CARBON STORAGE AND ALLOCATION PATTERN IN PLANT BIOMASS UNDER DROUGHT STRESS AND NITROGEN SUPPLY

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CERTIFICATE

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DEDICATION

I dedicate this thesis to my beloved mother who

is the reason behind my every achievement and

my respected and loveable father who stood

beside me at every moment in my life


بسم الله الرحمن الرحيم

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Saira Kanwal

Contents

LIST OF ABBREVIATIONS

CO_2	Carbon dioxide
IPCC	Intergovernmental Panel on Climate Change
GHGs	Greenhouse Gases
RuBisCO	Ribulose-1, 5-bisphosphate carboxylase/oxygenase
N	Nitrogen
D	Drought
P_n	Photosynthetic rate
Tr	Transpiration rate
Gs	Stomatal conductance
Ci	Intercellular carbon dioxide concentration
R/S	Root to Shoot Ratio
RWC	Relative Water Content
WUE	Water Use Efficiency
L/FR	Leaf to Fine Root Mass
FC	Field Capacity
TOC	Total Organic Carbon
FAS	Ferrous Ammonium Sulphate
NH_4-N	Ammonium Nitrogen
C	Carbon Content
LAR	Leaf Area Ratio
SLA	Specific Leaf Area
ANOVA	Analysis of Variance

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ABSTRACT

Climate change may have multi-faceted adverse effects on forests worldwide such as pest outbreaks, fires, heat waves and drought. These stresses including changes in water and nutrient availability, cause imbalance in carbon uptake by plants. In this study, two species *Eucalyptus camaldulensis* (evergreen) and *Populus deltoides* (deciduous) were selected for carbon content and allocation analysis with the application of nitrogen fertilizer and water stress treatments. Pot experiment was done by planting 2 years old seedlings in 5kg pots in a glasshouse for four weeks. Experiment was a 2-factor factorial completely randomized design having three water stress levels D0, D1, D2 (1000, 500 and 250 mL) and three nitrogen treatments N0, N1, N2 (0, 0.5 and 1 $\mathrm{gNkg^{-1}}$). Significant and non-significant Nitrogen into Drought interactions (NxD) were observed for each treatment. Results showed that in *Populus deltoides*, at N2D2 treatment, shoot carbon content increased up to 63% to 75%. Whereas in *Eucalyptus camaldulensis*, shoot carbon content increased up to 51% to 52% at N0D2 treatment. Leaf carbon content increased 23% to 44% in *E. camaldulensis* and 0.3% to 4% in *P. deltoides*, at N1D1 treatment respectively. Dry shoot biomass increased 3.8g to 7g at N2D2 treatment in *E. camaldulensis* whereas 45g to 81g at N1D2 in *P. deltoides.* Increased root biomass production was observed in N1D0 of *P. deltoides* (31.96g) and *E. camaldulensis* (2.73g). Leaf biomass was more observable in *E. camaldulensis*, at N1D2, up to 4.72g and in *P. deltoides* at N2D1 up to 3.4g. A significant increase at NxD interactions was observed in root carbon content, shoot length, root length, root biomass and Relative Water Content (RWC) in *E. camaldulensis*. Likewise, root length, shoot biomass, root biomass, Water Use Efficiency (WUE) and RWC was significantly increased in *P. deltoides* at NxD interactions. These significant improvements related to carbon allocation and physiological growth, with NxD interactions, can be attributed to improved acquisition of nutrients by these species in the drought stressed environments.

Chapter 1

INTRODUCTION

1.1 Climate Change

Climate change is the global apprehension and most important challenge in the recent era. Variation in climatic conditions not only causes disturbance in carbon cycle but also has key role in changing the favourable conditions for soil, water and agro forestry (Nyirambangutse et al., 2017). Carbon dioxide (CO_2) being the main GHG, causes noticeable rise in temperature that results in global warming (Field et al., 2014). CO_2 concentration in environment has reached up to 400 ppm (Oreskes, 2018) with consequent impacts such as sea-level rise, unpredictable weather patterns, temperature extremes, seasonal variations and damage to vegetation cover (Fischer & Knutti, 2015). Increase in CO_2 emissions are due to change in vegetation cover and anthropogenic activities like burning of fossil fuels, land use for agricultural aspects and emissions from livestock (Cavin et al., 2013). Intergovernmental Panel on Climate Change (IPCC) also specifies these human activities the prime cause of observed climate change (IPCC, 2014). These anthropogenic activities are major source of producing Greenhouse Gases (GHGs) in the atmosphere (Mackey et al., 2013) which increase the earth's surface temperature by 1.5^oC (Lindenmayer et al., 2012).

1.2 Carbon Sequestration

Carbon sequestration is a process in which CO_2 is removed from the atmosphere and stored in a reservoir (Wennersten et al., 2015). Tree capture CO_2 from atmosphere in a process called photosynthesis to make their own food. Biomass of a tree contains half the dry weight of carbon in it (Kirilenko & Sedjo, 2007). Trees need CO_2 for growth and stability, to prevent from harsh climatic conditions, by absorbing CO_2 during photosynthesis process and produce oxygen as by-product that ultimately results in storage of CO_2 in biomass (Spash, 2010). Carbon storage in trees may coup up with various kinds of stresses such as water and nitrogen stresses (Niinemets, 2010). Stress conditions lead to morphological, biochemical and physiological changes that may damage tree parts and disturbs production of biomass, leaf gas exchange and water use efficiency (Hernández & Bosch, 2004). Low water availability to tree species causes reduction in lateral branching, total dry matter and repressed rate of leaf, shoot and cell expansion (Tuomela et al., 2001).

1.3 Adaptation of Tress During Drought

When plants are subjected to water stress, stomatal response, metabolic changes, photosynthesis and Reactive Oxidative Species (ROS) scavenging mechanism affected (Fig. 1.1). As a result of this collective response, there is an adjustment in the plant growth rate which acts as an adaptation for survival (Osakabe et al., 2014). Ribulose-1, 5-bisphosphate carboxylase / oxygenase (RuBisCO) is available for carbon fixation in plants. During carbon fixation, RuBisCo catalyzes carboxylation reaction in which CO_2 is converted to energy rich molecules such as glucose (Xu et al., 2015). RuBisCo compete for CO_2 and O_2 i.e. for carboxylation and respiration (Long, 1991). Moreover, Nitrogen (N) is one of the major component of RuBisCo, a Nitrogen rich photosynthetic enzyme. It not only stores N but also keep it fixed in plants for large time-period (Leakey et al., 2009). Allocation of more carbon in biomass of the trees may improve by enhancing the efficiency of RuBisCo active site. N fertilizer enhances the efficiency of trees to work effectively and compensate under challenging circumstances. Tree response to limited water supply increases when fertilizer is applied (Ewers et al., 2000). In addition, it improves water use efficiency and growth patterns of plants (Laird et al., 2010). Some seedlings exhibit adaptation to the availability of higher amount of N while others showed more compassion to various forms of N (Maseda & Fernández, 2015). N supply enhances plant productivity under drought by improving water-use efficiency, assimilation rate and growth patterns while slight decrease in stomatal conductance (Granath et al., 2012).

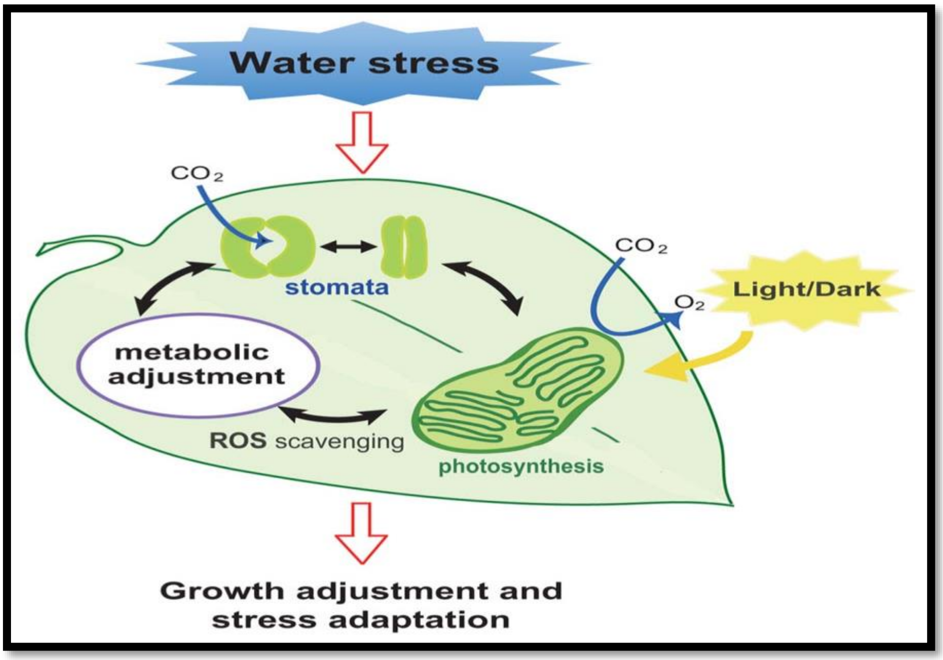


Figure 1.1: Schematic diagram of response and metabolic adjustment of plants during water stress conditions (Osakabe et al., 2014).

