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CARBON STORAGE AND ALLOCATION PATTERN IN PLANT BIOMASS UNDER DROUGHT STRESS AND NITROGEN SUPPLY IN *EUCALYPTUS CAMALDULENSIS* AND *POPULUS DELTOIDES*

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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 an 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. A pot experiment was done by planting 2 years old seedlings in 5kg pots in a glasshouse for four weeks. The 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 $gNkg^{-1}$). 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 was increased up to 63% to 75%. Whereas in *Eucalyptus camaldulensis*, shoot carbon content was increased up to 51% to 52% at N0D2 treatment. Leaf carbon contents were increased 23% to 44% in *E. camaldulensis* and 0.3% to 4% in *P. deltoides*, at N1D1 treatment respectively. Dry shoot biomass was 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 the improved acquisition of nutrients by these species in the drought-stressed environments.

Key words: Carbon allocation, Water stress, Nitrogen, *Populus deltoides*, *Eucalyptus camaldulensis*, Water use efficiency, Biomass.

Introduction

Climate change: Climate change is the global apprehension and most important challenge in the recent era. Variation in climatic conditions not only causes the disturbance in carbon cycle but also has a key role in changing the favorable conditions for soil, water and agroforestry (Nyirambangutse *et al*., 2017). Carbon dioxide (CO_2) being the main greengouse gas (GHG), causes a noticeable rise in temperature that results in global warming (Field *et al*., 2014). CO_2 concentration in the 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 is due to change in vegetation cover and anthropogenic activities like the 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 (Anon., 2014). These anthropogenic activities are the major source of producing Greenhouse Gases (GHGs) in the atmosphere (Mackey *et al*., 2013) which increase the earth's surface temperature by 1.5ºC (Lindenmayer *et al*., 2012).

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 the 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 a 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 disturb production of biomass, leaf gas exchange and water use efficiency (Hernández & Bosch, 2004). Low water availability to tree species causes a reduction in lateral branching, total dry matter and repressed rate of leaf, shoot and cell expansion (Tuomela *et al.*, 2001).

Adaptation of tress during drought: When plants are subjected to water stress, stomatal response, metabolic changes, photosynthesis and reactive oxidative species scavenging mechanism is affected (Fig. 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 competes for CO_2 and O_2 i.e. for carboxylation and respiration (Long, 1991). Moreover, Nitrogen (N) is one of the major components of RuBisCo, an N 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 a slight decrease in stomatal conductance (Granath *et al*., 2012).

Tree species that tend to store carbon in their different parts like leaves, branches, stem, bark and roots may tolerate water stress conditions (Villagra & Cavagnaro, 2006). Roots are not as much drought sensitive as compared to leaves because they have increased access to water (Cheng & Zhong, 2012) (Fig. 1). An increasing amount of water stress to tree seedlings cause a reduction in biomass and has an effect on growth. On the other hand, with the 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 varied in growing seedlings of *E. camaldulensis* (evergreen) and *P. deltoides* (deciduous) and also to examine growth parameters, primary production (biomass) in each tree seedling under water stress and N supply.

Materials and Methods

Experimental setup: This section outlines all the procedures that were used to identify carbon content and biomass production and the 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 the same size were placed in a glasshouse (10x12 feet) for 4 weeks with 9 treatments (Fig. 2). The 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.

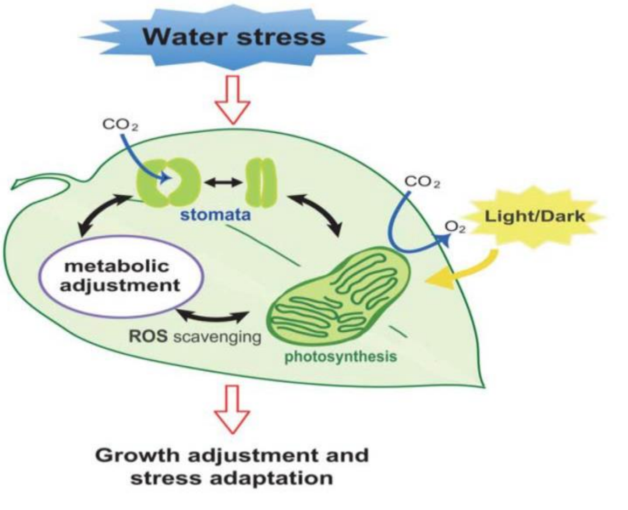

Fig. 1. Schematic diagram of response and metabolic adjustment of plants during water stress conditions (Osakabe *et al*., 2014).

Fig. 2. Experimental setup of *Eucalyptus camaldulensis* and *Populus deltoids*.

