature creates a positive response towards growth at elevated CO_2. Studies by Oliveira et al. (2012), reported accumulation of biomass and enhanced grain yield in wheat under elevated CO_2, combined with high-temperature treatment. The same authors imply that the combination of high temperature and elevated CO_2 will lessen the awful effects of terminal drought.

Even though reports on biomass responses of grasslands are there, the impacts of elevated CO_2 on individual morphology of grasses at experimental setup have not received much attention in research to date. In the current study, eight morphological traits were assessed and examined under conditions with elevated CO_2, as morphological features have a greater influence on the physiological and metabolic processes associated with plants. Enhancement in plant morphology will give insights into the overall development pattern of grass species in elevated CO_2 environments.

2.2.2 Elevated carbon dioxide - intercellular CO_2 concentrations and stomatal conductance

Elevated carbon dioxide levels change intercellular CO_2 concentrations (Ci) and stomatal conductance (g_s), which in turn affects how grasses respond physiologically. Gaining an understanding of these effects is crucial to improving plant development and adaptability to shifting environmental circumstances. Stomatal responses occur due to the intensity of intercellular CO_2 concentration, preceded by mesophyll CO_2 demands (Mott, 1988). With the rise in atmospheric CO_2, Ci increases (Kirkham et al., 1991). C_3 plants have a higher Ci than C_4 plants. C_4 plants maintain a lower Ci due to its CO_2 concentrating mechanism with a special arrangement of bundle sheath cells and mesophyll cells (kranz anatomy). Few studies on C_3 and C_4 grasses measured Ci and an increase is evident in both cases, while stomatal conductance decreased. From the literature, it is evident that grasses undergone experimentation under elevated CO_2 so far showed a decreased range of stomatal conductance, between 35-55% (**Table 2.3**). Previous reviews on plant CO_2 responses support this view (Curtis & Wang, 1998; Wand et al., 1999; Ainsworth et al., 2002; Ainsworth & Rogers, 2007; Aranda et al., 2020). In elevated CO_2 environments, plants often lower their stomatal conductance to prevent water loss and retain enough CO_2 uptake for photosynthesis (Manderscheid et al., 2016). A feedback mechanism works to decrease stomatal conductance under elevated CO_2 in most plants. It has been implicit that guard cells sense the concentration of CO_2 in the intercellular spaces, and as the mesophyll requirement for CO_2 increases, Ci decreases, causing stomatal opening and increasing Ci (Mott, 1988). Depolarization of the guard cell membrane is the main requirement for stomatal closure (Assmann, 1999). At elevated CO_2, depolarization happens to a larger extent, which leads to reduced stomatal aperture (Ainsworth & Rogers, 2007). Reduced stomatal conductance shown by plants at long-term elevated CO_2 exposure is ascribed to the changes in stomatal aperture or stomatal index or to stomatal density (Ainsworth & Rogers, 2007).

2.2.3 Elevated carbon dioxide and photosynthetic responses in C_3 / C_4 pathways

Photosynthetic responses of plants are considered an important measure of carbon capture efficiency in CO_2 sequestration studies. Different photosynthetic pathways found in grass species is indicative of their ability to adapt to different

conditions. For grass species, the C3 and C4 pathways are the main pathways for photosynthesis (Morgan et al., 1980). Each pathway shows distinct environmental adaptations that impact productivity and growth. Many species of grass, especially those found in temperate climates, use the C3 pathway, whereas the C4 system has evolved modifications that maximize photosynthesis in warm, arid environments (Christin et al., 2013). The enzyme Ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) catalyzes a single metabolic process in C3 plants to fix carbon to produce a three-carbon molecule. On the other hand, C4 plants use a complex method that uses mesophyll and bundle sheath cells, two different kinds of cells. When temperatures and light intensities are high, this route facilitates a more effective carbon fixation process, reducing photorespiration and increasing productivity (Christin et al., 2013).

A majority of previous studies proved that augmentation in CO_2 levels leads to increased photosynthetic rate (Ziska et al., 1999; Aranda et al., 2020). A comparative evaluation of the CO_2 elevation experiment conducted in *Poa pratensis* and *Andropogon gerardii* (Kirkham et al., 1991) reveals a rise in the photosynthetic rate in *Poa pratensis* (C_3) owing to the domination of carboxylation activity of RuBisCO enzyme under high CO_2 environment. RuBisCO catalyzes the oxygenation of Ribulose-1, 5-bisphosphate (RubP), a reaction that is competitively inhibited by CO_2 (Drake et al., 1997). This phenomenon eliminates energy-losing photorespiration under a doubled CO_2 environment in C_3 plants and growth is stimulated to a range of 40-45%, whereas in C_4 plants, growth only to a range of 10-20% (Ghannoum et al., 2000). Thus CO_2 elevation is more advantageous to C_3 plants than C_4 (Waggoner, 1984).

Contrary to the C_3 advantage, a positive photosynthetic response is shown by C_4 grasses. Arid environments with temperature ascending and limited nutrients favor C_4 plants and eventually exhibit positive responses at CO_2 elevation (Ghannoum et al., 2000; Sage & Kubein, 2003). Even though the CO_2 concentrating mechanism makes C_4 plants insensitive to elevated CO_2, superior photosynthetic nitrogen use efficiency (Sage & Kubein, 2003) is advantageous to them at elevated CO_2 than C_3 plants. RuBisCO accounts for about 30% of leaf nitrogen content in C_3 plants, while only 4 - 21% in C_4 species (Sage et al., 1987; Evans & Von Caemmerer, 2000). 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). In the case of C_3 plants like *Panicum laxum* and *Arundo donax*, reduction in assimilation is attributed to decreased RuBisCO activity (Ghannoum et al., 1997). Photosynthetic acclimatization and downregulation reported for long-term exposure of plants to elevated CO_2 are attributed to this decline in RuBisCO activity (Fredeen et al., 1995; Sicher & Bunce, 1997). Enhanced cellular carbohydrate levels may down-regulate genes transcribing RuBisCO and other photosynthetic proteins (Stitt, 1991; Krapp et al., 1991). Another observation states that an increased requisite of ATP (required for RuBP regeneration) for increased carbon fixation before CO_2 elevation results in a decline in RuBisCO activation state. Such reduction was also observed in previous studies (Sage et al., 1988; Cen & Sage, 2005). Besides augmented levels of CO_2, nutrient conditions and other factors of microenvironmental conditions such as temperature, light intensity, and treatment duration also influence the photosynthetic responses of grasses. **Table 2.4** represents published data from earlier research, such as the duration of time that different grass species were grown in elevated CO_2 and the percentage changes in the rate of photosynthesis (Pn).

Table 2.3: Changes in intercellular CO_2 concentration (Ci) and stomatal conductance (g_s) of grass species at elevated CO_2

Sl. No.	Plant species	Duration of elevated CO_2 & supplementary treatments	Ci	g_s	References
1	*Andropogon gerardii* (C_4)	40 days	180% increase	N.st	Kirkham et al., 1992
2	*Poa pratensis*(C_3)	40 days	67.79% increase	N.st	Kirkham et al., 1992
3	*Andropogon gerardii* (C_4)	2 growing seasons one with water stress	N.st	52% decrease	Knapp et al., 1993
4	*Andropogon gerardii* (C_4)	6 months	N.st	54.4% decrease	Bremer, 1996
5	*Sorghastrum nutans* (C_4)	6 months	N.st	39.6% decrease	Bremer,1996
6	*Panicum laxum* (C_3)	1.5 months (low light/high light)	N.st	50% decrease	Ghannoum et al., 1997

7	*Panicum antidotale* (C_4)	1.5 months (low light/high light)	N.st	50% decrease	Ghannoum et al., 1997
8	*Saccharum officinarum* (C_4)	50 weeks	N.st	37% decrease	De Souza et al., 2008
9	*Panicum maximum* (C_4)	2 growing seasons	Increased	Increased	Bhatt et al., 2010
10	*Agrostis stolonifera* (C_3)	84 days		40% decrease	Burgess & Huang., 2014
11	*Arundo donax* (C_3)	78days (28days drought)	Increased	Decreased	Nackley et al., 2014
12	*Calomagrostis arundinacea* (C_3)	3years	N.st	Decreased	Klem et al., 2018
13	*Urochloa brizantha* (C_4)	75 day (temperature)	N.st	Increased	Faria et al., 2018
14	*Megathyrsus maximus* (C_4)	75 days (temperature)	N.st	Increased	Faria et al., 2018
15	*Cenchrus pedicellatus* (C_4)	6 8days (wet & dry condition)	N.st	40% decrease	Tom Dery et al., 2018
16	*Stipa baicalensis* (C_3)	3 months	Increase from 72.3% to 129.6%	N.st	Wang et al., 2019

N.st, not studied

Table 2.4 Changes in photosynthetic rate (Pn) of various grass species at elevated CO_2

Sl. No.	Plant species	Duration of elevated CO_2 & supplementary treatments in brackets	Pn	References
1	*Andropogon gerardii* (C_4)	40 days	8.75% increase	Kirkham et al., 1992
2	*Poa pratensis* (C_3)	40 days	141% increase	Kirkham et al., 1992
3	*Lolium perenne* (C_3)	49 days	50% increase	Ryle et al., 1992
4	*Andropogon gerardii* (C_4)	2 growing seasons one with water stress	Increased	Knapp et al., 1993
5	*Agrostis capillaris* (C_3)	79 days	Increased	Baxter et al., 1994
6	*Avena barbata* (C_3)	One growing season	20% increase	Jackson et al., 1995
7	*Panicum laxum* (C_3)	1.5 months (low light/high light)	18% increase in low light	Ghannoum et al., 1997
8	*Panicum antidotale* (C_4)	1.5 months (low light/high light)	10% increase in high light	Ghannoum et al., 1997

9	*Triticum aestivum* (C_4)	2 growing seasons	30% increase	Oijen et al., 1999
10	*Agrostis capillaries* (C_3)	2 years (low & moderate nutrient supply)	38% increase at low nutrient supply. 12% increase at moderate nutrient supply.	Davey et al., 1999
11	*Pascopyrum smithii* (C_3)	2 years (low & moderate nutrient supply)	No significant change	Davey et al., 1999
12	*Bouteloua gracilis* (C_4)	7 months	Increased	Morgan et al., 2001
13	*Pascopyrum smithii* (C_3)	7 months	Increased	Morgan et al., 2001
14	*Saccharum officinarum* (C_4)	50 weeks	30% increase	De Souza et al., 2008
15	*Panicum maximum* (C_4)	2 growing seasons	53% increase	Bhatt et al., 2010
16	*Agrostis stolonifera* (C_3)	84 days	21% increase	Burgess & Huang., 2014
17	*Arundo donax* (C_3)	78 days (28 days drought)	Decreased but not significant	Nackley et al., 2014
18	*Poa pratensis* (C_3)	2 weeks(temperature)	Increased	Song et al., 2014
19	*Calomagrostis arundinacea* (C_3)	3years	Increased	Klem et al., 2017
20	*Urochloa brizantha* (C_4)	75 days (temperature)	Increased	Faria et al., 2018
21	*Megathyrsus maximus* (C_4)	75 days (temperature)	Increased	Faria et al., 2018
22	*Stipa baicalensis* (C_3)	3 months	Increase from 93.4% to 158%	Wang et al., 2019

2.2.4 Elevated carbon dioxide - transpiration rate and water use efficiency

Various species of grasses respond differently to elevated carbon dioxide (CO_2) concentrations, which have important effects on transpiration rates (E) and water usage efficiency. Elevated CO_2 experiments in grass species show a decrease in transpiration rates which is attributed to increased chamber temperature (Bhatt et al., 2010). Declining transpiration is a stress protection method that limits water loss and minimizes damage linked with desiccation. Plants that perform better photosynthesis in an environment with higher levels of carbon dioxide may close their stomata to limit water loss while still producing sufficient photosynthetic activity, which lowers transpiration rates (Tom-Dery et al., 2018). It was previously mentioned that grass

species with higher CO_2 levels have reduced stomatal conductance. Plants and ecosystems under drought conditions experience greater WUE (Water Use Efficiency) (Field et al., 1997; Arp et al., 1998). Increased WUE is also said to be a result of morphological adjustments rather than stomatal (Norby & O'Neill, 1991) under elevated CO_2. Studies indicate that crop plants grown at higher CO_2 environments have larger and highly branched root systems, which increases the capacity for resource acquirement, but at lesser efficiency (Pritchard & Rogers, 2000). Day et al. (1996) observed higher root production towards nutrient-available surfaces and water-available depths in a sandy nutrient-poor Oak-palmetto system. Upon review of the literature on root growth under elevated CO_2, Wullschleger et al. (2002) found that elevated CO_2 increases root growth and leads to enhanced water uptake and improved water balance, thus helping to evade water deficits. Yet when soil moisture is sufficient to meet transpiration loss of water, the CO_2 effect on root volume is seemingly extraneous, thus this mechanism could be detected only under specific conditions (Wullschleger et al., 2002). Since the rise in atmospheric CO_2 usually upholds WUE, there will be a tendency for plants to tolerate draught in the future (Beerling et al., 1996). The magnitude of the responses of various grass species regarding transpiration rates and water use efficiency is depicted in **Table 2.5.**

Table 2.5: Changes in transpiration rate (E) and water use efficiency (WUE) of various grass species at elevated CO_2

Sl. No.	Plant species	Duration of elevated CO_2 & supplementary treatments	E	WUE	References
1	*Andropogon gerardii* (C_4)	40 days	54% decrease	41.6% increase	Kirkham et al., 1992
2	*Poa pratensis* (C_3)	40 days	7% decrease	158% increase	Kirkham et al., 1992
3	*Lolium perenne* (C_3)	49 days	N.st	N.st	Ryle et al., 1992
4	*Andropogon gerardii* (C_4)	6 months	18% decrease	N.st	Bremer, 1996
5	*Sorghastrum nutans* (C_4)	6 months	22% decrease	N.st	Bremer, 1996
6	*Saccharum officinarum* (C_4)	50 weeks	N.st	62% increase	De Souza et al., 2008
7	*Panicum maximum* (C_4)	2 growing seasons	Increased	Increased	Bhatt et al., 2010
8	*Agrostis stolonifera* (C_3)	84 days	40% decrease	30% increase	Burgess & Huang, 2014

9	*Arundo donax* (C_3)	78days(28days drought)	100% decrease	Increased	Nackley et al., 2014
10	*Calomagrostis arundinacea* (C_3)	3years	Decreased	Increased	Klem et al., 2017
11	*Urochloa brizantha* (C_4)	75days (temperature)	N.st	Increased	Faria et al., 2018
12	*Megathyrsus maximus* (C_4)	75 days (temperature)	N.st	Increased	Faria et al., 2018
13	*Stipa baicalensis* (C_3)	3 months	N.st	87.2% increase	Wang et al., 2019

N.st, not studied

2.2.5 Elevated carbon dioxide and total non-structural carbohydrates

Elevated CO_2 levels encourage a proportionate rise in carbon availability, which builds up on total nonstructural carbohydrates and secondary metabolites that contain carbon (Faria et al., 2018). The principal non-structural carbohydrates present in leaves are total soluble sugars and starch. In grasses, photosynthesis usually increases with elevated CO_2 levels, increasing the amount of carbohydrates produced. Several previous investigations revealed that augmented levels of soluble sugars and starch contents in leaves are due to increased assimilation rates (Delucia et al., 1985; Long & Drake, 1992; Moore et al., 1997; Teng et al., 2006). In a study on the physiological, and biochemical responses of three grass species to elevated CO_2, Zheng et al. (2017) established that the enzyme RuBisCO functions better at increased CO_2 concentrations, and is primarily responsible for this improved carbon fixation. Consequently, grasses can transform a greater amount of atmospheric carbon into compounds that are high in energy, which increases plant growth and non-structural carbohydrate levels. The resource allocation of plants is affected by elevated CO_2. Moreover, grasses may allocate more resources to carbon storage in the presence of elevated carbon availability as opposed to other uses, such as growth or reproductive development (Zheng et al., 2017).

Growth and development of grasses thrive with an accumulation of non-structural carbohydrates; which are also related to environmental stress tolerance (Moraes et al., 2013). Elevated CO_2 exposure creates a considerable increase in soluble sugar and starch content (Teng et al., 2006). Elevated CO_2-based increase in sugar levels may also be due to an indirect effect of declined nitrogen concentrations

leading to lowered respiration rates (Oijen et al., 1999). Storage of non-structural carbohydrates such as starch and fructans is common among some grasses (Morvan-Bertrand et al., 2001; Xue et al., 2009). Temperate climate grasses usually store carbohydrates as fructans (Halford et al., 2011). Fructans are formed from imported sucrose and the site of synthesis is vacuolar lumen (Pollock et al., 2003). A limited number of grasses use starch as a carbohydrate storage pool and is synthesized in plastids or amyloplasts (Slewinski, 2012). The storage of carbohydrate polymers increases the sequestration capability of grasses. Drought stress tolerance of non-structural carbohydrates is hypothesized by previous authors (Moraes et al., 2013). The low hydraulic conductance of grass stems due to the accumulation of soluble sugars facilitates easy conductance of water from soil to plant parts (Fu et al., 2011). Along with carbon sequestration capability, this adaptation for drought tolerance will also be advantageous for grasses in facing future environmental challenges. Previously reported elevated CO_2 experimental details and responses of various grass species regarding total non-structural carbohydrates (TNC) were depicted in **Table 2.6**.

Table 2.6: Changes in total non-structural carbohydrates of various grass species at elevated CO_2

Sl. No.	Plant species	Duration of elevated CO_2 & supplementary treatments	TNC	References
1	*Agrostis capillaris* (C_3)	79 days	Increased	Baxter et al., 1994
2	*Festuca vivipara* (C_3)	189 days	Increased	Baxter et al., 1994
3	*Lolium perenne*-Root (C_3)	One growing season	46.9% increase	Jongen et al., 1995
4	*Panicum laxum* (C_3)	1.5 months (low light/high light)	No significant change	Ghannoum et al., 1997
5	*Panicum antidotale* (C_4)	1.5 months (low light/high light)	No significant change	Ghannoum et al., 1997
6	*Triticum aestivum* (C_4)	2 growing seasons	Increased	Oijen et al., 1999
7	*Agrostis stolonifera* (C_3)	65 days	Increased	Goverde et al., 2002
8	*Anthoxanthum odoratum* (C_3)	65 days	Increased	Goverde et al., 2002
9	*Festuca rubra* (C_3)	65 days	Increased	Goverde et al., 2002

10	*Poa pratensis* (C_3)	65 days	Increased	Goverde et al., 2002
11	*Poa pratensis* (C_3)	2 weeks (temperature)	38% increase at 25-35°C	Song et al., 2014
12	*Urochloa brizantha* (C_4)	75 days (temperature)	Increased	Faria et al., 2018
13	*Megathyrsus maximus* (C_4)	75 days (temperature)	Increased	Faria et al., 2018

2.2.6 Elevated carbon dioxide and nitrogen concentration in plant parts

Several physiological and biochemical factors influence the complicated mechanism of reduced nitrogen availability in plants exposed to elevated CO_2. These mechanisms may include modifications to soil nitrogen dynamics, adjustments to plant nitrogen metabolism, and adjustments to resource allocation brought on by enhanced carbon assimilation. A reduction in total leaf nitrogen content has been reported in early experiments by Davey et al. (1999). Formerly Cotrufo et al. (1998) reviewed experiments regarding elevated CO_2 effects on nitrogen concentration of plants including grasses and found a statistically significant decline in nitrogen concentration in plants grown under elevated CO_2 than control. Due to increased assimilation at elevated CO_2, up to 30% of leaf nitrogen is transported to RuBisCO (Evans, 1989). At elevated CO_2, a major portion of leaf nitrogen is reallocated to other photosynthetic and non-photosynthetic processes (Sage, 1994). The actual mechanism responsible for the reduction of nitrogen under elevated CO_2 is not yet established; however, there are substantial hypotheses explaining this phenomenon (Taub & Wang, 2008). The same authors opined that increased carbohydrate levels and decreased nitrogen uptake as key mechanisms affecting plant nitrogen concentration at elevated CO_2. The same study states that the plants with high WUE possess morphological, allocational, and physiological root traits, thus there is a low transpiration-mediated nitrogen supply, compared with the rate of carbon accumulation. There are further reports on the inhibition of nitrogen uptake and transport under CO_2 elevation due to reduced transpiration rates (Correia et al., 2005; Taub, 2010; Jauregui et al., 2016; Igarashi et al., 2021). A recent report (Padhan et al., 2020) illustrates the genetic mechanisms behind nitrogen reduction under elevated CO_2. Their CO_2 elevation experiment on bread wheat had shown the downregulation of ammonia assimilating genes and upregulation of reactive oxygen species at CO_2 enrichment. **Table 2.7** shows the percentage change in nitrogen

concentration of several species of grass under elevated CO_2 treatment recorded during the last years.

Table 2.7: Changes in nitrogen concentration at elevated CO_2

Sl. No.	Plant species	Duration of elevated CO_2 & supplementary treatments	N	References
1	*Lolium perenne*(C_3)	49 days	Decreased	Ryle et al., 1992
2	*Agrostis capillaris* (C_3)	79 days	No significant change	Baxter et al., 1994
3	*Poa alpinia* (C_3)	105 days	No significant change	Baxter et al., 1994
4	*Festuca vivipara* (C_3)	189 days	Decreased	Baxter et al., 1994
5	*Avena barbata* (C_3)	One growing season	25% decrease	Jackson et al., 1995
6	*Lolium perenne*–Root (C_3)	One growing season	Decreased	Jongen et al., 1995
7	*Panicum laxum* (C_3)	1.5 months (low light/high light)	No significant change	Ghannoum et al., 1997
8	*Panicum antidotale* (C_4)	1.5 months (low light/high light)	No significant change	Ghannoum et al., 1997
9	*Triticum aestivum* (C_4)	2 growing seasons	Decreased	Oijen et al., 1999
10	*Bouteloua gracilis* (C_4)	7 months	Decreased	Morgan et al., 2001
11	*Pascopyrum smithii* (C_3)	7 months	Decreased	Morgan et al., 2001
12	*Agrostis stolonifera* (C_3)	65 days	Decreased	Goverde et al., 2002
13	*Anthoxanthum odoratum* (C_3)	65 days	Decreased	Goverde et al., 2002
14	*Festuca rubra* (C_3)	65 days	Decreased	Goverde et al., 2002
15	*Poa pratensis* (C_3)	65 days	Decreased	Goverde et al., 2002
16	*Stipa baicalensis* (C_3)	3 months	9.7% decrease	Wang et al., 2019

2.2.7 Soil carbon storage and changes in soil properties under elevated CO_2 environment

Elevated CO_2 levels have a significant impact on grass soils. These changes primarily influence soil microbial populations (Cheng et al., 2011), nutrient cycling, carbon sequestration, and ecosystem function. Understanding these modifications is critical for estimating the impact of climate change on grassland ecosystems. Increased atmospheric CO_2 can increase net primary productivity (NPP), resulting in more organic matter in the soil and influencing the chemical makeup and nutrient dynamics of the soil ecosystem (Polley et al., 2019). For example, studies show that increasing biomass due to elevated CO_2 levels may alter nitrogen (N) and phosphorus (P) dynamics. Savannas and grasslands have a much influential role in carbon and nutrient cycles. Grass species have large, fibrous root systems that makeup about 60–80% of the total carbon in grassland ecosystems. These roots can grow several meters deep, adding a lot of organic carbon to the soil. This not only helps store carbon but also boosts soil fertility by building up organic matter (Meunier & Sutheimer 2022). Compared to tree species, grasses are often more resilient to environmental disturbances. Their capacity to quickly regenerate following grazing or fire contributes to the maintenance of their biomass and, in turn, their soil organic carbon contributions. After disturbances, grasses can regenerate and eventually improve soil carbon storage (Meunier & Sutheimer 2022). Globally soils are projected to have a sequestration potential of 0.4 to 1.2 Gt annually, of which 0.01 to 0.30 Gt come from grasslands (Ghosh & Mahanta, 2014). An estimated 194 GT of C, or about 8% of the world's SC, is stored in grassland soils worldwide (Jansson et al., 2010). Further, the grass species influences the global land-atmosphere energy balance (Mishra & Young, 2020).

Identifying the most effective grass species for carbon sequestration and climate mitigation requires understanding how grasses respond to elevated levels of CO_2. By analyzing the relative responses of morphological, biomass, biochemical, and associated soil characteristics to elevated CO_2, species that are better suited for carbon storage and resilience in changing climate could be identified. In this context, relative responses of grass species such as *Megathyrsus maximus, Saccharum arundinaceum*, *Cymbopogon flexuosus*, *Chrysopogon zizanioides*, *Arundo donax,* and *Pennicetum pedicellatum* to elevated levels of carbon dioxide are studied and explained in detail.

2.3. MATERIALS AND METHODS

All matters concerning the design of the experimental system, including the installation of chambers, and the facilities associated with them are explained in detail in Chapter I. The selection of grass species and the nursery trials for their multiplication and maintenance up to the desired growth stage for experimentation were also explained. The details regarding experimentation with the grass species and the stages of assessment of the microclimatic conditions together with the assessment of the CO_2 flux with and without plants (standardization studies) were also discussed in Chapter I. The present chapter (Chapter II) is a continuation of Chapter I where attempts have been made to evaluate the changes in growth and biochemical attributes of plants that have been subjected to elevated CO_2 levels. This chapter also deals with the modifications brought about by carbon enrichment in the soil properties related to the plants. Below is a summary of the experimental procedure undertaken.

As stated in Chapter I, six grass species, ie. *Megathyrsus maximus, Saccharum arundinaceum, Cymbopogon flexuosus, Chrysopogon zizanioides, Arundo donax,* and *Pennicetum pedicellatum* were selected for experimentation and were multiplied and grown for 6-7 months. For each study, 2 sets of 3 plants were selected and one set was maintained in the control chamber (CC) and the other in the treatment chamber (TC). Both the chambers were sealed from the outside to prevent the exchange of air. Ambient air was pumped into the CC in the morning (9 a.m.). Similar to this, an elevated CO_2 concentration was ensured in TC by supplying the CO_2-air mixture for about 15 minutes at the same time (9 a.m.). Using an automated CO_2 analyzer (Fuji Electric NDIR type Infrared Gas Analyzer), the CO_2 concentration in the CC as well as the TC was monitored in the morning (9 a.m.) and evening hours (6 p.m.). The temperature (^oC) and humidity (%) in the chambers were also recorded (Billion Bag digital wireless electronic Hygrothermometer) in the morning and evening hours. The amount of CO_2 retained in the chamber in the evening was subtracted from the amount of CO_2 in the morning to determine the day flux of CO_2. The experiment was continued and on the next day (9 a.m.), the night flux was computed by deducting the amounts of CO_2 that was remaining in the chamber on the next morning from the amount that was maintained in the previous day's evening. Assessments were repeated for 15 days. Standardization studies were also carried out in empty chambers with the same

procedure. The results of the experiments are discussed and conclusions are drawn in chapter I. This chapter (Chapter II) deals with the changes in growth and biochemical attributes associated with the plants and the changes in soil properties brought about by them after experimentation.

The growth measurements were carried out on the first and final day of experimentation. The leaf metabolites, minerals, and nutrients were analyzed on 1, 5, 10, and 15 days of experimentation. The growth attributes 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. The biochemical parameters analyzed include pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids), metabolites (carbohydrate, protein, and phenol), and plant nutrients (carbon, nitrogen, calcium, magnesium, sodium, and potassium). The soil characteristics studied include pH, moisture, total organic carbon (TOC), and nitrogen. **Figure 2.1** shows the outline of the experiment. Experimental trials with plant spices are depicted in **Figure 2.2**.

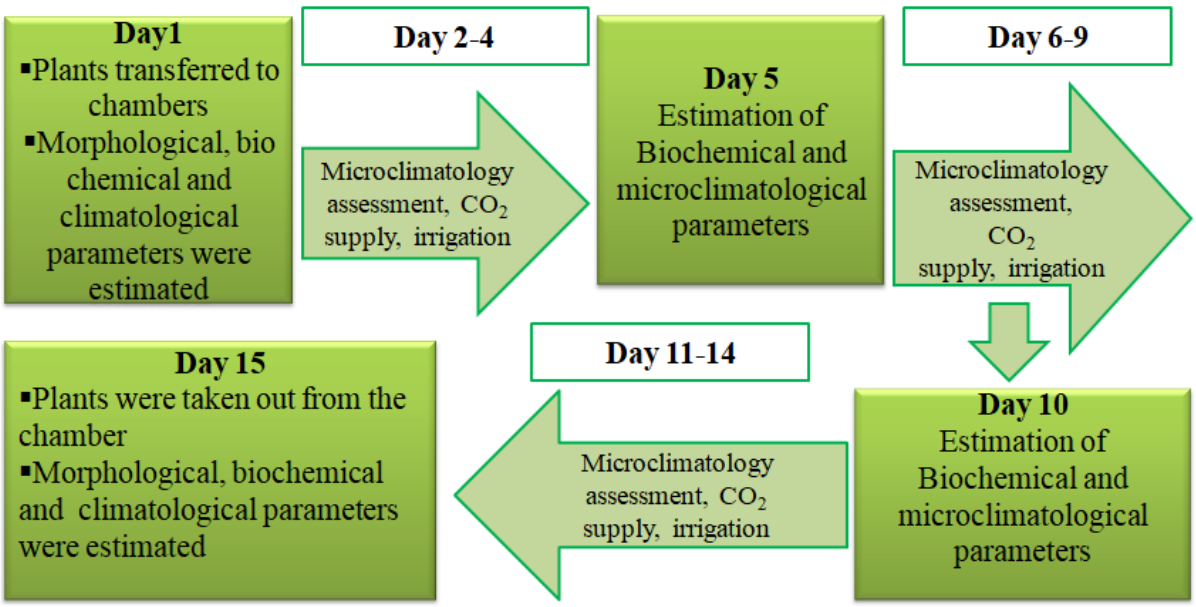


Figure 2.1: Outline of the CO_2 enrichment experiment on grass species conducted for 15 days

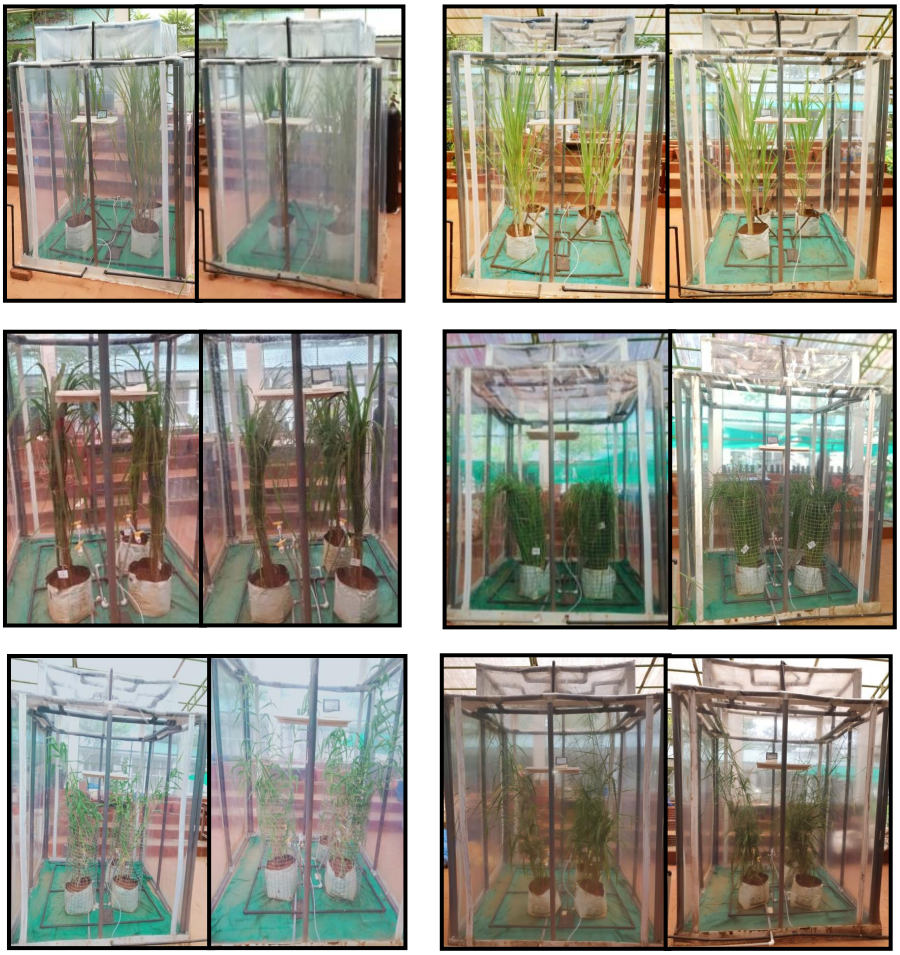


Figure 2.2. Experimental setup for various grass species

(As outlined in Chapter I)

2.3.1 Measurement of growth attributes under controlled experimental conditions

Plant height and Tiller height

Plant height of *Megathyrsus maximus*, *Saccharum arundinaceum*, *Cymbopogon flexuosus*, *Chrysopogon zizanioides*, *Arundo donax* and *Pennicetum pedicellatum* were assessed at two stages of experimentation, one on the initial day (1DOT) before introducing the plant into the chambers and the other on the final day of treatment (15DOT). Plant height (cm) was assessed from the level of soil to the tip of the tallest blade, using a measuring tape. Above ground, below ground, and the total height of all six plants were recorded. Mean values were then calculated.

A tiller is a shoot that emerges from the base of the grass plant. Above ground portion of the tiller is measured from the soil surface to the highest point of the tiller

that is to the tip of the tallest blade. The belowground portion, including roots and any part of the tiller that may be underground, is not included in the tiller height measurement.

Number of tillers

The number of tillers was calculated manually by counting the total number of tillers in the plant. Newly emerged small tillers are also considered for counting.

Number of leaves

The number of leaves was calculated by counting the total number of leaves in the plant. Both the young and old leaves of the plants were considered for counting.

Leaf length

Small, medium, and large-sized leaves of plants were selected and marked. The length of the leaf (cm) was measured from end to end. The mean values were calculated from the small, medium, and large leaves.

Leaf breadth

The breadth of the selected leaves of plants (small, medium, large) was measured from tip to tip at the widest portion of the lamina. The breadth of leaves is measured in such a way that the measurement from the widest portion and the middle of the leaf is taken. The mean values are then calculated.

Leaf area

For the calculation of leaf area Kemp's formula (Kemp, 1960) is used. Leaf area (m^2) was calculated using leaf length, breadth, number of leaves, and leaf area constant/Kemp's constant. The mean values were calculated and expressed in m^2. Kemp's formula is as follows:

Leaf area = leaf length x leaf breadth x K

K= Kemp's constant, it is 0.9 for monocots

Culm diameter

The culm is the main stem of the grass plant. Culm diameter (cm) was measured from the base of the culm from all the plants using a screw gauge. Reading in screw gauge was taken by observing the main scale reading and the circular scale reading. Reading in a circular scale is calculated from the number of divisions in a circular scale and the least count of screw gauge (0.001cm). The total reading is calculated by adding the main scale and circular scale reading.

Plant biomass

To estimate the plant biomass for 1DOT, one set from the ambient condition was uprooted, washed, cleaned, and blotted. The above-ground and below-ground portions were separated and the fresh weight was determined. For the dry weight measurement, biomass samples were dried in an oven set to a constant temperature (around 60-70°C) until the samples reached a constant weight. Total biomass was calculated from the above-ground and below-ground weights of plants. On the final day of experimentation (15DOT), the representative plants from the control and treatment chambers were uprooted, and the same procedures were repeated. The total biomass of the control and treated plants was calculated from the measurements of above-ground and below-ground portions of the plant (**Figure 2.3**).

Derivation of a formula for gram CO_2 uptake per gram of plant biomass

To know the efficiency of each grass species for CO_2 uptake relative to their biomass, gram CO_2 uptake per gram dry weight of plant biomass was calculated for the plants in the CO_2-treated chamber. A formula was derived for the calculation of gram CO_2 uptake per gram of plant biomass as follows (specifically for closed chamber studies).

To calculate the amount of CO_2 uptake per gram of biomass in the chamber, a formula is derived using the variables such as:

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

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

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.

The following assumptions were made for the derivation of the formula for the amount of CO_2 uptake per gram of biomass (in grams of CO_2 per gram of biomass).

- *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. 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).

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

- *The molecular weight of CO_2 is 44 g / mol*

***Derivation**:*

1. Convert CO_2 concentration from ppm to volume in the chamber:

The volume of CO_2 (vCO_2) in the chamber in cubic meters is:

$$vCO_2 = \frac{C}{10^6} \times V\ m^3$$

2. Convert the volume of CO_2 to moles:

Using the ideal gas law at STP, 1 mole of any gas occupies 22.4 L = 0.0224 m^3.

The number of moles of CO_2 (nCO_2) in the chamber is:

$$nCO_2 = \frac{Volume\ of\ CO_2}{0.0224} = \frac{\frac{C}{10^6} \times V\ m^3}{0.0224} \text{ moles}$$

3. Convert moles of CO_2 to grams:

The mass of CO_2 in the chamber is calculated by multiplying the number of moles by the molecular weight of CO_2:

$$nCO_2 \times 44 = \frac{\frac{C}{10^6} \times V\ m^3}{0.0224} \times 44\ g$$

Simplifying,

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

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

4. Calculation of the CO_2 uptake per gram of biomass

The total CO_2 uptake by the plants is the mass of CO_2 calculated above. Therefore, the CO_2 uptake per gram of biomass (U) is:

$$U = \frac{Mass\ of\ CO_2}{W}$$

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

Simplifying the equation,

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

The final equation for the CO_2 uptake per gram of biomass is:

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

Where U represents the CO_2 uptake per gram of biomass, C is the average CO_2 uptake by the plants (ppm), V is the volume of the chamber (m^3), and W is the total dry weight of the plants (g).