Tree species that tend to store carbon in their parts like leaves, branches, stem, bark and roots may tolerate water stress conditions (Villagra & Cavagnaro, 2006). Roots are fewer droughts sensitive as compared to leaves as they have increased access to water (Cheng & Zhong, 2012). Increasing amount of water stress to tree seedlings cause reduction in biomass and has effect on growth. On the other hand, with application of N, trees may survive during harsh climatic conditions and water stress would not retard their growth pattern (Li et al., 2015).

Hence, the objectives of the study were to assess how carbon storage and allocation pattern varies in growing seedlings of *E. camaldulensis* (evergreen) and *P. deltoides* (deciduous) and to examine growth parameters, primary production (biomass) in each tree seedling under water stress and N supply.

1.4 Significance of Study

Many studies have been conducted to evaluate impacts of drought and nitrogen availability on growth of plants but little information is available on comparison between carbon storing ability of *E. camaldulensis* and *P. deltoides* This study determined response of deciduous and evergreen species as well as carbon sequestration under different water and nitrogen regimes. In past, studies related to drought and nitrogen availability were investigated among different tree species at different time period but no study has investigated carbon content, primary production, growth parameters as interactive effects of drought and nitrogen supply.

1.5 Hypothesis

Based on literature review, it was hypothesized that carbon content of species may change with change with both Nitrogen and drought levels and Physiological characteristics may improve with Nitrogen supply to survive under water stress conditions.

1.6 Objectives of Study

Specific objectives of study were to:

a. Evaluate how carbon storage and allocation varies within each tree species (*Eucalyptus camaldulensis* and *Populus deltoides*).

b. Examine growth parameters and primary production (biomass) in each tree seedling under water stress condition and nutrient supplementation.

Chapter 2

LITERATURE REVIEW

2.1 Climate Change Affecting Forest Cover

Climate change and global warming are the two well-known issues; directly and indirectly related to anthropogenic activities (Obani, 2017). Intense temperatures, altered weather patterns are resulting in flash floods, frequent droughts, melting of glacier and many climate-related disasters (Altieri & Nicholls, 2017). Water is the major factor limiting plant productivity. It effects the plant growth more than any other environmental factor. Changes in average annual temperatures of earth are affecting the hydrological cycle. This ultimately results in water scarcity or drought when prolonged (Van Loon, 2015). Furthermore, tree growth is mostly restricted by availability of low nitrogen (N) (Jarvis and Linder 2000). Consequently, N fertilization normally increases tree growth by increasing foliar biomass, transpiration and water uptake (Saarsalmi and Mälkönen 2001). Schlyter et al. (2006) reported that the sensitivity of trees to drought stress is likely to increase due to N availability through deposition or fertilization.

Arid regions of the world are facing low primary productivity, scarce water conditions and less availability of nutrients which are needed for better survival of vegetation cover (Demuzere et al., 2014). To recover and restore the efficiency and productivity of vegetation cover is always an issue. Demuzere et al. (2014) suggested in their study to sequester more carbon in vegetation cover by changing nutrient availability regimes (Mandal & Van Laake, 2005) among different kind of tree species. In an another study reported by Jyske et al. (2010), an effect of water stress on height, wood density, tracheid and radial properties of *Picea abies* under N- fertilization experiments in Heinola and Sahalahti areas were observed. No interaction was found in N-fertilization and drought treatments in wood density at the start of the experiment. Height was significantly decreased in both unfertilized and fertilized species by 8–28% in Sahalahti and 24–42% in Heinola.

2.2 Carbon Allocation in Trees

Carbon allocation is removal of CO_2 from the environment and storing it in biosphere (Tubiello et al., 2015). Excess CO_2 may be removed by various means and it may be stored in oceans, geological establishment and terrestrial ecosystem like vegetative cover, sediments and in soil etc. (Poorter et al., 2012). Trees may store 50% of carbon in their woody parts e.g. stem, roots and branches (Poorter et al., 2012). Furthermore, trees are the most important source of capturing CO_2 as they not only store it but also use CO_2 for their growth, maintenance and adaptation until maturity (Ryan et al., 2010). Leaves capture the energy during photosynthesis and convert half of the CO_2 from the atmosphere into sugars to regulate metabolism and remaining CO_2 is used to build roots, wood and new leaves as trees grow. According to FAO (2013), mature forest contains about 60% of the carbon in dead and live trees and about 40% in forest and soil litter (Fig. 2.1). McKiernan et al. (2016) reported that trees are storing 4.1 billion hectares of the world major carbon stock and 60% of the world's CO_2 with soil carbon sequestration.

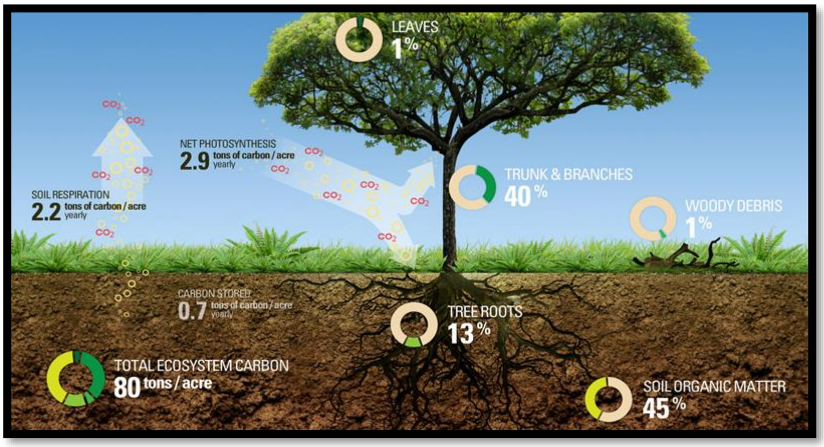


Figure 2.1: Carbon storage in an ecosystem (http://treespiritproject.com/carbon/)

Eucalyptus species are reported as sensitive to water stress by McKiernan et al. (2016) while it is the limiting factor in establishment of vegetative parts of trees but they may store carbon in a very large amount. Chen et al. (2011) worked on *Eucalyptus globulus, Eucalyptus saligna, and Eucalyptus urophylla* and found out that many Eucalyptus species are well known to tolerate water stress conditions, yet differs from species to species because of different osmotic potential.

Fang et al. (2007) worked on Poplar clones (I-69, NL-80351 and I-72) and found that carbon sequestration potential in *Populus deltoides* was 24.8% higher and stem wood carbon allocation was 45-46% increased. Fortier et al. (2010) reported that carbon storage and nutrient showed modifications in hybrid poplar buffers in four agricultural sites. Above ground carbon allocation was observed to be increased from 31 to 37%, 29 to 41% and 30 to 38% in different sites in Populus species. Fast growing poplar plantations (strong carbon sinks) are sensitive to the changes in environmental conditions. It was also reported that these species have much potential in sequestering carbon in comparison to unmanaged forestry adaptations.

Interactive effect of N and drought on ectomycorrhiza of Norway spruce trees were investigated with three levels of N e.g. 5, 27 and 82 kg $ha^{-1}yr^{-1}$ and water levels e.g. N0 (control), N5 (amount of water 5 times of N) and N15 (amount of water 15 times of N) for five years (Nilsen et al., 1997). According to Nilsen et al. (1997), drought and N interaction decreased mycorrhizal colonization significantly. N treatment alone did not show any significant effect either on mycorrhizal colonization or mycorrhizal types.

2.3 Trees Adaptation during Stress

Ripullone et al. (2004) worked on *Pseudotsuga menziesii* and *Populus euroamericana*. *P. menziesii* plants were treated with Low N (0.006 g), Medium N (0.02 g) and High N (0.04 g) whereas *P. euroamericana* plants were treated with Low N (0.285 g), Medium N (0.95 g) and High N (1.9 g) with water use efficiency (WUE) for short-time and long-time. In both species, biomass production was increased but there was there was no interactive effect of N supply and WUE of both species. Mechanisms related to the response of N supply and WUE showed a positive effect on photosynthetic rates, increased biomass production and suggested Poplar as an adaptive as well as stress tolerant species.

Another study related to the interaction within soil pH (5 and 7), N supply (ammonium sulphate and calcium nitrate) and water stress (85% and 40% field capacity) was done by DesRochers et al. (2007) to assess growth parameters and physiology of three Poplar clones (*Populus deltoides*, *Populus petrowskyana* and *Populus balsamifera*). The study suggested that decreased rate was observed in growth, photosynthesis and stomatal conductance with N fertilization at soil pH 7. Interaction between drought and N reduced growth of the trees (12%) but it increased carbon content by 0.55%. Moreover, Poplar species are better in adaptation to take up N rapidly for survival during drought periods.