Soil analysis: Glass electrode method was used to determine pH and electrical conductivity (EC) of the soil sample (Page, 1982). Soil samples were air dried to determine the water-holding capacity by using the method described by Israelsen & West, (1922). For calculation of total organic carbon (TOC) in the soil, ferrous ammonium sulfate (FAS) titration method was used (Bremner & Mulvaney, 1982). Total nitrogen involved was measured by Kjeldahl apparatus as well as digestion and distillation methods (Bremner & Mulvaney, 1982). Nitrate nitrogen by the salicylic acid method and ammonium nitrogen (NH_4-N) by using the 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 taken on a spectrophotometer at 410 nm wavelength (Olsen *et al*., 1982).

Plant analysis

Carbon content: In the present study, the 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.

$$C = 0.475 \times B$$

Total plant biomass determination: At the start of the experiment, five equal sized seedlings for both species were harvested for initial biomass measurements. Harvest method for biomass determination was done by taking the sum of the root, shoot, and leaf biomass. Readings of each part of individual 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 a measuring tape (Flombaum & Sala, 2007).

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

Water use efficiency (WUE): WUE was determined by using the following formula described by Wu *et al*., (2008);

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

Relative water content: Relative water content (RWC) of leaf calculated by using the 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 the refrigerator for 24 hours and then weighed for turgid weight and in the 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

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.

Results

Temperature variation and characteristics of soil: There was an observable difference between indoor and outdoor temperatures of the glasshouse. Highest observed indoor temperature was up to 34°C whereas outdoor temperature was 27°C while the lowest indoor temperature was observed at 12th day due to cloudy weather (Fig. 3). Soil analysis values including pH, EC, water-holding capacity, TOC and total N, P, K, and NO_3-N are given in Table 1.

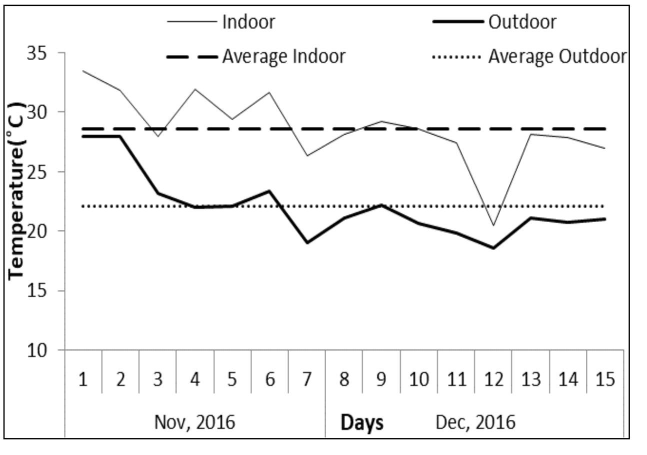

Fig. 3. Indoor and outdoor temperature for 15 days recorded (8hours each day randomly).

Carbon content

Shoot and root carbon content: There were reports that carbon content constitutes between 45-50% of all dry matter of tree species (Selva *et al*., 2007). 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). N2 allocated more carbon in their shoots 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. 5). 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.

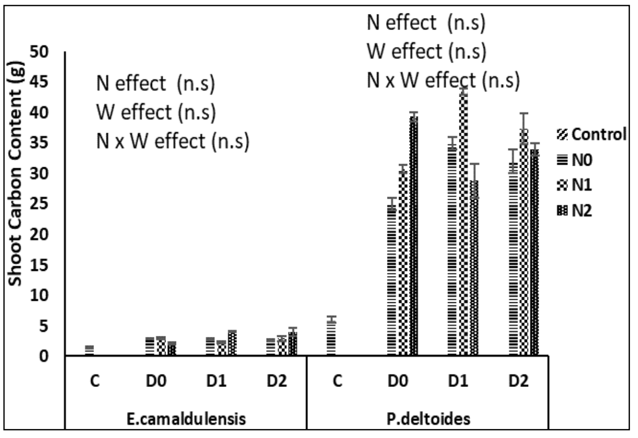

Fig. 4. Shoot carbon content measurements in *E. camaldulensis* and *P. deltoides* after one-month fertilizer application. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three waters levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment;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

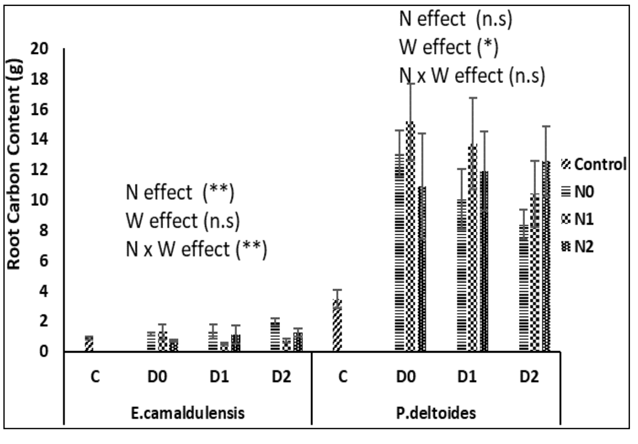

Fig. 5. Root carbon content measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing $p>0.05$, *$p<0.05$ and **$p<0.01$ (Bars indicate SD).