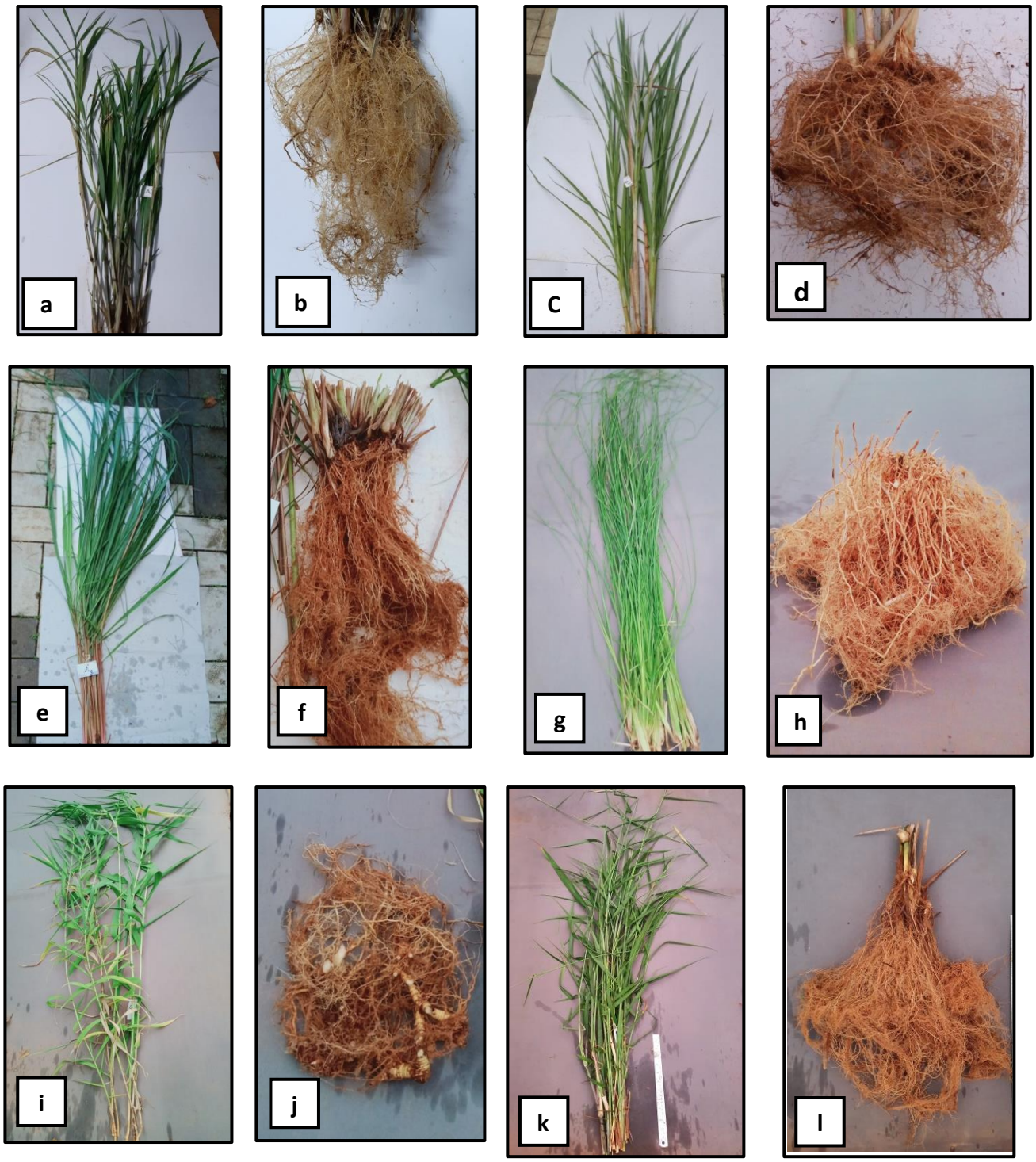


Figure 2.3. Above-ground and below-ground portions of *M. maximus* (a, b), *S. arundinaceum* (c, d), *C. flexuosus* (e, f), *C. zizanioides* (g, h), *A. donax* (i, j) and *P. pedicellatum* (k, l)

2.3.2 Estimation of Biochemical Parameters

Changes in the biochemical parameters associated with *Megathyrsus maximus*, *Saccharum arundinaceum*, *Cymbopogon flexuosus*, *Chrysopogon zizanioides*, *Arundo donax* and *Pennicetum pedicellatum,* consequent to CO_2 treatments are recorded at 4 stages (1DOT, 5DOT, 11DOT, and 15DOT). Three leaves of different age groups were collected and homogenized for this purpose. Fresh leaves were used for the analysis of pigments, protein, carbohydrates, and phenol content. Oven-dried leaf samples were subjected to acid digestion to estimate their concentration of minerals and other nutrients. The methodology followed is detailed below:

Estimation of Pigments

Pigments were analyzed using the DMSO method. 0.025g of leaf tissue samples were taken in each test tube, followed by 7 ml of DMSO reagent. The test tubes were maintained in the dark before being placed in an oven at 60°C for 1 hour. The test tubes were removed from the oven and allowed to cool in darkness. Absorbance at various optical density (OD) levels was measured using a UV-visible spectrophotometer (Shimadzu, Japan). The formulae used to calculate chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were as follows:

Chlorophyll a = ((12.7×OD 663) - (2.69×OD 645)) ×V×DF/1000×W×1

Chlorophyll b = ((22.9×OD 645) - (4.68×OD 663)) ×V×DF/1000×W×1

Total chlorophyll = ((20.2×OD 645) + (8.02× OD 663)) ×V×DF/1000×W×1

Carotenoids = OD480+ ((0.114×OD 663) - (0.638×OD645)) ×V× DF/1000×W×1

Where V is the volume of the solution, W is the weight of the plant, and DF is the dilution factor.

Estimation of Carbohydrates

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. Sodium carbonate powder was used to neutralize the samples until the effervescence subsided. They were then cooled to room

temperature. After being neutralized, the samples were diluted to 100 ml and centrifuged for 5 minutes at 4000 rpm. A 0.5 ml supernatant was extracted from each sample and placed in individual test tubes. Distilled water was then added to make the final volume of 1 ml. At the same time a glucose standard with concentrations of 0.2, 0.4, 0.6, 0.8, and 1 ml was made. Distilled water was added to all test tubes, including the sample, to bring the volume up to 1 ml. Then 4 ml of anthrone reagent was added to the tubes, and they were heated for 8 minutes. After that, they were quickly cooled by submerging the test tube holder in a tray filled with water. A UV-visible spectrophotometer was used to measure the absorbance at 630 nm. Standard graph of glucose is depicted in **Annexure 1**. Using the following formula, the carbohydrate concentration (mg/g) was determined:

$$\frac{OD\ of\ sample\ \times average\ cocentration\ of\ standard \times total\ volume \times DF}{Average\ OD\ of\ standard\ \times Aliquote\ volume \times weight\ of\ sample}$$

Protein

The method of Lowry et al. (1951) was used to estimate protein. For this, a mortar and pestle were used to homogenize 0.5 g of fresh leaf samples in 10 ml of phosphate buffer. The homogenate was centrifuged for 15 minutes at 6000 rpm. Protein estimation was performed using the supernatant obtained after centrifugation. Working standards of BSA (Bovine Serum Albumin) in volumes of 0.2, 0.4, 0.6, 0.8, and 1 ml were pipetted into test tubes to prepare the standards. In another test tube, 0.1 ml of the sample extract was added. Then distilled water was added to each test tube to bring the volume up to 1 ml. The blank was a tube containing 1ml of water. 5 ml of reagent C (2% Na_2CO_3 and 0.1 N NaOH along with an adequate concentration of 0.5% copper sulphate and 1% potassium sodium tartarate) was added to each test tube containing the standards and blank. After thorough mixing, it was let to stand for 10 minutes. Reagent D, which is distilled water diluted to 1:1 with folin phenol reagent, was added to an extent of 0.5 ml to each test tube. It was then kept for 30 minutes at room temperature in the dark after thorough mixing. The resultant blue colour was measured at 660 nm using a UV-visible spectrophotometer. Standard graph of BSA is depicted in **Annexure 2**. The following formula was used to determine the protein concentration (mg/g):

$$\frac{OD\ of\ sample\ \times average\ concentration\ of\ standard \times total\ volume \times DF}{Average\ OD\ of\ stadard \times Aliquot\ volume \times weight\ of\ sample}$$

Phenol

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. The homogenate was centrifuged at 10,000 rpm for 20 min. The centrifugation and extraction were repeated using 80% ethanol. The supernatants were collected, pooled, and evaporated until dry. The residue was then dissolved in a known volume of distilled water. From this, aliquots were pipetted out and each tube containing aliquots was made up to 3.0 ml of distilled water.

To prepare the catechol standard, 0.01 g of catechol was dissolved in 10 ml of distilled water. After 3 minutes, the Folin-Ciocalteau reagent (0.5 ml) was added to all the test tubes including the sample, followed by 2 ml of 20% sodium carbonate. The tube contents were mixed and all the tubes were placed in a hot water bath for precisely one minute. They were then cooled and the absorbance was measured in a spectrophotometer at 650nm against a reagent blank. Standard graph of catechole is depicted in **Annexure 3**. The phenol concentration (mg/g) was estimated using the following equation:

$$\frac{OD\ of\ sample\ \times average\ concentration\ of\ standard \times total\ volume \times DF}{Average\ OD\ of\ stadard \times Aliquot\ volume \times weight\ of\ sample}$$

Estimation of mineral nutrients

Acid digestion of leaf samples

The leaf samples were dried in an oven at 65°C. A 0.25 g dried leaf sample was then placed in a 100 ml Kjeldahl flask, containing 60% of 0.25 ml perchloric acid, 1.25 ml nitric acid, and 0.125 ml sulphuric acid for acid digestion. The mixture was heated at a low temperature and allowed to digest for 10 to 15 minutes until the appearance of white fumes. The sample was then cooled, filtered, and made up to 25 ml following multiple washings of the filter paper. This acid-digested sample solution was tested for mineral nutrients.

Calcium (EDTA titration method)

Each leaf sample was acid-digested, and 2.5 ml of the acid-digested solution was taken in a conical flask for the estimation of calcium content. It was then mixed with 50 ml of distilled water, 5ml of sodium hydroxide (1.0 N) solution, and 100 – 200 mg of murexide indicator. This pink colour mixture was then titrated with 0.01 M EDTA solution until it turned purple. The following formula was used to determine the calcium content of the solution:

Ca (%) = A×400.8×V/v×10000 ×S

A = Volume of EDTA used

V = Total volume of ash solution

v = Volume of ash solution titrated

S = Weight of plant tissue in g

Magnesium (EDTA titration method)

A 2.5 ml acid-digested solution was collected and diluted with 50 ml of distilled water for the estimation of magnesium content. To this mixture, 7.5 ml of ammonia buffer and 100–200 mg of Eriochrome black T indicator were added. The solution was titrated with EDTA (0.01 M) until the colour turned blue. The following formula was used to get the percentage of magnesium in the sample solution:

Mg (%) = (B-A) ×400.8×V/ v×1.645×10000×S

A = Volume of EDTA for determining calcium alone

B = Volume of EDTA for calcium and magnesium

V = Total volume of ash solution

V = Volume of ash solution titrated

S = Weight of plant material taken (mg/g)

Sodium and Potassium

A flame photometer (Systronics, 128) was used to estimate the concentration of sodium and potassium in the leaf samples. Acid-digested solutions (ash solution) of the leaf samples were used for the estimation of sodium and potassium. To make sodium standards, dissolve 0.252 g of sodium in 100 ml of distilled water (1000 ppm). To prepare working standards, this was then diluted proportionately. Similarly, 1.907g of potassium was dissolved in 100 ml of distilled water to make standards of potassium (1000 ppm). To create working standards, this was suitably diluted. The instrument was calibrated using the respective standards of sodium and potassium. The samples were then aspirated to obtain the corresponding concentrations in ppm levels. Percentage sodium and potassium content of the sample were then calculated using the following equation:

$$\text{Na \%} = \frac{\boldsymbol{mg\ Na/L\ of\ ash\ solution \times V}}{\boldsymbol{10000 \times S}}$$

$$\text{K \%} = \frac{\boldsymbol{mg\ K/L\ of\ ash\ solution \times V}}{\boldsymbol{10000 \times S}}$$

Where obtained ppm values were directly substituted to mg/L, since for water-based solutions ppm values are equivalent to mg/L.

Carbon and Nitrogen

Leaves were collected from each chamber at 1DOT and 15DOT of the study. Leaves were dried and ground into a homogeneous powder to measure the carbon and nitrogen content of the plants. A CHNS organic elemental analyzer (Thermo Scientific - FLASH 2000), was used to determine the carbon and nitrogen contents of each sample. The CHNS peaks obtained represent the levels of hydrogen and sulphur in the samples. While Carbon and Nitrogen content was only considered for the study.

Fourier- Transform Infrared Spectroscopic Analysis (FTIR)

For analyzing the changes in chemical composition, infrared analysis was performed on each plant sample using the equipment at CSIF, Calicut University. The equipment used is an Agilent Technologies Cary 620 model, which has chemical

imaging capabilities, a wide field of view, and excellent spatial resolution. The leaves of *M. maximus*, *S. arundinaceum*, *C. flexuosus*, *C. zizanioides*, *A. donax*, and *P. pedicellatum* were dried and ground into powder and 1mg dried leaf powder was mixed with KBr salt and was crushed to form a uniformly sized pellet. The infrared spectrum of each plant material was recorded in the mid-infrared range (4000-500 cm-1) at room temperature. Origin Pro software was used to visualize the FTIR spectrum of leaf samples of each plant.

2.3.3 Estimation of soil characteristics

Soil samples were collected from each grow bag on 1DOT and 15 DOT of the experimentation for the estimation of pH, moisture, TOC, and available nitrogen.

Soil moisture

Soil samples were collected from both control and treatment chambers. The fresh weight of these samples was immediately measured, and to determine the dry weight of the soil, the samples were heated in an oven set at 60˚C until the samples reached a constant weight. The percentage of soil moisture content is calculated using the difference between the fresh weight and the dry weight. The formula used for the calculation of soil moisture is:

$$\frac{Fresh\ weight\ of\ soil - Dry\ weight\ of\ soil}{Fresh\ weight\ of\ soil}$$

Soil pH

Soil solution was prepared (1:10 ratio) and the soil pH was estimated using a digital pH meter (MK V1).

Soil carbon (Total Organic Carbon-TOC)

The soils associated with respective grass species from the control and CO_2-treated ones were examined to assess the organic carbon content using the Walkley and Black method (Krishnan and Bharathi, 2009). For this 1 g of dry soil was taken in a conical flask. This was mixed with 10 ml of 1N potassium dichromate and 20 ml of concentrated sulphuric acid that had silver sulphate dissolved in it. After being kept for thirty minutes, the reaction product was diluted with 200 ml of distilled water. It was

combined with 10 ml of phosphoric acid and 1 ml of diphenylamine indicator. After the colour change to bluish-purple, the mixture was titrated with ferrous ammonium sulphate (FAS) until the blue colour turned brilliant green. The same procedure was followed for a blank (distilled water). The following formula was used to determine the total organic carbon content of the soil:

$$\text{Total organic carbon (\% C)} = \frac{(V1-V2)\times A\times 100}{W}$$

Where;

V1 = Volume of FAS used up for blank titration

V2 = Volume of FAS used for sample titration

A = Normality of potassium dichromate

W = Weight of sample

Percentage of organic matter (% OM) = %C×1.724

Available Nitrogen

The Alkaline Permanganate method is used to assess the amount of available nitrogen in the soil. To accomplish this, excess alkaline permanganate is applied to a known weight of soil (20 g) and the mixture is distilled. In the presence of KMnO4 and NaOH, the organic matter in the soil is oxidized, resulting in the release of ammonia. This released ammonia is absorbed and transformed into ammonium borate in a known volume of boric acid (2%) that contains a double indicator. This ammonium borate is titrated with 0.02 N H_2SO_4. The following formula was used to determine relative levels of available nitrogen in soils.

$$\text{Available Nitrogen (mg/kg)} = \frac{V\times N\times 14\times 1000}{w}$$

Where,

V = Volume of H_2SO_4

N = Normality of H_2SO_4

14 = Atomic weight of Nitrogen

W = Weight of soil

2.3.4 Statistical analysis

To validate the significance of the outcomes obtained, a Paired samples T-test was conducted. The test was carried out to validate the significance of changes in plant traits during the experimentation. The mean difference in the plant traits between the initial day and final day, control, and treatments were visualized using boxplots. All these analysis was carried out using Jamovi ver.2.3.28. In certain cases, even if there are visible changes in plant traits, the T-test results were insignificant. It was comprehended to be due to insufficient sample size. In chamber studies like the present study, there are limitations in increasing the sample size due to insufficient space for incorporating more plants. In this context, for expressing the size of the difference between two groups (here 1DOT and 15DOT), 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. It enables the comparison of effect sizes between samples. It accounts for sample size bias, making it more accurate than comparable metrics such as Cohen's d, particularly with small sample sizes. 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, t-SNE algorithm (t-Distributed Stochastic Neighbor Embedding) with Hedges g values was plotted. Analysis was done using *'Matplotlib'*, *'seaborn'* libraries in python. Important outcomes derived from the study with specific conclusions were made with these analysis and visualization tools.

2.4. RESULTS

2.4.1 Outcomes of plant growth measurements

Growth attributes such as plant height and tiller height, number of tillers, number of leaves, leaf length, leaf breadth, leaf area, culm diameter, and plant biomass were measured and results were analyzed. Visible changes were noticed in the morphological traits and biomass of plants when treated under elevated CO_2 levels. To validate the significance of these changes paired samples T-test was carried out for control and treatment plant groups. Also, to comprehend the magnitude and direction of the change, Hedges g or effect size values were found. Hedges g is a statistical tool that measures the degree of variation between two groups or conditions while accounting for inter-group variability and eliminating the possibility for bias resulting from small sample numbers. Hedges g values or effect size normally range from 0.2 to 0.8, where 0.2 is a small effect size which indicates a small difference between two groups, 0.5 is a medium difference, and 0.8 is a large difference. The effect size value may sometimes go beyond 1.00 or below 0.2 based on the magnitude of the difference between the two groups. Hedges g value may be positive or negative based on positive or negative variation. Paired samples T-test results and Hedges g values regarding morphological traits of grass species are depicted in **Table 2.8**.

Plant Height and Tiller height

Visible morphometric changes were noticed in the total plant height of *M. maximus, S. arundinaceum, C. zizanioides,* and *P. pedicellatum* (**Table 2.9**). To validate the significance of these changes, a paired samples T-test was carried out, in which measurements of 1DOT and 15 DOT were considered as two groups, and the test was done separately for control and treatment plants. Significant results were obtained for *S. arundinaceum* ($p=0.009$) grown at elevated CO_2 (TC). In the other four species, changes were not statistically significant. In this context, to find out the strength and direction of these changes regarding plant height under the influence of elevated CO_2, Hedges g analysis was carried out. From Hedges g analysis it was understood that the 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; Hedges g values of control and treatment groups of each plant is depicted in Table

2.8. In *M. maximus*, the average plant height in TC at 15DOT is 232.67±11.06 cm, which is 7.50% greater than 1DOT (216.67±6.11 cm). *S. arundinaceum* exhibited an increase in plant height of 11.70% in TC (1DOT=237.33±10.79 cm; 15 DOT=265.17±14.78 cm), whereas *C. zizanioides* shows a minor increase of 0.60% in TC (1DOT=233±9.17 cm; 15DOT= 234.33±7.2 cm). In *P. pedicellatum* Hedges g value for CC was 0.624 and for TC, it was 0.276, since lower effect size in TC there is not much influence of elevated CO_2 on plant height. There is no discernible change was noticed in the height of the plants of *C. flexuosus* and *A. donax* treated under elevated CO_2 and the respective Hedges g values for TC are 0.009 and 0.115. Box plots represented (**Figure 2.4**) show mean differences in plant height of each treatment group.

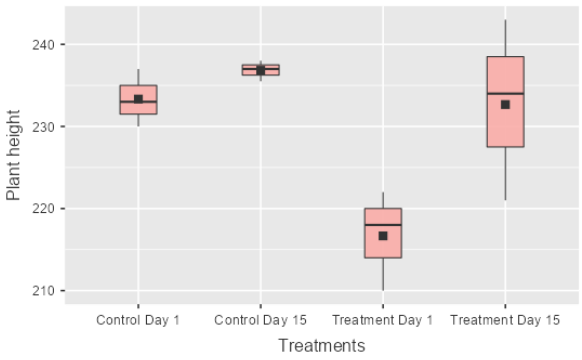
A tiller is a shoot that emerges from the base of the grass plant. The mean tiller height of a single grass plant is calculated by pooling the heights of 3 tillers from that plant, one small-sized, one medium-sized, and one large-sized. Changes were noticed in the tiller heights of grass species under study. Paired samples T-test results show a significant change in the tiller height of plants of *M. maximus* and *S. arundinaceum* in TC, but a significant change is also there in CC, thus the change in tiller height in TC was not considered as a result of elevated CO_2. Hedges g analyses were carried out to find out the direction and strength of the change, and the effect sizes obtained revealed a positive change in all species but its strength varies. Higher effect size with TC is shown only by *C. zizanioides* (CC=0.295; TC=0.612) and *P. pedicellatum* (CC=1.628; TC=2.753). In *C. zizanioides*, the mean tiller height in TC at 15 DOT is 167.44±8.32 cm, which is 3.41% higher than 1DOT (161.89±6.05 cm). Here in CC average percentage change in tiller height during the study period is 1.48%. In *P. pedicellatum* an increase of 24.83% in the mean height of tillers was reported in TC. Noticeable mean differences in the tiller height between control and CO_2-treated plants were depicted in Box plot representations (**Figure 2.5**). **Table 2.10** depicts variations in the tiller height of CC and TC on 1DOT and 15DOT.

Table 2.8: Paired T-test significance values and Hedges g values of Morphological traits

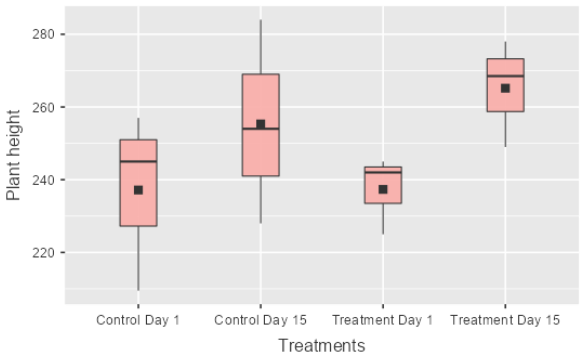
Morphological traits	Species	Paired samples T-test (p value)		Hedges g (effect size)	
		Control	Treatment	Control	Treatment
Plant height	*M. maximus*	0.277	0.214	1.061	1.432
	S. arundinaceum	0.073	0.009*	0.550	1.720
	C. flexuosus	0.184	0.423	0.278	0.009
	C. zizanioides	NaN	0.423	0.000	0.130
	A. donax	0.270	0.184	0.557	0.115
	P. pedicellatum	0.153	0.044*	0.624	0.276
Tiller height	*M. maximus*	0.017*	0.013*	2.673	2.099
	S. arundinaceum	0.015*	0.048*	1.793	1.228
	C. flexuosus	0.423	0.423	0.483	0.296
	C. zizanioides	0.270	0.138	0.295	0.612
	A. donax	0.171	0.368	0.679	0.255
	P. pedicellatum	0.161	0.073	1.628	2.753
Number of tillers	*M. maximus*	0.423	0.423	0.102	0.197
	S. arundinaceum	NaN	NaN	0.000	0.000
	C. flexuosus	0.120	0.138	1.901	1.056
	C. zizanioides	NaN	0.267	0.240	0.254
	A. donax	0.225	0.225	0.223	0.249
	P. pedicellatum	0.145	NaN	1.810	-0.231
Number of leaves	*M. maximus*	0.287	0.264	0.812	0.644
	S. arundinaceum	0.757	0.120	-0.149	-1.047
	C. flexuosus	0.083	0.191	-1.106	-1.123
	C. zizanioides	0.439	0.460	-0.279	-0.243
	A. donax	0.064	0.031*	-0.303	-0.712
	P. pedicellatum	0.040*	0.706	1.578	-0.190
Leaf length	*M. maximus*	0.423	0.094	0.054	0.056
	S. arundinaceum	0.215	0.244	0.082	1.129
	C. flexuosus	0.026*	0.060	3.282	1.864
	C. zizanioides	0.061	0.174	1.137	1.262
	A. donax	0.021*	0.266	1.022	0.876
	P. pedicellatum	NaN	NaN	0.000	0.000
Leaf breadth	*M. maximus*	0.102	0.199	0.761	0.094
	S. arundinaceum	0.423	0.255	0.059	0.846
	C. flexuosus	0.360	0.423	0.454	0.450
	C. zizanioides	0.060	0.506	1.348	0.232
	A. donax	0.861	0.235	0.158	0.451
	P. pedicellatum	0.240	0.250	1.480	0.437
Leaf area	*M. maximus*	0.097	0.232	1.157	0.437
	S. arundinaceum	0.923	0.360	-0.025	0.721
	C. flexuosus	0.971	0.982	0.021	0.016
	C. zizanioides	0.035*	0.227	0.388	0.249
	A. donax	0.535	0.050*	-0.081	-0.544
	P. pedicellatum	0.034*	0.847	1.163	-0.113
Culm diameter	*M. maximus*	0.057	0.103	1.306	1.078
	S. arundinaceum	0.423	0.423	0.043	0.078
	C. flexuosus	0.915	0.235	-0.094	0.548
	C. zizanioides	0.547	0.063	0.269	0.552
	A. donax	0.184	0.195	0.234	0.342
	P. pedicellatum	0.423	0.184	0.037	0.662

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

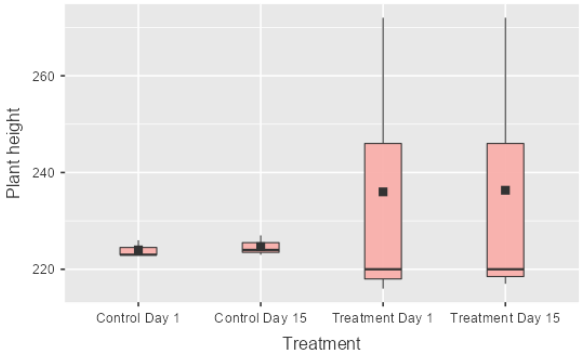
M. maximus



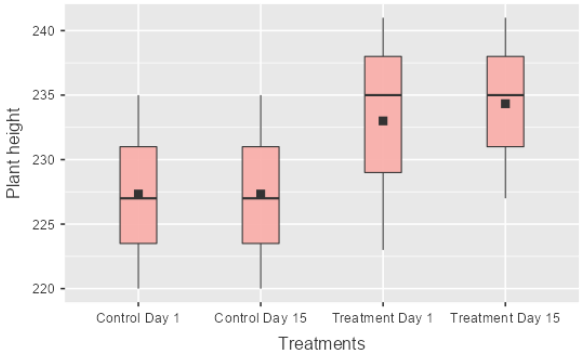
S. arundinaceum



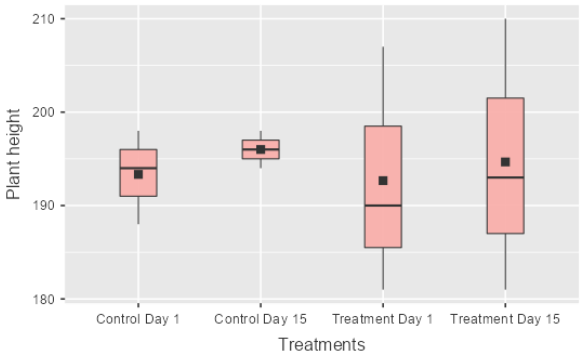
C. flexuosus



C. zizanioides



A. donax



P. pedicellatum

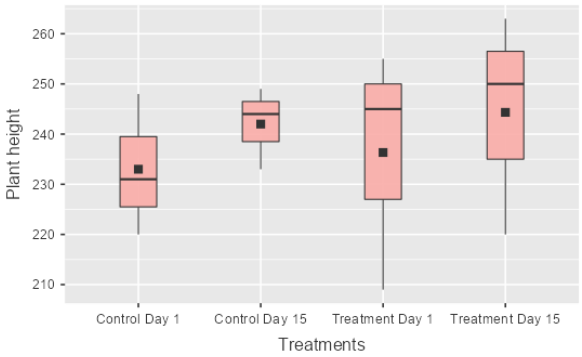
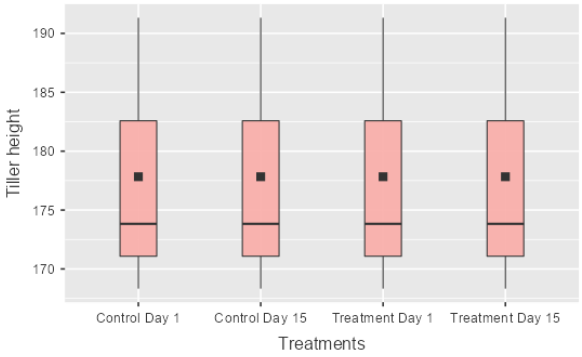


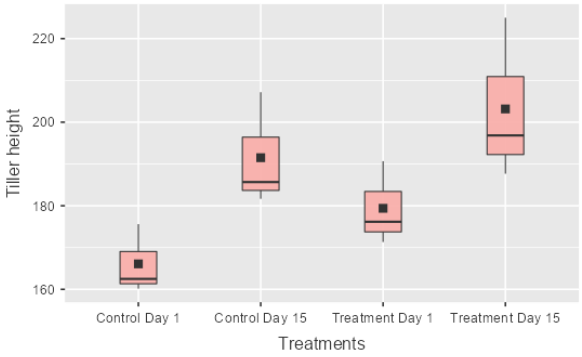
Figure 2.4: Box plot representations of changes in the plant height

Table 2.9: Variation in the Plant height (cm) under elevated levels of CO_2

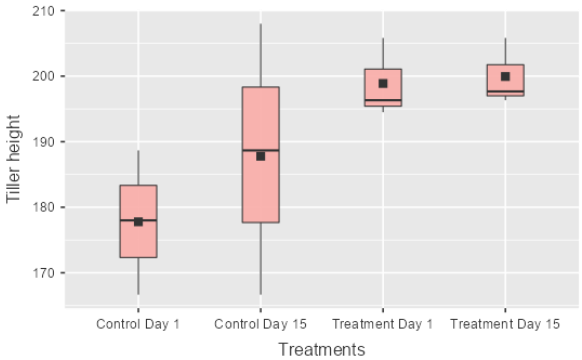
Control	D1	D15	Treatment	D1	D15
Megathyrsus maximus					
C1	230	238	T1	218	221
C2	233	235.5	T2	210	243
C3	237	237	T3	222	234
Mean	233.33	236.83	Mean	216.67	232.67
STDEV	3.51	1.26	STDEV	6.11	11.06
% Change	1.52		% Change	7.50	
Saccharum arundinaceum					
C1	245	254	T1	245	278
C2	209.5	228	T2	225	249
C3	257	284	T3	242	268.5
Mean	237.17	255.33	Mean	237.33	265.17
STDEV	24.70	28.02	STDEV	10.79	14.78
% Change	7.67		% Change	11.70	
Cymbopogon flexuosus					
C1	223	224	T1	272	272
C2	226	227	T2	220	220
C3	223	223	T3	216	217
Mean	224	224.67	Mean	236	236.33
STDEV	1.73	2.08	STDEV	31.24	30.92
% Change	0.30		% Change	0.15	
Chrysopogon zizanioides					
C1	227	227	T1	235	235
C2	220	220	T2	241	241
C3	235	235	T3	223	227
Mean	227.33	227.33	Mean	233	234.33
STDEV	7.51	7.51	STDEV	9.17	7.02
% Change	0.00		% Change	0.60	
Arundo donax					
C1	194	196	T1	207	210
C2	198	198	T2	190	193
C3	188	194	T3	181	181
Mean	193.33	196	Mean	192.67	194.67
STDEV	5.03	2.00	STDEV	13.20	14.57
% Change	1.41		% Change	1.01	
Pennisetum pedicellatum					
C1	220	233	T1	209	220
C2	248	249	T2	245	250
C3	231	244	T3	255	263
Mean	233	242	Mean	236.33	244.33
STDEV	14.11	8.19	STDEV	24.19	22.05
% Change	3.98		% Change	3.48	

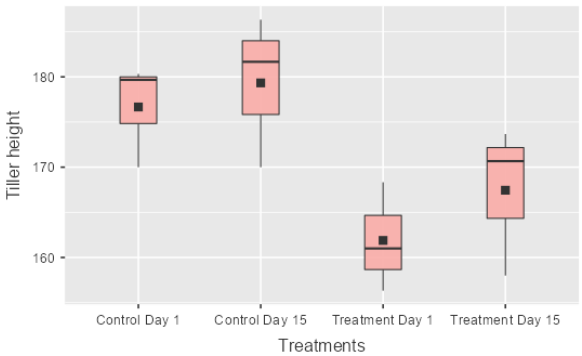
M. maximus ***S. arundinaceum***



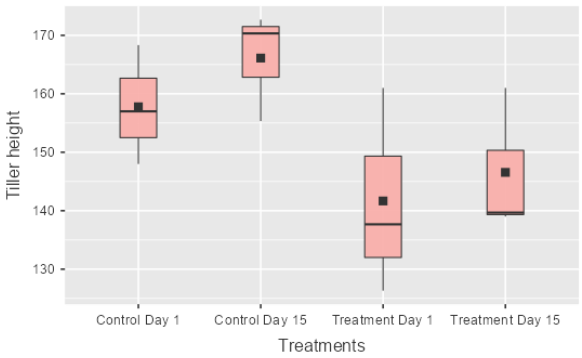


C. flexuosus ***C. zizanioides***





A. donax ***P. pedicellatum***



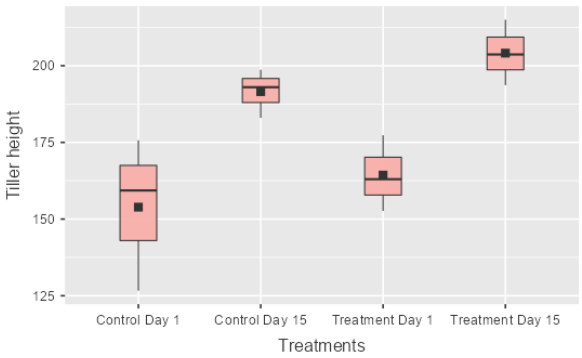


Figure 2.5: Box plot representations of changes in the tiller height

Table 2.10: Variation in tiller height (cm) of plants under elevated levels of CO_2

Control	D1	D15	Treatment	D1	D15
Megathyrsus maximus					
C1	160.50	183.00	T1	141.33	173.83
C2	143.43	174.50	T2	146.33	168.33
C3	156.33	192.50	T3	160.00	191.33
Mean	153.42	183.33	Mean	149.22	177.83
STDEV	8.90	9.00	STDEV	9.66	12.01
% Change	19.60		% Change	19.20	
Saccharum arundinaceum					
C1	162.50	185.67	T1	176.17	196.83
C2	160.17	181.67	T2	171.33	187.67
C3	175.60	207.17	T3	190.67	225.00
Mean	166.09	191.50	Mean	179.39	203.17
STDEV	8.32	13.71	STDEV	10.06	19.46
% Change	15.22		% Change	13.09	
Cymbopogon flexuosus					
C1	178	208	T1	194.50	197.67
C2	166.67	166.67	T2	205.83	205.83
C3	188.67	188.67	T3	196.33	196.33
Mean	177.78	187.78	Mean	198.89	199.94
STDEV	11.00	20.68	STDEV	6.08	5.14
% Change	5.62		% Change	0.54	
Chrysopogon zizanioides					
C1	170.00	170.00	T1	161.00	170.67
C2	179.67	181.67	T2	156.33	158.00
C3	180.33	186.33	T3	168.33	173.67
Mean	176.67	179.33	Mean	161.89	167.44
STDEV	5.78	8.41	STDEV	6.05	8.32
% Change	1.48		% Change	3.41	
Arundo donax					
C1	148.00	155.33	T1	137.67	139.00
C2	157	172.67	T2	126.33	139.67
C3	168.33	170.33	T3	161.00	161.00
Mean	157.78	166.11	Mean	141.67	146.56
STDEV	10.19	9.41	STDEV	17.68	12.51
% Change	5.37		% Change	3.84	
Pennisetum pedicellatum					
C1	126.67	198.67	T1	163.00	193.67
C2	175.67	193.00	T2	152.67	215.00
C3	159.33	183.00	T3	177.33	203.67
Mean	153.89	191.56	Mean	164.33	204.11
STDEV	24.95	7.93	STDEV	12.39	10.67
% Change	27.19		% Change	24.83	

Number of tillers

Even though new tillers were developed in *M. maximus* (0–2), *C. flexuosus* (2–9), *C. zizanioides* (0–7) and *A. donax* (0–2), the change was not statistically significant in TC compared to CC. The paired samples T-test result shows p value>0.05 in all species. Hedges g values regarding CC and TC of all species except *P. pedicellatum* indicate a smaller difference in the number of tillers between 1DOT and 15 DOT (Table 2.8). In *P. pedicellatum* Hedges g values of CC and TC respectively were 1.810 and -0.231. Here a decline in the tiller number was observed in TC (-5.09%) and the effect size obtained was smaller. No new tillers were developed in both CC and TC of *S. arundinaceum*. Box plot representations of mean differences in the number of tillers are depicted (**Figure 2.6**). **Table 2.11** represents variations in the number of tillers in CC and CC during the study.