2.4 Primary Production and Growth Characteristics

A study was done by Wu et al. (2008) in *Sophora dividii* seedlings in which two-month-old seedlings were subjected to three water stress levels (80, 40 and 20% FC) and three N supply regimes e.g. N0 (0 mg N kg^{-1} of soil), Nl (92 mg N kg^{-1} of soil) and Nh (184 mg N kg^{-1} soil). Wu et al. (2008) reported that seedlings height, diameter, leaf area and number, root height and primary production (biomass) decreased for N to drought interactions. Maximum Root to Shoot Ratio (R/S) was observed in below-ground biomass. Interaction between Nitrogen and drought stress significantly increased Water Use Efficiency (WUE) in Nl among all three water conditions whereas non-significant variations were observed in N0 and Nh. It was reported that tree seedlings revealed strong response to nitrogen supply also while there are inconsistent variations among responses to different nitrogen levels. Study showed that, low nitrogen supply (92 $mgNkg^{-1}$ of soil) increased diameter, height, leaf area, leaf area ratio, leaf number, ratio of ass (L/FR) and relative water content as well as biomass production even under severe water stress which was at 20% Field Capacity (FC). While, high nitrogen supply (184 $mgNkg^{-1}$ of soil) decreased biomass production as well as growth characteristics of trees. Furthermore, suitable or less availability of nitrogen suggested to stimulate growth, WUE and fading drought stress. Growth of seedlings under dry condition may be attained by avoiding high nitrogen level (Wu et al., 2008).

Another study was conducted by Wu et al. (2017) to understand the interaction between N and water on *Populus canadensis.* Effect of N was observed with 0, 1, 5, 10, and 15 mmol L^{-1} NH_4NO_3 inoculation at 75% FC to determine plant growth, nutrient uptake, photosynthesis, water status and leaf anatomical properties of *Populus canadensis* in natural soil. Plant height and stem diameter were significantly affected by N fertilization and water stress treatment. Significant increase in WUE (29.3%) and RWC (4.8%) was observed at various N levels (0, 1, 5, 10, and 15 mmol L^{-1} NH_4NO_3, respectively) which suggested that RWC and WUE significantly affected by N fertilization under 75% FC.

Several physiological characteristics were observed in *Eucalyptus camaldulensis* by Siddique et al. (2008) with N (0.92g per pot), Phosphorus (5.75g per pot) and water levels e.g. -0.8 MPa (well-watered) and -0.3 MPa (water stress). Nitrogen and water stress did not significantly increase biomass of the leaves. Root mass per plant mass ratio was not obvious by water stress in control, whereas it was higher in both fertilization treatments (31%) in stressed plants in comparison to well-watered plants.

2.5 Related Research Work at IESE, SCEE, NUST

No work had been done previously at IESE-SCEE-NUST to investigate the interactive effect of Drought and Nitrogen supply. Present study not only worked on tree species but also focused on growth variability under increase drought and nutrient supply.

Chapter 3

MATERIALS AND METHODS

3.1 Experimental Setup

This section outlines all the procedures that were used to identify carbon content and biomass production and impact of nitrogen and water treatment on the selected tree seedlings. All these methods were carried out at Environmental Biotechnology Lab of Institute of Environmental Sciences and Engineering, School of Civil and Environmental Engineering, National University of Sciences and Technology, Islamabad Pakistan. Two year grown seedlings of same size were placed in glasshouse (10x12 feet) for 4 weeks with 9 treatments (Fig. 3.1). Experimental design was two factor factorials with five replicates for each treatment. Three Nitrogen supply regimes N0 (0 gNkg^{-1}) N1 (0.5 gNkg^{-1}) and N2 (1 gNkg^{-1}) with three water stress levels D0 (1000 mL), D1 (500 mL) and D2 (250 mL) were maintained for each treatment.

Figure 3.1: Experimental Setup of *Eucalyptus camaldulensis* and *Populus deltoides*

3.2 Soil Analysis

Glass electrode method was used to determine pH and Electrical Conductivity (EC) of soil sample (Page, 1982). Soil samples were air dried and used to determine water-holding capacity of soil by using method described by Israelsen & West, (1922). For calculation of Total Organic Carbon (TOC) in soil, Ferrous Ammonium Sulfate (FAS) titration method was used (Bremner & Mulvaney, 1982). Total Nitrogen (N) involved Kjeldahl apparatus as well as digestion and distillation methods (Bremner & Mulvaney, 1982). Nitrate Nitrogen by Salicylic acid method and Ammonium Nitrogen (NH_4-N) by using method described by Keeney & Nelson, (1982). Total P determination was done by using Perchloric acid digestion method. Reagents and standard stock solutions were prepared. Readings were measured on spectrophotometer at 410 nm wavelength (Olsen et al., 1982).

3.3. Plant Analysis

3.3.1 Carbon Content

Information in literature suggested that carbon content constitutes between 45-50% of all dry matter of tree species (Selva et al., 2007). In the present study, carbon content was calculated by taking the percentage of biomass (B) and multiplying it with 0.475 factor (Magnussen & Reed, 2004) where C is the carbon content and B is oven-dried biomass.

$$C = 0.475 \times B$$

3.3.2 Total Plant Biomass Determination

At start of the experiment, five equally size seedlings for both species were harvested for initial biomass measurements (Fig. 3.2). Harvest Method for biomass determination was done by taking sum of root, shoot and leaf biomass. Readings of each part of species were calculated such as Leaf Area Ratio (LAR), Root to Shoot Ratio (R/S) and Specific Leaf Area (SLA). Root and shoot length was measured manually by using measuring tape (Flombaum & Sala, 2007).

Figure 3.2: Harvesting and biomass measurements of *Populus deltoides* and *Eucalyptus camaldulensis*

3.3.3 Leaf Area

Leaf area was calculated by using HP jet Scanner 200 and ImageJ software (Varma & Osuri 2013). Fully expanded leaves were placed in scanner to obtain correct area of image. Scanned image of leaves was attached in ImageJ software and hence leaf area was determined (Wu *et al.* 2008).

3.3.4 Water Use Efficiency (WUE)

WUE was determined as shown in Figure 3.3 and 3.4 by using the following formula described by Wu et al. (2008);

$$\text{WUE (gL}^{-1}\text{)} = \text{Total plant biomass / Water used}$$

3.3.5 Relative Water Content

Relative water content (RWC) of leaf calculated by using saturated weighing process described by Ehleringer et al. (1986). For fresh weight calculation, fresh green leaves were selected and placed in water for 4 hours to become fully turgid. When the leaves were fully turgid with water, leaves surfaces were dried with filter paper softly. Leaves were placed in refrigerator for 24 hours and then weighed for turgid weight and in oven for 48 hours for dry weight measurement. Following formula was used for further calculation;

$$\text{RWC (\%)} = [(\text{LFW} - \text{LDW}) / (\text{LTW} - \text{LDW})] * 100$$

LFW = Leaf Fresh Weight

LDW = Leaf Dry Weight

LTW = Leaf Turgid Weight

3.4 Statistical Analysis

Differences between the values of control and treatment data sets were analyzed by using R software. Multivariate ANOVA (Analysis of Variance) test was done to identify statistically significant variations between treatments values and it was based on probabilities of $P<0.05$. Statistical analyses were done using R-programming.

Figure 3.3: Plants were treated with Nitrogen Fertilizer and Water.

Figure 3.4: Water Use Efficiency was measured by taking Biomass of the plant increased at the end of the experiment divided by water used by the plant throughout treatment. Pots were covered loosely with polythene bags to prevent water loss from the soil.

Chapter 4

RESULTS AND DISCUSSION

4.1 Temperature Variation and Characteristics of Soil

There was observable difference between indoor and outdoor temperatures of glasshouse. Highest observed indoor temperature was up to 34°C whereas outdoor temperature was 27°C while lowest indoor temperature was observed at 12th day due to cloudy weather as shown in Figure 4.1. Soil analysis values including pH, EC, water-holding capacity, TOC and total N, P, K and NO_3-N are given (Table 1).

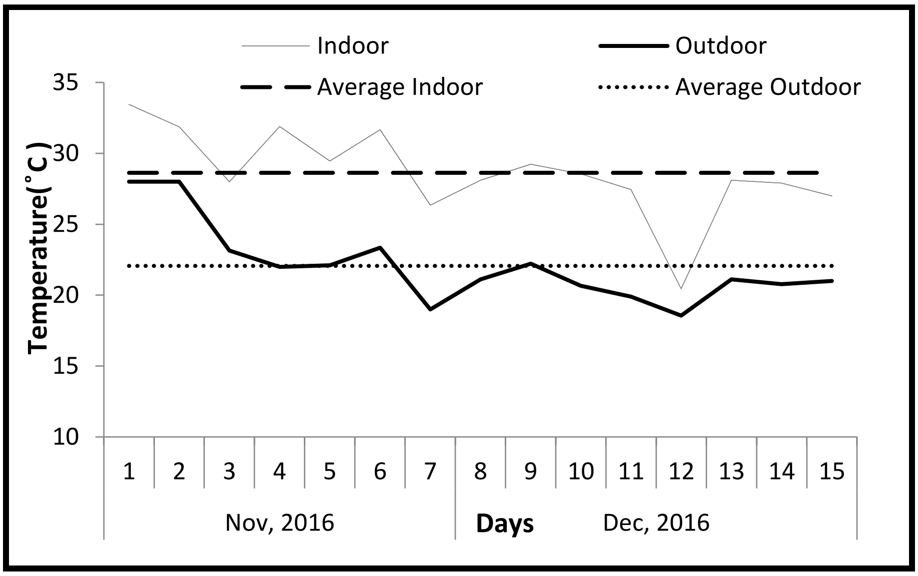


Figure 4.1: Readings of indoor and outdoor temperature recorded for 15 days (8hours each day) randomly.