Number of leaves

Elevated CO_2 treatment resulted in a decline in the number of leaves in the grass species except *M. maximus* and the differences between control and treatment groups were visible. New leaves were developed in *M. maximus*, where the mean leaf number in TC at 15DOT is 81.67±15.57, which is 12.28% higher compared to that of 1DOT (72.33±5.13). In *P. pedicellatum*, the number of leaves has increased in CC (24.56%) but 7.36% of leaves decreased in TC. To validate these observations, a paired samples T-test was performed, and significant change was shown only by *A. donax* (p value= 0.031). Hedges g analysis was also carried out to validate the direction of the change and to find out the effect size of treatment groups. Concerning the plants of TC, a higher effect size was also shown by *A. donax* and the value is -0.303 for CC and -0.712 for TC. The mean leaf number in TC regarding *A. donax* at 15 DOT was 141.67±52.37, which was 28.21% lower than 1DOT (196.33±69.29). CC regarding this species showed a decrease in the number of leaves of 15.07%. In *P. pedicellatum* direction of the changes in leaf number was different as mentioned and the Hedges g values were 1.578 for CC and -1.190 for TC. In boxplot representations (**Figure 2.7**) noticeable mean differences are observed among treatment groups. Variation in the number of leaves concerning CC and TC is depicted in **Table 2.12**.

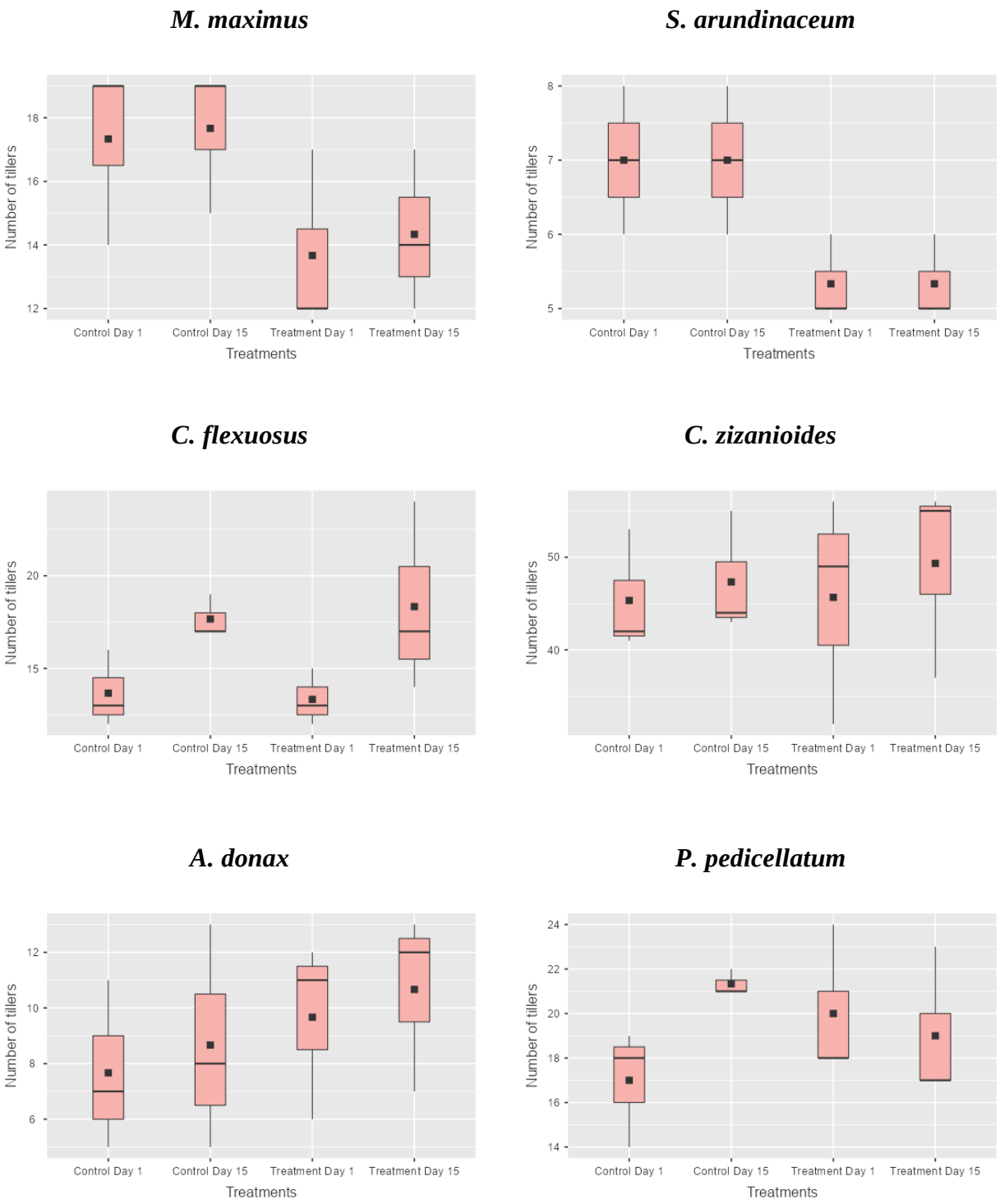


Figure 2.6: Box plot representations of changes in the number of tillers

Table 2.11: Variation in the number of tillers under elevated levels of CO_2

Control	D1	D15	Treatment	D1	D15
Megathyrsus maximus					
C1	14	15	T1	17	17
C2	19	19	T2	12	12
C3	19	19	T3	12	14
Mean	17.33	17.67	Mean	13.67	14.33
STDEV	2.89	2.31	STDEV	2.89	2.52
% Change	2.38		% Change	5.56	
Saccharum arundinaceum					
C1	6	6	T1	5	5
C2	8	8	T2	6	6
C3	7	7	T3	5	5
Mean	7	7	Mean	5.33	5.33
STDEV	1.00	1.00	STDEV	0.58	0.58
% Change	0.00		% Change	0.00	
Cymbopogon flexuosus					
C1	16	17	T1	15	24
C2	13	19	T2	13	17
C3	12	17	T3	12	14
Mean	13.67	17.67	Mean	13.33	18.33
STDEV	2.08	1.15	STDEV	1.53	5.13
% Change	31.36		% Change	35.81	
Chrysopogon zizanioides					
C1	41	43	T1	32	37
C2	42	44	T2	49	56
C3	53	55	T3	56	55
Mean	45.33	47.33	Mean	45.67	49.33
STDEV	6.66	6.66	STDEV	12.34	10.69
% Change	4.47		% Change	9.38	
Arundo donax					
C1	5	5	T1	12	12
C2	7	8	T2	11	13
C3	11	13	T3	6	7
Mean	7.67	8.67	Mean	9.67	10.67
STDEV	3.06	4.04	STDEV	3.21	3.21
% Change	10.82		% Change	11.62	
Pennisetum pedicellatum					
C1	18	21	T1	24	23
C2	14	22	T2	18	17
C3	19	21	T3	18	17
Mean	17	21.33	Mean	20	19
STDEV	2.65	0.58	STDEV	3.46	3.46
% Change	28.11		% Change	-5.09	

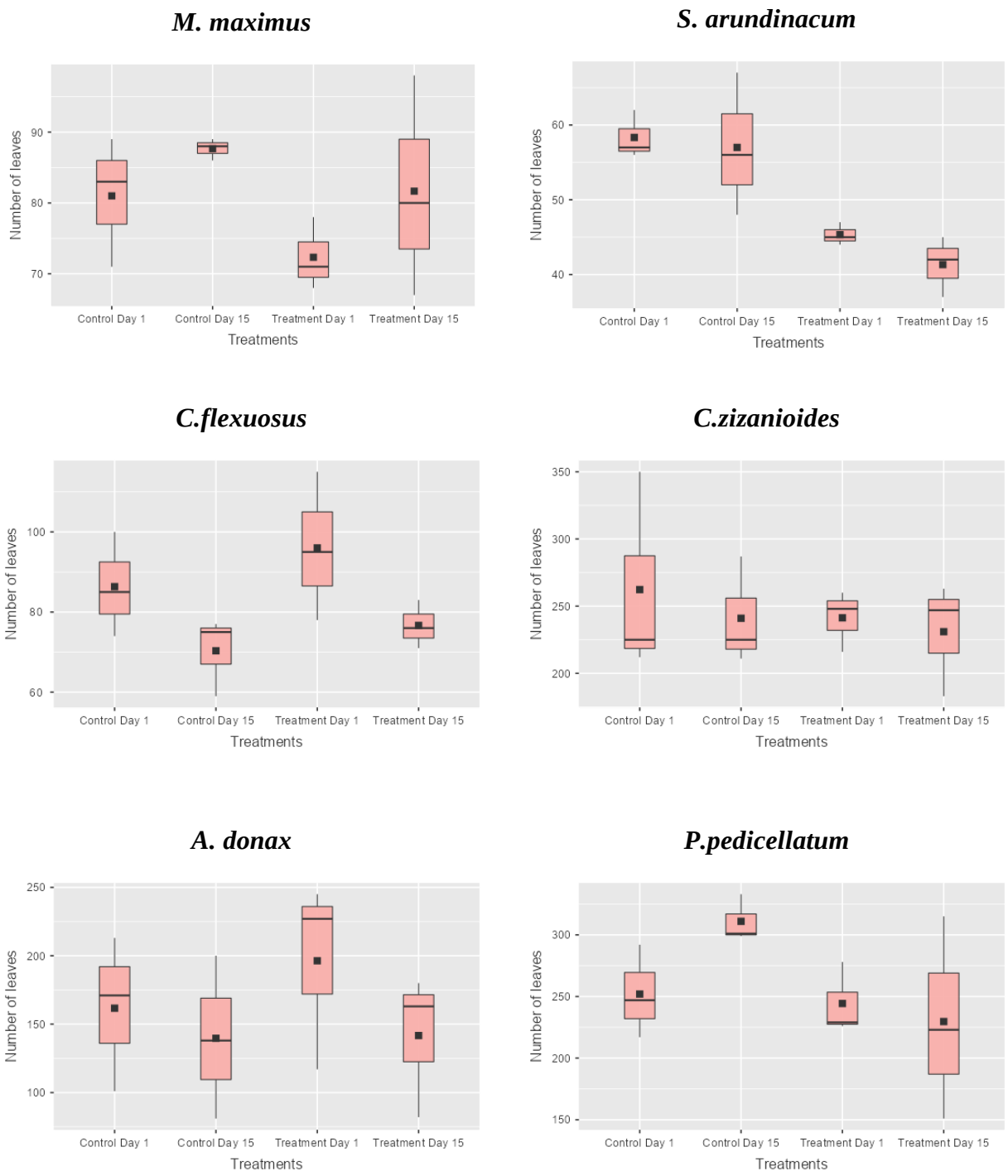


Figure 2.7: Box plot representations of changes in the number of leaves

Table 2.12: Variation in the number of leaves of plants under elevated levels of CO_2

Control	D1	D15	Treatment	D1	D15
Megathyrsus maximus					
C1	83	89	T1	78	98
C2	71	86	T2	68	67
C3	89	88	T3	71	80
Mean	81	87.67	Mean	72.33	81.67
STDEV	9.17	1.53	STDEV	5.13	15.57
% Change	9.08		% Change	12.28	
Saccharum arundinaceum					
C1	57	56	T1	44	37
C2	62	67	T2	45	42
C3	56	48	T3	47	45
Mean	58.33	57	Mean	45.33	41.33
STDEV	3.21	9.54	STDEV	1.53	4.04
% Change	-2.66		% Change	-8.94	
Cymbopogon flexuosus					
C1	100	75	T1	95	83
C2	85	77	T2	115	76
C3	74	59	T3	78	71
Mean	86.33	70.33	Mean	96	76.67
STDEV	13.05	9.87	STDEV	18.52	6.03
% Change	-18.23		% Change	-18.51	
Chrysopogon zizanioides					
C1	225	211	T1	216	183
C2	212	225	T2	248	247
C3	350	287	T3	260	263
Mean	262.33	241	Mean	241.33	231
STDEV	76.20	40.45	STDEV	22.74	42.33
% Change	-6.03		% Change	-4.84	
Arundo donax					
C1	101	81	T1	245	180
C2	171	138	T2	227	163
C3	213	200	T3	117	82
Mean	161.67	139.67	Mean	196.33	141.67
STDEV	56.58	59.52	STDEV	69.29	52.37
% Change	-15.07		% Change	-28.21	
Pennisetum pedicellatum					
C1	292	333	T1	229	151
C2	217	299	T2	226	223
C3	247	301	T3	278	315
Mean	252	311	Mean	244.33	229.67
STDEV	37.75	19.08	STDEV	29.19	82.20
% Change	24.56		% Change	-7.36	

Leaf length

Visible morphometric changes were observed in the leaf length of experimented plants except *P. pedicellatum*. The leaf length of *S. arundinaceum* in TC at 15 DOT is 95.53±15.64 cm. and is 20.09% higher than 1DOT (79.70±2.61 cm). In this species control plants showed only an increase of 0.44% at 15DOT from 1 DOT. To validate the statistical significance of these differences in treatment groups a paired samples T test was carried out. There were no significant results obtained in TC while considering the effect size values. The magnitude of the effect varied with treatment groups regarding *S. arundinaceum* (CC= 0.082; TC= 1.129) and *A. donax* (CC=1.022; TC=0.876). Thus in *S. arundinaceum*, the elevated CO_2 effect on the leaf length is higher compared to other plants. Considering *A. donax* the effect size is higher in CC than in TC and the percentage change in leaf length was 12.48% and 5.94% respectively in CC and TC. Thus it is assumed that elevated CO_2 may result in lowering the change in leaf length in TC concerning *A.donax*. According to Hedges g values, differences between the initial and final days of treatments were negligible in *M. maximus* (CC= 0.054; TC= 0.056). Regarding *C. flexuosus* and *C. zizanioides* higher effect size values were obtained in both CC and TC, thus the change in leaf length in this case is not considered to be an elevated CO_2 effect. In *P. pedicellatum* no changes were noticed in the leaf length of plants in treatment groups. Mean differences in leaf length changes of treated and control plants were shown in boxplot representations (**Figure 2.8**). **Table 2.13** depicts variations in leaf length in grass species at experimental conditions.

Leaf breadth

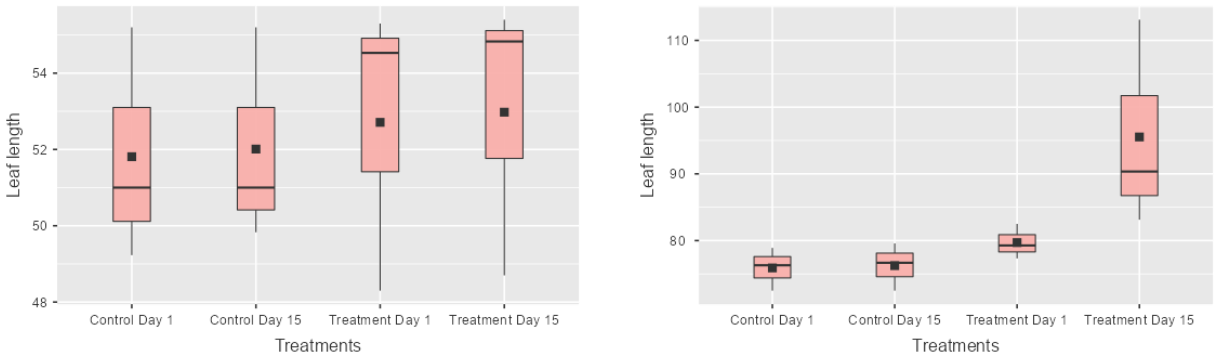
Changes were observed in the leaf breadth of experimented plants and to validate the statistical significance of these changes paired samples T-test was carried out. Significant changes were not noticed, while positive and various magnitudes of changes were noticed concerning Hedges g statistical analysis. Considering the species *S. arundinaceum* higher effect size was shown by TC (0.846) than CC (0.059), thus the elevated CO_2 effect on the leaf breadth was higher in this species. Here the mean leaf breadth of TC plants of *S. arundinaceum* at 15DOT is 2.99±0.74 cm, which is 22.05% greater than 1DOT (2.43±0.12 cm) and there is only a 0.48% increase in the mean leaf

length of plants in CC. Similarly, a medium higher effect size was shown by plants in TC of *A. donax* (0.451) than CC (0.158). The percentage of increase in the leaf length of *A. donax* in TC is 5.37% and that of CC is 2.18%. Regarding the species *M. maximus* (CC=0.761; TC=0.094), *C. zizanioides* (CC=1.348; TC=0.232), and *P. pedicellatum* (CC=1.480; TC=0.437), Hedges g values were lower in TC compared to CC. Thus this comparatively lower effect size in TC was assumed to be an effect of enrichment in CO_2 levels inside the chamber which lowers the leaf breadth of plants. Even though these outcomes were statistically insignificant ($p>0.05$), noticeable mean differences between control and treatment groups were reported in boxplot representations (**Figure 2.9**). **Table 2.14** depicts the leaf breadth measurements of control and treatment plants at the initial and final days of treatment.

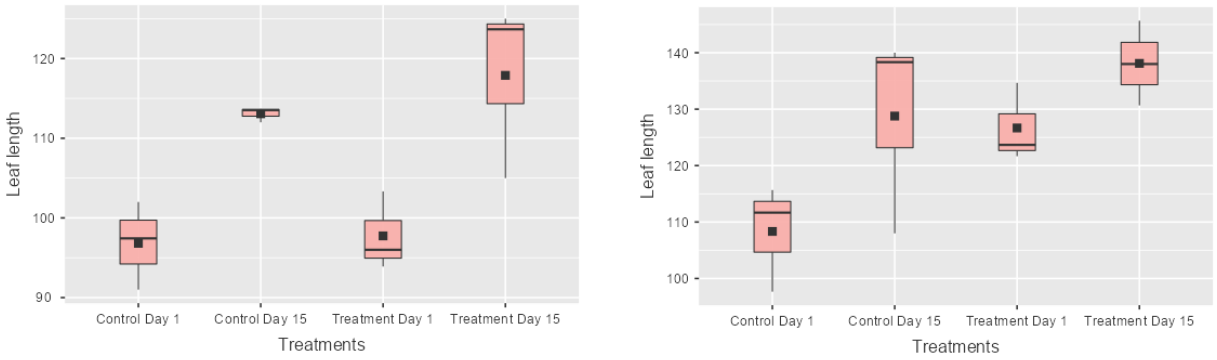
Leaf area

Changes were evident in the leaf area of plants in treated groups. Paired samples T-test results revealed a significant change in treated plants of *A. donax* (p value=0.05). Here a decrease of 19.87% in the leaf area was reported in plants treated at elevated CO_2 condition (TC), compared to a decrease of 3.22% in CC. Hedges g values also prove this higher negative effect of elevated CO_2 on the leaf area of *A. donax* with an effect size of -0.544 in TC than CC (-0.081). Elevated CO_2-induced increase in the leaf area was shown only by *S. arundinaceum* with an effect size of 0.721 in TC compared to an effect size of -0.025 in CC. Thus here the enriched CO_2 environment positively influenced the leaf area. In this species average leaf area of plants in TC at 15DOT is 1.10±0.49 m^2, which is 37.87% higher than 1 DOT (0.79±0.03 m^2) and as mentioned the control plants, showed a decline in leaf area by 1.79%. Considering the species *P. pedicellatum* to treatment groups, the direction of effect size was different and the magnitude was highly varied (CC=1.163; TC=-0.113). Here the lowered leaf area in TC (-4.50%) compared to CC (30.80) was assumed to be a result of the effect of elevated CO_2. In *C. flexuosus* and *C. zizanioides* the effect size was smaller in both CC and TC indicating a smaller difference between the leaf area of plants at 1 DOT and 15DOT. Thus it was assumed that elevated CO_2 doesn't influence much in the leaf area of these plants. All plants exhibited obvious mean differences between control and treatment groups as shown by box plot representations (**Figure 2.10**). Variations in the leaf area in CC and TC of grass species under experimentation were depicted in **Table 2.15**.

M. maximus ***S. arundinacum***



C. flexuosus ***C. zizanioides***



A. donax ***P.pedicellatum***

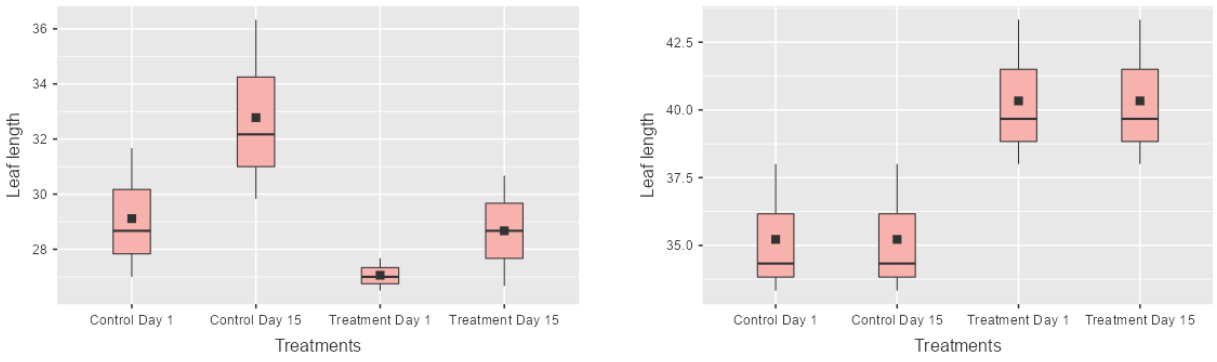


Figure 2.8: Box plot representations of changes in leaf length

Table 2.13: Variation in the Leaf length (cm) of plants under elevated levels of CO_2

Control	D1	D15	Treatment	D1	D15
Megathyrsus maximus					
C1	51.00	51.00	T1	55.30	55.40
C2	49.23	49.83	T2	54.53	54.83
C3	55.20	55.20	T3	48.30	48.70
Mean	51.81	52.01	Mean	52.71	52.98
STDEV	3.06	2.82	STDEV	3.84	3.72
% Change	0.41		% Change	0.52	
Saccharum arundinaceum					
C1	76.30	76.67	T1	82.50	83.13
C2	78.90	79.57	T2	79.27	113.10
C3	72.50	72.50	T3	77.33	90.35
Mean	75.90	76.24	Mean	79.70	95.53
STDEV	3.22	3.55	STDEV	2.61	15.64
% Change	0.44		% Change	20.09	
Cymbopogon flexuosus					
C1	102	113.67	T1	93.90	105
C2	97.43	113.53	T2	103.33	123.67
C3	91	112	T3	96	125
Mean	96.81	113.07	Mean	97.74	117.89
STDEV	5.53	0.93	STDEV	4.95	11.18
% Change	17.01		% Change	20.57	
Chrysopogon zizanioides					
C1	115.67	138.33	T1	121.67	130.67
C2	97.67	108.00	T2	123.67	145.67
C3	111.67	140.00	T3	134.67	138.00
Mean	108.33	128.78	Mean	126.67	138.11
STDEV	9.45	18.01	STDEV	7.00	7.50
% Change	18.52		% Change	9.22	
Arundo donax					
C1	27.00	29.83	T1	27.00	30.67
C2	31.67	36.33	T2	26.50	26.67
C3	28.67	32.17	T3	27.67	28.67
Mean	29.11	32.78	Mean	27.06	28.67
STDEV	2.36	3.29	STDEV	0.59	2.00
% Change	12.48		% Change	5.94	
Pennisetum pedicellatum					
C1	38.00	38.00	T1	38.00	38.00
C2	33.33	33.33	T2	43.33	43.33
C3	34.33	34.33	T3	39.67	39.67
Mean	35.22	35.22	Mean	40.33	40.33
STDEV	2.46	2.46	STDEV	2.73	2.73
% Change	0.00		% Change	0.00	

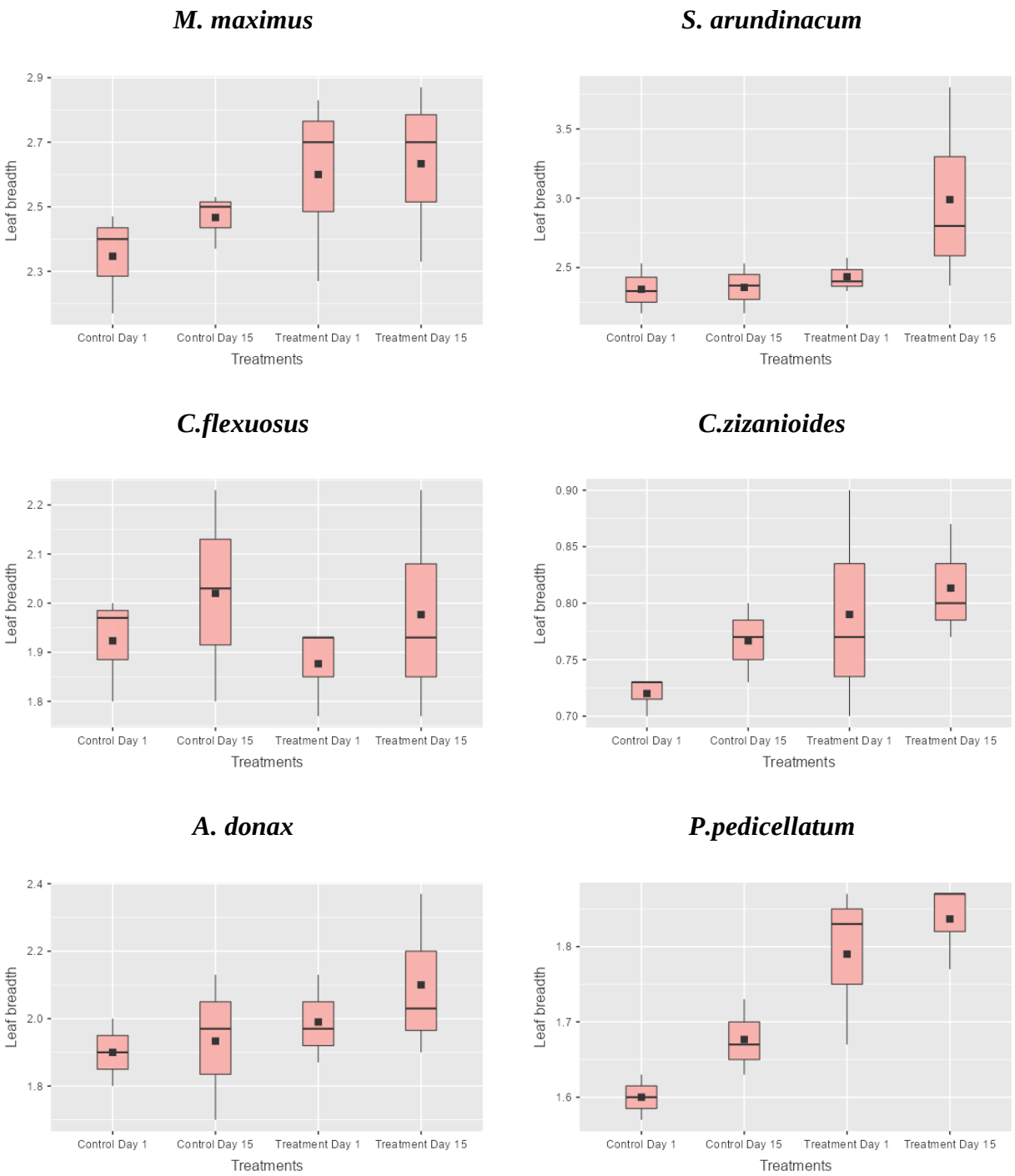
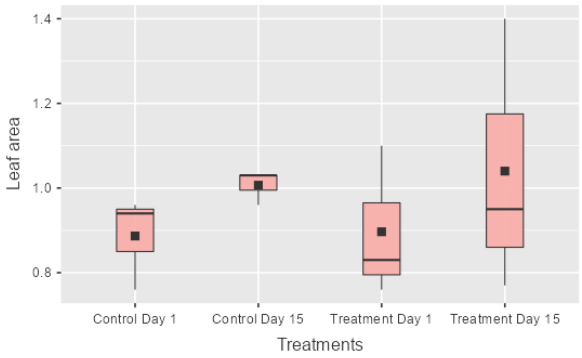


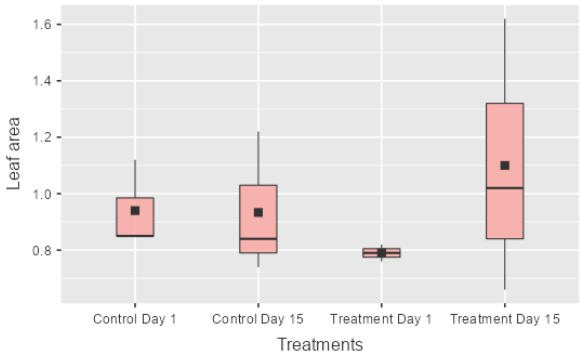
Figure 2.9: Box plot representations of changes in leaf breadth

Table 2.14: Variation in the Leaf breadth (cm) of plants under elevated levels of CO_2

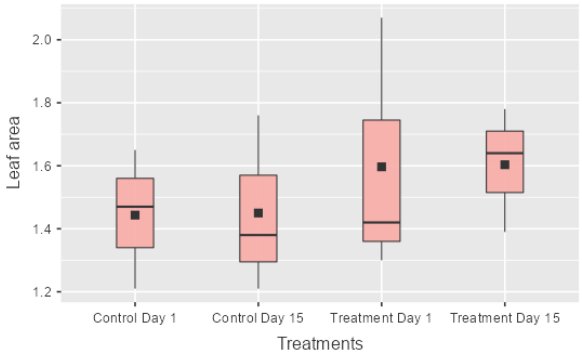
Control	D1	D15	Treatment	D1	D15
Megathyrsus maximus					
C1	2.47	2.53	T1	2.83	2.87
C2	2.40	2.50	T2	2.27	2.33
C3	2.17	2.37	T3	2.70	2.70
Mean	2.34	2.47	Mean	2.60	2.63
STDEV	0.16	0.09	STDEV	0.30	0.27
% Change	5.37		% Change	1.37	
Saccharum arundinaceum					
C1	2.17	2.17	T1	2.33	2.37
C2	2.53	2.53	T2	2.57	3.80
C3	2.33	2.37	T3	2.40	2.80
Mean	2.34	2.36	Mean	2.43	2.99
STDEV	0.18	0.18	STDEV	0.12	0.74
% Change	0.48		% Change	22.05	
Cymbopogon flexuosus					
C1	1.80	1.80	T1	1.77	1.77
C2	1.97	2.23	T2	1.93	1.93
C3	2.00	2.03	T3	1.93	2.23
Mean	1.92	2.02	Mean	1.88	1.98
STDEV	0.11	0.22	STDEV	0.10	0.24
% Change	5.08		% Change	5.17	
Chrysopogon zizanioides					
C1	0.70	0.73	T1	0.70	0.77
C2	0.73	0.80	T2	0.90	0.87
C3	0.73	0.77	T3	0.77	0.80
Mean	0.72	0.77	Mean	0.79	0.81
STDEV	0.02	0.03	STDEV	0.10	0.05
% Change	6.13		% Change	3.39	
Arundo donax					
C1	1.80	1.97	T1	1.97	2.03
C2	1.90	2.13	T2	1.87	1.90
C3	2.00	1.70	T3	2.13	2.37
Mean	1.90	1.93	Mean	1.99	2.10
STDEV	0.10	0.22	STDEV	0.13	0.24
% Change	2.18		% Change	5.37	
Pennisetum pedicellatum					
C1	1.63	1.63	T1	1.87	1.87
C2	1.60	1.67	T2	1.83	1.87
C3	1.57	1.73	T3	1.67	1.77
Mean	1.60	1.68	Mean	1.79	1.83
STDEV	0.03	0.05	STDEV	0.11	0.06
% Change	4.93		% Change	2.61	

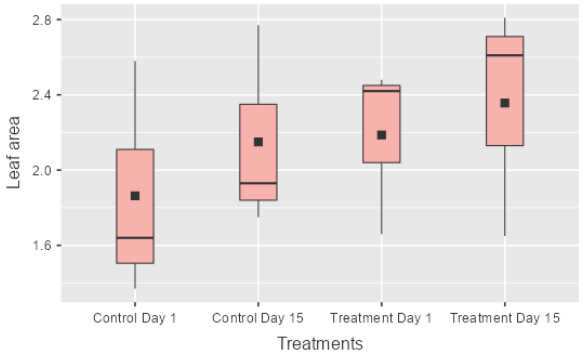
M. maximus ***S. arundinaceum***



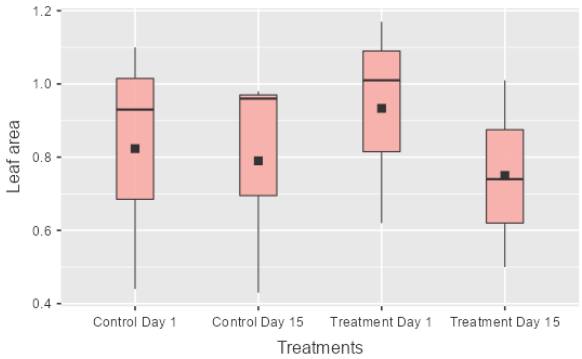


C.flexuosus ***C. zizanioides***





A. donax ***P.pedicellatum***



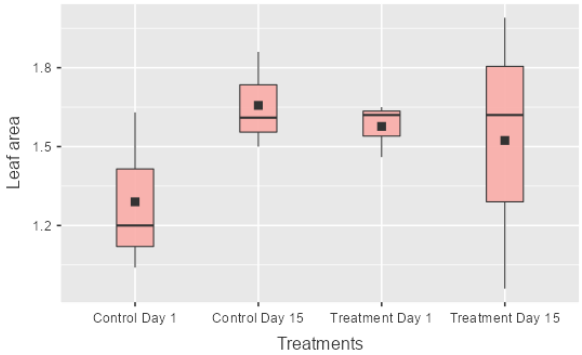


Figure 2.10: Box plot representations of changes in leaf area

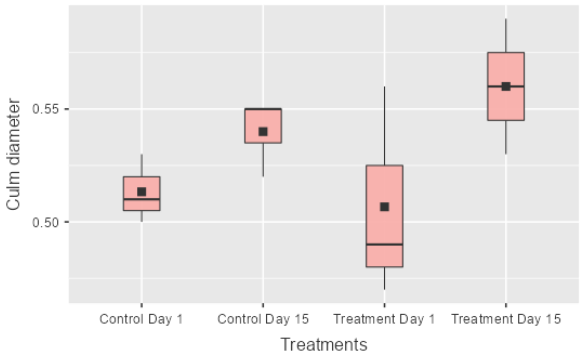
Table 2.15: Variation in the Leaf area (m^2) of plants under elevated levels of CO_2

Control	D1	D15	Treatment	D1	D15
Megathyrsus maximus					
C1	0.94	1.03	T1	1.10	1.40
C2	0.76	0.96	T2	0.76	0.77
C3	0.96	1.03	T3	0.83	0.95
Mean	0.88	1.01	Mean	0.90	1.04
STDEV	0.11	0.04	STDEV	0.18	0.32
% Change	15.05		% Change	14.60	
Saccharum arundinaceum					
C1	0.85	0.84	T1	0.76	0.66
C2	1.12	1.22	T2	0.82	1.62
C3	0.85	0.74	T3	0.79	1.02
Mean	0.94	0.93	Mean	0.79	1.10
STDEV	0.15	0.25	STDEV	0.03	0.49
% Change	-1.79		% Change	37.87	
Cymbopogon flexuosus					
C1	1.65	1.38	T1	1.42	1.39
C2	1.47	1.76	T2	2.07	1.64
C3	1.21	1.21	T3	1.30	1.78
Mean	1.44	1.45	Mean	1.60	1.60
STDEV	0.22	0.28	STDEV	0.41	0.20
% Change	1.07		% Change	4.57	
Chrysopogon zizanioides					
C1	1.64	1.93	T1	1.66	1.65
C2	1.37	1.75	T2	2.48	2.81
C3	2.58	2.77	T3	2.42	2.61
Mean	1.86	2.15	Mean	2.19	2.36
STDEV	0.64	0.55	STDEV	0.46	0.62
% Change	17.67		% Change	6.93	
Arundo donax					
C1	0.44	0.43	T1	1.17	1.01
C2	0.93	0.96	T2	1.01	0.74
C3	1.10	0.98	T3	0.62	0.50
Mean	0.82	0.79	Mean	0.93	0.75
STDEV	0.34	0.32	STDEV	0.28	0.25
% Change	-3.22		% Change	-19.87	
Pennisetum pedicellatum					
C1	1.63	1.86	T1	1.46	0.96
C2	1.04	1.50	T2	1.62	1.62
C3	1.20	1.61	T3	1.65	1.99
Mean	1.29	1.66	Mean	1.58	1.52
STDEV	0.31	0.19	STDEV	0.10	0.52
% Change	30.80		% Change	-4.50	

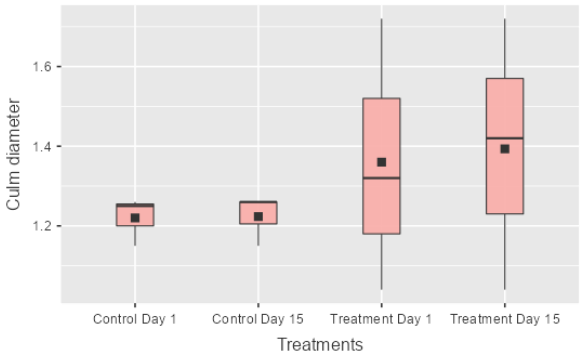
Culm diameter

Elevated CO_2 environment-induced enhancement in the culm diameter was observed in all species, while statistically, this was not significant as per the paired samples T-test. Since there were observable changes, Hedges g analysis was performed to find out the direction and magnitude of these changes. An increase in culm diameter was noticed and the magnitude of the change considerably varies among the treatment groups in the species such as *C. flexuosus* (CC=-0.094; TC=0.548), *C. zizanioides* (CC=0.269; TC=0.552) and *P. pedicellatum* (CC=0.037; TC=0.662). The average culm diameter reported for the plants in TC regarding *C. flexuosus* was 0.55±0.06 cm at 1DOT and 0.59±0.04 cm at 15DOT, thus 7.14% increase was reported, and a decrease of 0.33% was reported in CC. Concerning the plants of TC regarding *C. zizanioides*, a 12.37% increase was observed compared to an increase of 5.51% in CC. The average culm diameter of the plants of TC regarding *A. donax* is 0.52±0.09 cm at 1 DOT and 0.56±0.09 cm at 15DOT (7.82% rise), whereas in CC it is 0.55±0.05 cm and 0.56±0.04 cm respectively at 1DOT and 15DOT (2.20% rise). Regarding the species *P. pedicellatum* an increase of 2.72% in TC and only 0.86% in CC occurred. Regarding *M. maximus*, higher effect size was obtained in both CC and TC thus it was assumed that the elevated CO_2 does not influence the change in the culm diameter in this species. Considering the species *S. arundinaceum*, negligible change was noticed in the culm diameter and the effect size values go below 0.1 in both CC (0.043) and TC (0.078). Thus here also the elevated CO_2 has no considerable effect on the culm diameter. As well regarding *A. donax*, a smaller effect size was noticed in CC (0.234) and TC (0.342) but the magnitude of change was similar and the elevated CO_2 influence on the change in culm diameter was rejected in this case also. Boxplots of the culm diameter of treatment groups were represented (**Figure 2.11**). Changes in the culm diameter of CC and TC plants at 1DOT and 15DOT were depicted in **Table 2.16**.

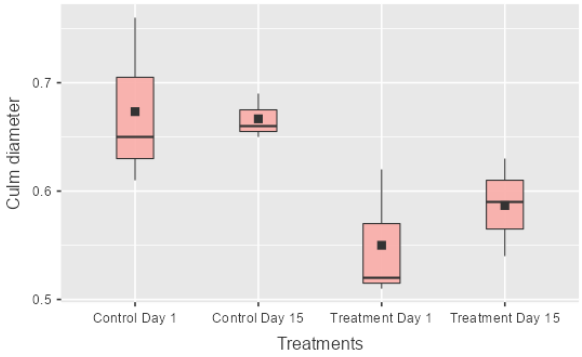
M. maximus



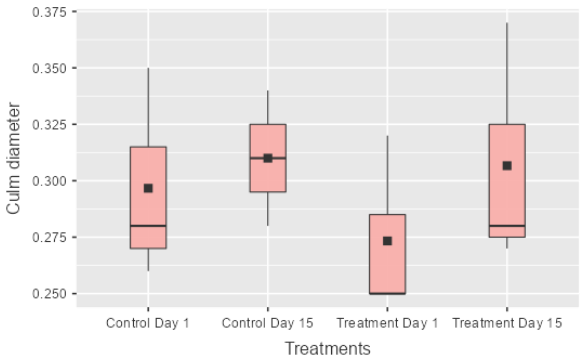
S. arundinaceum



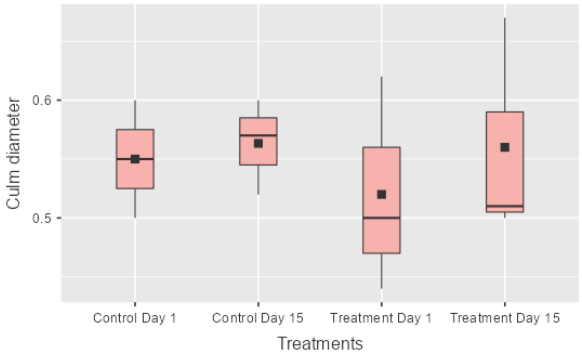
C. flexuosus



C. zizanioides



A. donax



P. pedicellatum

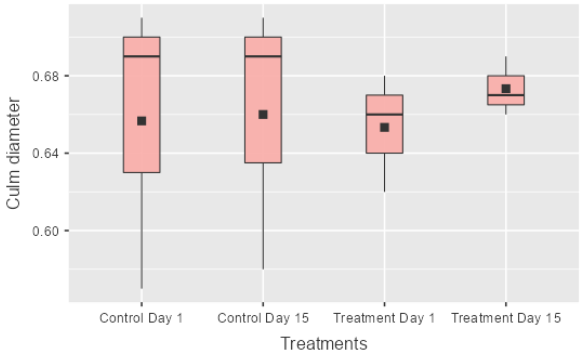


Figure 2.11: Box plot representations of changes in culm diameter

Table 2.16: Variation in culm diameter (cm) of plants under elevated levels of CO_2

Control	D1	D15	Treatment	D1	D15
Megathyrsus maximus					
C1	0.53	0.55	T1	0.47	0.56
C2	0.51	0.55	T2	0.49	0.53
C3	0.50	0.52	T3	0.56	0.59
Mean	0.51	0.54	Mean	0.51	0.56
STDEV	0.02	0.01	STDEV	0.04	0.03
% Change	5.34		% Change	10.97	
Saccharum arundinaceum					
C1	1.25	1.26	T1	1.32	1.42
C2	1.26	1.26	T2	1.04	1.04
C3	1.15	1.15	T3	1.72	1.72
Mean	1.22	1.22	Mean	1.36	1.39
STDEV	0.06	0.06	STDEV	0.34	0.34
% Change	0.10		% Change	2.33	
Cymbopogon flexuosus					
C1	0.61	0.69	T1	0.52	0.54
C2	0.65	0.66	T2	0.62	0.63
C3	0.76	0.65	T3	0.51	0.59
Mean	0.68	0.67	Mean	0.55	0.59
STDEV	0.08	0.02	STDEV	0.06	0.04
% Change	-0.33		% Change	7.14	
Chrysopogon zizanioides					
C1	0.28	0.28	T1	0.25	0.27
C2	0.26	0.31	T2	0.25	0.28
C3	0.35	0.34	T3	0.32	0.37
Mean	0.30	0.31	Mean	0.27	0.31
STDEV	0.05	0.03	STDEV	0.04	0.05
% Change	5.51		% Change	12.37	
Arundo donax					
C1	0.50	0.52	T1	0.62	0.67
C2	0.60	0.60	T2	0.50	0.50
C3	0.55	0.57	T3	0.44	0.51
Mean	0.55	0.56	Mean	0.52	0.56
STDEV	0.05	0.04	STDEV	0.09	0.09
% Change	2.20		% Change	7.82	
Pennisetum pedicellatum					
C1	0.57	0.58	T1	0.62	0.66
C2	0.71	0.71	T2	0.66	0.67
C3	0.69	0.69	T3	0.68	0.69
Mean	0.66	0.66	Mean	0.65	0.67
STDEV	0.07	0.07	STDEV	0.03	0.02
% Change	0.86		% Change	2.72	

The total effect of elevated CO_2 on the overall morphology of plants based on t-SNE plots and Hedges g.

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. Here Hedges g values (effect size) of overall morphological parameters associated with the grass species were also depicted in the t-SNE plot of CC (**Figure 2.12**) and TC (**Figure 2.13**).

Considering both plots, there are no clusters of data points, thus it was obvious that there is no similarity between the six grass species in morphological traits. When comparing the three diagonal points concerning each species in CC, diagonal points indicating the species were not considerably changed its position in all species except *P. pedicellatum*, thus there is only negligible change was happened in overall morphological traits of control plants. 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. The overall effect size or the Hedges g values of morphological traits of the plant species are depicted in **Table 2.17**.

Table 2.17: Overall effect size or the Hedges g values of morphological traits

Species	Hedges g (CC)	Hedges g (TC)
M. maximus	2.36	4.34
S. arundinaceum	1.62	3.31
C. flexuosus	0.78	0.21
C. zizanioides	0.05	0.24
A. donax	-0.09	-0.71
P. pedicellatum	7.46	0.38

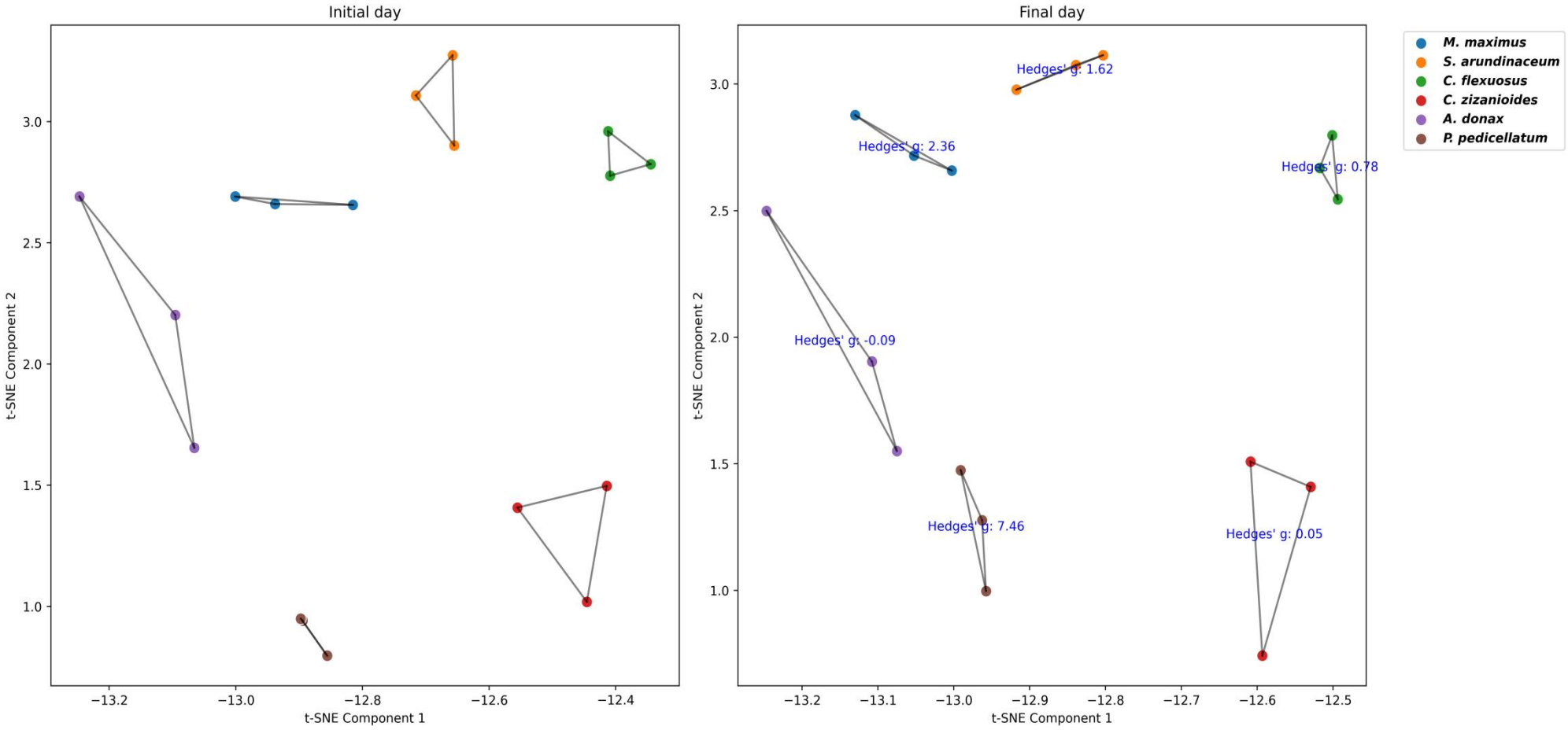


Figure 2.12: t-SNE plot of morphological parameters in CC

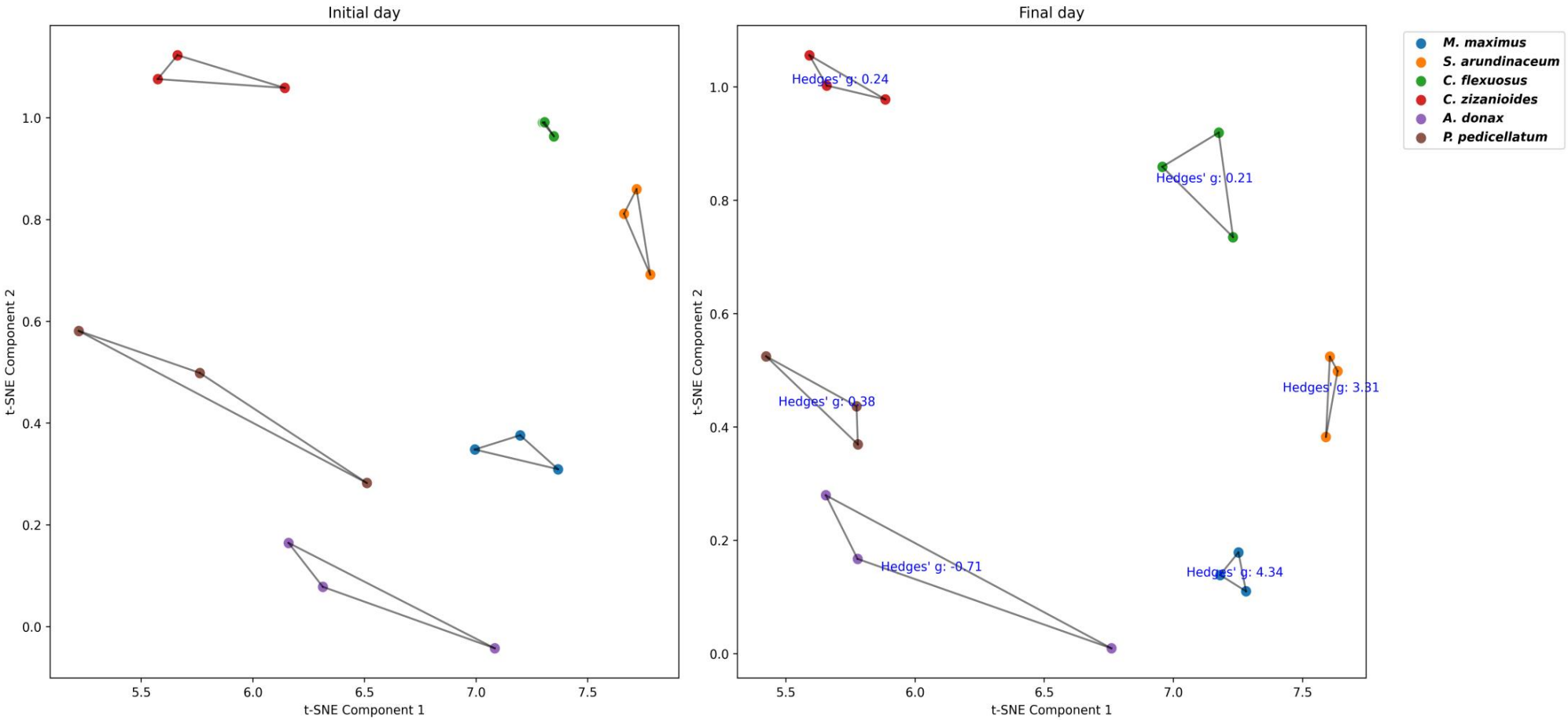


Figure 2.13: t-SNE plot of morphological parameters in TC

Plant biomass

The biomass of the plants was measured both on a dry and fresh weight basis. Regarding net day flux *S. arundinaceum* comes first followed by *M. maximus*, *A. donax*, *P. pedicellatum*, *C. flexuosus* and the least was *C. zizanioides*. While the biomass concerned, *M. maximus* and *C. zizanioides* come first with 825g on a dry weight basis, followed by *S. arundinaceum* (525g) and *P. edicellatum* (525g) and the least was with *C. flexuosus* (450g) and *A. donax* (450g).

CO_2 uptake per gram dry weight of each species under elevated CO_2 treatment was calculated. For this, net day CO_2 flux, volume of the chamber, and dry weight of the plant samples were considered. The calculation of CO_2 uptake per gram dry weight 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. For this, a formula was derived as follows (Derivation of the formula is explained in detail in the materials and methods section):

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

Based on CO_2 uptake per gram dry weight, *S. arundinaceum* was more efficient, followed by *A. donax*, *C. flexuosus*, *P. pedicellatum*, *M. maximus*, and *C. zizanioides*. The results are depicted in **Table 2.18**.

Table 2.18: CO_2 uptake potential per gram of plant biomass

Grass species	DF(N)	Total Biomass of CO_2-treated plants (Dry weight in g)	CO_2 uptake potential per gram of plant biomass (g)	CO_2 uptake potential per gram of plant biomass (ppm)
M. maximus	321.07	825	4.829×10^{-3}	0.389
S. arundinaceum	338.64	525	8.004×10^{-3}	0.645
C. flexuosus	240.86	450	6.642×10^{-3}	0.535
C. zizanioides	222.93	825	3.353×10^{-3}	0.270
A. donax	257.86	450	7.110×10^{-3}	0.573
P. pedicellatum	245.5	525	5.803×10^{-3}	0.467

DF (N): Net day flux

2.4.2 Outcomes of the analysis of plant pigments

Plant pigments such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were analyzed at 1DOT and 15DOT. Changes were noticed in the concentration of pigments at the final day of CO_2 treatment from that of initial day. To validate the significance of changes in TC with respect to CC paired samples T test was carried out. Hedges g analysis was carried out to find out the direction and magnitude of changes occurred in various pigment concentration during the study.

Concerning chlorophyll a content, an enhancement at elevated CO_2 environment was shown by *S. arundinaceum* (effect size, CC=0.670; TC=0.827) and *P. pedicellatum* (effect size, CC=2.958; TC=5.248). All other grass species exhibited a decline in the concentration of chlorophyll a in TC. Regarding *P. pedicellatum* the enhancement is statistically significant in both chambers ($p<0.05$), thus the change couldn't be considered as an effect of elevated CO_2. Concerned with *S. arundinaceum* the effect size in CC indicates a medium difference between 1DOT and 15 DOT while a large difference in the case of TC. Regarding chlorophyll content a significant change was happened in TC of *C. flexuosus* (p- value=0.008) and *A. donax* (p-value=0.008). These significant changes were a reduction in chlorophyll a content at elevated CO_2, where *C. flexuosus* exhibited a reduction of -38.79 % in the control plants and -45.16% in CO_2 treated plants. In *A. donax*, mean chlorophyll a content in TC at 1DOT is 1.911±0.384 mg g^{-1} and 33.59%, which reduced during the experimental period and was reported to be 1.274±0.286 mg g^{-1} at 15DOT. Whereas in CC it was 1.678±0.197 mg g^{-1} at 1DOT and 1.315±0.379 mg g^{-1} at 15 DOT, i.e the reduction in chlorophyll a content was only 21.59%. Concerning the species *M. maximus* a reduction in chlorophyll a content was noticed in both chambers with similar magnitude (effect size, CC=-1.119; TC=-1.219), thus elevated CO_2 has not much influence on the chlorophyll a content of this species. Boxplots of the changes in chlorophyll a content in the treatment groups of 6 grass species are represented (**Figure 2.14**). **Table 2.19** represents changes in the chlorophyll a content during the study period.

As the chlorophyll b is concerned, a reduction was noticed in all plants except *S. arundinaceum*, where an increase was reported. Paired samples T test shows $p<0.05$ in TC regarding *C. flexuosus*, *C. zizanioides*, *A. donax* and *P. pedicellatum*. However

regarding *C. zizanioides*, *A. donax* and *P. pedicellatum*, control plants also exhibited significant changes. Thus the reduced chlorophyll b content in the TC of these species couldn't be assumed as a result of elevated CO_2. In *C. zizanioides* higher reduction was noticed in TC where the chlorophyll b content at 15 DOT is 0.129±0.75 mg g^{-1}, which is 68.53% lower than 1DOT (0.416±0.030 mg g^{-1}). While in CC it is 0.161±0.020 mg g^{-1} at 15 DOT, 52.33% lower than the mean chlorophyll b content in CC (0.353±0.080 mg g^{-1}). Likewise in *A. donax* higher reduction of chlorophyll b content was noticed in TC (-32.50%) than in CC (-29.63%). Control plants of *P. pedicellatum* exhibited a mean chlorophyll b content of 0.100±0.058 mg g^{-1} in TC and 0.075±0.087 mg g^{-1} in CC at 15DOT; it was a reduction of 81.90% and 73.32% from initial day (CC=0.443±0.040 mg g^{-1}; TC=0.405±0.067 mg g^{-1}) in CC and TC respectively. Concerning *M. maximus,* the chlorophyll b content in TC at 15DOT is 0.421±0.054 mg g^{-1}, which was 61.73% lower than 1DOT (1.392±0.698 mg g^{-1}). Likewise in CC, a reduction in chlorophyll b content is noticed but a higher reduction than TC (-78.70%). As mentioned, *C. flexuosus* only exhibited a significant change in chlorophyll b content as a result of the influence of elevated CO_2 (TC, p-value=0.011). Concerning the Hedges g values of chlorophyll b content, all species in both treatments show a higher effect. Mean differences of the variations in chlorophyll b content of the treatment group concerning the grass species are represented (**Figure 2.15**). **Table 2.20** depicts the variations in chlorophyl b content of plants under experimentation.

Regarding total chlorophyll, an augmentation was noticed only in *S. arundinaceum* and *P. pedicellatum.* In all other species, total chlorophyll content decreased in both treatments. While differences were noticed between treatment groups. Effect sizes obtained indicate higher differences between initial and final days and regarding *C. flexuosus*, *C. zizanioides*, *A. donax,* and *P. pedicellatum,* effect sizes were higher in TC than CC. As well the changes in the total chlorophyll content of elevated CO_2-treated plants of *C. flexuosus* and *A. donax* were significant according to paired samples T-test. The mean total chlorophyll content of CC plants of *C. flexuosus* was 1.569±0.588 mg g^{-1} and 2.892±0.714 mg g^{-1} at 1DOT and 15DOT respectively (39.94% reduced). Here in TC, compared to 1DOT (1.551±0.281 mg g^{-1}) 44.90 % were deceased at 15DOT (2.806±0.303 mg g^{-1}). Concerning the species *A. donax,* the mean total chlorophyll content in CC at 1DOT was 2.157±0.269 mg g^{-1} and at 15DOT it was 1.656±0.480 mg g^{-1} (23.63% reduction). While in TC, the mean values are 2.390±0.503

mg g^{-1} and 1.599±0.379 mg g^{-1} respectively at 1DOT and 15DOT (33.37% reduction). As mentioned, augmentation was noticed only in *S. arundinaceum* and *P. pedicellatum*, where in the former the increase was lower in TC plants (14.29%), compared to CC (16.74%). However, in the later, the percentage increase in total chlorophyll content was almost double that of CC, where the mean total chlorophyll content in TC at 15DOT is 2.170±0.197 mg g^{-1}, which is 31.84% higher compared to initial content (1.659±0.116 mg g^{-1}), and in CC only an increase of 14.05% was noticed. In *M. maximus* mean total chlorophyll content in TC is 3.529±0.924 mg g^{-1} and 2.214±0.258 mg g^{-1} at 1DOT and 15DOT respectively (33.53% decreased). Whereas in CC compared to TC, a higher reduction in total chlorophyll content was noticed, it was 4.549±0.500 mg g^{-1} and 2.274±0.682 mg g-^{1} respectively at 1DOT and 15DOT (49.56% reduction). Control plants of *M. maximus* show significant change while no significance is with CO_2 treated plants. Thus, in this case, the elevated CO_2 effect may contribute to different performance in TC compared to CC. Changes in the total chlorophyll content in treatment groups were depicted (**Table 2.21**) and mean differences in the total chlorophyll content in treatment groups regarding experimented grass species were represented in box plots (**Figure 2.16**).

Changes were noticed in the carotenoid content of all grass species under study. Effect sizes were found out through Hedges g analysis and the values indicate a decrease in the total carotenoid content of *M. maximus, C. flexuosus, C. zizanioides,* and *A.donax*, while it was increased in *S. arundinaeum* and *P. pedicellatum*. A significant decrease in the carotenoid content was noticed in *C. flexuosus* ($p < 0.05$) and *A. donax* ($p < 0.05$) compared to an insignificant change in their control plants. The total carotenoid content of *C. flexuosus* in TC at 15 DOT was 0.061±0.008 mg g^{-1}, which was 34.29% lower than 1DOT (0.093±0.014 mg g^{-1}); here the decline in carotenoid content associated with CC was 31.54%. Likewise in *A. donax*, carotenoid content in TC at 1DOT was 0.110±0.020 mg g^{-1}, from which 33.62% decreased due to high CO_2 condition, and the amount reported on the final day of treatment was 0.073±0.014 mg g^{-1}. Only a 19.03% decrease was noted in CC during the experimental period. As mentioned in species *S. arundinaeum* and *P. pedicellatum*, carotenoid content increased and was significant in both control and treatment ($p > 0.05$). Hedges g values indicate higher and similar changes regarding the carotenoid content in both CC (4.477) and TC (3.262) of *S. arundinaceum* and *P. pedicellatum* (CC=2.959; TC=4.401). Thus the

changes noticed in these species can't be considered as a result of elevated CO_2 effect. Significant changes were not noticed in *M. maximus* and *C. zizanioides* treated under elevated CO_2 conditions. Box plot representations of the carotenoid content of grasses in treatment groups were illustrated (**figure 2.17**). Variations in the carotenoid content of CC and TC are depicted in **Table 2.22**. Paired T-test significance values and Hedges g (effect size) values regarding pigments are depicted in **Table 2.23**.

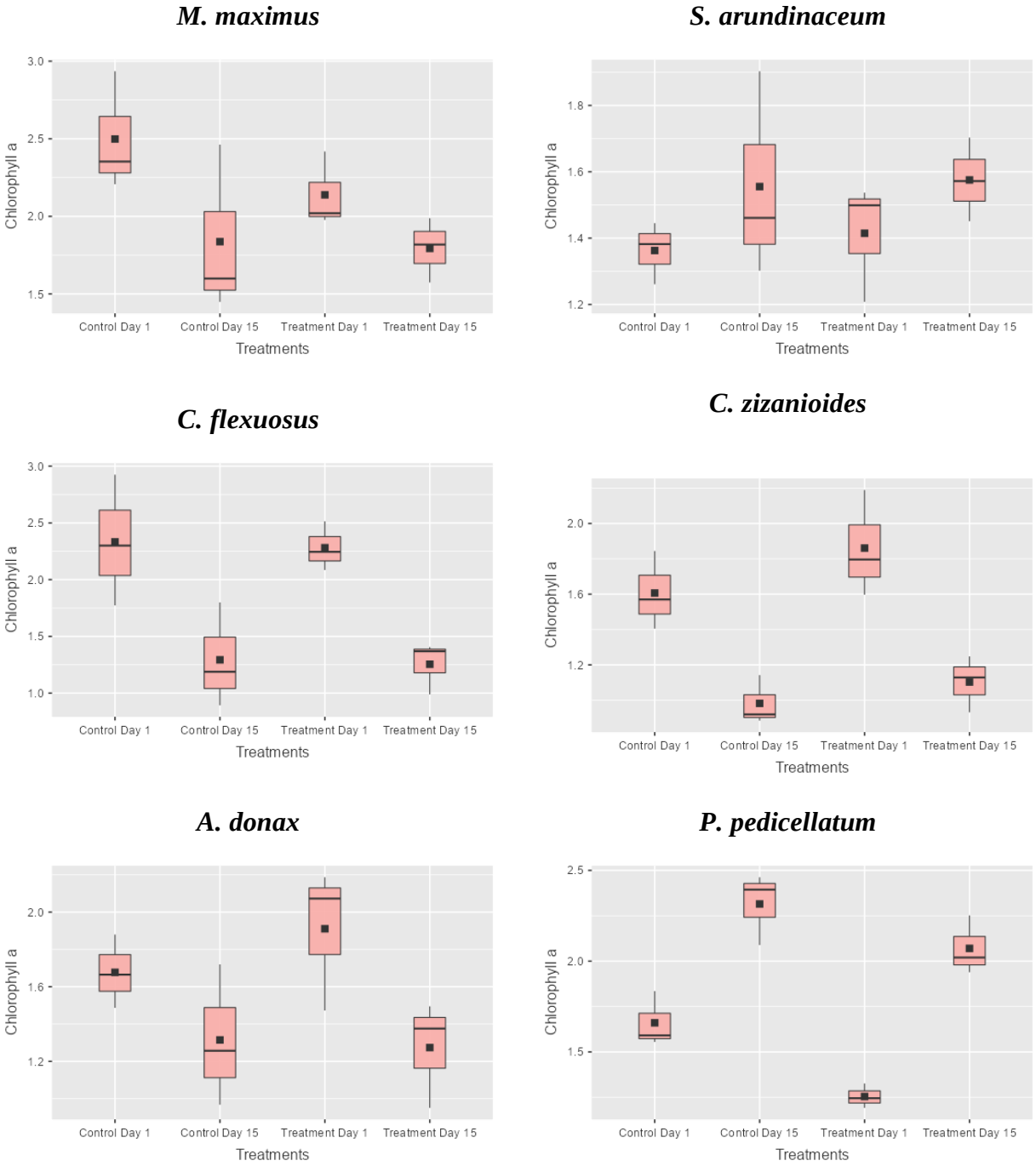


Figure 2.14: Box plot representations of changes in Chlorophyll a

Table 2.19: Variations in Chlorophyll a (mg g^{-1}) of plants under elevated levels of CO_2

Control	D1	D5	D10	D15	Treatment	D1	D5	D10	D15
Megathyrsus maximus									
C1	2.207	1.005	1.277	1.449	T1	2.418	1.944	1.154	1.574
C2	2.935	1.042	1.472	1.599	T2	1.977	1.242	2.069	1.818
C3	2.353	1.059	1.271	2.462	T3	2.020	2.420	1.127	1.988
Mean	2.498	1.035	1.340	1.837	Mean	2.138	1.869	1.450	1.793
STDEV	0.385	0.028	0.114	0.547	STDEV	0.243	0.593	0.536	0.208
% Change	-25.077				% Change	-14.844			
Saccharum arundinaceum									
C1	1.382	1.559	1.649	1.903	T1	1.208	1.281	1.411	1.703
C2	1.445	1.655	1.509	1.302	T2	1.537	1.550	1.487	1.572
C3	1.261	1.394	1.790	1.461	T3	1.499	1.263	1.471	1.451
Mean	1.363	1.536	1.649	1.555	Mean	1.414	1.364	1.457	1.575
STDEV	0.094	0.132	0.141	0.311	STDEV	0.180	0.161	0.040	0.126
% Change	14.563				% Change	13.354			
Cymbopogon flexuosus									
C1	2.300	2.558	1.464	1.188	T1	2.514	2.578	1.215	1.369
C2	2.925	2.024	1.769	0.893	T2	2.085	1.971	1.132	0.989
C3	1.773	2.814	2.372	1.799	T3	2.245	2.585	1.711	1.405
Mean	2.333	2.466	1.868	1.293	Mean	2.281	2.378	1.352	1.255
STDEV	0.577	0.403	0.462	0.462	STDEV	0.217	0.353	0.313	0.230
% Change	-38.792				% Change	-45.162			
Chrysopogon zizanioides									
C1	1.570	1.122	0.944	0.885	T1	1.597	1.223	1.484	1.129
C2	1.844	1.428	1.553	1.142	T2	2.189	1.075	1.015	0.932
C3	1.405	1.454	1.036	0.920	T3	1.796	0.956	0.951	1.248
Mean	1.606	1.335	1.178	0.982	Mean	1.861	1.085	1.150	1.103
STDEV	0.222	0.184	0.329	0.140	STDEV	0.301	0.134	0.291	0.160
% Change	-38.738				% Change	-39.088			
Arundo donax									
C1	1.487	1.774	1.296	0.968	T1	2.187	2.129	1.741	1.495
C2	1.665	1.871	1.698	1.720	T2	2.073	1.612	1.541	1.376
C3	1.880	1.518	1.685	1.257	T3	1.473	1.674	1.630	0.951
Mean	1.678	1.721	1.560	1.315	Mean	1.911	1.805	1.637	1.274
STDEV	0.197	0.182	0.228	0.379	STDEV	0.384	0.282	0.100	0.286
% Change	-21.588				% Change	-33.588			
Pennisetum pedicellatum									
C1	1.591	2.317	1.731	2.462	T1	1.245	1.950	1.880	2.020
C2	1.835	2.334	2.117	2.394	T2	1.326	2.054	1.705	1.939
C3	1.555	2.876	2.306	2.089	T3	1.192	2.868	1.911	2.252
Mean	1.660	2.509	2.051	2.315	Mean	1.254	2.291	1.832	2.070
STDEV	0.152	0.318	0.293	0.199	STDEV	0.067	0.503	0.111	0.163
% Change	39.836				% Change	65.800			

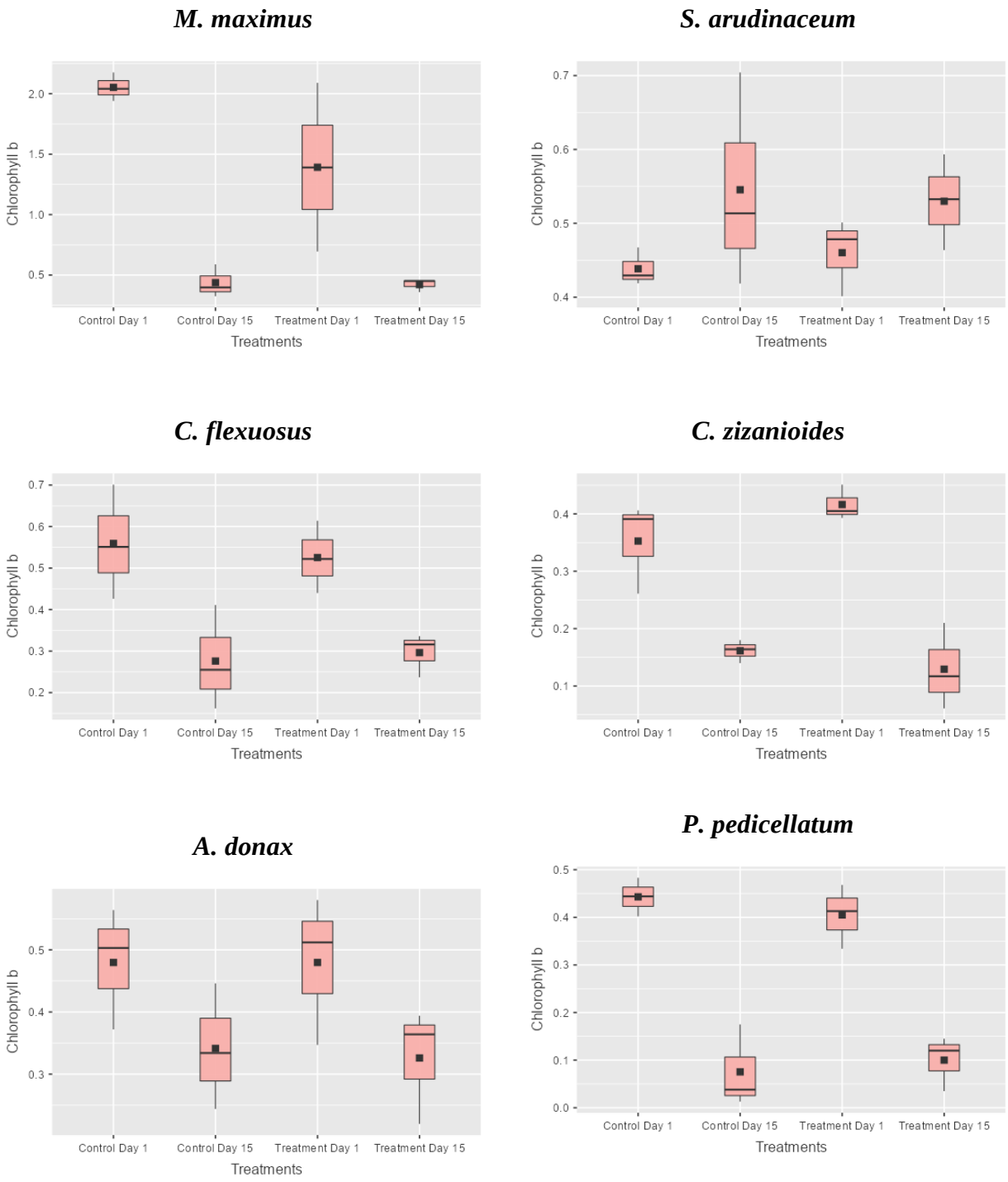


Figure 2.15: Box plot representations of changes in Chlorophyll b

Table 2.20: Variations in Chlorophyll b (mg g^{-1}) of plants under elevated levels of CO_2