Table 1: **Physical and Chemical Soil Characteristics**

Parameters	Average ± SD
pH	8.03 ± 0.2
Water holding capacity (%)	52.9 ± 3.3
Moisture content (%)	16 ± 2.3
Total organic carbon (%)	0.08 ± 0.1
NO_3-N (mg kg^{-1})	188.29 ± 123.2
Total P (mg kg^{-1})	42.23 ± 13.5
Total K (mg kg^{-1})	92.1 ± 0.8

4.2 Carbon Content

4.2.1 Shoot and root carbon content

Carbon content in shoot, root and leaf varied at different water stress and N levels. Results showed that there were non-significant variations between NxD interactions ($p>0.05$) of shoot carbon content in both species. More shoot carbon content was observed in N2D1 (4.07g) of *E. camaldulensis* and N1D1 (43.5g) of *P. deltoides* (Fig. 4.2). N2 allocated more carbon in their shoot with D1 and D2 water stress levels. In severe drought conditions, seedlings of *E. camaldulensis* showed more carbon storage in N2 while in *P. deltoides* more carbon was allocated in N1. Relatively, root carbon content in *E. camaldulensis* showed signification NxD interactions ($p<0.05$) while highest observed values were in N0D2 (1.33g) of *E. camaldulensis* and N1D0 (15.18g) in *P. deltoides* (Fig. 4.3). Seedlings of *E. camaldulensis* stored carbon in N1 even during minimum water stress conditions. Availability of N enhanced carbon allocation in roots of N1 in contrast to N2.

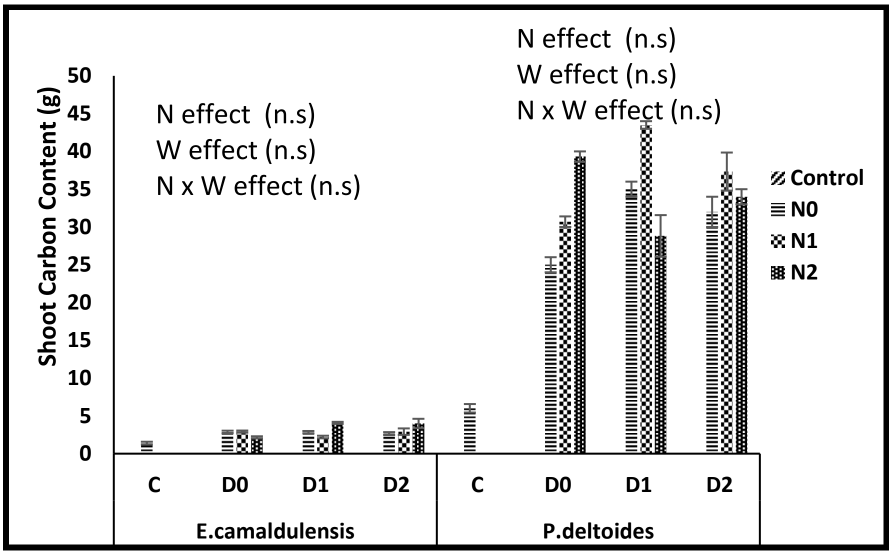


Figure 4.2: Shoot carbon content measurements in *E. camaldulensis* and *P. deltoides* after one-month fertilizer application. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three waters levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing P>0.05, *P<0.05 and **P<0.01 (Bars indicate SD).

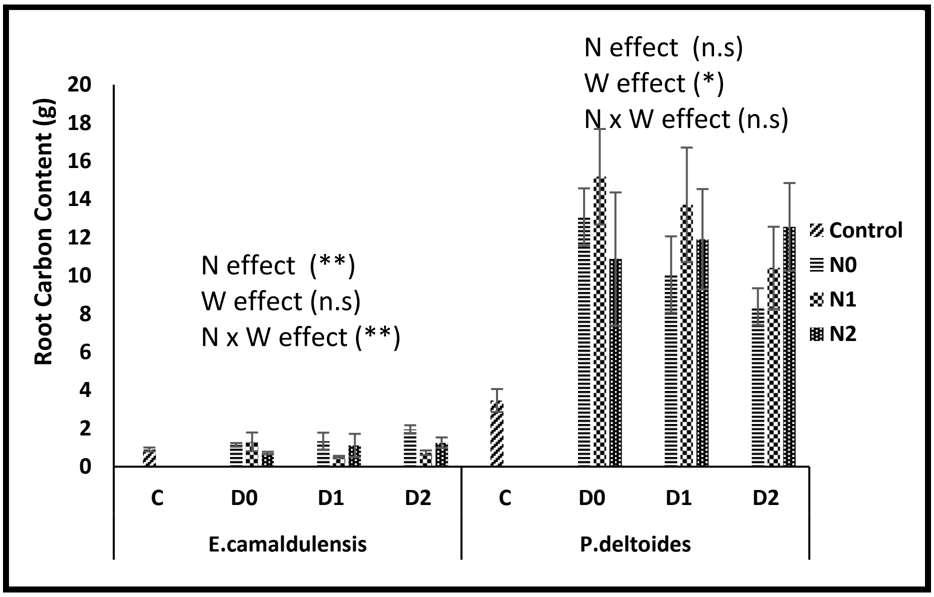


Figure 4.3: Root carbon content measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing P>0.05, *P<0.05 and **P<0.01 (Bars indicate SD).

4.2.2 Leaf carbon content

Shoot and root carbon content in *E. camaldulensis* were less observable as compared to *P. deltoides* but leaf carbon content was more in *E. camaldulensis* (Fig. 4.4). Leaf carbon content showed non-significant NxD interactions ($p<0.05$). Seedlings of *P. deltoides* suppress their leaf growth more in D0 even in the availability of N. In contrast, seedlings of *E. camaldulensis* allocated more carbon in their leaves in D0 water stress level. Moreover, increase in carbon content of leaves in stressed seedlings showed that seedlings response was positive as N played a key role in compensating the stress conditions. Maximum leaf carbon content during limited N supply in *E. camaldulensis* (2.55g) *P. deltoides* (2.24g) species showed that with the slight increase in N level, these species tend to store more carbon in their leaf biomass as compared to N0 and N2. Results in both species showed insignificant effect among treatments.