Control	D1	D5	D10	D15	Treatment	D1	D5	D10	D15
Megathyrsus maximus									
C1	1.940	0.280	0.302	0.325	T1	2.090	1.903	0.286	0.359
C2	2.176	0.617	0.359	0.398	T2	0.695	0.315	0.451	0.453
C3	2.041	0.285	0.274	0.589	T3	1.390	2.047	0.284	0.451
Mean	2.052	0.394	0.312	0.437	Mean	1.392	1.422	0.340	0.421
STDEV	0.118	0.193	0.043	0.136	STDEV	0.698	0.961	0.096	0.054
% Change	-78.700					-61.732			
Saccharum arundinaceum									
C1	0.4294	0.5419	0.5886	0.5135	T1	0.4013	0.4174	0.4983	0.5933
C2	0.4673	0.5065	0.6833	0.7042	T2	0.5011	0.4779	0.5745	0.4637
C3	0.4188	0.4741	0.5490	0.4185	T3	0.4784	0.4303	0.5375	0.5326
Mean	0.4385	0.5075	0.6070	0.5454	Mean	0.4603	0.4419	0.5368	0.5298
STDEV	0.0255	0.0339	0.0690	0.1455	STDEV	0.0523	0.0319	0.0381	0.0648
% Change	23.4016				% Change	17.2294			
Cymbopogon flexuosus									
C1	0.551	0.376	0.252	0.255	T1	0.614	0.381	0.289	0.336
C2	0.701	0.322	0.379	0.162	T2	0.440	0.194	0.239	0.237
C3	0.426	0.398	0.574	0.411	T3	0.522	0.354	0.498	0.316
Mean	0.559	0.365	0.401	0.276	Mean	0.525	0.310	0.342	0.296
STDEV	0.138	0.040	0.162	0.126	STDEV	0.087	0.101	0.138	0.053
% Change	-44.747				% Change	-43.637			
Chrysopogon zizanioides									
C1	0.391	0.237	0.192	0.140	T1	0.393	0.273	0.343	0.210
C2	0.406	0.241	0.274	0.180	T2	0.451	0.229	0.197	0.117
C3	0.261	0.280	0.189	0.164	T3	0.405	0.206	0.183	0.061
Mean	0.353	0.253	0.218	0.161	Mean	0.416	0.236	0.241	0.129
STDEV	0.080	0.024	0.048	0.020	STDEV	0.030	0.034	0.088	0.075
% Change	-52.333				% Change	-68.530			
Arundo donax									
C1	0.372	0.450	0.289	0.244	T1	0.580	0.559	0.444	0.394
C2	0.564	0.439	0.460	0.446	T2	0.512	0.412	0.377	0.364
C3	0.503	0.397	0.459	0.334	T3	0.347	0.408	0.399	0.220
Mean	0.480	0.429	0.403	0.342	Mean	0.480	0.460	0.407	0.326
STDEV	0.098	0.028	0.098	0.101	STDEV	0.120	0.086	0.034	0.093
% Change	-29.627				% Change	-32.500			
Pennisetum pedicellatum									
C1	0.444	0.434	0.380	0.013	T1	0.468	0.024	0.037	0.035
C2	0.483	0.140	0.054	0.038	T2	0.413	0.010	0.200	0.120
C3	0.402	0.363	0.009	0.175	T3	0.334	0.286	0.038	0.145
Mean	0.443	0.312	0.148	0.075	Mean	0.405	0.107	0.092	0.100
STDEV	0.040	0.154	0.203	0.087	STDEV	0.067	0.156	0.094	0.058
% Change	-81.901				% Change	-73.323			

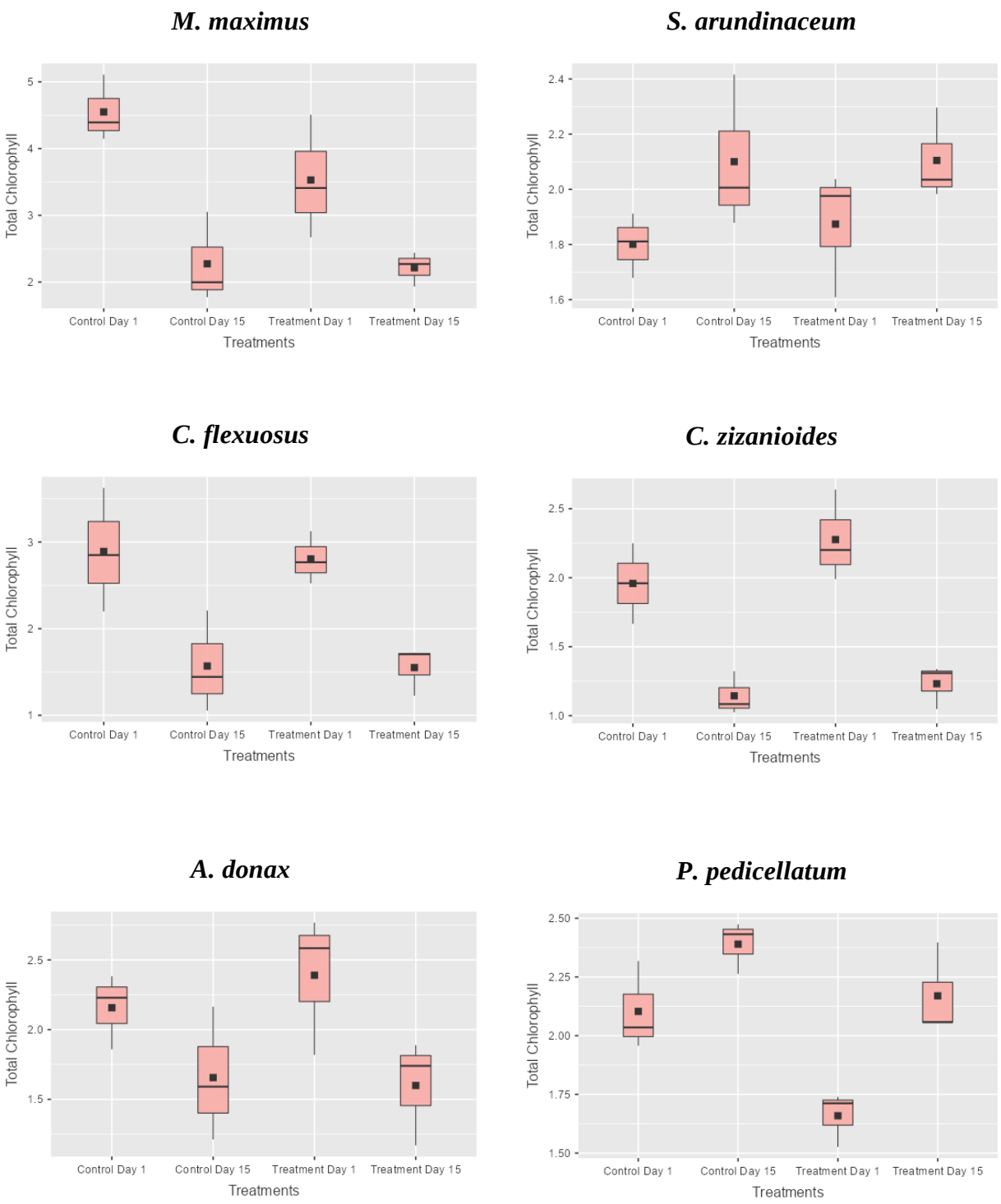


Figure 2.16: Box plot representations of changes in Total Chlorophyll

Table 2.21: Variations in Total Chlorophyll (mg g^{-1}) of plants under elevated levels of CO_2

Control	D1	D5	D10	D15	Treatment	D1	D5	D10	D15
Megathyrsus maximus									
C1	4.147	1.284	1.579	1.773	T1	4.507	3.846	1.439	1.932
C2	5.109	1.326	1.831	1.997	T2	2.671	1.557	2.521	2.270
C3	4.392	1.343	1.544	3.051	T3	3.409	4.465	1.411	2.439
Mean	4.549	1.318	1.651	2.274	Mean	3.529	3.289	1.790	2.214
STDEV	0.500	0.030	0.157	0.682	STDEV	0.924	1.532	0.633	0.258
% Change	-49.564				% Change	-33.534			
Saccharum arundinaceum									
C1	1.811	2.101	2.237	2.416	T1	1.609	1.698	1.909	2.296
C2	1.912	2.161	2.191	2.006	T2	2.037	2.027	2.061	2.035
C3	1.679	1.867	2.338	1.879	T3	1.976	1.693	2.008	1.983
Mean	1.801	2.043	2.255	2.100	Mean	1.874	1.806	1.993	2.105
STDEV	0.117	0.155	0.075	0.280	STDEV	0.232	0.192	0.077	0.167
% Change	16.739				% Change	14.296			
Cymbopogon flexuosus									
C1	2.850	2.934	1.715	1.443	T1	3.126	2.959	1.503	1.705
C2	3.625	2.345	2.147	1.054	T2	2.524	2.164	1.371	1.226
C3	2.199	3.212	2.945	2.209	T3	2.767	2.938	2.208	1.721
Mean	2.892	2.830	2.269	1.569	Mean	2.806	2.687	1.694	1.551
STDEV	0.714	0.443	0.624	0.588	STDEV	0.303	0.453	0.450	0.281
% Change	-39.944				% Change	-44.903			
Chrysopogon zizanioides									
C1	1.960	1.359	1.135	1.025	T1	1.990	1.496	1.826	1.338
C2	2.250	1.668	1.827	1.322	T2	2.639	1.304	1.211	1.048
C3	1.666	1.734	1.224	1.084	T3	2.201	1.161	1.134	1.309
Mean	1.959	1.587	1.396	1.144	Mean	2.277	1.321	1.390	1.232
STDEV	0.292	0.200	0.376	0.157	STDEV	0.331	0.168	0.379	0.160
% Change	-41.299				% Change	-44.511			
Arundo donax									
C1	1.859	2.224	1.585	1.212	T1	2.767	2.687	2.185	1.888
C2	2.229	2.309	2.158	2.165	T2	2.584	2.023	1.917	1.740
C3	2.383	1.915	2.143	1.591	T3	1.819	2.081	2.029	1.170
Mean	2.157	2.150	1.962	1.656	Mean	2.390	2.264	2.044	1.599
STDEV	0.269	0.207	0.326	0.480	STDEV	0.503	0.368	0.134	0.379
% Change	-23.626				% Change	-33.372			
Pennisetum pedicellatum									
C1	2.035	2.751	2.111	2.474	T1	1.712	1.973	1.917	2.054
C2	2.318	2.474	2.170	2.432	T2	1.739	2.063	1.904	2.058
C3	1.957	3.239	2.314	2.263	T3	1.526	3.154	1.949	2.397
Mean	2.103	2.821	2.199	2.390	Mean	1.659	2.397	1.923	2.170
STDEV	0.190	0.387	0.105	0.112	STDEV	0.116	0.657	0.023	0.197
% Change	14.055				% Change	31.840			

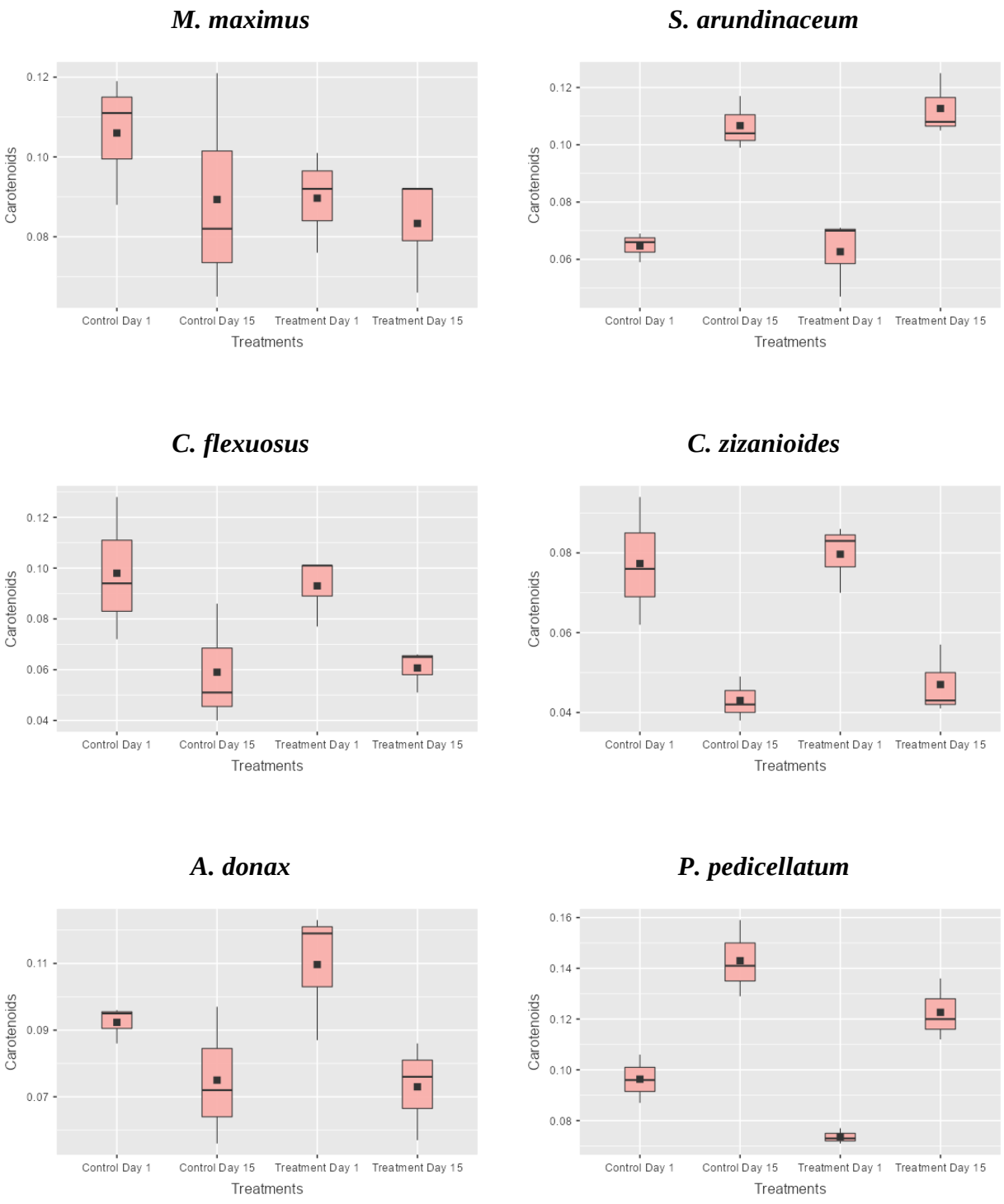


Figure 2.17: Box plot representations of changes in Carotenoids

Table 2.22: Variations in Carotenoids of plants (mg g^{-1}) under elevated CO_2

Control	D1	D5	D10	D15	Treatment	D1	D5	D10	D15
Megathyrsus maximus									
C1	0.111	0.038	0.062	0.065	T1	0.076	0.098	0.052	0.066
C2	0.119	0.042	0.068	0.082	T2	0.092	0.062	0.098	0.092
C3	0.088	0.052	0.053	0.121	T3	0.101	0.107	0.056	0.092
Mean	0.106	0.044	0.061	0.090	Mean	0.090	0.089	0.069	0.083
STDEV	0.016	0.007	0.008	0.028	STDEV	0.013	0.024	0.025	0.015
% Change	-11.184				% Change	-7.389			
Saccharum arundinaceum									
C1	0.066	0.102	0.122	0.117	T1	0.047	0.057	0.087	0.108
C2	0.069	0.106	0.137	0.104	T2	0.070	0.062	0.096	0.105
C3	0.059	0.097	0.121	0.099	T3	0.071	0.072	0.122	0.125
Mean	0.065	0.101	0.126	0.107	Mean	0.063	0.064	0.102	0.113
STDEV	0.005	0.005	0.009	0.009	STDEV	0.014	0.008	0.018	0.010
% Change	65.414				% Change	85.321			
Cymbopogon flexuosus									
C1	0.094	0.111	0.063	0.051	T1	0.101	0.114	0.057	0.066
C2	0.128	0.089	0.081	0.040	T2	0.077	0.082	0.051	0.051
C3	0.072	0.126	0.110	0.086	T3	0.101	0.116	0.076	0.065
Mean	0.098	0.108	0.085	0.059	Mean	0.093	0.104	0.061	0.061
STDEV	0.028	0.019	0.023	0.024	STDEV	0.014	0.019	0.013	0.008
% Change	-31.543				% Change	-34.289			
Chrysopogon zizanioides									
C1	0.076	0.055	0.045	0.038	T1	0.070	0.059	0.072	0.057
C2	0.094	0.064	0.073	0.049	T2	0.083	0.057	0.052	0.041
C3	0.062	0.072	0.050	0.042	T3	0.086	0.052	0.050	0.043
Mean	0.077	0.064	0.056	0.043	Mean	0.080	0.056	0.058	0.047
STDEV	0.016	0.008	0.015	0.006	STDEV	0.009	0.004	0.012	0.009
% Change	-43.615				% Change	-39.769			
Arundo donax									
C1	0.086	0.108	0.072	0.056	T1	0.123	0.122	0.098	0.086
C2	0.095	0.109	0.093	0.097	T2	0.119	0.096	0.084	0.076
C3	0.096	0.092	0.097	0.072	T3	0.087	0.096	0.090	0.057
Mean	0.092	0.103	0.087	0.075	Mean	0.110	0.105	0.091	0.073
STDEV	0.006	0.010	0.013	0.020	STDEV	0.020	0.015	0.007	0.014
% Change	-19.026				% Change	-33.617			
Pennisetum pedicellatum									
C1	0.096	0.145	0.107	0.159	T1	0.073	0.127	0.118	0.120
C2	0.106	0.146	0.138	0.141	T2	0.077	0.137	0.110	0.112
C3	0.087	0.163	0.151	0.129	T3	0.071	0.174	0.116	0.136
Mean	0.096	0.151	0.132	0.143	Mean	0.073	0.146	0.115	0.123
STDEV	0.009	0.011	0.023	0.015	STDEV	0.003	0.024	0.004	0.012
% Change	48.973				% Change	68.035			

2.4.3 Outcomes of the analysis of plant metabolites

Major metabolites such as carbohydrates, protein, and phenol were measured on 1DOT and 15 DOT of the experiment. Changes were analyzed and validated via paired samples T-test. To find out the direction and magnitude of changes Hedges g statistics were performed for control and treatment groups, separately. Hedges g values or effect size normally range from 0.2 to 0.8, where 0.2 is a small effect size which indicates a small difference between two groups, 0.5 a medium difference, and 0.8 a large difference. The effect size value may sometimes go beyond 1.00 or below 0.2 based on the magnitude of the difference between the two groups. Hedges g value may be positive or negative based on positive or negative variation. Significance values of paired samples, T-test, and Hedges g values (effect size) were depicted in **Table 2.23**.

Changes were detected in the concentration of carbohydrates in grass plants subjected to 15 days of experimentation. To validate the significance of these changes, paired samples T-tests were conducted, in which carbohydrate concentrations of 1DOT and 15DOT were considered as two groups. No significance was noticed in the T-test results in the concentration of carbohydrates at 1DOT and 15DOT. Since there were detectable changes, to find out the direction and magnitude of these changes Hedges g statistics were performed for control and treatment groups separately. Regarding carbohydrate concentration, *M. maximus*, *C. zizanioides*, and *A. donax* show a higher positive effect size in TC (0.918, 0.957, and 1.909, respectively) compared to CC (0.662, 0.292, and -0.266, respectively), which indicates the greater influence of elevated CO_2 levels on the carbohydrate concentration of these species. Among these three species, *A. donax* exhibited an extremely high positive effect size in TC compared to a smaller negative effect size in CC. Here the mean carbohydrate concentration in TC on the final day of treatment was reported to be 46.50±2.26 mg g^{-1}, which was 25.58% greater than the initial day (37.50±4.83 mg g^{-1}), and in CC carbohydrate concentration decreased by 1.05%. In *S. arundinaceum* and *C. flexuosus*, carbohydrate concentration decreased in both treatments, where higher negative effect sizes were noticed in both treatment groups (CC=-1.508; TC=-1.499) of *S. arundinaceum*, while the effect size in TC (-0.624) of *C. flexuosus* was lower than CC (-1.924). In *P. pedicellatum*, higher positive effect size in CC (6.303) compared to TC (0.210), i.e. only 2.40% was increased in TC compared to CC (21.93%). Thus it is assumed that the

elevated CO_2 effect may contribute to this lower change in the mean carbohydrate concentration of plants in TC compared to CC. Mean differences in carbohydrate concentration of treatment groups were represented in boxplots (**Figure 2.18**). Changes in carbohydrate concentration in treatment groups during various days of treatment were also depicted (**Table 2.24**).

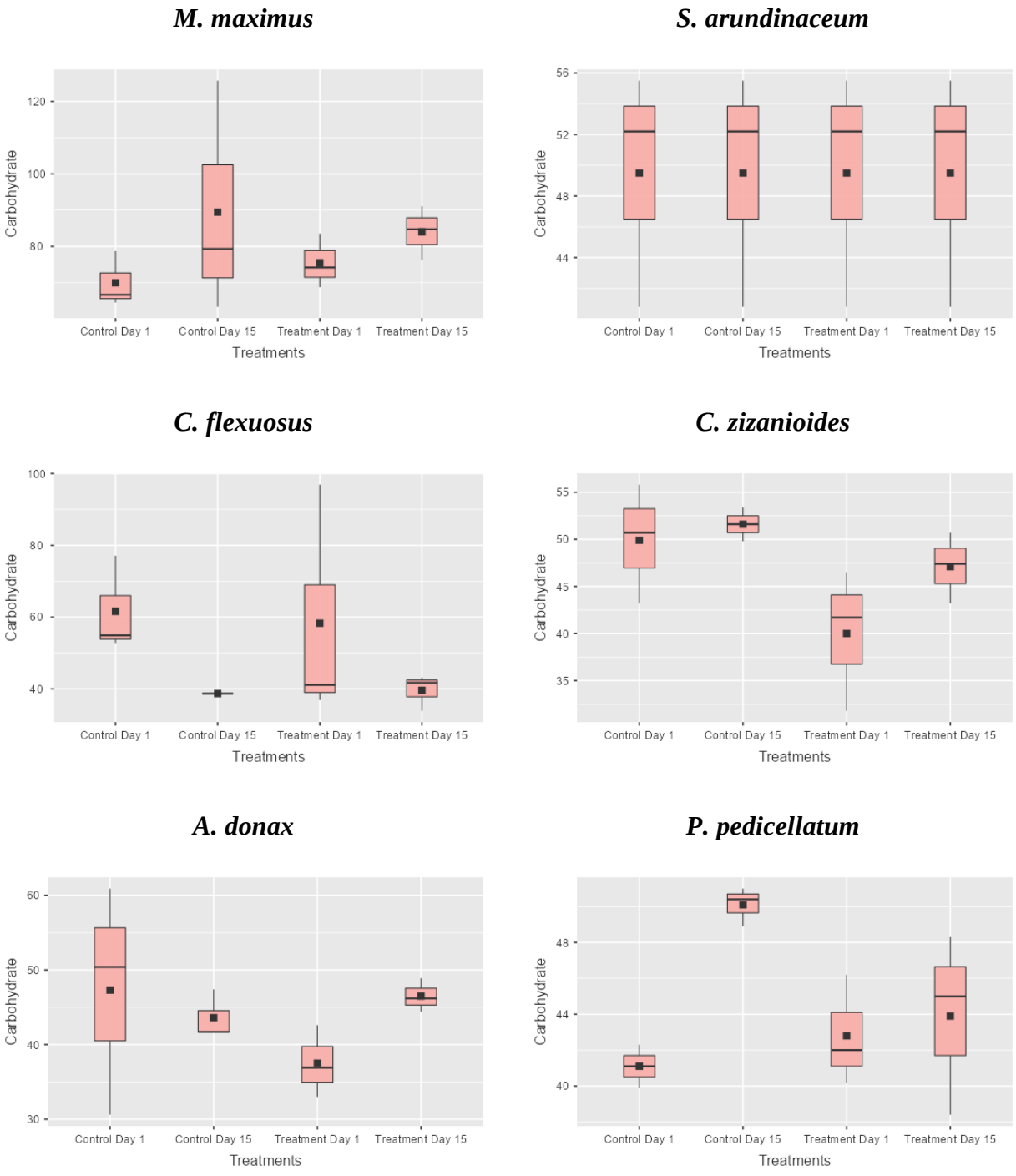


Figure 2.18: Box plot representations of changes in Carbohydrates

Table 2.23: Paired samples T-test significance values and Hedges g values regarding plant pigments and metabolites

Pigments & metabolites	Species	Paired samples T-test (p value)		Hedges g (effect size)	
		Control	Treatment	Control	Treatment
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

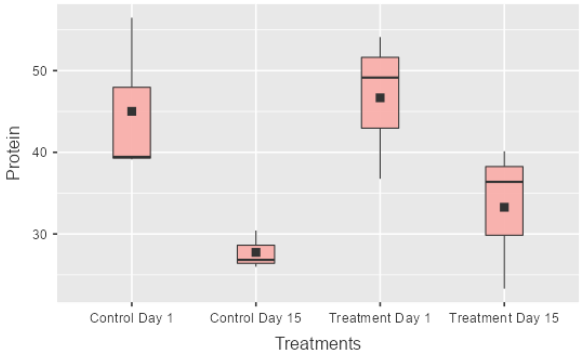
Table 2.24: Variations in Carbohydrate (mg g^{-1}) of plants under elevated levels of CO_2

Control	D1	D5	D10	D15	Treatment	D1	D5	D10	D15
Megathyrsus maximus									
C1	66.63	60.90	28.04	79.30	T1	74.17	62.41	28.04	76.28
C2	64.52	54.57	41.01	63.32	T2	68.74	60.30	28.04	84.72
C3	78.69	60.00	32.86	125.73	T3	83.52	50.35	31.96	91.06
Mean	69.95	58.49	33.97	89.45	Mean	75.48	57.69	29.35	84.02
STDEV	7.65	3.42	6.55	32.42	STDEV	7.47	6.44	2.26	7.41
% Change	25.64				% Change	11.71			
Saccharum arundinaceum									
C1	61.2	61.2	47.4	44.4	T1	67.2	73.5	48.9	40.8
C2	57.3	70.5	47.1	38.4	T2	58.2	52.8	54.6	55.5
C3	88.2	67.2	57.6	51.6	T3	59.4	59.1	52.5	52.2
Mean	68.90	66.30	50.70	44.80	Mean	61.60	61.80	52.00	49.50
STDEV	16.83	4.71	5.98	6.61	STDEV	4.89	10.61	2.88	7.71
% Change	-33.98				% Change	-18.68			
Cymbopogon flexuosus									
C1	77.1	48	44.1	38.7	T1	41.1	42.9	40.2	33.9
C2	52.8	53.4	54.3	38.7	T2	36.9	44.1	40.2	43.2
C3	54.9	60.6	35.1	38.7	T3	96.9	37.5	58.2	41.7
Mean	61.60	54.00	44.50	38.70	Mean	58.30	41.50	46.20	39.60
STDEV	13.46	6.32	9.61	0.00	STDEV	33.49	3.52	10.39	4.99
% Change	-35.34				% Change	-19.14			
Chrysopogon zizanioides									
C1	55.80	32.70	46.20	53.40	T1	41.70	37.50	36.30	47.40
C2	43.20	41.70	27.90	49.80	T2	31.80	36.60	42.90	43.20
C3	50.70	41.40	38.70	51.60	T3	46.50	37.80	48.60	50.70
Mean	49.90	38.60	37.60	51.60	Mean	40.00	37.30	42.60	47.10
STDEV	6.34	5.11	9.20	1.80	STDEV	7.50	0.62	6.16	3.76
% Change	4.25				% Change	19.52			
Arundo donax									
C1	60.90	50.40	46.80	47.40	T1	33.00	44.40	41.40	46.20
C2	30.60	39.60	43.20	41.70	T2	36.90	47.70	45.90	48.90
C3	50.40	46.20	42.30	41.70	T3	42.60	47.70	41.10	44.40
Mean	47.30	45.40	44.10	43.60	Mean	37.50	46.60	42.80	46.50
STDEV	15.39	5.44	2.38	3.29	STDEV	4.83	1.91	2.69	2.26
% Change	-1.05				% Change	25.58			
Pennisetum pedicellatum									
C1	41.10	42.30	44.10	51.00	T1	42.00	42.60	49.20	45.00
C2	39.90	45.60	54.60	48.90	T2	40.20	43.50	49.20	38.40
C3	42.30	45.90	62.40	50.40	T3	46.20	45.60	49.80	48.30
Mean	41.10	44.60	53.70	50.10	Mean	42.80	43.90	49.40	43.90
STDEV	1.20	2.00	9.18	1.08	STDEV	3.08	1.54	0.35	5.04
% Change	21.93				% Change	2.40			

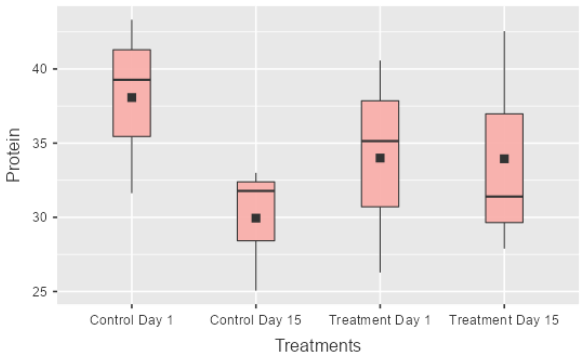
Protein concentration was decreased in *M. maximus*, *S. arundinaceum*, and *C. flexuosus* at elevated CO_2 treatment. Effect size values of both treatments of *M. maximus* (CC=-1.915; CC=-1.206) and *C. flexuosus* (CC=-2.484; TC=-1.060) indicate higher differences between initial and final days, thus the changes were not considered to be caused by elevated CO_2. This assumption was also supported by significant T-test results in both CC (p-value, 0.050 and 0.010 respectively) and TC (p-value, 0.033 and 0.008 respectively). Protein concentrations of *A. donax* and *P. pedicellatum* were increased, but effect sizes were similar in both CC and TC, and the elevated CO_2 effect was also rejected. In *C. zizaanioides,* protein concentration was increased by 1.83% in TC, compared to a decrease of 16% in CC. Thus considering the 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, which could be considered to be an influence of elevated CO_2. Mean differences in protein concentration of grass species in CC and TC were illustrated in **Figure 2.19**. **Table 2.25** shows variations in protein concentration that occurred during the study.

Considering the concentration of total phenol, significant change due to elevated CO_2 was shown only by *C. Zizanioides* (p value=0.011). Hedges g analysis was carried out to find out the direction and magnitude of changes and it was noticed that the phenol concentration decreased in *M. maximus, C. flexuosus* and *C. zizanioides*. In *C. zizanioides* a significant decrease in the concentration of phenol was noticed during the 15 days of the experiment in TC, where the mean phenol content was reported to be 16.67±2.97 mg g^{-1} in TC on the final day of the experiment, which was 35.49% lower than initial day (25.75±2.63 mg g^{-1}). In the other two species mentioned, the decrease has happened at a similar rate in both CC and TC, thus this change can't be considered as an effect of elevated CO_2 concentration in the chamber atmosphere. *S. arundinaceum, A. donax,* and *P. pedicellatum* exhibited an increase in the concentration of phenol. Here in *S. arundinaceum* the Hedges g value of CC was -2.033 and in TC it was 0.0310. Thus the effect size was extremely low indicating a meager difference between 1DOT and 15DOT in the concentration of phenol. Regarding *A. donax* and *P. pedicellatum,* extremely higher effect size, as well as percentage difference, was noticed in both CC and TC, thus this change also can't be considered as an effect of elevated CO_2 concentration. Box plots of the phenol content of the control and treatment groups are represented in **Figure 2.20**. **Table 2.26** depicts variations in the phenol content during the study.

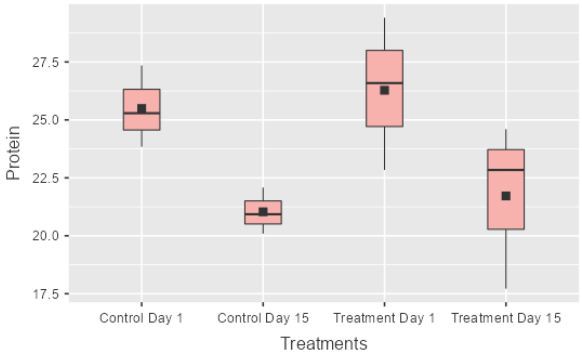
M. maximus



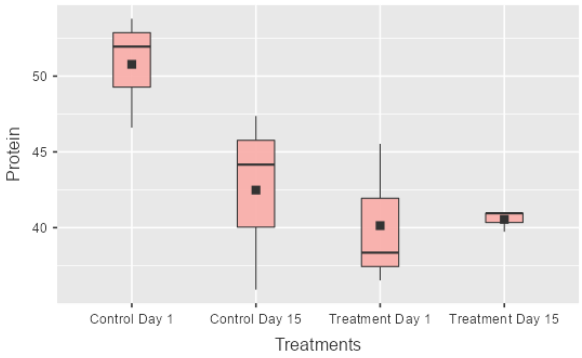
S. arundinaceum



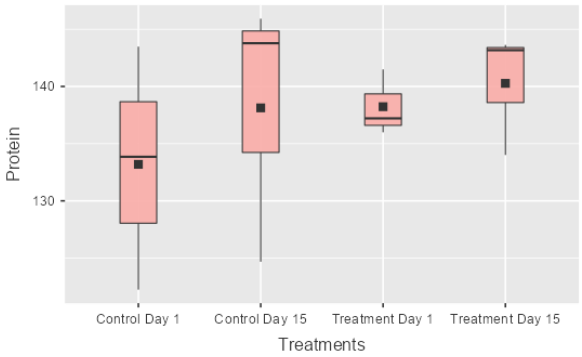
C. flexuosus



C. zizanioides



A.donax



P. pedicellatum

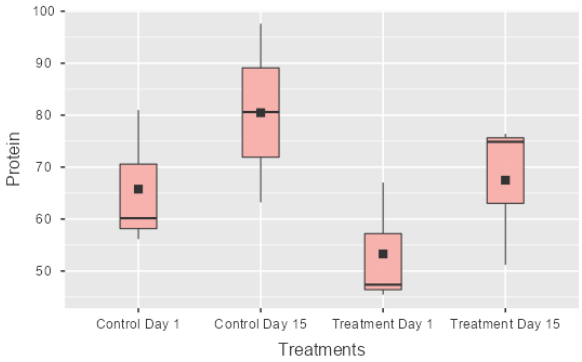
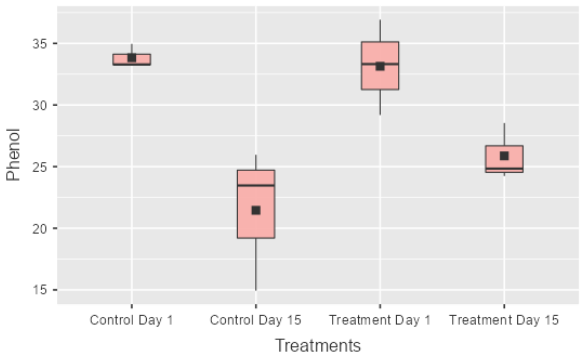


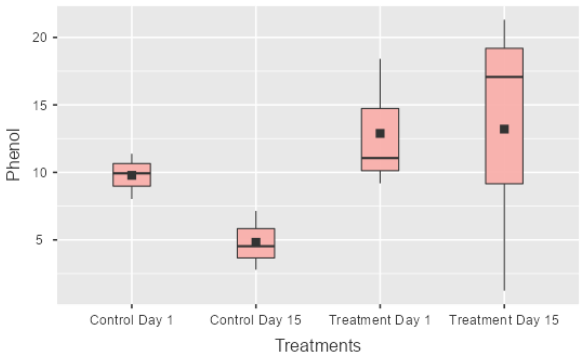
Figure 2.19: Box plot representations of changes in Protein

Table 2.25: Variations in Protein (mg g^{-1}) of plants under elevated levels of CO_2

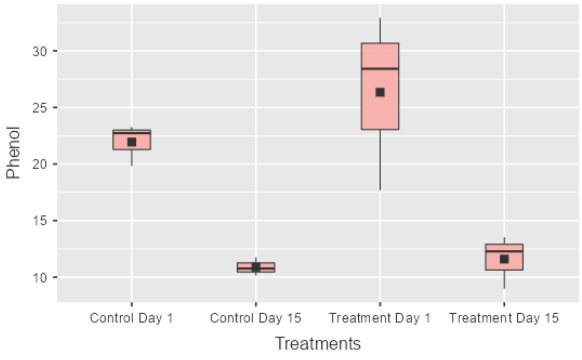
Control	D1	D5	D10	D15	Treatment	D1	D5	D10	D15
Megathyrsus maximus									
C1	39.44	38.75	40.97	25.99	T1	36.76	29.73	38.29	23.31
C2	39.13	36.08	36.23	26.83	T2	49.15	34.62	36.69	40.13
C3	56.48	37.38	38.37	30.42	T3	54.11	37.15	45.63	36.38
Mean	45.02	37.40	38.52	27.75	Mean	46.68	33.83	40.20	33.27
STDEV	9.93	1.34	2.37	2.35	STDEV	8.94	3.77	4.77	8.83
% Change	-37.23				%Change	-29.24			
Saccharum arundinaceum									
C1	39.2696	27.504	28.7264	31.7824	T1	35.144	22.7672	33.6924	42.5548
C2	31.6296	28.8792	28.4972	25.0592	T2	26.2816	17.8776	26.0524	31.4004
C3	43.3188	26.1288	21.01	33.0048	T3	40.5684	24.1424	28.1152	27.886
Mean	38.07	27.50	26.08	29.95	Mean	34.00	21.60	29.29	33.95
STDEV	5.94	1.38	4.39	4.28	STDEV	7.21	3.29	3.95	7.66
% Change	-21.22				%Change	3.10			
Cymbopogon flexuosus									
C1	23.8368	30.0252	22.3088	20.0932	T1	22.8436	23.302	20.7044	17.7248
C2	27.3512	27.122	29.032	22.0796	T2	26.5872	28.4972	23.9132	22.8436
C3	25.2884	28.7264	26.4344	20.9336	T3	29.414	25.6704	27.504	24.6008
Mean	25.49	28.62	25.93	21.04	Mean	26.28	25.82	24.04	21.72
STDEV	1.77	1.45	3.39	1.00	STDEV	3.30	2.60	3.40	3.57
% Change	-17.40				%Change	-17.62			
Chrysopogon zizanioides									
C1	46.60	40.03	42.02	44.16	T1	36.52	39.88	40.34	39.73
C2	51.95	34.53	35.76	35.91	T2	38.35	45.99	38.81	40.95
C3	53.79	36.98	39.12	47.37	T3	45.53	20.78	48.44	40.95
Mean	50.78	37.18	38.96	42.48	Mean	40.14	35.55	42.53	40.54
STDEV	3.73	2.76	3.14	5.91	STDEV	4.76	13.15	5.17	0.71
% Change	-16.02				%Change	1.83			
Arundo donax									
C1	143.48	128.66	105.89	124.68	T1	141.49	136.30	135.53	143.63
C2	122.24	123.77	121.48	145.92	T2	137.21	128.50	117.66	134.01
C3	133.85	89.39	110.32	143.78	T3	135.99	132.48	124.38	143.17
Mean	133.19	113.94	112.56	138.13	Mean	138.23	132.43	125.86	140.27
STDEV	10.64	21.40	8.03	11.69	STDEV	2.89	3.90	9.03	5.43
% Change	4.57				%Change	1.48			
Pennisetum pedicellatum									
C1	80.98	76.78	66.85	80.60	T1	47.37	34.76	55.20	51.19
C2	56.15	69.52	70.67	97.60	T2	45.46	64.18	71.05	74.87
C3	60.17	73.15	69.14	63.22	T3	67.04	55.77	55.77	76.40
Mean	65.77	73.15	68.89	80.47	Mean	53.29	51.57	60.67	67.49
STDEV	13.33	3.63	1.92	17.19	STDEV	11.95	15.15	8.99	14.14
% Change	26.14				%Change	28.91			