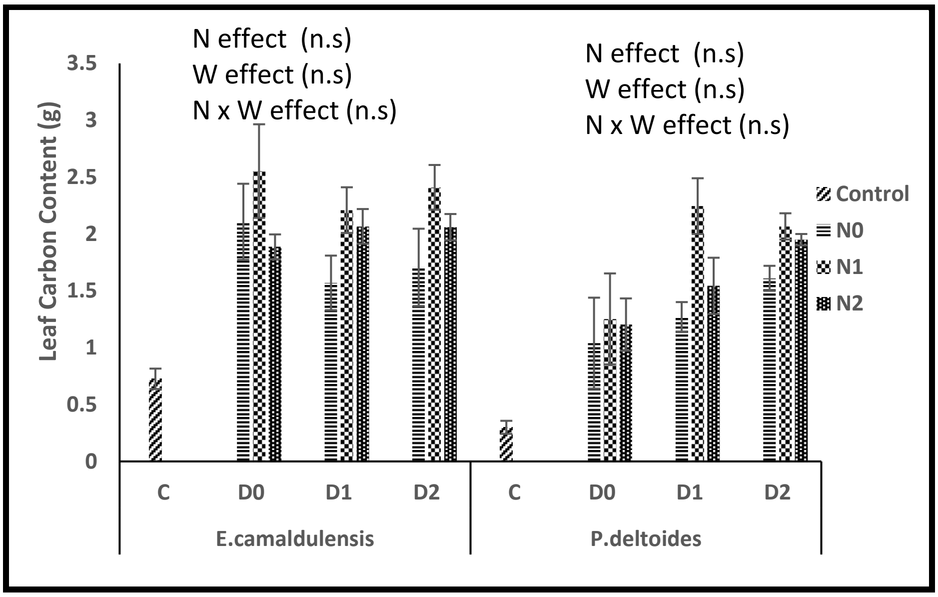
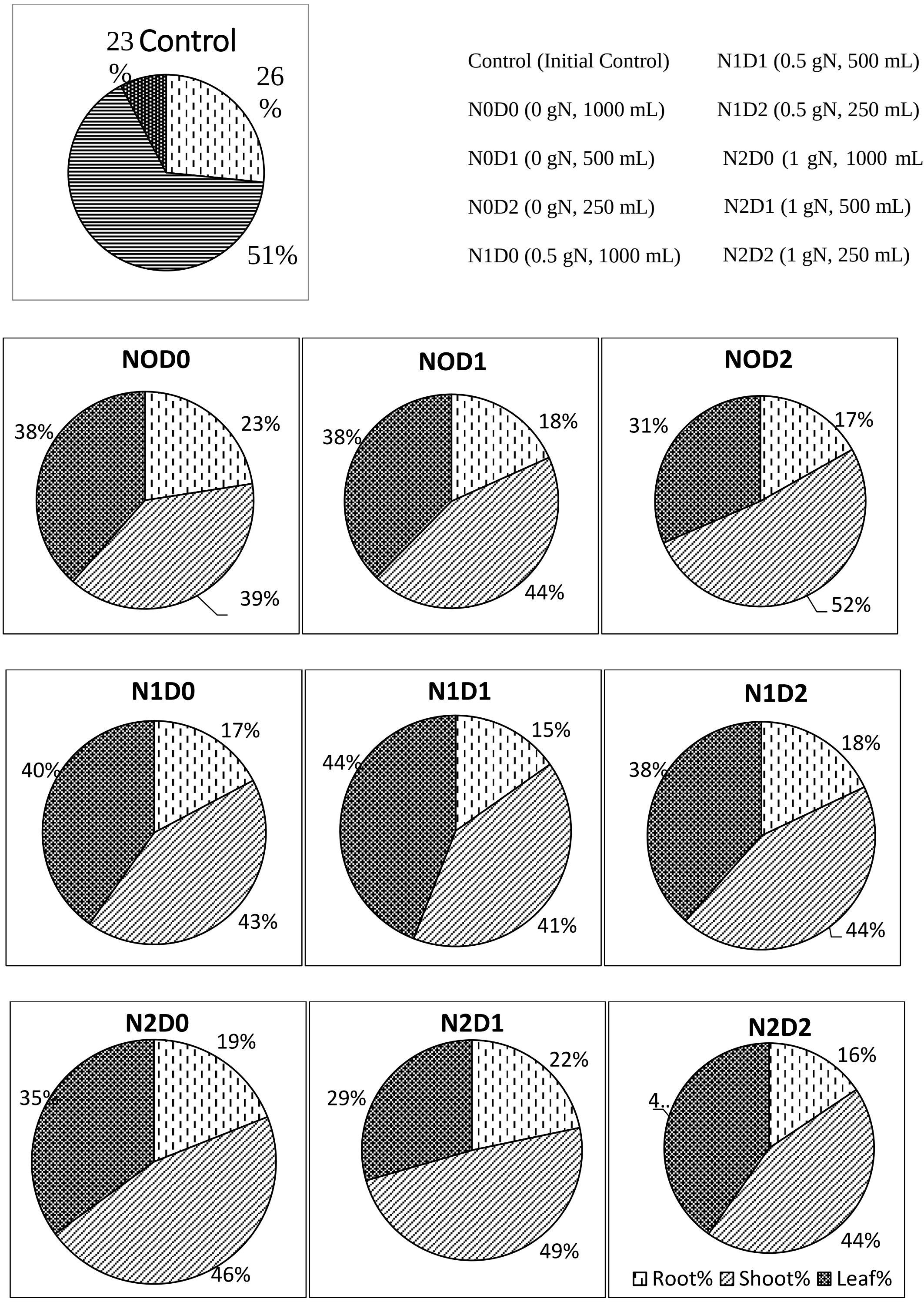


Figure 4.4: Leaf carbon content measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing P>0.05, *P<0.05 and **P<0.01 (Bars indicate SD).

4.2.3 Carbon content percentages in *E. camaldulensis* and *P. deltoides*

Carbon content in *E. camaldulensis* (Fig. 4.5) and in *P. deltoides* (Fig. 4.6), of all treatments, are separately shown to give an overview about percentage carbon content in shoot, root and leaves of each seedling. *E. camaldulensis* showed 51% shoot carbon content in control, 52% increase in N0D2 and 44% in leaf carbon content at D2 water stress level. In contrast, *P. deltoides* percentage shoot carbon content increased 75% in N2D2 supply regime while root carbon content was more in N0 and N1 supply regime and leaf carbon content in N1.



Control (Initial Control)

N0D0 (0 gN, 1000 mL)

N0D1 (0 gN, 500 mL)

N0D2 (0 gN, 250 mL)

N1D0 (0.5 gN, 1000 mL)

N1D1 (0.5 gN, 500 mL)

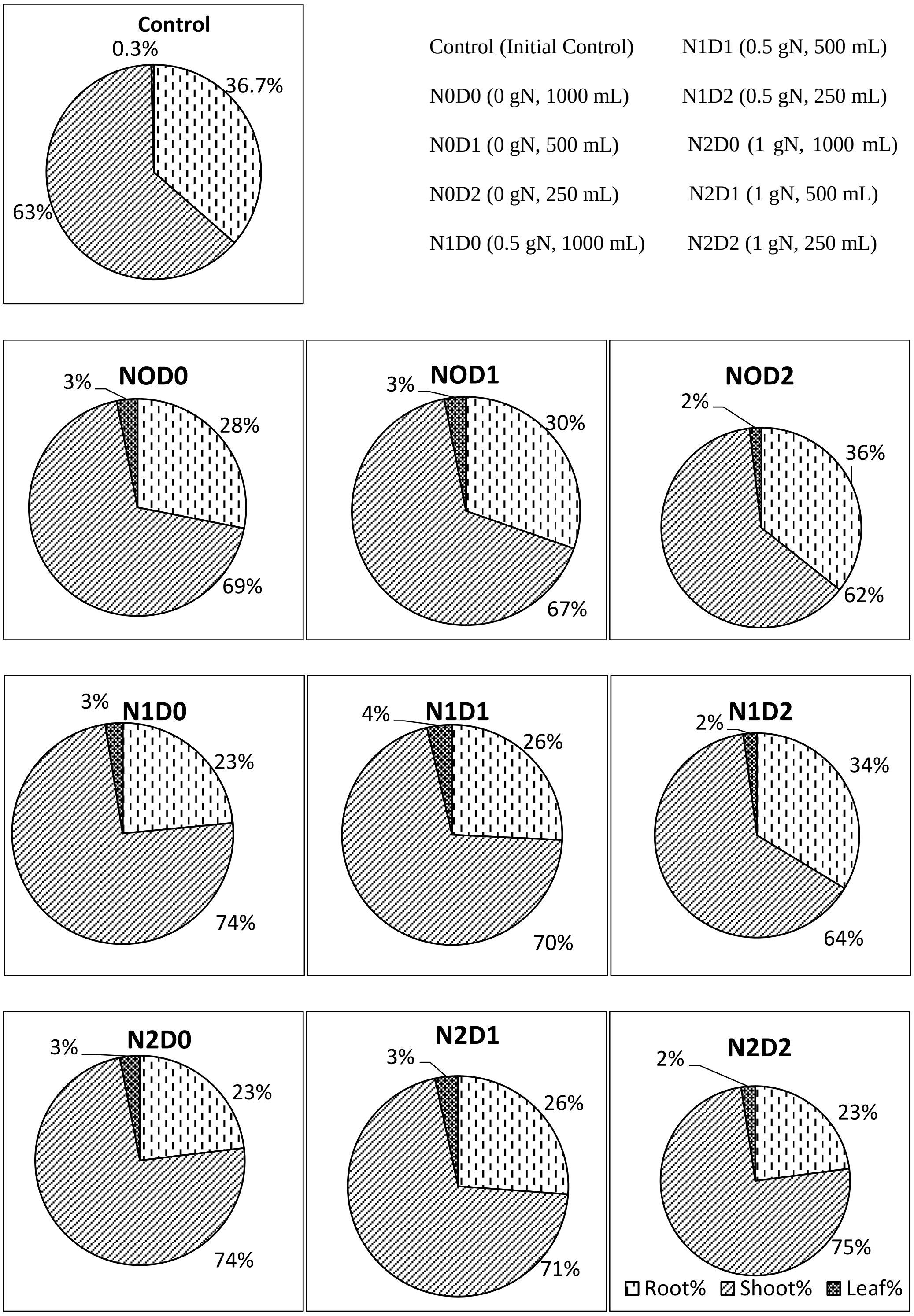
N1D2 (0.5 gN, 250 mL)

N2D0 (1 gN, 1000 mL)

N2D1 (1 gN, 500 mL)

N2D2 (1 gN, 250 mL)

Figure 4.5: Carbon content percentages of *E. camaldulensis* in all 9 treatments.



Control (Initial Control)
N0D0 (0 gN, 1000 mL)
N0D1 (0 gN, 500 mL)
N0D2 (0 gN, 250 mL)
N1D0 (0.5 gN, 1000 mL)
N1D1 (0.5 gN, 500 mL)
N1D2 (0.5 gN, 250 mL)
N2D0 (1 gN, 1000 mL)
N2D1 (1 gN, 500 mL)
N2D2 (1 gN, 250 mL)

Figure 4.6: Carbon content percentages of *P. deltoides* in all 9 treatments.

4.3 Physical Characteristics

4.3.1 Shoot length

Shoot length of both seedlings showed slight differences under drought stress as compared to control at different water levels as shown in Fig. 4.7. Significant differences were observed in shoot length (p <0.05) within the interaction of NxW at different water stress levels in *E. camaldulensis* and due to N effect in *P. deltoides*. Increased height was observed in shoot of *E. camaldulensis* (in N0D0 40.33cm) and *P. deltoides* (in N2D2 57.66cm). At D1 and D2 water stress level, they slow down their response rate but N played a vital role in stability of the growth parameters of these seedlings. As N2 was applied, there was swift response in *E. camaldulensis* seedlings while *P. deltoides* seedlings showed variation at N1 and N2 application.