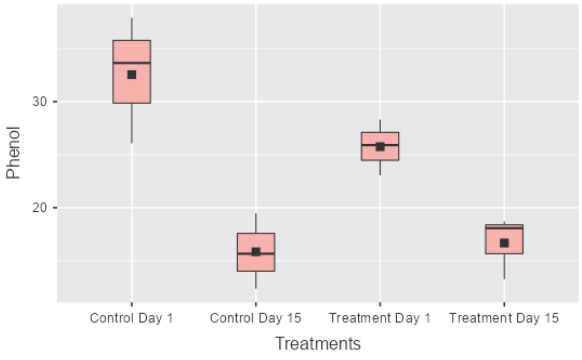
M. maximus ***S. arundinaceum***



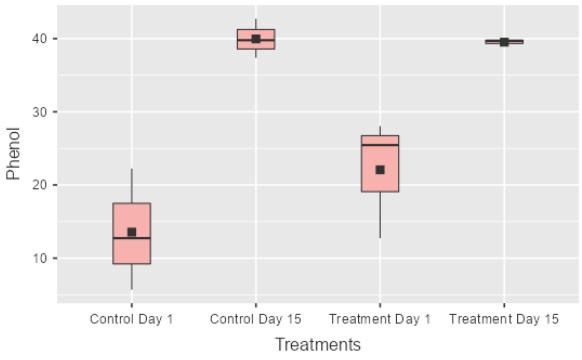


C. flexuosus ***C. zizanioides***





A. donax ***P. pedicellatum***



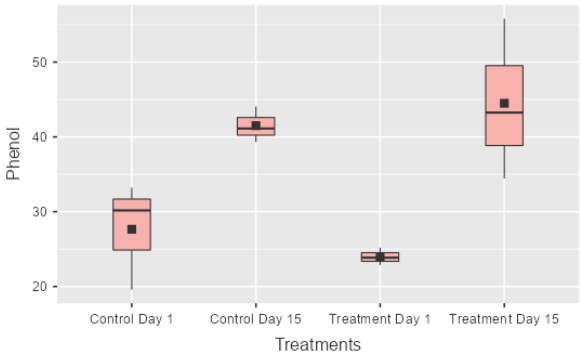


Figure 2.20: Box plot representations of changes in Phenol

Table 2.26: Variations in Phenol (mg g^{-1}) of plants under elevated levels of CO_2

Control	D1	D5	D10	D15	Treatment	D1	D5	D10	D15
Megathyrsus maximus									
C1	34.96	41.85	45.17	25.96	T1	36.92	33.61	38.94	24.24
C2	33.25	36.66	41.94	14.94	T2	33.32	50.35	36.39	28.54
C3	33.29	46.17	33.08	23.47	T3	29.19	41.40	35.00	24.84
Mean	33.83	41.56	40.06	21.46	Mean	33.14	41.79	36.78	25.87
STDEV	0.98	4.76	6.26	5.78	STDEV	3.87	8.38	2.00	2.33
% Change	-36.77				% Change	-21.20			
Saccharum arundinaceum									
C1	9.93	15.29	25.49	4.53	T1	18.41	13.40	16.54	17.07
C2	8.03	21.46	19.09	7.14	T2	9.19	8.24	23.92	1.24
C3	11.38	15.65	23.24	2.79	T3	11.06	9.84	15.59	21.31
Mean	9.78	17.47	22.61	4.82	Mean	12.88	10.49	18.68	13.21
STDEV	1.68	3.46	3.25	2.19	STDEV	4.87	2.64	4.56	10.58
% Change	-46.97				% Change	-0.31			
Cymbopogon flexuosus									
C1	19.83	19.15	16.20	10.15	T1	32.93	11.26	23.59	13.53
C2	23.27	18.79	21.09	10.77	T2	17.67	10.02	15.75	8.99
C3	22.73	31.00	13.26	11.75	T3	28.42	22.53	23.14	12.28
Mean	21.94	22.98	16.85	10.89	Mean	26.34	14.60	20.83	11.60
STDEV	1.85	6.95	3.96	0.81	STDEV	7.84	6.89	4.40	2.35
% Change	-50.29				% Change	-54.94			
Chrysopogon zizanioides									
C1	33.64	24.56	19.14	15.66	T1	28.30	15.49	19.49	18.07
C2	37.91	15.13	15.40	12.37	T2	23.05	13.44	26.17	13.26
C3	26.08	30.17	11.13	19.49	T3	25.90	12.10	21.54	18.69
Mean	32.54	23.29	15.22	15.84	Mean	25.75	13.68	22.40	16.67
STDEV	5.99	7.60	4.01	3.56	STDEV	2.63	1.70	3.42	2.97
% Change	-48.69				% Change	-35.49			
Arundo donax									
C1	5.70	35.69	28.75	37.38	T1	28.04	17.71	35.96	38.98
C2	22.25	21.89	34.18	39.78	T2	25.45	31.06	33.73	39.87
C3	12.73	12.28	27.41	42.72	T3	12.73	28.12	32.22	39.69
Mean	13.56	23.29	30.11	39.96	Mean	22.07	25.63	33.97	39.52
STDEV	8.31	11.77	3.58	2.67	STDEV	8.20	7.02	1.88	0.47
% Change	290.24				% Change	102.53			
Pennisetum pedicellatum									
C1	33.20	39.25	25.54	41.12	T1	23.85	10.15	23.59	34.44
C2	19.58	19.94	25.54	44.06	T2	22.87	35.42	41.56	55.80
C3	30.17	38.63	29.55	39.34	T3	25.19	15.93	22.87	43.25
Mean	27.65	32.60	26.88	41.50	Mean	23.97	20.50	29.34	44.50
STDEV	7.15	10.97	2.31	2.38	STDEV	1.16	13.24	10.59	10.73
% Change	59.75				% Change	86.70			

2.4.4 Outcomes of the analysis of plant nutrients

To assess the effect of elevated CO_2 on plant nutrients, the concentration of nutrients such as carbon, nitrogen, calcium, magnesium, potassium, and sodium were measured on 1DOT and 15 DOT of the experiment. Powdered leaf samples were used to measure carbon and nitrogen concentration using a CHNS analyzer. Dried leaves were acid digested to check calcium and magnesium by EDTA titration method and sodium and potassium using a flame photometer.

Carbon and Nitrogen

Carbon content was increased in the leaf samples of *M. maximus*, *C. flexuosus* and *C. zizanioides* in the TC compared to CC. In *S. arundinaceum* and *P. pedicellatum* an increase was noticed in TC but the percentage increase was lesser than CC. A decrease in the carbon content was noticed in *A. donax* (CC=-2.92%; TC=-2.37%). Regarding the carbon content of *M. maximus*, TC plants exhibited an increase of 0.94% greater than CC; it was 1% in *C. flexuosus* and 1.72% in *C. zizanioides*. Thus it was confirmed that the carbon content of the leaves was increased at elevated CO_2 conditions in all grass species except *A. donax*.

Concerning the nitrogen content, the direction of change varied with species and treatment. Control plants of *C. flexuosus* and *C. zizanioides* exhibited a decrease in the nitrogen content (8.42% and 3.96% respectively), while an increase was noticed in CO_2-treated plants (18.48% and 24.73% respectively). Regarding *S. arundinaceum* and *P. pedicellatum*, nitrogen content decreased in both treatment groups. In *A. donax*, a higher percentage decrease was noticed in the concentration of nitrogen in plants of TC (40.01%) due to the elevated CO_2 effect compared to the decrease in control (15.58%). **Table 2.27** depicts the carbon and nitrogen content of plant samples on initial and final days of experimentation. Figures below represent the CHNS peaks for Nitrogen and Carbon in the leaf samples (**Fig 2.21**: *M. maximus*, **Fig 2.22**: *S. arundinaceum*, **Fig 2.23**: *C. flexuosus*: **Fig 2.24**: *C. zizanioides*, **Fig 2.25**: *A. donax*, **Fig 2.26**: *P. pedicellatum*).

Table 2.27: Variations in the concentration of plant Carbon and Nitrogen

Grass Species	Nitrogen (%)					
	Control			Treatment		
	D1	D15	% Change	D1	D15	% Change
M. maximus	0.92	1.26	36.84	0.79	1.03	30.62
S. arundinaceum	0.94	0.93	-0.83	0.83	0.81	-1.60
C. flexuosus	1.52	1.39	-8.42	1.12	1.32	18.48
C. zizanioides	0.53	0.51	-3.96	0.64	0.80	24.73
A. donax	2.20	1.86	-15.58	2.73	1.64	-40.01
P. pedicellatum	2.35	2.08	-11.47	2.03	1.76	-13.37
	Carbon (%)					
	Control			Treatment		
	D1	D15	% Change	D1	D15	% Change
M. maximus	41.79	42.33	1.29	41.36	42.29	2.23
S. arundinaceum	44.36	44.41	0.12	44.27	44.32	0.11
C. flexuosus	42.67	42.81	0.33	42.11	42.67	1.33
C. zizanioides	43.88	44.47	1.34	44.13	45.48	3.06
A. donax	46.79	45.43	-2.92	45.85	44.76	-2.37
P. pedicellatum	42.38	44.27	4.47	42.40	43.36	2.27

M. maximus

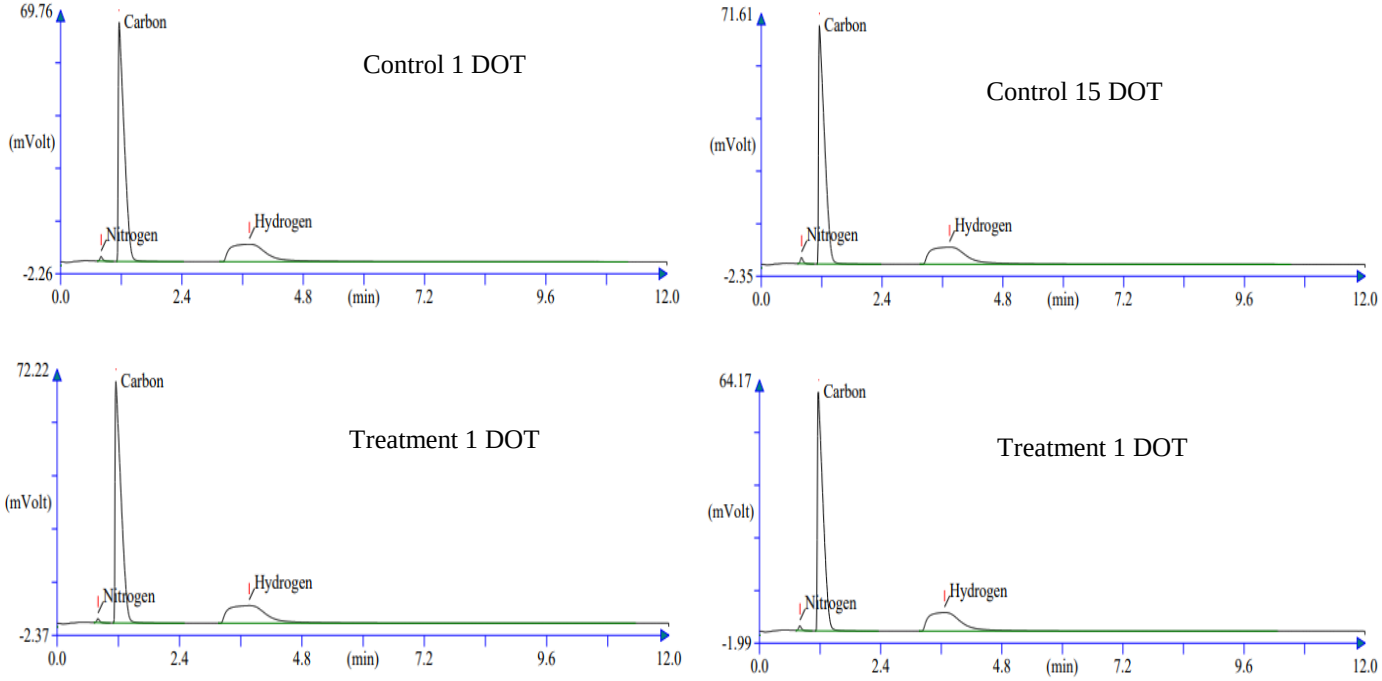


Figure 2.21: Peaks for Nitrogen and Carbon in the control and treatment leaf samples of *M. maximus*

S. arundinaceum

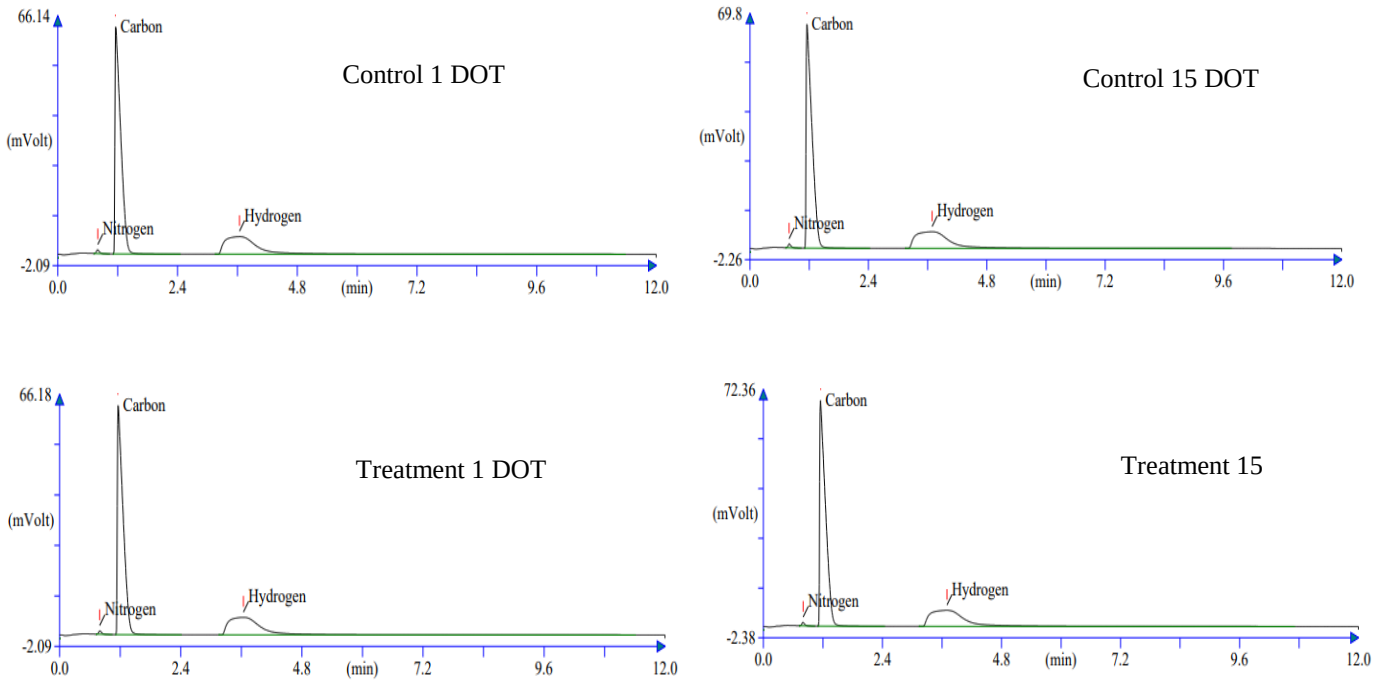


Figure 2.22: Peaks for Nitrogen and Carbon in the control and treatment leaf samples of *S. arundinaceum*

C. flexuosus

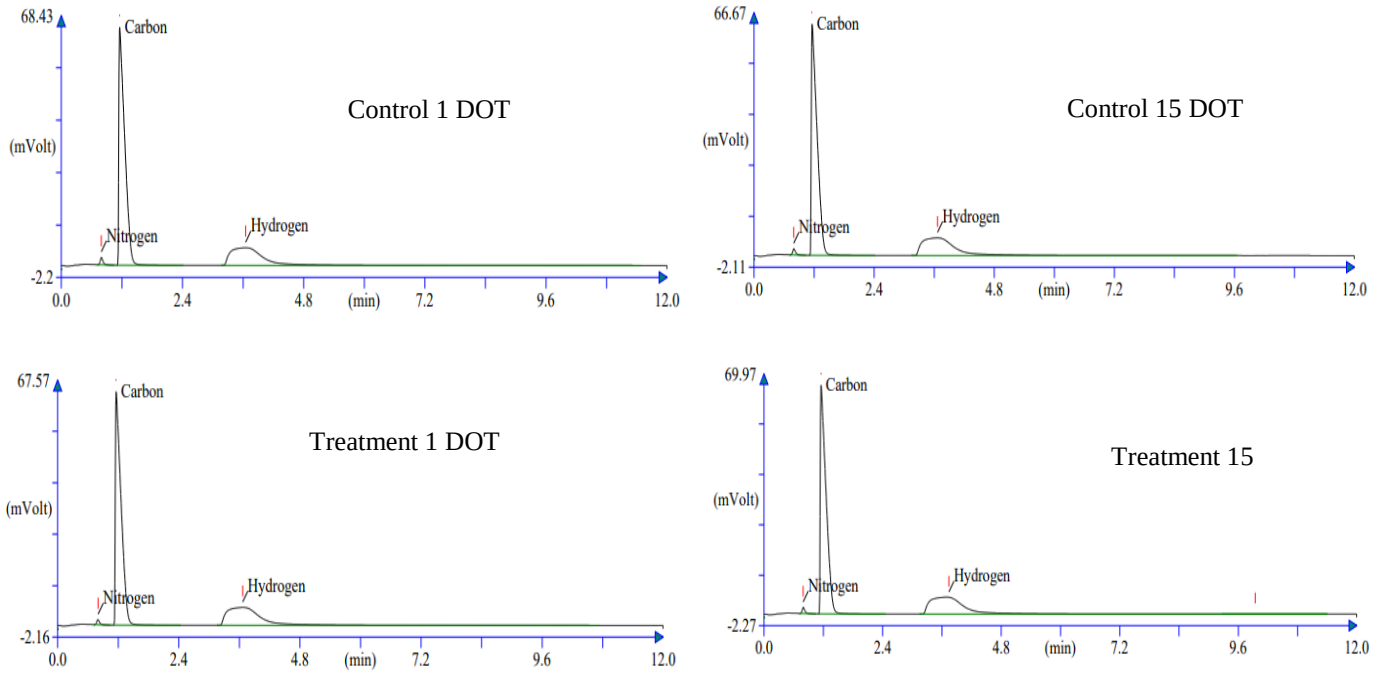


Figure 2.23: Peaks for Nitrogen and Carbon in the control and treatment leaf samples of *C. flexuosus*

C. zizanioides

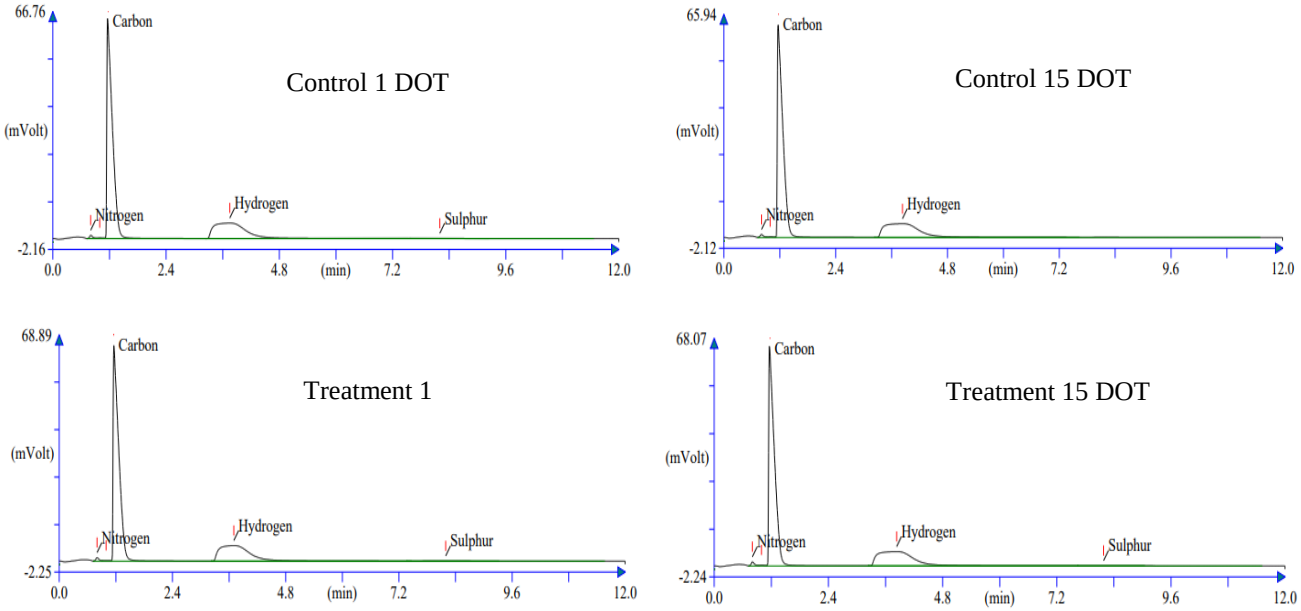


Figure 2.24: Peaks for Nitrogen and Carbon in the control and treatment leaf samples of *C. zizanioides*

A. donax

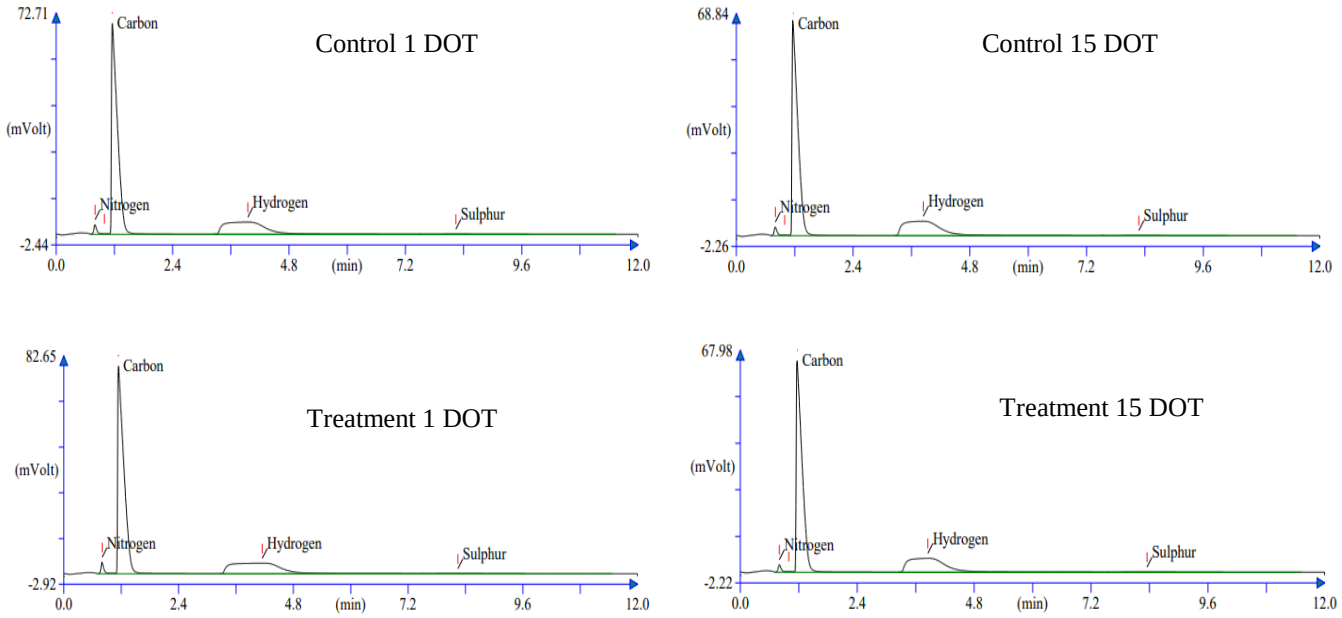


Figure 2.25: Peaks for Nitrogen and Carbon in the control and treatment leaf samples of *A. donax*

P. pedicellatum

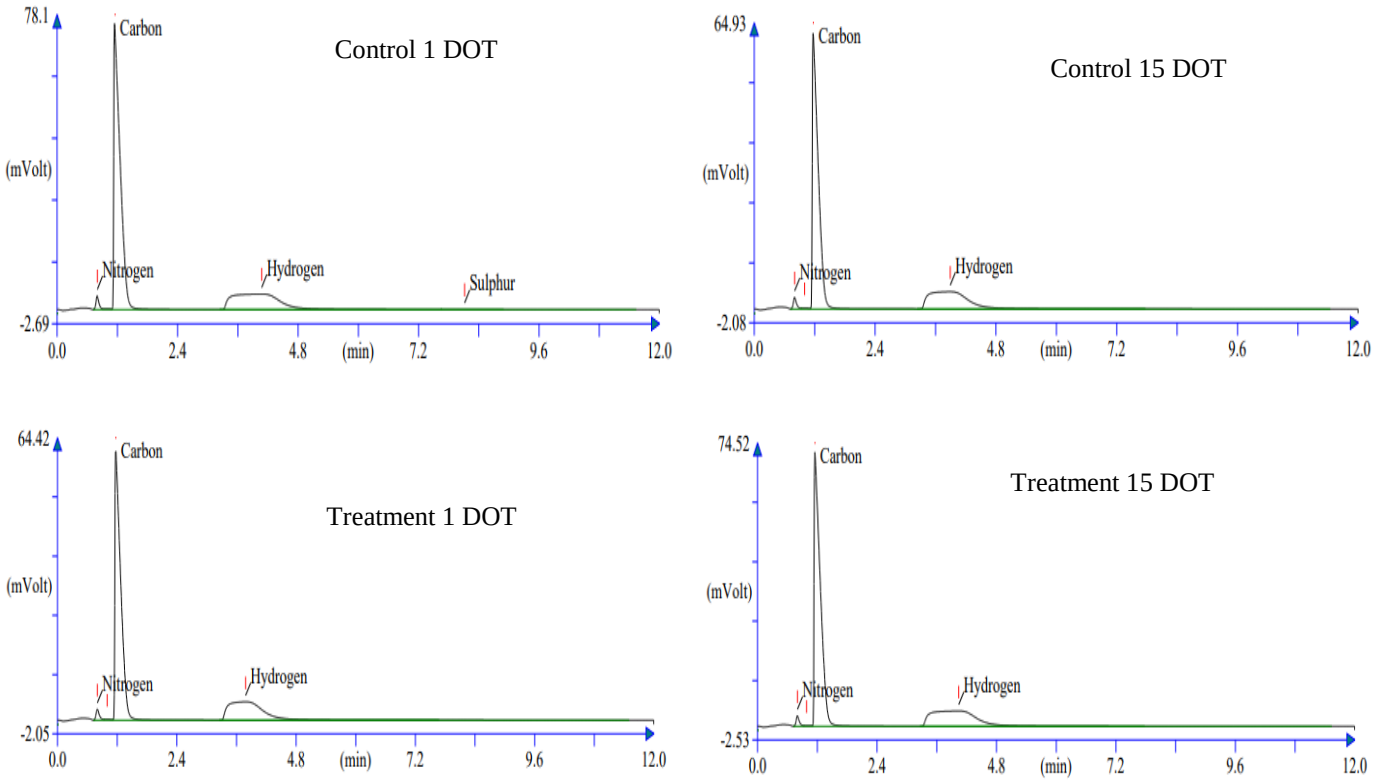


Figure 2.26: Peaks for Nitrogen and Carbon in the control and treatment leaf samples of *P. pedicellatum*

Calcium and Magnesium

As mentioned above, calcium and magnesium content were analyzed by the EDTA titration method. Calcium content has decreased in TC of all grass species under elevated CO_2 treatment, except *P. pedicellatum*. It was increased by 22.22% in *P. pedicellatum* compared to a decrease of 5% in control. However, paired samples T-test results were insignificant for the change in calcium content of control and treatment of all species, and this may be due to an insufficient sample size. As mentioned earlier, in chamber studies there are limitations in retaining more plants. *S. arundinaceum* exhibited a decrease (10.56%) in the calcium content of the plants under TC, while an increase (11.11%) in CC was noticed. Regarding *C. flexuosus*, a meager increase of 1.67% in CC and a decrease of 13.33% in TC were noticed. Thus in these cases elevated CO_2 may assumed to influence the calcium content since the direction of the

change was different in TC compared to CC. Concerning *M. maximus*, *C. zizanioides*, and *A. donax*, both treatments showed a decline in the calcium content, where the decrease is prominent in the CO_2-treated plants of *C. zizanioides* and *A. donax* compared to their control plants.

Magnesium content decreased in all species at elevated CO_2 treatment. *A. donax* treated with elevated CO_2 exhibited a significant decrease in the magnesium content with a p-value<0.05 (0.023). Here the magnesium content decreased by 57.14% in TC. In *C. zizanioides* and *P. pedicellatum*, an enhancement in the magnesium content of the plants of CC was noticed, while a decline in TC, thus the elevated CO_2 effect on the reduction of the magnesium content of plants is evident. *M. maximus* exhibited a similar rate of decrease in both treatments, while magnesium reduced at a higher rate in TC plants (28.17%) of *S. arundinaciun* than in C C (11.11%). However, magnesium decreased at a lower rate in TC (27.78%) with *C. flexuosus* than it's CC (42.68%).

Sodium and Potassium

Significant changes were noticed in the sodium content of *S. arundinaceum* ($p<0.05$) and *C. flexuosus* ($p<0.001$), and the change was an increase of 56.89% and a decrease of 58.64% respectively in the TC plants of *S. arundinaceum* and *C. flexuosus*. A significant decrease was also noticed with the TC of *M. maximus*, but a significant decrease was noticed in the CC also, thus which may not be an elevated CO_2 effect. As well a decrease in the sodium content was also evident in *P. pedicellatum*. While it has increased with elevated CO_2 treatment in *C. zizanioides* and *A. donax* compared to a decrease in their CC.

Concerning the percentage change in potassium content during the study, an increase was shown only by *S. arundinaceum* and *C. flexuosus.* Compared to CC the change was smaller in the TC of *C. flexuosus* (CC=34.26%; TC=7.14%) and it was higher in the TC of *S. arundinaceum* (CC=1.54%; TC=20.55%). Potassium content was decreased by 19.60% in the TC of *M. maximus* compared to a decrease of 6.87% in CC. Regarding *C. zizanioides* the percentage decrease was similar in CC and TC. Concerned with the species *A. donax*, an increase of 4.60% in CC and a decrease of 2.34% in TC were noticed, thus an elevated CO_2 effect on lowering potassium content

was evident in this species. Treatments on *P. pedicellatum* showed a decrease in the potassium content (CC=12.20%; TC=9.95%). The paired samples' T-test significance values regarding plant nutrients are depicted in **Table 2.28**. Variations in the concentration of plant nutrients such as calcium, magnesium, sodium, and potassium are depicted in **Table 2.29.**

Table 2.28: Paired samples T-test significance values of plant nutrient analysis

Plant Nutrients	Species	Paired samples T-test (p value)	
		Control	Treatment
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
Sodium	*M. maximus*	<0.001**	0.003*
	S. arundinaceum	0.081	0.008*
	C. flexuosus	0.128	<0.001*
	C. zizanioides	0.322	0.155
	A. donax	0.393	0.668
	P. pedicellatum	0.003*	0.085
Potassium	*M. maximus*	0.618	0.212
	S. arundinaceum	0.803	0.449
	C. flexuosus	0.590	0.423
	C. zizanioides	0.003*	0.024*
	A. donax	0.906	0.728
	P. pedicellatum	0.048*	0.336

*significance at p<0.05; ** significance at p<0.001

Table 2.29: Changes in the concentration of plant nutrients

Days	*M. maximus*		*S. arundinaceum*		*C. flexuosus*		*C. zizanioides*		*A. donax*		*P. pedicellatum*	
	CC	TC	CC	TC	CC	TC	CC	TC	CC	TC	CC	TC
Calcium												
1 DOT	0.77±0.05	0.77±0.12	0.27±0.05	0.32±0.08	0.69±0.09	0.75±0.24	0.53±0.24	0.59±0.09	1.66±0.37	1.18±0.09	0.75±0.09	0.64±0.16
5 DOT	0.86±0.20	0.67±0.12	0.21±0.05	0.19±0.05	0.64±0.28	0.48±0.00	0.64±0.00	0.59±0.09	0.91±0.09	0.86±0.37	0.80±0.16	0.80±0.16
10 DOT	0.59±0.05	0.51±0.05	0.24±0.08	0.32±0.08	0.69±0.09	0.64±0.16	0.48±0.00	0.32±0.00	1.28±0.42	1.44±0.42	0.91±0.24	0.80±0.28
15 DOT	0.37±0.12	0.43±0.12	0.29±0.05	0.27±0.05	0.69±0.09	0.59±0.09	0.48±0.16	0.48±0.16	1.39±0.52	0.80±0.16	0.69±0.09	0.75±0.09
Magnesium												
1 DOT	0.19±0.05	0.24±0.08	0.26±0.03	0.34±0.05	1.01±0.2	0.81±0.06	0.32±0.2	0.29±0.00	0.71±0.31	0.75±0.2	0.1±0.00	0.19±0.1
5 DOT	0.18±0.06	0.23±0.12	0.24±0.05	0.31±0.06	0.45±0.06	0.52±0.06	0.52±0.06	0.62±0.2	0.39±0.1	0.52±0.15	0.19±0.17	0.29±0.26
10 DOT	0.31±0.07	0.29±0.08	0.34±0.13	0.19±0.08	0.62±0.06	0.62±0.06	0.42±0.06	0.42±0.15	0.26±0.15	0.23±0.06	0.13±0.06	0.1±0.00
15 DOT	0.05±0.00	0.05±0.00	0.23±0.06	0.24±0.05	0.55±0.15	0.58±0.1	0.29±0.1	0.23±0.11	0.19±0.1	0.32±0.15	0.13±0.06	0.13±0.06
Sodium												
1 DOT	0.35±0.00	0.33±0.02	0.23±0.03	0.2±0.00	5.58±1.6	7.17±0.19	1.55±0.25	0.77±0.22	0.77±0.82	0.39±0.32	1.27±0.05	0.98±0.34
5 DOT	0.35±0.03	0.31±0.03	0.35±0.04	0.3±0.05	3.07±0.31	3.86±0.42	0.77±0.58	1.91±0.14	0.93±0.2	0.37±0.23	1.07±0.04	1.01±0.01
10 DOT	0.24±0.01	0.22±0.1	0.36±0.1	0.27±0.03	2.72±0.48	2.37±1.52	0.94±0.55	1.02±0.99	0.39±0.15	0.45±0.34	1.26±0.16	1.26±0.1
15 DOT	0.17±0.01	0.12±0.01	0.31±0.01	0.31±0.01	2.73±0.37	2.97±0.21	1.32±0.2	1.23±0.07	0.35±0.19	0.52±0.31	0.07±0.08	0.14±0.11
Potassium												
1 DOT	0.22±0.02	0.22±0.02	0.26±0.00	0.21±0.07	9.94±3.43	10.71±0.34	11.74±1.40	12.44±2.54	3.24±0.25	3.17±0.74	13.81±1.42	12.21±0.94
5 DOT	0.21±0.02	0.21±0.03	0.23±0.02	0.22±0.05	13.4±1.92	12.07±1.11	5.43±0.57	13.23±2.01	4.44±0.23	3.85±1.62	12.28±0.36	12.35±1.05
10 DOT	0.21±0.01	0.2±0.03	0.36±0.02	0.35±0.01	11.9±0.73	11.31±0.08	6.21±0.80	6.19±0.57	4.21±0.81	3.45±0.20	12.09±0.21	13.41±1.38
15 DOT	0.2±0.03	0.18±0.05	0.27±0.06	0.24±0.05	11.77±2.14	11.45±1.00	5.8±0.93	6.33±1.02	3.33±1.00	2.89±0.56	12.17±2.05	10.97±1.66

2.4.5 Outcomes of FTIR analysis of plant samples

The FTIR analysis was used to determine if elevated CO_2 levels caused any noticeable changes in the chemical compounds of grass species, as reflected in their spectral peaks. While no prominent new peaks emerged in the treated samples, subtle differences between the control and treated conditions were observed. The control spectra remained almost identical over the 15 days, suggesting minimal chemical changes without CO_2 treatment. Key spectral peaks were identified around, 2900 cm^{-1}, linked to C-H stretching (indicating aliphatic hydrocarbons), 2350 $cm^{-}1$, associated with stretching vibrations of CO_2 1650 cm^{-1}, corresponding to C=O stretching (indicating carbonyl groups), and 1050 cm^{-1}, related to C-O stretching (indicating carbohydrates). In contrast, the spectra of CO_2-treated leaf samples revealed significant deviations from the control. Notably, there was a reduction in the intensity of peaks in the O-H and C-H stretching regions, along with shifts and changes in the fingerprint region. These alterations suggest that the CO_2 treatment induces chemical changes, such as the breakdown of certain molecular structures or the formation of new compounds. The overall FTIR spectra of the grass species indicate that CO_2 treatment leads to significant chemical changes over time. These changes include a decrease in the presence of hydroxyl, aliphatic, and carbonyl groups, hinting at degradation processes. Additionally, there is evidence of new chemical structures forming or the reorganization of existing ones, particularly within the fingerprint region. These findings imply that the CO_2 treatment not only alters the chemical composition of the plant material but might also affect its physical properties. FTIR spectra of leaf samples of CC and TC of grass species were represented (**Fig 2.27**: *M. maximus*, **Fig 2.28**: *S. arundinaceum*, **Fig 2.29**: *C. flexuosus*, **Fig 2.30**: *C. zizanioides*, **Fig 2.31**: *A. donax*, **Fig 2.32** *P. pedicellatum*).

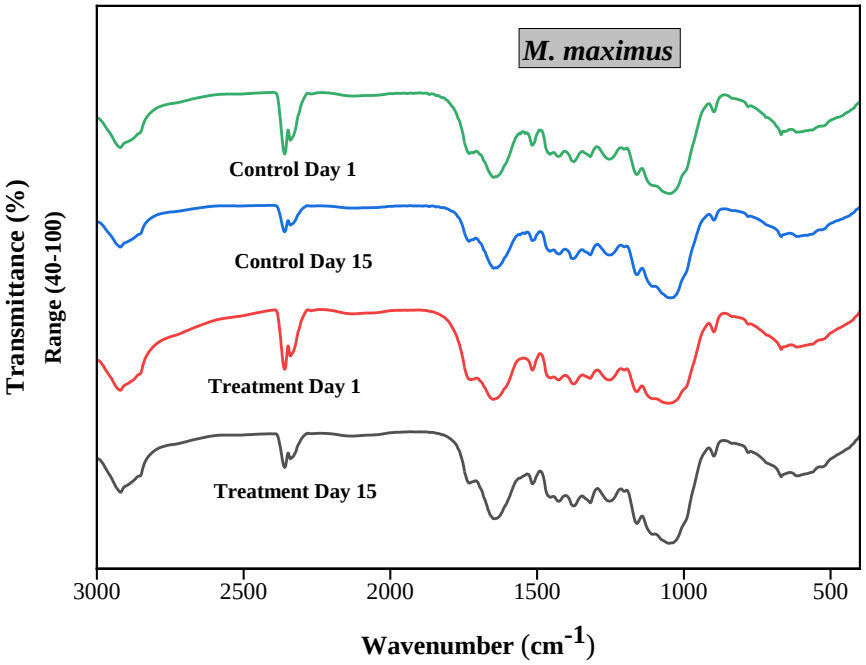


Figure 2.27: FTIR spectrum of *M. maximus*

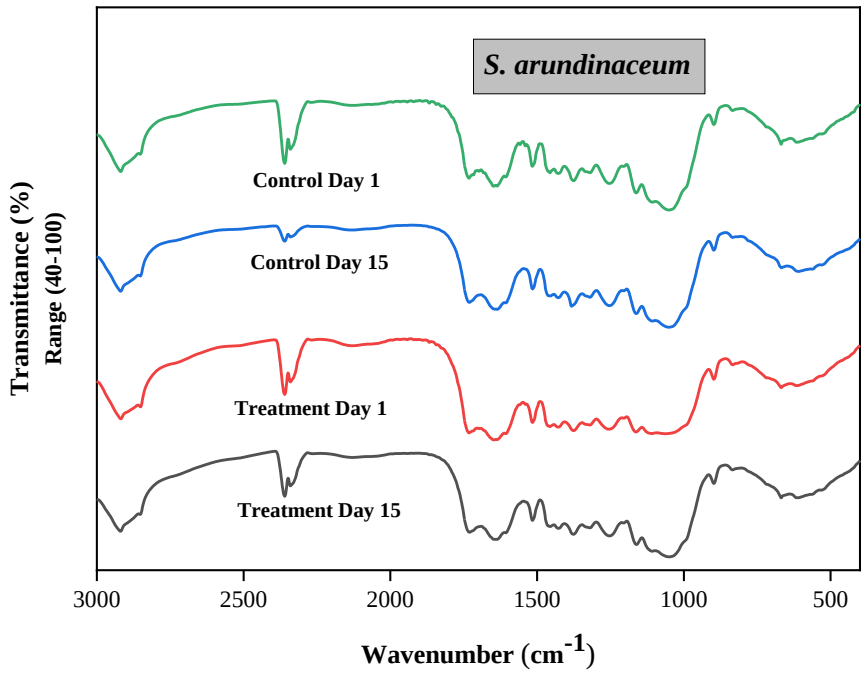


Figure 2.28: FTIR spectrum of *S. arundinaceum*

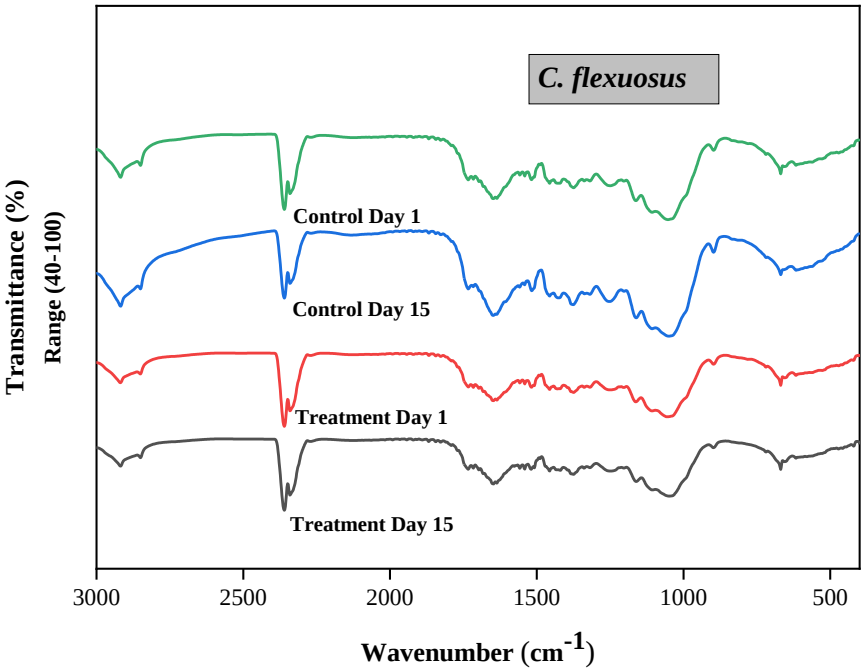


Figure 2.29: FTIR spectrum of *C. flexuosus*

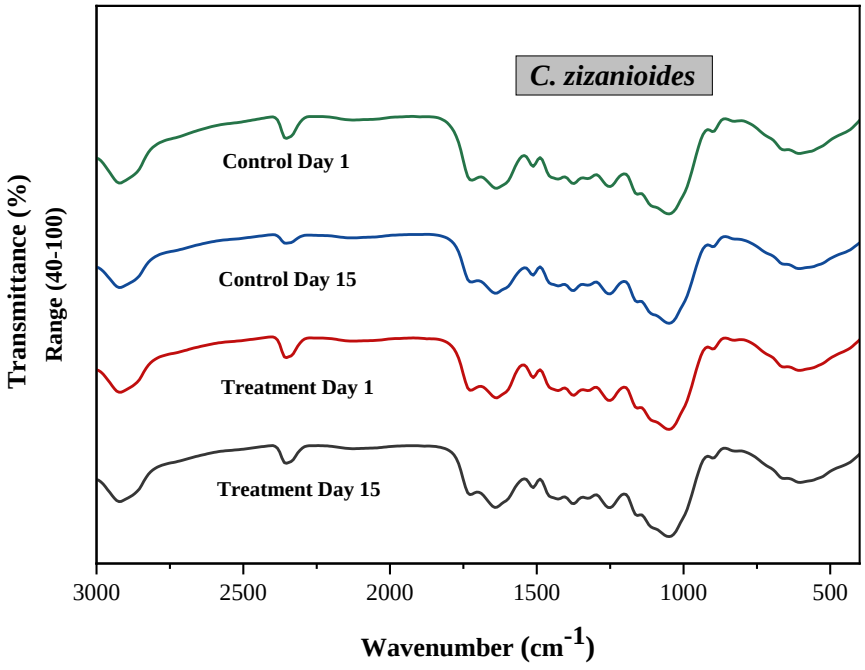


Figure 2.30: FTIR spectrum of *C. zizanioides*

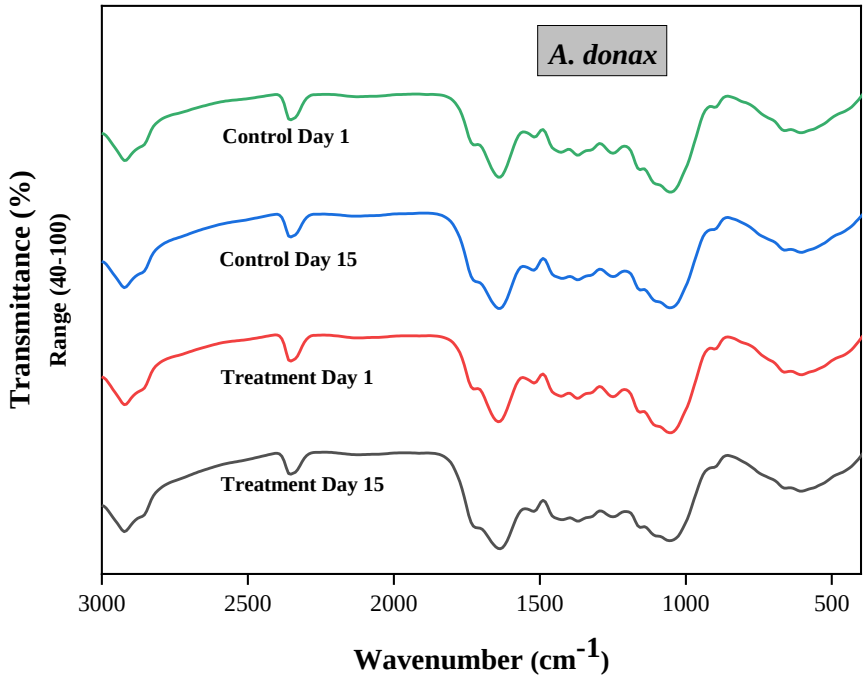


Figure 2.31: FTIR spectrum of *A. donax*

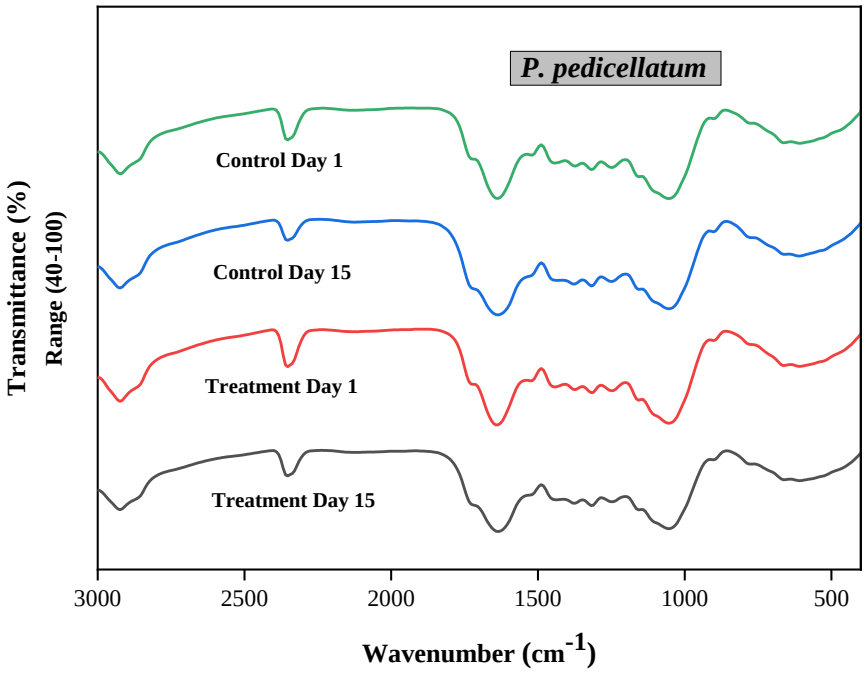


Figure 2.32: FTIR spectrum of *P. pedicellatum*

2.4.6 Outcomes of the analysis of soil characteristics

Soil characters were analyzed on the initial and final days of treatment. Soil moisture, pH, TOC, and nitrogen were measured. Paired samples T-test was carried out to validate the changes and Hedges g values were found to know the magnitude and direction of the changes in soil characteristics during the study (**Table 2.30**). Soil moisture was increased in *M. maximus, A. donax,* and *P. pedicellatum* and was decreased in *S. arundinaceum, C. flexuosus,* and *C. zizanioides* under elevated CO_2 conditions at 15DOT. Compared to CC, *C. zizanioides* plants in TC show a high negative Hedges g value, thus a high negative effect on the soil moisture content was assumed to be imposed by elevated CO_2 conditions of TC. Similarly in *S. arundinaceum*, the soil moisture content of TC was decreased (Hedges g=-0.027) compared to CC (Hedges g= 0.416). As well the decrease in the soil moisture in both chambers of *M. maximus* and *C. flexuosus* was statistically significant, and thus couldn't be considered as an effect of elevated CO_2. The soil moisture content of CC and TC on the initial and final days of the experiment is depicted in **Table 2.31**.

The pH values show that the soil turns more acidic in TC of *S. arundinaceum* and *C. zizanioides* than that in CC. The pH change in TC from the initial day to the final day regarding *C. zizanioides* was statistically significant (p-value =0.007), here the soil pH changed from a neutral condition to a moderately acidic condition. *P. pedicellatum* exhibited a change from an alkaline condition to a moderately acidic condition. Regarding *M. maximus* and *C. flexuosus* soil turns from moderately acidic to neutral condition. **Table 2.32** shows the pH of soils in the CC and TC on 1 DOT and 15 DOT associated with the experimented plants.

Considering the TOC of soil, a significant increase was noticed in *C. zizanioides* under elevated CO_2 treatment (p value=0.027), and here percentage increase was much higher in TC (377.78%) than that of CC (29.63%). In *M. maximus* Hedges g value of CC and TC regarding TOC was 0.187 and 1.325 respectively, a higher effect size of TC indicates a higher rate of increase of TOC in TC (24.33%) than CC (4.01%). In *A. donax* also TOC increased, while the percentage increase was less in TC (112.15), compared to CC (128.63%). A decrease was noticed in *S. arundinaceum* (effect size, CC=2.309; TC=-0.448), *C. flexuosus* (effect size, CC=-2.437; TC=-2.077) and *P.*

pedicellatum (effect size, CC=-0.951; TC=-0.493). Box plots regarding the TOC of each plant are represented (**Figure 2.33**). **Table 2.33** depicts variations of TOC in CC and TC reported during the experiment.

Table 2.30: Paired samples T-test significance values and Hedges g values of soil analysis

Soil characteristics	Species	Paired samples T-test (p value)		Hedges g (effect size)	
		Control	Treatment	Control	Treatment
Soil Moisture	*M. maximus*	0.007*	0.002*	2.520	3.497
	S. arundinaceum	0.410	0.819	0.416	-0.027
	C. flexuosus	0.025*	0.008*	-4.434	-6.638
	C. zizanioides	0.525	0.261	0.364	-1.076
	A. donax	0.249	0.103	1.172	2.259
	P. pedicellatum	0.249	0.302	1.154	1.211
TOC	*M. maximus*	0.691	0.195	0.187	1.325
	S. arundinaceum	0.109	0.296	2.309	-0.448
	C. flexuosus	0.024*	0.118	-2.437	-2.077
	C. zizanioides	0.321	0.027*	1.182	5.258
	A. donax	NaN	0.184	1.386	0.653
	P. pedicellatum	0.368	0.529	-0.951	-0.493
pH	*M. maximus*	0.003*	0.115	2.498	1.680
	S. arundinaceum	0.600	0.060	0.229	-2.508
	C. flexuosus	0.705	0.145	0.234	1.343
	C. zizanioides	0.399	0.007*	-0.451	-2.702
	A. donax	0.009*	0.165	-4.495	-0.897
	P. pedicellatum	<0.001**	0.011*	-4.272	-3.952

*Significance at p<0.05; ** significance at p<0.001; (-), decrease; NaN (Not a Number), Zero variance between samples

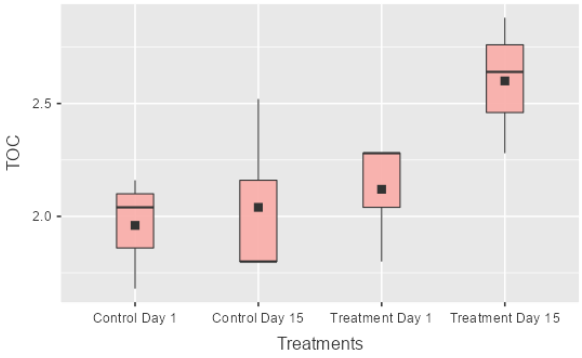
Table 2.31: Variations in the soil moisture (%)

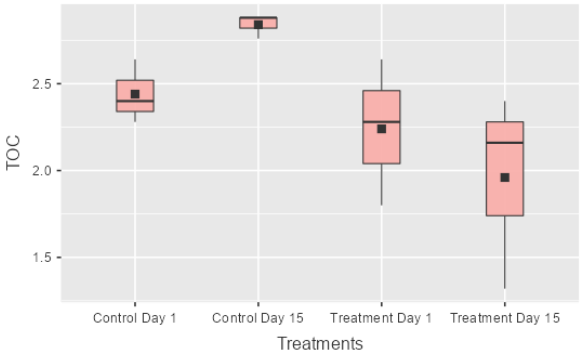
Control	D1	D15	Treatment	D1	D15
Megathyrsus maximus					
C1	15.24	23.32	T1	13.86	25.55
C2	20.39	27.75	T2	18.87	29.61
C3	18.52	28.29	T3	18.52	28.58
Mean	18.05	26.45	Mean	17.08	27.91
STDEV	2.61	2.73	STDEV	2.80	2.11
% Change	47.31		% Change	65.20	
Saccharum arundinaceum					
C1	21.05	20.46	T1	19.18	18.61
C2	18.64	19.70	T2	23.09	23.54
C3	20.62	22.12	T3	21.60	21.49
Mean	20.10	20.76	Mean	21.29	21.22
STDEV	1.29	1.24	STDEV	1.97	2.48
% Change	3.37		% Change	-0.50	
Cymbopogon flexuosus					
C1	13.55	8.06	T1	14.20	7.83
C2	13.53	7.30	T2	15.30	7.01
C3	13.88	4.69	T3	13.18	6.77
Mean	13.65	6.69	Mean	14.22	7.21
STDEV	0.20	1.77	STDEV	1.06	0.56
% Change	-50.91		% Change	-49.21	
Chrysopogon zizanioides					
C1	13.82	13.52	T1	15.75	14.81
C2	10.12	13.57	T2	15.67	15.39
C3	15.58	15.29	T3	15.90	12.10
Mean	13.17	14.13	Mean	15.77	14.10
STDEV	2.79	1.01	STDEV	0.12	1.76
% Change	10.01		% Change	-10.53	
Arundo donax					
C1	16.76	17.46	T1	14.07	17.52
C2	16.45	16.56	T2	16.09	16.95
C3	15.19	17.40	T3	13.55	17.51
Mean	16.13	17.14	Mean	14.57	17.33
STDEV	0.83	0.50	STDEV	1.34	0.33
% Change	6.47		% Change	19.74	
Pennisetum pedicellatum					
C1	15.64	27.55	T1	15.56	19.66
C2	15.62	18.85	T2	18.47	18.19
C3	17.47	18.42	T3	17.26	18.71
Mean	16.24	21.61	Mean	17.10	18.85
STDEV	1.06	5.15	STDEV	1.46	0.75
% Change	34.07		% Change	11.09	

Table 2.32: Variations in soil pH

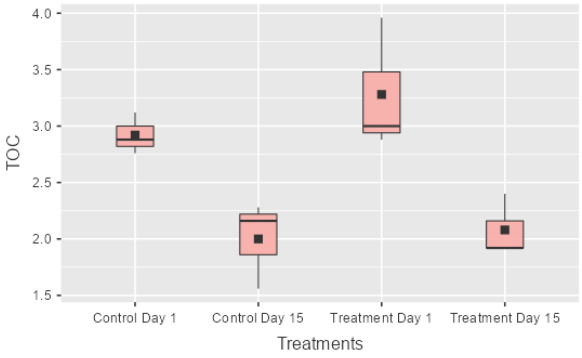
Control	D1	D15	Treatment	D1	D15
Megathyrsus maximus					
C1	5.97	6.6	T1	5.85	6.2
C2	6.04	6.65	T2	5.91	6.5
C3	5.6	6.32	T3	6.2	6.33
Saccharum arundinaceum					
C1	7.12	7.11	T1	7.24	6.97
C2	6.72	6.58	T2	7.26	6.61
C3	7.19	7.72	T3	7.54	6.82
Cymbopogon flexuosus					
C1	5.92	6.38	T1	6.4	6.56
C2	5.92	5.54	T2	4.95	6.38
C3	5.43	5.68	T3	5.5	6.54
Chrysopogon zizanioides					
C1	6.14	5.73	T1	6.3	6.01
C2	5.54	5.69	T2	6.34	5.96
C3	6.32	6.04	T3	6.51	6.14
Arundo donax					
C1	6.24	5.36	T1	6.4	5.82
C2	6.5	5.52	T2	5.46	5.43
C3	6.4	5.7	T3	6.05	5.4
Pennisetum pedicellatum					
C1	11.34	5.44	T1	10.52	6.31
C2	12.51	6.22	T2	10.73	6.34
C3	12.28	6.09	T3	11.73	5.95

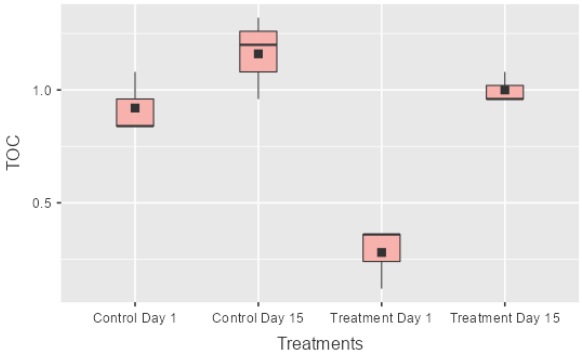
M. maximus ***S. arundinaceum***



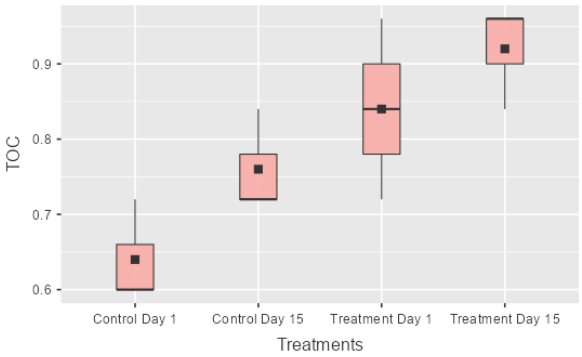


C.flexuosus ***C. zizanioides***





A. donax ***P. pedicellatum***



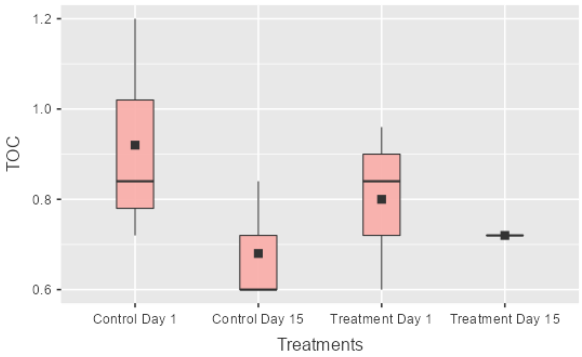


Figure 2.33: Boxplots of soil TOC in control and treatment plants

Table 2.33: Variations in soil TOC (%)

Control	D1	D15	Treatment	D1	D15
Megathyrsus maximus					
C1	2.16	2.52	T1	1.80	2.64
C2	1.68	1.80	T2	2.28	2.88
C3	2.04	1.80	T3	2.28	2.28
Mean	1.96	2.04	Mean	2.12	2.60
STDEV	0.25	0.42	STDEV	0.28	0.30
% Change	4.01		% Change	24.33	
Saccharum arundinaceum					
C1	2.28	2.88	T1	1.80	1.32
C2	2.40	2.88	T2	2.28	2.40
C3	2.64	2.76	T3	2.64	2.16
Mean	2.44	2.84	Mean	2.24	1.96
STDEV	0.18	0.07	STDEV	0.42	0.57
% Change	16.95		% Change	-13.20	
Cymbopogon flexuosus					
C1	2.88	2.16	T1	3.00	1.92
C2	3.12	2.28	T2	2.88	2.40
C3	2.76	1.56	T3	3.96	1.92
Mean	2.92	2.00	Mean	3.28	2.08
STDEV	0.18	0.39	STDEV	0.59	0.28
% Change	-31.80		% Change	-34.73	
Chrysopogon zizanioides					
C1	0.84	1.32	T1	0.12	1.08
C2	1.08	0.96	T2	0.36	0.96
C3	0.84	1.20	T3	0.36	0.96
Mean	0.92	1.16	Mean	0.28	1.00
STDEV	0.14	0.18	STDEV	0.14	0.07
% Change	29.63		% Change	377.78	
Arundo donax					
C1	0.60	0.72	T1	0.84	0.96
C2	0.72	0.84	T2	0.72	0.84
C3	0.60	0.72	T3	0.96	0.96
Mean	0.64	0.76	Mean	0.84	0.92
STDEV	0.07	0.07	STDEV	0.12	0.07
% Change	128.63		% Change	112.15	
Pennisetum pedicellatum					
C1	1.20	0.60	T1	0.96	0.72
C2	0.72	0.84	T2	0.84	0.72
C3	0.84	0.60	T3	0.60	0.72
Mean	0.92	0.68	Mean	0.80	0.72
STDEV	0.25	0.14	STDEV	0.18	0.00
% Change	-20.63		% Change	-6.43	

Nitrogen levels for the growth of plants normally range from 5.5 to 103.7 mg/kg of dry soil (Sunaga et al., 2008). All grass species under the study exhibited a normal range before and after the experiment. Nitrogen content decreased in soils associated with TC of all species. Regarding *C. flexuosus*, *A. donax*, and *P. pedicellatum* a decline in the soil nitrogen was noticed in both CC and TC. Here concerned with *C. flexuosus* the percentage decrease of soil nitrogen in CC was less (6.25%) compared to TC (28.57%). In *A. donax* and *P. pedicellatum*, the percentage decline was greater and similar in both CC and TC. A higher percentage decrease in the soil nitrogen among the species under study was noticed in *A. donax*. Regarding *M. maximus* and *S. arundinaceum*, nitrogen increased in CC contrary to the decrease in TC. *C. zizanioides* exhibited a decrease of 5.88% in TC, the least among the species under study. While no change was noticed in soil nitrogen associated with CC plants of *C. zizanioides*. **Table 2.34** depicts the concentration of nitrogen and the percentage change happened during the experiment.

Table 2.34: Variations in the soil nitrogen concentration

Species	Control (mg/Kg)			Treatment (mg/Kg)		
	D1	D15	Percentage change (%)	D1	D15	Percentage change (%)
M. maximus	92.40	100.58	8.85	92.40	77.44	-16.19
S. arundinaceum	92.40	104.72	13.33	92.40	86.24	-6.67
C. flexuosus	86.24	61.60	-28.57	98.56	92.40	-6.25
C. zizanioides	104.72	104.72	0.00	104.72	98.56	-5.88
A. donax	92.40	55.44	-40.00	117.04	73.92	-36.84
P. pedicellatum	92.40	80.08	-13.33	86.24	73.92	-14.29

2.5. DISCUSSION

The primary aim of the study is to assess the changes in microclimate brought about by the growth and metabolism of selected grass species in an environment having elevated levels of carbon dioxide which is detailed in Chapter 1. Furthermore, the changes in morphology, growth, biochemical constituents, and nutrient profile of plants subjected to elevated levels of CO_2 conditions are significant and such details are given in Chapter II. Understanding the relative capabilities of plant species to capture atmospheric CO_2 and their relative growth performances at high levels of CO_2 can provide inputs for the selection of better species for CO_2 sequestration initiatives. Thus the morphological, biochemical, biomass, and soil characteristics owing to an increase in atmospheric CO_2 levels were measured with standard methodologies, and the results obtained were statistically analyzed to get the necessary outcomes and are depicted in this chapter.

2.5.1 Changes in growth attributes of grass species under elevated CO_2 conditions

Growth attributes such as plant height and tiller height, number of tillers, number of leaves, leaf length, leaf breadth, leaf area, culm diameter, and plant biomass were measured and the results were statistically validated, and the impact of elevated CO_2 on overall morphology was analyzed. The growth and development of each plant at elevated CO_2 was different. Plant growth in high CO_2 environments modifies the primary and secondary meristems of roots and shoots (Pritchard et al., 1999). According to them differential expression of genes involved in cell cycling or cell expansion has an impact on cell division, expansion, and patterning (Pritchard et al., 1999). Growth in an elevated CO_2 often modifies the size and morphology of plant leaves, regardless of the specific mechanisms at play. However, the extent of these alterations, which tends to diminish with leaf maturity, depends on several factors including temperature, phenology, nutrient availability, and genetic character of the plant. According to Hager et al. (2016) increased atmospheric CO_2 is known to change many plant characteristics and commonly lead to increased plant growth. Previous studies have shown that the response of plants to elevated CO_2 was mainly influenced by the plant's functional group (C3, C4, or CAM). In the present study among the six grass species selected, five species represented the C4 group and one species was of C3

type. These factors were taken into account when discussing their relative performances to elevated levels of carbon dioxide.

All the morphological traits had shown a change in elevated CO_2 condition, with most of the changes in a positive direction. Regarding the plant height, an augmentation was noticed in *S. arundinaceum* ($p<0.05$), *M. maximus*, and *C. zizanioides*. Reports revealed that independent of their photosynthetic pathway (C3/C4), grasses showed improved growth responses under increasing CO_2, including increased intercellular CO_2 concentration, water use efficiency, photosynthesis, total non-structural carbohydrates, and total biomass (Sashna et al., 2022). Furthermore, previous studies on grasslands showed that when CO_2 levels are raised, the overall aboveground biomass of grasslands increases dramatically (Andresen et al., 2018). Dominant grass species show a positive reaction to this condition, which causes the total aboveground biomass to rise by 15% annually. Plant photosynthesis is accelerated by elevated CO_2 was evident from previous reports (Kimball et al., 2002; Poorter & Navas, 2003; Ainsworth & Long, 2005; Leakey et al., 2009). As previously mentioned, *M. maximus* and *C. zizanioides* increased plant height as well as carbohydrate concentration at elevated CO_2. Increased carbon availability for the Calvin cycle leads to a higher yield of sugars and other carbohydrates, which are vital for plant growth, when CO_2 levels are higher (Smith & Stitt, 2007). Plants that produce more carbohydrates may grow taller and produce more biomass. As well the mechanisms for plant development start from meristems which include cell division, cell expansion, and cell differentiation (Taylor, 1997). According to Korner (1991), all these processes were controlled by specific genetically programmed and scheduled ontogenetic events which determine the rate of production, shape, and number of plant organs based on environmental signals (Kerstetter & Hake, 1997). The benefits of higher CO_2 on plant growth are more noticeable in ideal conditions such as soil with sufficient nutrients, water availability, optimum temperature, and light.

Contrary to the above-mentioned facts, even though the carbohydrate concentration was decreased in *S. arundinaceum*, its height showed an increasing trend. According to reports by Dijkstra et al, (2010) and Lamichaney et al. (2021), plants may modify their strategy for allocating resources in response to increasing CO_2. Even though some tissues may have lower quantities of carbohydrates than others, this

does not always mean that growth has slowed down overall. Different plant species respond differently to elevated CO_2 levels because of differences in their genetic composition and physiological adaptations. *S. arundinaceum* may possess certain characteristics that allow it to benefit from elevated CO_2 for height development, even if this results in an accumulation of carbohydrates in other tissues (De Souza et al., 2008). In other species such as *C. flexuosus*, *A. donax,* and *P. pedicelltum*, a higher percentage of net day flux (DF (N)) was noticed and in *A. donax,* carbohydrate concentration increased under elevated CO_2 while plant height was not considerably increased in all those species. In these species elevated CO_2 may cause morphological changes that mostly include increases in cell volume rather than cell number, and these alterations may not have a substantial impact on height (Sashna et al., 2022). *A. donax* as a C3 grass shows higher DF (N) in the present study and exhibits increased carbohydrate concentrations under elevated CO_2 may be due to enhanced photosynthesis and improved water use efficiency (Sánchez et al., 2021). While the lack of a noticeable height increase in *A. donax* could be attributed to morphological responses and nutrient limitations as the plant adapts to changing environmental conditions (Sashna et al., 2022).

A tiller is a shoot that emerges from the base of the grass plant. Tiller height was not significantly increased in the plants under experimentation even though there was a total plant height increase. According to Burgess and Huang (2014), although more CO_2 may result in more biomass overall, the main stem and leaves may receive more resources (such as nutrients and carbohydrates) than the tillers. Plants may grow higher as a result of this without noticeably increasing tiller height. As well the number of tillers was not significantly changed in all the grass species under study. In grasses, tiller production is not always influenced by CO_2 levels, but rather by species, time, and their interactions. Hager et al. (2016) proposed that for most species, the number of tillers increased between 7 and 14 weeks. In the present study, plants older than 14 weeks (almost 4 months) were taken and this may be the reason for a lack of considerable tiller production in the present study.

Regarding the number of leaves except for *M. maximus,* all other species exhibited a decline at elevated CO_2 conditions, with a significant decline in *A. donax* ($p<0.05$). Reduced stomatal conductance, decreased nitrogen content, and altered

biomass allocation are the main causes of the decline in leaf count in grasses under increased CO_2 treatment (Li et al., 2023). Together, these elements make it more difficult for the plant to sustain leaf production and health, which lowers the quantity of leaves. Likewise in *A. donax*, leave nitrogen was highly reduced at elevated CO_2 conditions (40.01%). While *M. maximus* experiences an increase in leaf number under elevated CO_2 conditions, which may mainly be due to improved water use efficiency, anatomical changes supporting leaf growth, enhanced photosynthesis, and dynamics of nutrient availability (Prior et al., 2004; Habermann et al., 2022). Enhanced photosynthesis under elevated CO_2 was evident from enhanced carbohydrate concentration of *M. maximus* (Hedges g= 0.918). All of these elements may work together to help the plant grow more leaves in a CO_2-rich environment.

A considerable augmentation was noticed in the leaf length and breadth of *S. arundinaceum*, while these traits were decreased in all other C4 species under elevated CO_2 treatment. Augmentation was also reported in the C3 species *A. donax*. The reduction in stomatal conductance at high CO_2 levels is one of the main causes of smaller leaves in C4 grasses (Al-Salman et al., 2023). Reduced stomatal opening can result from elevated CO_2, which can restrict transpiration and gas exchange. Less water may be available for leaf expansion as a result of this decrease in transpiration, which could ultimately result in reduced leaf dimensions (Taylor et al., 2018). In a study conducted on *Saccharum* species, De Souza et al. (2008) reported that different gene expressions associated with development and photosynthesis have been induced by elevated CO_2. According to them, increased growth and leaf elongation are specifically caused by the up-regulation of 22 genes linked to these processes at increasing CO_2 levels. Increased leaf length may be made possible by these genetic alterations that improve leaf structure and function, such genetic mechanism was assumed to be the reason for increased leaf dimensions in *S. arundinaceum*. Moreover, *A. donax* is among the several C3 species that often show increased growth responses in the presence of increasing CO_2 (Taub, 2010). Increased atmospheric CO_2 concentration enhances the gradient of carbon concentration, enabling more effective fixation of carbon during photosynthesis. Likewise, higher carbohydrate concentration was noticed in *A. donax* as a result of its enhanced photosynthetic efficiency, and this is correlated with the development of larger, thicker leaves as the plant takes in more light and carbon (Net DF (DF(N) =-257.86±115.64) for its growth.

As the leaf area is concerned, *A. donax* exhibited a significant decline ($p<0.05$). Even though the species exhibited an increase in leaf dimensions such as leaf length and breadth the decreased leaf area was assumed to be due to a significant decrease in the number of leaves under elevated CO_2. For *Arundo donax*, increased photosynthesis and biomass accumulation are responsible for the increase in leaf length and breadth at higher CO_2 levels (Nackley et al., 2017). *A. donax* produces higher concentrations of non-structural carbohydrates per unit leaf area, such as sugars and starches, at higher CO_2 levels. In a study with *A. donax*, Nackley et al. (2017) reported that in reaction to increased CO_2, this rise typically ranges from 30 to 40%, driven by increased photosynthetic activity. Here the percentage rise in carbohydrates was 25.58% and this could result in increased leaf dimensions. Under conditions of elevated CO_2, there seems to be a trade-off between the number and size of leaves in *A. donax*. There is a decrease in the total number of leaves as individual leaves grow larger thus the leaf area was considerably decreased at this 15-day CO_2 treatment. This is a reaction to strategic resource allocation as explained by Nackley et al. (2017). From their study in *A. donax*, it was evident that in these environments, the plant favors allocation to rhizomes, improving its ability to compete in its surroundings. Despite the rise in leaf size, this shift in biomass allocation results in a lower total investment in making new leaves, which in turn leads to a fall in leaf quantity, thereby a fall in leaf area. In the present study, elevated CO_2-induced rise in the leaf area was shown only by *S. arundinaceum*. As mentioned above in *S. arundinaceum* leaf length and breadth were increased under elevated CO_2 even though the number of leaves declined. *S. arundinaceum* strategically utilizes its resources to take advantage of the increased CO_2 availability, as seen by the trade-off between leaf size and number. Despite having fewer leaves overall, the plant increases the efficiency of photosynthesis by investing in larger leaves. In situations where resource management is essential, this strategy might be necessary to preserve sustainability and competition (De Souza et al., 2008). Even though the number of leaves decreased in this species, due to various physiological changes as mentioned above induced by elevated CO_2, the larger leaves contribute to the overall increase in leaf area to absorb light and CO_2.