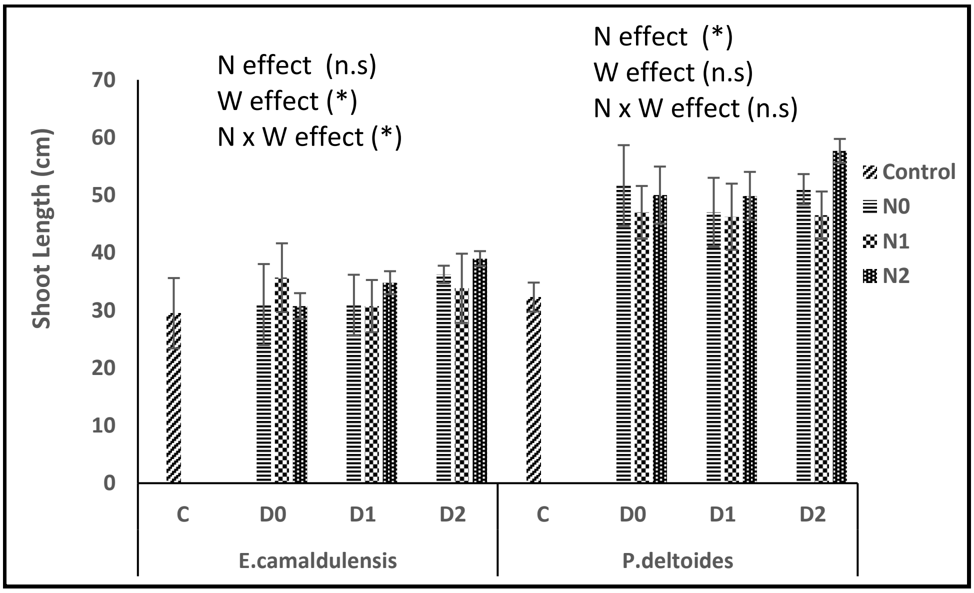


Figure 4.7: Shoot length measurements in *E. camaldulensis* and *P. deltoides* after one-month fertilizer application. Control (C) placed along with treatments. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing P>0.05, *P<0.05 and **P<0.01 (Bars indicate SD).

4.3.2 Root length

Results showed significant variations between treatments in combine effect of NxW ($p<0.05$) in root length of both seedlings but there were significant observations for W effect in *P. deltoides* also (Fig. 4.8). Root length of *E. camaldulensis* showed increasing response to N1D2 (8.92cm). In contrast, maximum root length in *P. deltoides* was observed in N0D2 (14.6cm). With an increase in N application, there was slight decrease in root length at D1 and D2 water stress level in *P. deltoides.*

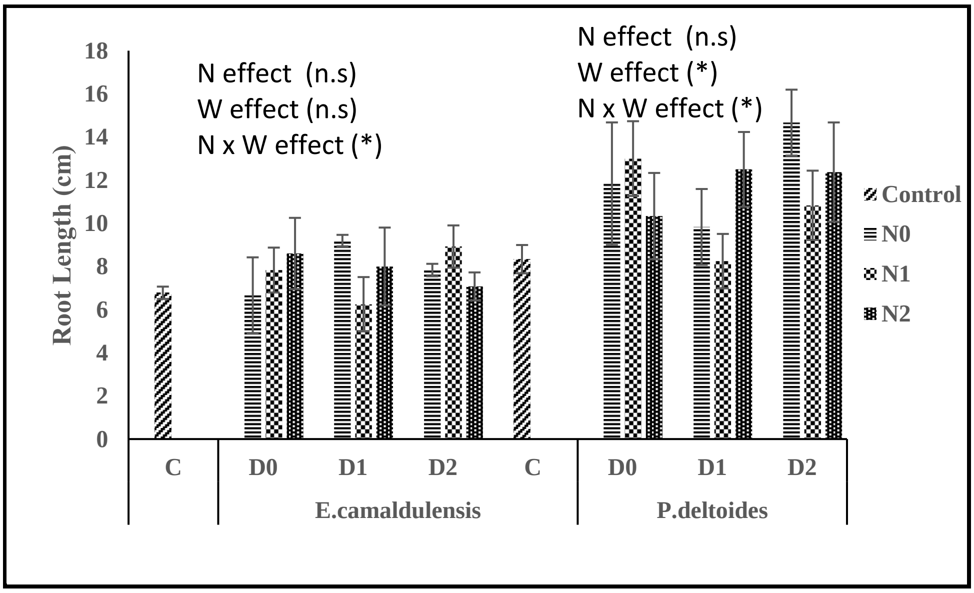


Figure 4.8: Root length measurements in *E. camaldulensis* and *P. deltoides* after one-month fertilizer application. Control (C) placed along with treatments. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing P>0.05, *P<0.05 and **P<0.01 (Bars indicate SD).

4.4. Biomass Production

4.4.1 Shoot biomass

Total biomass production influences carbon storage in tree parts within the availability of N. Biomass of three parts of each seedling i.e. shoot, root and leaf was observed which showed variation in readings (Fig. 4.9). Significant effect was observed in *E. camaldulensis* (W effect) and *P. deltoides* (NxW effect). Shoot biomass showed positive results during stress making our hypothesis strong that *P. deltoides* seedlings may work better during N1 and D2 level. It was highest in N1D2 (81.24g) of *P. deltoides* and N2D2 (7.07g) of *E. camaldulensis* with no obvious response in shoot growth. Moreover, observations showed slight decrease with an increase in N regime at D1 water stress level but growth sustained in N1 and N2 in *E. camaldulensis* seedlings even under D2 water stress level.

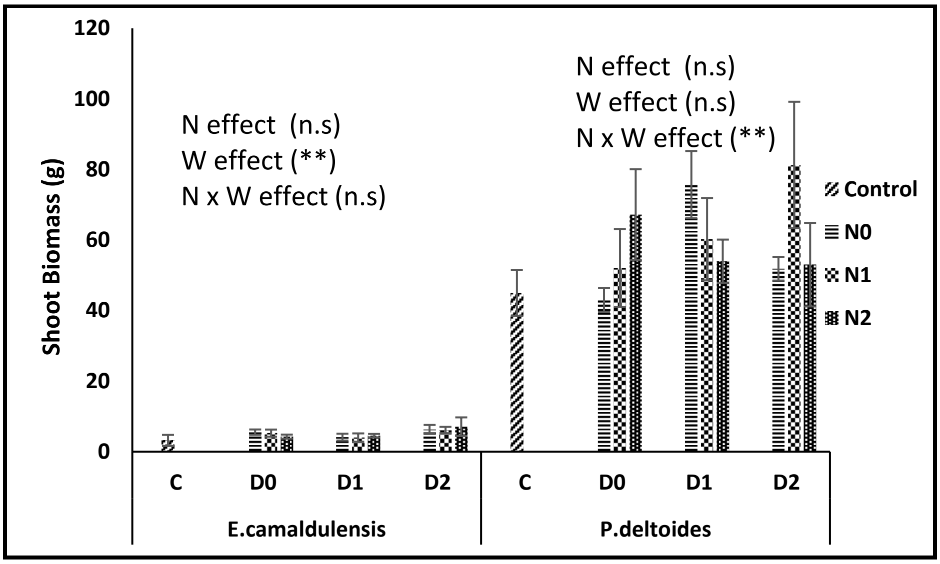


Figure 4.9: Shoot biomass measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Control (C) was also placed along with treatments. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing P>0.05, *P<0.05 and **P<0.01 (Bars indicate SD).

4.4.2 Root biomass

N1 maintained root biomass in *P. deltoides* but was less observed in *E. camaldulensis* (Fig. 4.10). Effect of N, W and NxW was significantly different in *E. camaldulensis* and W and NxW effect in *P. deltoides.* Moreover, N2 of *E. camaldulensis* restricted their root growth with an increase in water stress level. Increased root biomass production was observed in N1D0 of *P. deltoides* (31.96g) and *E. camaldulensis* (2.73g).