In other C4 grasses under study, the difference between CC and TC was negligible or the TC plants showed minimal responses regarding leaf area. Physiological systems in many C4 grasses have already adapted to effectively use low

ambient CO_2 levels; this is particularly applicable for those grasses examined under enriched CO_2 environments. Accordingly, Hager et al. (2016) suggested that as their photosynthetic pathways are nearly saturated under the current atmospheric conditions, these species may exhibit modest responses in leaf length, width, area, and number when exposed to elevated CO_2. The authors also ascertain that elevated CO_2 does not affect all C4 grass species equally; some show increases in specific features while others do not. This variation can be explained by genetic and ecological variation among species, implying that certain C4 species may exhibit minimal responses, while others may attain improved development through alternative means. Accordingly, species such as *C. flexuosus*, *C. zizanioides,* and *P. pedicellatum* responded to elevated CO_2 by an increase in culm diameter, while *S. aundinaceum* exhibited negligible increase where the resource allocation was more concentrated towards the leaves. According to Song and Huang (2014) when grasses are exposed to high CO_2, their physiological response mechanisms cause changes in cell expansion and structural development. The thicker and more robust stem development that results from the improved carbon environment is partly responsible for the increase in culm diameter.

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. In Chapter I high CO_2 exchange potential of *A. donax* was revealed. However, 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.

A relation between the average net day flux and biomass of the plant was established in the present study. For this a formula for the calculation of gram CO_2 uptake per gram dry weight of plant was derived using the variables such as net day CO_2 flux (average CO_2 uptake by plant species), dry weight of the plant samples, and volume of the chamber. By using this formula CO_2 uptake (in grams) by 1 gram 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, total biomass growing by 60%, 15%, and 30% for tall fescue, perennial ryegrass, and Kentucky bluegrass, respectively were noticed. 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 222.93ppm, 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 gram of plant biomass (8.004×10^{-3}g CO_2). The biomass increase and CO_2 absorption efficiency of different grass species vary in response to elevated CO_2. When compared to C4 grasses, C3 grasses typically respond to higher

CO_2 conditions more robustly. In fact, C3 species often show considerable increases in biomass and photosynthesis with rates of carbon uptake (Pendall et al., 2011). Numerous studies have demonstrated that C4 plant species have lower CO_2 sensitivity than C3 plant species (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.

2.5.2 Changes in the pigment levels of grass species under elevated CO_2 conditions

A considerable increase in the pigment content at elevated CO_2 condition was noticed in *S. arundinaceum* and regarding *P. pedicellatum* except chlorophyll b, other pigments such as chlorophyll a, total chlorophyll, and carotenoids were increased. All other species exhibited a decline in the pigment content. 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 several 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, this 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, the plant 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 the 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. Also, *A. donax* exhibited an enhanced carbohydrate production (25.58% increase) and a 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*.

Carotenoid content decreased in experimented grass species except *S. arundinaceum* and *P. pedicellatum*. *C. flexuosus* and *A. donax* exhibited a significant decrease. According to a meta-analysis of several studies, carotenoid concentrations in plants can drop by 6% to 26% when CO_2 levels are raised (Loladze et al., 2019). A decrease of 34.29% and 33.62% in the carotenoid content was noticed with *C. flexuosus* and *A. donax*, respectively. Increased biomass and growth rates lead to a diluting effect, which is one of the suggested causes for the decrease in carotenoid

levels at increasing CO_2. Increased CO_2 availability promotes photosynthesis and growth in plants, increasing biomass without increasing carotenoid concentrations in tandem (Holley et al., 2022). A drop in the total carotenoid concentration per unit of biomass could result from this diluting effect. But in other circumstances, if chlorophyll production is also proportionately increased, resulting in an overall rise in both pigments, the increased biomass may not considerably dilute the carotenoid level (Netto et al., 2005). Accordingly, carotenoid content was increased in *S. arundinaceum* and *P. pedicellatum* at elevated CO_2 conditions. A balanced ratio is important because more chlorophyll concentration can be a sign of better photosynthetic capacity, which promotes the production of carotenoid pigments (He et al., 2023). Interconnected pathways of metabolism in plants regulate the synthesis of chlorophyll and carotenoids. Increased CO_2 could stimulate several regulatory mechanisms that simultaneously increase the production of chlorophyll and carotenoids. Under conditions of elevated CO_2, the interaction of these metabolic processes implies a coordinated adjustment in pigment production that optimizes plant growth and adaptation (Niroula et al., 2019; Taub, 2010). As they function as antioxidants and provide photoprotection, carotenoids are essential to the health of plants. The increased trend in carotenoid levels in *S. arundinaceum* and *P. pedicellatum* may improve nutritional quality, especially in environments where greater CO_2 concentrations promote growth as suggested by Niroula et al. (2019).

Elevated CO_2-induced enhancement in photosynthetic activity influences the biosynthesis of pigments. The process of producing 5-aminolevulinic acid (ALA) from glutamate is the first step in the biosynthesis of chlorophyll. Chlorophyllide is the resultant product of this process, and chlorophyll is finally produced by esterifying chlorophyllide. For instance, the conversion of ALA and its associated intermediates into chlorophyllide is eased by the increased photosynthetic rates under increasing CO_2, which provide more energy and precursors required for chlorophyll production (Chatterjee & Kundu, 2015). Accumulation of chlorophyll and carotenoid pigments in *S. arundinaceum* and *P. pedicellatum* was in a similar way as mentioned which can be explained by the interdependence of biosynthetic pathway of both pigments. 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. A similar reduction in both pigments in *M. maximus*, *C. flexuosus*, *C. zizanioides*, and *A. donax* might also happen due to this interdependence of biosynthetic pathways of chlorophyll and carotenoids.

2.5.3 Changes in plant metabolites under elevated CO_2 conditions

The responses of various grass species to increased CO_2 levels vary, which affects the accumulation of carbohydrates. Here species such as *M. maximus*, *C. zizanioides*, and *A. donax* show a rise in carbohydrate concentration. 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 undergoes 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*. *P. pedicellatum* experiences a decrease in carbohydrate content upon exposure to elevated CO_2, in contrast to an increase in chlorophylls and carotenoids. Cellular and metabolic adaptations are often included in the physiological mechanisms behind these alterations. Improved overall photosynthetic capacity can be attributed to the production of carotenoid and chlorophyll synthesis and better absorption of light. On the other hand, changes in leaf area and carbohydrate accumulation indicate a reallocation of energy and resources meant to maximize development in response to high CO_2 settings, which can entail giving preference to some growth forms over others (Lupitu et al., 2022).

As mentioned above three species exhibited an enhancement in carbohydrate levels at elevated CO_2, in which *A. donax* is a C3 species and it was previously reported that elevated CO_2 increases the photosynthetic efficiency of C3 plants by facilitating improved carboxylase activity of the Rubisco enzyme. 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* as discussed in Chapter I was an indication of lowered respiration rates due to declined nitrogen concentration. In C3 plants due to its substrate limitation by the present atmospheric CO_2 concentrations, the RuBisCO enzyme can react to rises in CO_2 concentration and can change the CO_2 flux during carbon absorption through the regulation of metabolism (Bernacchi et al., 2003; Long et al., 2004). However, at low ambient CO_2 levels, photosynthetic carbon absorption in the C4 species is either CO_2-saturated or nearly so. Here the enzyme PEP carboxylase is not sensitive to variations in the CO_2:O_2 ratio because O_2 does not bind to its catalytic site; instead, it uses HCO_3 as its substrate instead of CO_2 (Lara & Andreo, 2011). 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. According to previous reports, different C4 species may exhibit distinct changes in their levels of carbohydrates and pigments; in fact, some circumstances may promote a greater accumulation of carbohydrates rather than enhanced production of pigments (Hager et al., 2016; Lara & Andreo, 2011). This variation displays how photosynthetic mechanisms and resource allocation schemes of different grass species interact intricately.

Regarding the protein content changes happened 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 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. Decreased protein concentration was noticed in *M. maximus*, *S. arundinaceum,* and *C. flexuosus*. However, protein concentration was increased in *A. donax* and *P. pedicellatum*. The decrease and increase of protein concentration in these species were noticed at similar rates in both CC and TC. In many plant species, elevated levels of CO_2 can cause a substantial decrease in protein concentration. 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 grass plants over time (Taub, 2010; Burgess & Huang, 2014).

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. Different species respond differently to increases in CO_2 levels, as evidenced by the variety of reactions observed. Certain studies imply that under certain conditions, elevated CO_2 levels stimulate phenolic production by increasing the total amount of phenolic compounds (Robinson et al, 2012; Rajashekar, 2018). The primary pathways in grasses that produce phenolic compounds are phenylpropanoid and shikimic acid (Lattanzio, 2013). Phenylalanine and tyrosine are the initial amino acids in these processes because they are essential for the synthesis of different phenolic compounds that support defense systems and plant growth. 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 has increased in CO_2 treatment while a considerable decrease has occurred in control. Likewise in the study of Castells et al,

(2002), it was shown that under elevated CO_2, two perennial grass species, *Dactylis glomerata* and *Bromus erectus*, significantly raised the contents of phenolic compounds; *Dactylis glomerata* showed a 15.2% increase, while *Bromus erectus* showed a startling 86.9% increase. According to Castells et al, (2002), as phenolic compounds often function as defense agents against herbivores, their elevated levels in grass species might increase their resistance to herbivory. Contrary to these observations, in the present study phenol content has 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). Under experimental conditions, increased CO_2 often encourages biomass accumulation and plant development. The allocation of resources may, however, shift due to this rapid expansion from primary growth activities to secondary metabolites, such as phenolic compounds (Bezemer et al., 2000; Rajashekar, 2018). These alterations may also cause the amounts of phenolic compounds in grass tissues to decrease. 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. 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.

2.5.4 Changes in plant nutrients 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. Studies reveal that elevated CO_2 concentrations promote photosynthetic rates and the synthesis of

carbohydrates, resulting in significant alterations to the nutritional composition of leaves. Regarding carbon content, the experimented grass species exhibited only a meager difference between CC and TC. Such responses might be influenced by the duration of the experiment (Taub, 2010; 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 the 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.

As far as the nitrogen content of plants is concerned, each plant responds differently. 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 N 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. Studies show that this phenomenon, in which nitrogen contents dramatically decrease in the presence of high CO_2, affects a variety of species, including major crops like wheat and rice (Taub, 2010). There are multiple physiological factors responsible for the decrease in nitrogen levels. Increased CO_2 has an adverse effect on 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,* and 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. As in Chapter I, this species showed increased CO_2 exchange, which resulted in improved photosynthesis and increased amounts of carbohydrates, however, the plant's overall morphology showed a negative response. Therefore it was certain that, *A. donax* was greatly impacted by the sharp drop in nitrogen concentration in an environment with higher CO_2.

Contrary to the common phenomenon of the reduction in nitrogen levels at elevated CO_2 conditions in plants, it was 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). Moreover according to Gorissen and Cotrufo (1999) increased CO_2 can change rhizosphere microbial activity, which may improve nitrogen availability and boost the uptake of nitrogen. These modifications to microbial processes may enable a more efficient uptake of nitrogen, hence increasing the amount of nitrogen in the plant's leaves. It was previously reported that the microbial growth that is stimulated by the root excretions of *C. zizanioides* aids in the breakdown of organic matter and increases the availability of vital nutrients including nitrogen (Chen et al., 2021). Moreover, the plant was noticed to enhance in total organic carbon (TOC) of soil. The breakdown of the plant's roots enhances the amounts of soil organic matter and TOC (Tessema et al., 2024). Microbial colonization is made possible by this process (Lakshmi & Sekhar, 2020). Further research is required on the changes in soil microflora at elevated CO_2 conditions because the rhizosphere and soil microorganisms were not taken into consideration in the current study.

The calcium content of the leaves of various grass species has been found to generally decrease with elevated atmospheric CO_2 levels. In this study, calcium content was decreased in TC of all grass species under elevated CO_2 treatment, 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. 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 the transformation of carbon dioxide and water into carbohydrates (Ishfaq et 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*.

Grass species under experimental conditions showed species-specific responses to sodium levels. Three species in the present study exhibited a reduction in sodium levels, such as *C. flexuosus* ($p<0.001$), *M. maximus*, and *P. pedicellatum*. After examining the combined impacts of elevated CO_2 and salinity on three grass species, Moxley (2012) concluded that when compared to grass species that were purely under saline stress, those exposed to higher CO_2 levels had reduced sodium levels. According to the author, lower sodium levels seem especially helpful for grasses under salt stress, where more CO_2 helps with a physiological recovery. This beneficial modification most likely lessens the negative consequences of elevated salt concentrations, improving overall plant health and growth in difficult environmental circumstances. Similar to the reduced salt content of the grass species in the current study, salt tolerance of Kentucky bluegrass in high CO_2 conditions was observed by Zhuang et al. (2019). Through the regulation of metabolomics, changes elevated CO_2 enhanced salt tolerance in Kentucky bluegrass, positively influencing the levels of sodium and potassium in the experimental chambers. Contrary to this, an increase in sodium content was noticed in *S. arundinaceum* ($p<0.05$), C. zizanioides, and *A. donax*. This increase might be due to a phenomenon called "ionome shift" which often takes place under elevated CO_2 conditions in plants as proposed by Loladze (2014). This shift, which can be caused by distinct transpiration rates and nutrient transport methods, refers to variations in the concentrations of different minerals and nutrients, including sodium, inside the plant tissue.

Potassium levels also exhibited species-specific responses. An increase was noticed in *S. arundinaceum* and *C. flexuosus*, a decrease was noticed in *M. maximus* and *A. donax*, and the changes were similar in both CC and TC of remaining grass species studied. A comprehensive study revealed that a variety of plant species may experience a deficiency in potassium and other important minerals as a result of elevated CO_2 concentrations. In particular, it was found that when plants were exposed to elevated CO_2 levels, their total potassium concentration decreased by about 8% (Loladze, 2014). Thus the decrease in potassium levels of *M. maximus* and *A. donax*

might be due to this general phenomenon. Furthermore, the decrease in the level of potassium could potentially be the result of the dilution effect brought on by an increase in biomass (Loladze, 2014; Baxter et al., 1994). The potassium level of *Poa alpina* was shown to be much lower in the study by Baxter et al. (1994) that focused on three grass species such as *Festuca vivipara*, *Agrostis capillaris*, and *Poa alpina*. Meanwhile, the growth of *A. capillaris* and *P. alpina* rose under increasing CO_2. When grown at higher CO_2 levels, on the other hand, *F. vivipara* showed a more noticeable decline in nutrient uptake efficiency, particularly potassium. Decreased potassium levels impair plant health and productivity by making the physiological reactions to CO_2, more complex. The benefits of enhanced biomass production that are usually associated with higher atmospheric CO_2 levels are severely impeded by potassium deficiency (Asif et al., 2017). This suggests that maintaining sufficient potassium levels is necessary for grass species to fully benefit from elevated CO_2 concentrations. Potassium is required for many physiological functions, such as osmoregulation and enzyme activation, both of which are critical for plants to use water efficiently. Because of their reduced ability to photosynthesize, when potassium levels are low, grasses are more vulnerable to abiotic stressors like drought and grow at slower rates (Asif et al., 2017).

2.5.5 Insights from FTIR spectra of grass leaves

The biochemical changes in grass leaves as a result of elevated CO_2 are examined using Fourier-transform infrared (FTIR) analysis. These changes reveal differences in substances like cellulose, hemicellulose, lignin, and other plant polymers that are essential for evaluating the productivity and health of plants in diverse environmental settings. More prominent peaks were not appeared in TC but some differences in the peaks between CC and TC were noted. When grasses are exposed to elevated CO_2, a range of biochemical and physiological changes occur, which can be observed as a reduction in hydroxyl, aliphatic, and carbonyl groups in their FTIR spectra after 15 days of treatment. Moreover, peaks were observed around 2300 cm^{-1}, which exhibits a unique asymmetric stretching vibration of CO_2. This peak is prominent and frequently detected in environmental and industrial assessments.

As CO_2 levels rise, plants boost their photosynthetic rates, leading them to prioritize carbohydrate production over secondary metabolites, which often contain these key functional groups. For instance, Zhuang (2012) found that elevated CO_2 can

alter the chemical composition of leaves, resulting in lower levels of certain organic molecules, including those with hydroxyl and carbonyl groups. As phenolic chemicals, usually produced from aliphatic precursors, may decline in production, this change can potentially affect the plant's defence systems. As well the phenol content was decreased in *M. maximus*, *C. flexuosus*, and *C. zizanioides* with a significant decrease in *C. zizanioides*. The reduction in these functional groups could be a sign of this decreased production in secondary metabolites such as phenolic compounds. Watling et al. (2000) suggest that these changes might be part of a broader metabolic reprogramming, triggered by the elevated CO_2, which could alter the plant's overall structure and function. According to Tooth and Leishman (2014), the observed decreases are likely the result of a complex interaction between the enhanced growth and the shifting metabolic priorities that occur in elevated CO_2 environments.

2.5.6 Changes in the characteristics of soil associated with grass species under experimentation

Elevated atmospheric CO_2 concentrations can have a significant effect on soil moisture levels in certain ecosystems. In controlled environments, such as experiment chambers, variations in plant water relations and the overall effectiveness of plant water usage may interfere with the dynamics of soil moisture (Nelson et al., 2004). The regulation of stomatal conductance is the main mechanism by which high CO_2 influences the water status of plants. Reduced transpiration rates may result from plants closing their stomata to retain moisture when CO_2 levels increase. Depending on the plant species and development conditions, this may have positive effects on leaf water potential but may also have complex implications on soil moisture retention (Nelson et al., 2004). Furthermore, according to Nelson et al. (2004), plants may produce more biomass with less water in CO_2-enriched environments, which can result in a 43% increase in water-use efficiency (WUE). This phenomenon further supports soil moisture retention. According to one study, soil moisture in CO_2-treated chambers increased by 4.88%, which is in line with the results from other studies involving other grass ecosystems (Nelson et al., 2004). Similarly in the present study, soil moisture increased in TC of *A. donax*. Contrary to a general trend of increased moisture content of soil associated with elevated CO_2, soil moisture level was found to be lower in the CO_2-treated *S. arundinaceum* and *C. zizanioides* in the current study. Both species had

higher below-ground biomass and more extensive root systems. Sometimes the increased root growth and biomass might result in a net increase in water consumption, which can lower the moisture content of the soil (Nelson et al., 2004; Madhu & Hatfield 2015). This notion is also explained by the fact that elevated CO_2 levels often lead grass species to grow and accumulate biomass faster, which can increase the rate at which water is absorbed from the soil. Because the transpiration rate increases as a result of the higher water demand of the plant, the amount of moisture in the soil may decrease in several plants (Seneweera et al., 1998). Soil moisture levels are also profoundly impacted by the way elevated CO_2 interacts with other climate factors like temperature and precipitation. For instance, when temperatures rise along with elevated CO_2, the higher evaporation rates can intensify moisture loss, leading to an even greater reduction in soil moisture content.

Soils were turned more acidic in the present study except for *M. maximus* and *C. flexuosus*. Different grass species can influence soil chemistry through processes like root exudation and litter decomposition. As a result, changes in soil pH are often closely tied to the specific type of grass being studied (Adiputra, 2022). Several mechanisms have been linked to soil acidification under elevated CO_2 conditions. When more carbon dioxide dissolves in soil moisture, it forms carbonic acid, which can considerably lower the pH of soil, making it more acidic (Xiao et al., 2017). Soil moisture was noticed to be increased in *M. maximus*, *A. donax,* and *P. pedicellatum*. Thus the observed acidification of soils associated with this species could be attributed to carbonic acid formation. The physiological changes in grasses triggered by increased CO_2 also influence how they absorb nutrients. For instance, according to Fay et al. (2002), plants may take up more cationic nutrients like potassium, calcium, and magnesium, as these base cations are removed from the soil, hydrogen ion levels increase, further contributing to soil acidification. Moreover, at elevated CO_2, triggered metabolic processes may cause the exudation of organic acids by grasses, and these organic acids contribute to an increase in hydrogen ion concentration and lead to acidification of soil. In the case of *M. maximus* and *C. flexuosus*, the soil turned from moderately acidic to neutral condition. This can be clarified by the view of Pastore et al., (2021), who proposed that the increase in primary production leads to more organic matter being added to the soil and as this organic matter decomposes, it releases valuable nutrients that can help buffer soil acidity, promoting a more neutral

pH. Moreover according to Suseela et al. (2017), grasses, with their enhanced root growth and increased interaction with the soil, also play a key role in nutrient cycling. They boost the availability of base cations like calcium and magnesium, which can help neutralize soil acidity by reducing hydrogen ion concentration and raising pH levels. As these plants grow and mature, they slow down the acidification process, gradually bringing the soil pH closer to neutral.

Grasses growing in high CO_2 environments usually show an increase in soil total organic carbon (TOC). This is because elevated CO_2 levels enhance plant growth, which in turn adds more organic matter to the soil through greater root biomass and litter decomposition (Xiao et al., 2009; Gill et al., 2006). Accordingly, TOC was noticed to be increased at elevated CO_2 in soils associated with *C. zizanioides* ($p=0.027$), *M. maximus*, and *A. donax*. As nitrogen availability gradually decreases, it becomes harder for the soil to store carbon, which can offset the gains from increased carbon inputs due to plant growth (Gill et al., 2006; Bills, 2009). For soil to effectively retain carbon in organic matter, there needs to be enough nitrogen present. The nitrogen range in the soils associated with all plant species was in the normal range, but with increased CO_2, all species showed a decline in nitrogen levels, with *C. zizanioides* showing the least reduction in nitrogen levels. This decline in nitrogen levels might be impacted by the decrease in the TOC of *S. arundinaceum*, *C. flexuosus*, and *P. pedicellatum*. Weber, (2023) was of the view that the overall responses of TOC under increasing CO_2 are influenced by many factors, including soil type, moisture availability, and the management strategies used. The total organic carbon (TOC) of soil concerning rising atmospheric CO_2 levels is a significant concern that requires emphasis. The actual ability for soil carbon sequestration depends largely on nitrogen availability and the fragile equilibrium between the organic matter input and the processes of decomposition. Since all of these factors were not extensively covered in the current study, it is important to understand these interactions to develop future strategies for successful soil carbon management.

Nitrogen is often regarded as one of the most important nutrients for plant growth, as it affects several growth characteristics, including plant height, chlorophyll content, and general vigor (Razaq et al., 2017). The nitrogen levels in the soils used for the growth of plants in the present study are within normal ranges. Nitrogen levels for the growth of plants normally range from 5.5 to 103.7 mg/kg of dry soil (Sunaga et al.,

2008). This range represents the amounts of nitrogen required by plants for their optimum growth and output. At elevated CO_2 conditions soils associated with all grass species shows a decline in nitrogen levels. The notable decline in TC compared to CC was shown by *M. maximus*, *S. arundinaceum*, and *C. flexuosus*. This could be explained by the fact that elevated CO_2 levels increase the need for nitrogen, which is essential for protein and nucleic acid synthesis. As a result, soil nitrogen levels may drop because increased plant biomass outpaces the natural replenishment of nitrogen in the soil, as grass species require more of the available nitrogen for their growth and development (Cotrufo & Gorissen, 1997). Moreover, as plants contribute more carbon through their roots; it can alter the microbial communities in the soil. If these microbes become less efficient at breaking down organic matter or releasing nitrogen, the total nitrogen in the soil could decrease, despite there is an increase in plant growth (Gill et al., 2006). *M. maximus* and *S. arundinaceum* displayed higher development among grass species with soil nitrogen levels lowered, implying that the aforementioned notions may be the cause of the reduction in soil nitrogen. While among the grass species under study *A. donax* exhibited higher reductions in soil nitrogen. The plant also exhibited a decrease in leaf nitrogen. While the plant exhibited an enhancement in the leaf length and breadth. The species may rapidly consume available nitrogen as a result, its better growth brought on by elevated CO_2. The faster uptake may cause a drop in soil nitrogen level, which will also affect the leaves even when the leaf length and breadth are increased. This quick uptake may decrease nitrogen resorption efficiency from the leaves, as they senesce, can result in a decrease in the nitrogen content of the leaves (Yue et al., 2023).

Results from the experiment suggest that over time, there may be significant changes in the soil characteristics due to the interaction between grass species and atmospheric CO_2 enrichment. Nevertheless, the direction and magnitude of this change might differ significantly based on the surrounding circumstances and the particular characteristics of the grass species under study. Because of these dynamics, it is clear that species-specific assessments in experimental chambers are necessary to fully understand the complexity of ecological responses to climate change. Moreover, the nutritional profile and microbiota of soil were not extensively covered in the current study; therefore, further research on these aspects will address changes in soil conditions within the given context.

2.6 SUMMARY AND CONCLUSION

Chapter II deals with the evaluation and subsequent discussion of the changes in growth attributes and biochemical characteristics of grass species under elevated CO_2 treatment. Objectives of this chapter include the evaluation of the growth/development and changes in plant biomass at different carbon dioxide supply levels; the evaluation of the biochemical responses of plants to different carbon dioxide concentrations; the listing of plants with the greatest potential for sequestering carbon dioxide; and optimizing conditions for the most efficient sequestration.

The growth measurements were carried out on the first and final day of experimentation. Likewise, leaf metabolites, minerals, and nutrients were analyzed on 1, 5, 10, and 15 days of the experiment. Growth attributes measured 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. The biochemical parameters analyzed include pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids); metabolites (carbohydrate, protein, and phenol), and plant nutrients (carbon, nitrogen, calcium, magnesium, sodium, and potassium). A CHNS organic elemental analyzer was used to determine the carbon and nitrogen contents of each sample. For analyzing the changes in chemical composition, Fourier - Transform Infrared Spectroscopic Analysis (FTIR) was performed on each plant sample. The soil characteristics studied include moisture, pH, total organic carbon (TOC), and nitrogen. To comprehend the magnitude and direction of the changes in morphological and biochemical attributes, Hedges g or effect size values were considered. Hedges g is a statistical tool that measures the degree of variation between two groups or conditions while accounting for inter-group variability and eliminating the possibility for bias resulting from small sample numbers.

To know the efficiency of each grass species for CO_2 uptake relative to their biomass, gram CO_2 uptake per gram dry weight of plant biomass was calculated for the TC. A formula was derived for the calculation of gram CO_2 uptake per gram of plant biomass as follows,

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

Where U represents the CO_2 uptake per gram of biomass, C is the average CO_2 uptake by the plants (ppm), V is the volume of the chamber (m^3), and W is the total dry weight of the plants (g).

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*. Elevated CO_2 treatment resulted in a decline in the number of leaves of all grass species except *M. maximus* where new leaves were developed. Leaf number noticeably decreased 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 concerning *M. maximus*, and *S. arundinaceum,* negligible change was noticed in the culm diameter. To analyze the effect of elevated CO_2 on the overall morphology of grass species, t-SNE algorithm (t-Distributed Stochastic Neighbor Embedding) with Hedges g values were plotted. Thus regarding overall morphological performance at elevated CO_2, *M. maximus* is superior, followed by *S. arundinaceum*, *P. pedicellatum*, *C. zizanioides*, *C. flexuosus,* and *A. donax.*

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). Based on CO_2 uptake per gram dry weight, *S. arundinaceum* was more efficient, followed by *A. donax*, *C. flexuosus*, *P. pedicellatum*, *M. maximus,* and *C. zizanioides*.

Changes were noticed in the concentration of pigments. Chlorophyll a content was increased in *S. arundinaceum* and significantly decreased in *A. donax*. As 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*, and 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*.

Changes were detected in the concentration of carbohydrates in grass plants subjected to 15 days of experimentation. *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 in 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*. 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. Calcium content was decreased in TC of all grass species under elevated CO_2 treatment, except *P. pedicellatum*. Magnesium content has decreased in all species at elevated CO_2 treatment. *A. donax* treated with elevated CO_2 exhibited a significant decrease in the magnesium content. Significant changes were noticed in the sodium content of *S. arundinaceum* (increase) and *C. flexuosus* (decrease). Concerning the percentage change in potassium content during the study, an increase was shown only by *S. arundinaceum* and *C. flexuosus.*

There is no prominent new peaks emerged in the FTIR spectra of CO_2-treated leaf samples, while subtle differences between the control and treated conditions were observed. The overall FTIR spectra of the grass species indicate that CO_2 treatment leads to significant chemical changes over time. These changes include a decrease in

the presence of hydroxyl, aliphatic, and carbonyl groups, hinting at degradation processes.

Soil moisture was increased in *M. maximus*, *A. donax,* and *P. pedicellatum* and was decreased in *S. arundinaceum*, *C. flexuosus,* and *C. zizanioides* under elevated CO_2 conditions. Regarding soil pH, soil turns more acidic in TC of *S. arundinaceum* and *C. zizanioides* compared to soil samples of its CC. *P. pedicellatum* exhibited a change from an alkaline condition to a moderately acidic condition. Regarding *M. maximus* and *C. flexuosus* soil turns from moderately acidic to neutral condition. Considering the TOC of the soil, a significant increase was noticed in *C. zizanioides* under elevated CO_2 treatment. Nitrogen content decreased in soils associated with TC of all species. A higher percentage decrease in the soil nitrogen among the species under study was noticed in *A. donax*.

Chapter I discussed the changes in microclimatic conditions brought about by the grass species. CO_2 uptake potential (Net Day flux) and net CO_2 exchange of each grass species were calculated and discussed in Chapter I. In Chapter II overall development and CO_2 uptake per gram biomass were 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 in several aspects. Thus *S. arundinaceum* and *M. maximus* could be recommended for CO_2 mitigation efforts. *A. donax*, the C3 species included in the study, even though better in net CO_2 exchange and higher CO_2 uptake per gram biomass, exhibited negative responses regarding overall morphology. This was due to the poor nitrogen cycling of the plant, a common phenomenon exhibited by C3 plants in elevated CO_2 environments. Since the plant has a higher potential for CO_2 mitigation it is advised that when nitrogen fertilizers are used, this species also will be able to grow well in elevated CO_2 environments and be utilized in CO_2 mitigation programmes.

GENERAL CONCLUSIONS

In the current study, CO_2 fluxes linked to each of the chosen grass species were worked out. By examining several factors, such as DF (N), Net CO_2 exchange, Overall development, and CO_2 uptake per biomass, the best grass species for reducing atmospheric CO_2 were identified.

As far as DF (N) is concerned *S. arundinaceum* was noticed to be best followed by *M. maximus*, *A. donax*, *P. pedicellatum*, *C. flexuosus*, and *C. zizanioides*. It was assumed that the species uses this amount of CO_2 for the photosynthetic process. While regarding the Net CO_2 exchange, that is the matric concerned with the balance between the amount of CO_2 that plants absorb during photosynthesis and the amount of CO_2 that they release during respiration, *A. donax* (C3) was noticed to be best followed by *C. zizanioides*, *M. maximus*, *P. pedicellatum*, *S. arundinaceum*, and *C. flexuosus*. The night flux or the CO_2 release during night time influences the overall balance of CO_2 exchange of plants that shows better DF (N), thus from the point of view of overall CO_2 exchange in the chamber *S. arundinaceum* occupies the 5^{th} position. While the overall development or overall morphological enhancement is concerned, *M. maximus* occupies the first position and *S. arundinaceum* occupies the second position. Thus it is obvious that these species enhanced their morphological characters (especially leaf number and leaf morphology) through enhanced metabolism in an elevated CO_2 environment. There is an interplay between respiratory release and the overall metabolism of plants, and this interplay might have been influenced by the net CO_2 exchange of *S. arundinaceum*. Based on CO_2 uptake per gram dry weight, *S. arundinaceum* was more efficient, followed by *A. donax*, *C. flexuosus*, *P. pedicellatum*, *M. maximus*, and *C. zizanioides*. Thus *S. arundinaceum* and *M. maximus* could be recommended for CO_2 mitigation efforts.

A. donax, the C3 species included in the study, even though better in net CO_2 exchange and higher CO_2 uptake per biomass, exhibited a negative response regarding overall morphology. It was due to the poor nitrogen cycling of the plant. As the plant has a higher potential for CO_2 mitigation (better DF(N), higher net CO_2 exchange, and CO_2 uptake per gram biomass), it is advised that an adequate supply of nitrogen fertilizer may benefit the plant to grow well in high CO_2 environments and can be utilized in CO_2 mitigation programs.

RECOMMENDATIONS

India's carbon mitigation initiatives can address climate change in an economical and environmentally responsible manner by incorporating vegetation-based solutions. To encourage the widespread use of nature-based solutions in India, future studies should concentrate on strengthening policy frameworks, refining methodologies for measuring sequestration and optimizing land management practices.

The current study offered an economical experimental setup to detect carbon dioxide fluxes ascribed to plants and provide information on the carbon sequestration efficiency of plants. By adding other facilities to the current experimental setup, it may be possible to obtain information that would help choose the best species among a variety of species.

Carbon mitigation efficiency (net day flux of CO_2, net CO_2 exchange), morphological changes, and CO_2 uptake per biomass of *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 evaluated in this study, and it was determined that *S. arundinaceum* and *M. maximus* could be strongly suggested for CO_2 mitigation programs.

According to earlier reports, *S. arundinaceum* is a viable feedstock for the production of bioethanol and other products with added value. Its use in bioenergy projects is consistent with India's ethanol blending policy, which attempts to reduce greenhouse gas emissions in the transportation industry. In addition to improving rural livelihoods, expanding its production on marginal and degraded soils can provide environmental and economic sustainability. *M. maximus* is primarily used as a crop for livestock feed. It makes it a good choice for sequestering carbon in agro-pastoral environments. By increasing livestock productivity and reducing the need for feed imports, improved fodder availability lowers the carbon footprint associated with animal husbandry.

Better net CO_2 exchange and more CO_2 uptake per plant biomass were observed in *A. donax*. When nitrogen is supplied appropriately, their high capability for CO_2

uptake makes them more suitable for carbon mitigation projects. The plant is previously reported to be a promising feedstock of biofuel due to its quick growth, high biomass output, and minimal input needs. Furthermore, the carbon sequestration efficiency of *A. donax* makes it even more suitable for India's carbon sequestration, and bioenergy initiatives.

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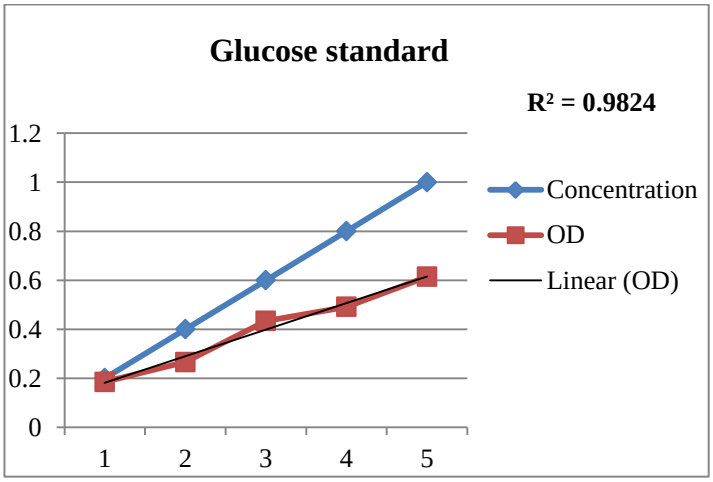
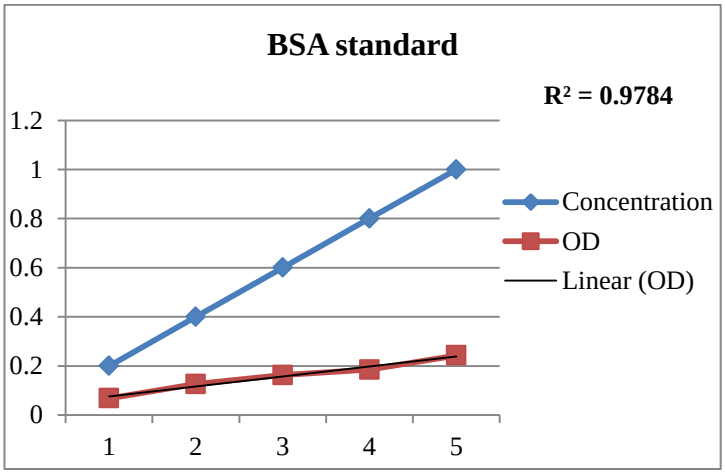
ANNEXURE

Annexure 1: Graph of Carbohydrate standard



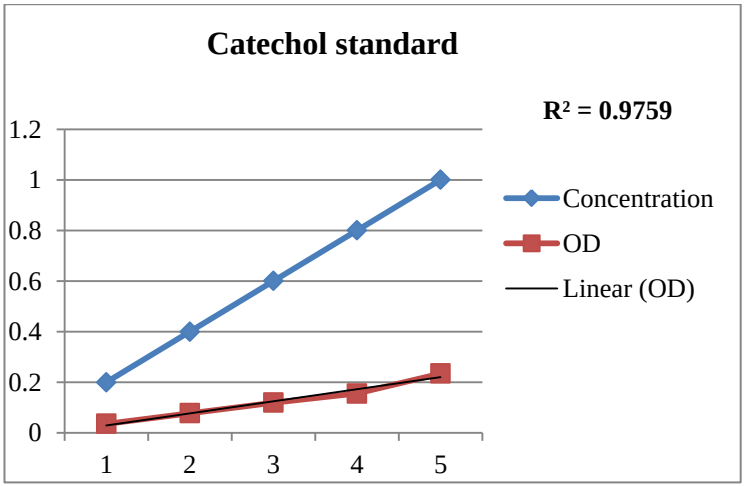
Note: OD, optical density

Annexure 2: Graph of Protein standard



Note: OD, optical density

Annexure 3: Graph of Phenol standard



Note: OD, optical density