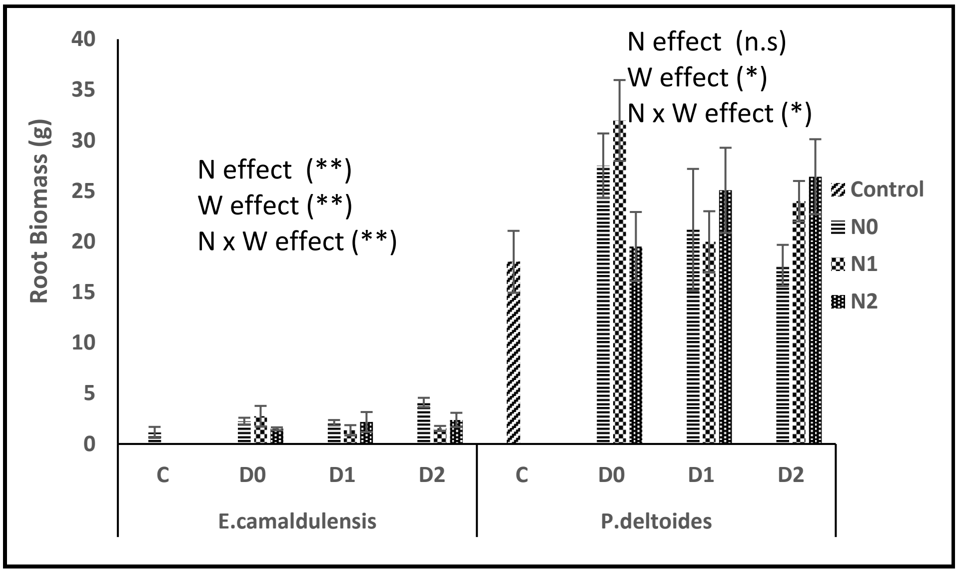


Figure 4.10: Root biomass measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Control (C) was also placed along with treatments. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing P>0.05, *P<0.05 and **P<0.01 (Bars indicate SD).

4.4.3 Leaf biomass

E. camaldulensis seedlings showed much better response in leaf biomass measurement in N1D2 (4.72g) as stress increased (Fig. 4.11). Non-significant results were observed in NxD interactions (p>0.05). Highest values were observed in N2D1 (3.24g) of *P. deltoides*. Results showed that N1 level incorporate in leaves to grow in severe drought in comparison to N0.

Leaves of *P. deltoides* were less in biomass, as they restrict their growth during water stress conditions.

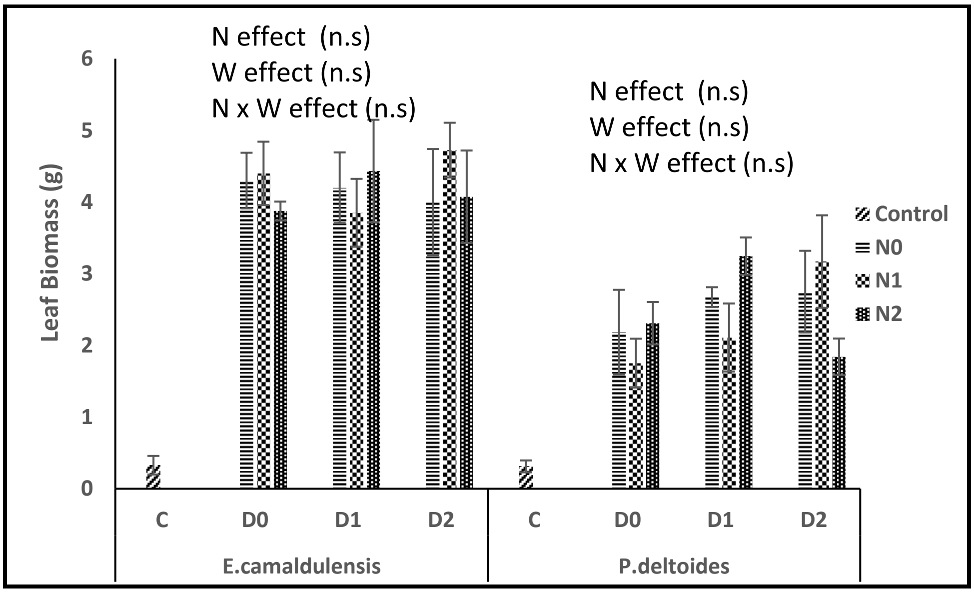


Figure 4.11: Leaf biomass measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Control (C) was also placed along with treatments. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing P>0.05, *P<0.05 and **P<0.01 (Bars indicate SD).

In comparison to different water stress and N levels, Root to Shoot Ratio (Root/ Shoot) was more in N0D0 in *E. camaldulensis* (0.37) and *P. deltoides* (0.71) as described in Table 2 and 3. After N application, Root/ Shoot was maximum as in N1D0 of *E. camaldulensis* (0.51) and N2D1 of *P. deltoides* (0.45) in comparison to LA, LAR and SLA in N1 and N2. Highest values of *P. deltoides* were also observed in N0 also.

Table 2: Measurements of Root to Shoot Ratio (Root/Shoot), Leaf Area (LA), Specific Leaf Area (SLA) and Leaf Area Ratio (LAR) of *P. deltoides.*

Treatments	R/S	LA (cm^2)	SLA (cm^2g^{-1})	LAR (cm^2g^{-1})
Control	0.71± 0.09	13.3± 0.92	140.33± 8.92	0.15± 0.03
N0D0	0.58± 0.03	12.29± 0.67	343.1± 37.17	0.14± 0.02
N0D1	0.47± 0.06	16.52± 0.66	376.98± 7.41	0.19± 0.04
N0D2	0.42± 0.09	17.82± 0.95	248.27± 25.82	0.21± 0.07
N1D0	0.42± 0.01	14.75± 0.63	296.53± 35.59	0.18± 0.03
N1D1	0.26± 0.03	19.49± 0.69	307.79± 45.63	0.18± 0.06
NID2	0.33± 0.07	15.83± 0.53	185.21± 15.23	0.17± 0.02
N2D0	0.36± 0.02	21.36± 1.21	299.95± 54.21	0.19± 0.02
N2D1	0.45± 0.01	18.6± 0.88	217.34± 24.77	0.15± 0.04
N2D2	0.25± 0.05	26.06± 1.42	379.67± 28.93	0.31± 0.01

Table 3: Measurements of Root to Shoot Ratio (Root/Shoot), Leaf Area (LA), Specific Leaf Area (SLA) and Leaf Area Ratio (LAR) of *E. camaldulensis.*

Treatments	R/S	Leaf Area (cm^2)	SLA (cm^2g^{-1})	LAR (cm^2g^{-1})
Control	0.37± 0.01	37.4± 0.88	214.12± 24.32	5.21± 1.20
N0D0	0.42± 0.03	31.72± 1.09	257.43± 33.83	2.42± 0.24
N0D1	0.52± 0.01	40.78± 1.23	170.38± 12.41	2.65± 0.16
N0D2	0.66± 0.04	28.5± 0.78	234.85± 39.20	1.74± 0.11
N1D0	0.51± 0.02	26.7± 1.89	168.22± 22.12	2.83± 0.32
N1D1	0.38± 0.09	36.47± 0.73	189.5± 11.52	3.43± 0.29
NID2	0.36± 0.12	44.51± 0.17	372.97± 35.42	4.12± 0.33
N2D0	0.36± 0.09	21.76± 1.26	126.07± 29.45	3.21± 0.29
N2D1	0.37± 0.04	28.75± 1.08	169.4± 35.54	1.42± 0.09
N2D2	0.35± 0.01	24.31± 0.77	144.45± 32.01	2.03± 0.26

4.5. Water Use Efficiency and Relative Water Content

Water use efficiency (WUE) was highest among N1D0 ($2.6gL^{-1}$) of *E. camaldulensis* and N1D2 of P. deltoides ($18.8gL^{-1}$) as shown in Figure 4.12. N effect and NxW effect had significant results in *P. deltoides* while for *E. camaldulensis* results were significantly different in effect of W. In *E. camaldulensis*, Relative Water Content (RWC) of leaf was highest among seedlings where there was no N application (in N0D2 48%) (Fig. 4.13). RWC increased in N1 in comparison to N2 in both seedlings. Values of seedlings were significantly different from each other in *E. camaldulensis* N effect and NxW effect. Likewise, *P. deltoides* showed maximum values in N1D2 (27%). Significant observations were noticed in N effect, W effect and NxW effect.

Correlation values of *E. camaldulensis* were assessed as shown in Table 4. SLA was positively correlated with LA while WUE showed negative correlation values for root carbon content and significant correlation values for Leaf Carbon Content (LCC). In contrast, Table 5 showing correlation values for *P. deltoides* in which RWC and RCC showed positive correlation values for WUE, SLA, SCC and LCC. WUE significantly increased with an increase in SCC and RCC respectively.

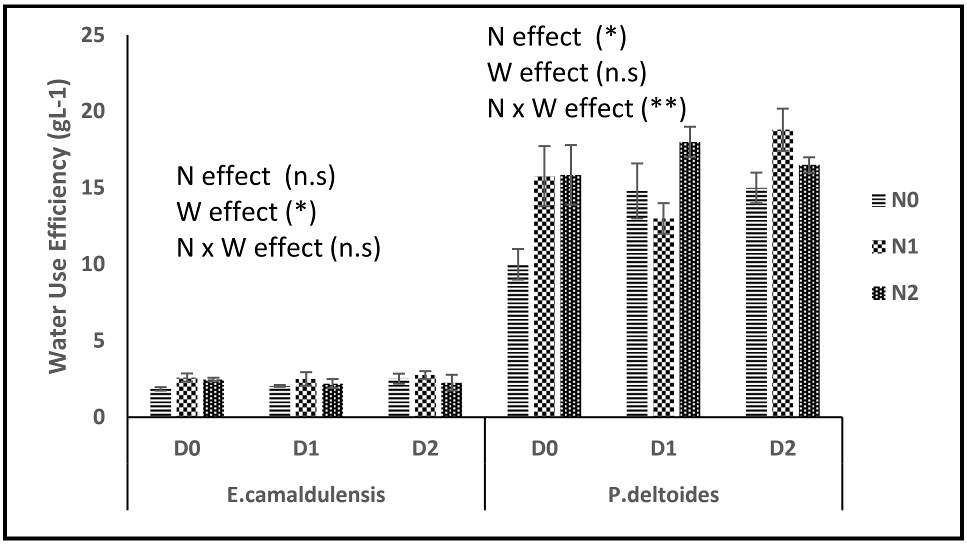


Figure 4.12: Water-use efficiency (WUE) measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing P>0.05, *P<0.05 and **P<0.01 (Bars indicate SD).

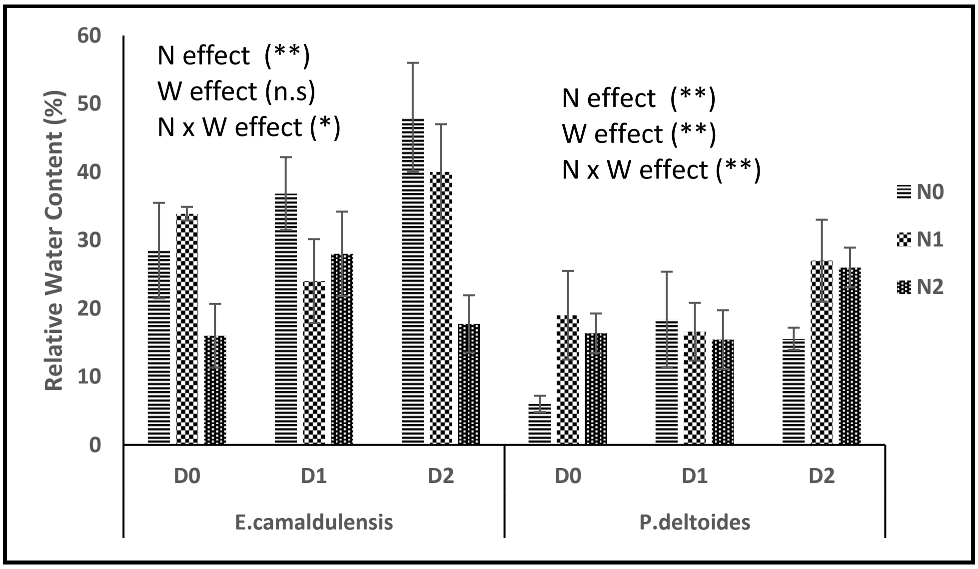


Figure 4.13: Relative Water Content (RWC) of leaf measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing P>0.05, *P<0.05 and **P<0.01 (Bars indicate SD).

Table 4: Correlation values for Relative water content (RWC), Leaf Area (LA), Specific Leaf Area (SLA), Leaf Area Ratio (LAR), Water Use Efficiency (WUE), Root Carbon Content RCC), Shoot Carbon Content (SCC) and Leaf Carbon Content (LCC) of *E. camaldulensis.*

	RWC	**LA**	**SLA**	**LAR**	**WUE**	**RCC**	**SCC**	**LCC**
RWC								
LA	0.21							
SLA	-0.13	0.39*						
LAR	0.27	0.41	0.24					
WUE	0.15	-0.04	0.06	-0.04				
RCC	0.11	0.25	0.07	-0.09	-0.36*			
SCC	0.15	0.1	-0.08	-0.18	-0.13	0.25		
LCC	0.05	0.27	0.02	0.08	-0.32	0.33*	0.05	

Table 5: Correlation values for Relative water content (RWC), Leaf Area (LA), Specific Leaf Area (SLA), Leaf Area Ratio (LAR), Water Use Efficiency (WUE), Root Carbon Content RCC), Shoot Carbon Content (SCC) and Leaf Carbon Content (LCC) of *P. deltoides.*

	RWC	**LA**	**SLA**	**LAR**	**WUE**	**RCC**	**SCC**	**LCC**
RWC								
LA	0.21							
SLA	0.41*	0.41*						
LAR	0.02	0.44**	0.32					
WUE	0.47**	-0.05	0.01	-0.49**				
RCC	0.1	-0.16	-0.03	-0.41*	0.47**			
SCC	0.42*	0.2	0.19	-0.19	0.42*	0.09		
LCC	0.33*	0.32	0.48	0.13	0.25	0.22	0.18	

DISCUSSION

In the present study, the interactive effect of Nitrogen and drought had more effect on root carbon allocation in Eucalyptus camaldulensis. Same results were observed by (Peng, 2009) where *E. camaldulensis* seedlings respond 56% increase in belowground biomass at different N levels. It depends on species physiological characteristics like photosynthetic activity, water-use efficiency, light-use efficiency and nutrient uptake with an increase in tree age (Peng, 2009). Results of the present study showed that shoot carbon allocation in seedlings of *P. deltoides* showed significant response in water stress level and N interaction. These results are in accordance to the results reported by Kaul et al. (2010) in which carbon allocation in shoot of *Populus deltoides, Eucalyptus tereticornis* and *Tectona grandis* ranges from 62 to 75%.

Adaptation in species morphological characteristics may coup up with environmental stresses mainly drought. During dry conditions, trees mainly restrict their growth pattern and biomass production rate (Hunter, 2001). Water stress and N both limited the root length of *E. camaldulensis* (Susiluoto & Berninger, 2007) while increase shoot length of *P. deltoides* (DesRochers et al., 2007). In the present study, *E. camaldulensis* showed positive increase in biomass results in N0 without any nitrogen treatment (Hunter, 2001) while in *P. deltoides* shoot biomass responded well, at N1D2, in comparison to control. Moreover, when N applied to *E. camaldulensis*, the root and shoot restricted their growth and more shoot biomass was observed in *P. deltoides* seedlings. Results are in relation to the study done by Fortunel et al. (2009) that Poplar seedlings shift more biomass to shoots as compared to root and leaf biomass when both N supply and water stress treatments were applied (Fortunel et al., 2009). A study by Wu et al. (2008) Concerning with leaf biomass, *E. camaldulensis* and *P. deltoides* both showed a much more increase in leaf biomass at N x D interactions. Leaves of *E. camaldulensis* were large as compared to *P. deltoides* and hence resulted in more leaf biomass even under water stress conditions.

Generally, deciduous tree species that accumulate major portion of their biomass in roots may cause increase in Root/ Shoot and hence considered as adaptive species to tolerate drought stress (Villagra & Cavagnaro, 2006). Our results indicated same response with more Root/Shoot in drought stressed seedlings. N1 showed increasing Root/Shoot in drought stressed seedlings and same results were observed by Ripullone et al. (2004) where more Root/ Shoot was found in *Pseudotsuga menziesii* and *Populus euroamericana* with low N fertilization. Many studies have shown that roots absorb more water during drought and hence WUE of the trees increase (Wikberg & Orgen, 2007). WUE is the vital characteristic to analyze during water stress conditions as it indicates water used by the tree and its whole biomass (Yin et al., 2005; Monclus et al., 2009). Previous studies demonstrated that WUE may improve in limited water supply (Liu et al., 2005) but some others have found that every species have different water- use efficiency depending on their strategy to water use (Clavel et al., 2005).

Researchers also reported that due to changes in tree morphology, the stressed seedlings would reduce leaf area, LAR and SLA as present study results showed while a slight increase in N may cause change in leaf morphology and high N may restrict leaf growth (Erice et al., 2010). Overall, more carbon allocation was observed in shoot of *P. deltoides* as compared to *E. camaldulensis.* Same results were shown by Saraswathi & Ezhilarasi (2012) in which highest amount of carbon content was observed in *Pongamia pinnata* under water stress and urea supplementation.

CONCLUSIONS AND RECOMMENDATIONS

Carbon storage in shoot of *P. deltoides* increased 75% (N2D2) and in *E. camaldulensis* up to 52% (N0D2) where there is N x D interactions. Carbon content was shifted more towards leaf in *E. camaldulensis* (23 to 44%) in N1D1 in comparison to *P. deltoides* in N1D1 (0.3 to 4%). Biomass production was more in shoot in comparison to root and control. Shoot biomass in *P. deltoides* was increased (45 to 81g) in N1D2 as compared to *E. camaldulensis* in N2D2 (3.8 to 7g). Shoot length was increased in *E. camaldulensis* in N2D2 (29.5 to 38.9cm) while in *P. deltoides* it was highest in N2D2 (32.3 to 57.6cm). Root length was highest in N1D2 (6.8 to 8.9cm) in *E. camaldulensis* and *P. deltoides* in N0D2 (8.3 to 12.3cm). Water use efficiency was highest in N1D2 of *E. camaldulensis* (2.75gL^{-1}) and *P. deltoides* (18.8g L^{-1}). Significant interactions were observed between treatments in water use efficiency and relative water content of leaf of *E. camaldulensis*. Results showed that N1 may counteract the effect of drought while N2 slows down tree growth as well as carbon storage capacity. Recommendations of the study are to examine growth response of these tree species for more than 3 months at different tree sizes and ages.

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