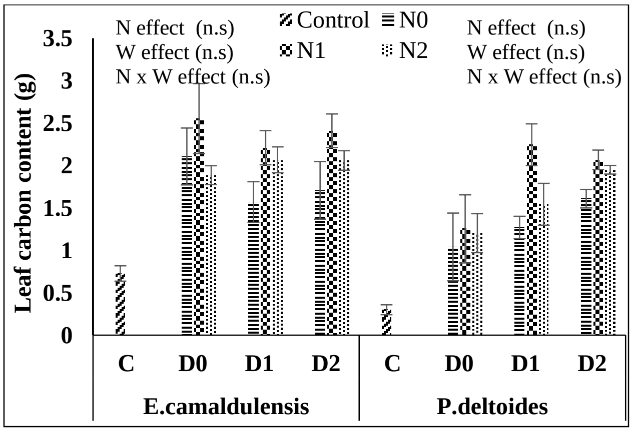

Fig. 6. Leaf carbon content measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing $p>0.05$, *$p<0.05$ and **$p<0.01$ (Bars indicate SD).

Leaf carbon content: Shoot and root carbon contents in *E. camaldulensis* were less observable as compared to *P. deltoides* but leaf carbon content was more in *E. camaldulensis* (Fig. 6). 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, an 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 tended to store more carbon in their leaf biomass as compared to N0 and N2. Results in both species showed an insignificant effect among treatments.

Carbon content percentages in *E. camaldulensis* and *P. deltoids:* Carbon content in *E. camaldulensis* (Fig. 7) and in *P. deltoides* (Fig. 8), of all treatments, are separately shown to give an overview about percentage carbon content in the 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.

Physical characteristics

Shoot length: Shoot length of both seedlings showed slight differences under drought stress as compared to control at different water levels (Fig. 9). 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 the shoots 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 the stability of the growth parameters of these seedlings. As N2 was applied, there was a swift response in *E. camaldulensis* seedlings while *P. deltoides* seedlings showed variation at N1 and N2 application.

Root length: Results showed significant variations between treatments in combined 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. 10). Root length of *E. camaldulensis* showed an 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 a slight decrease in root length at D1 and D2 water stress level in *P. deltoides.*

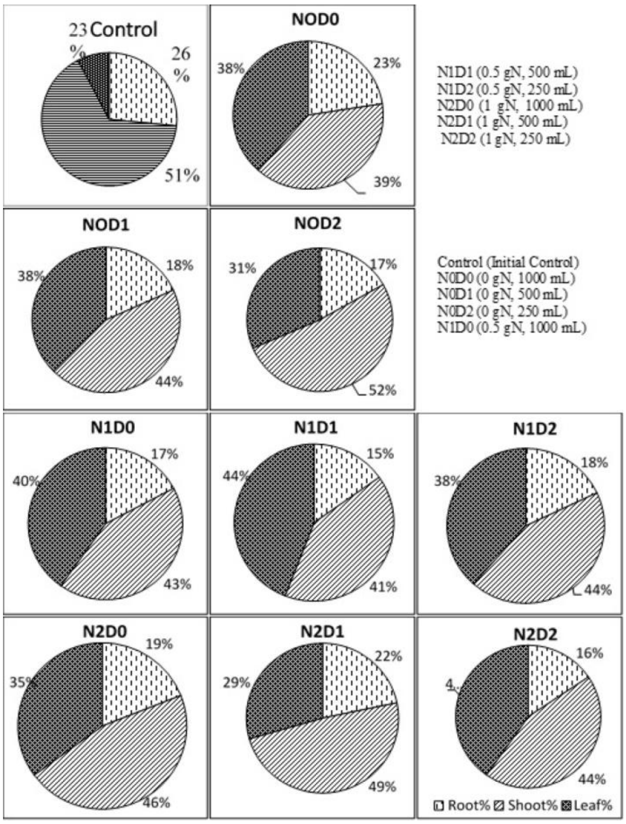

Fig. 7. Carbon content percentages of *E. camaldulensis* in all 9 treatments.

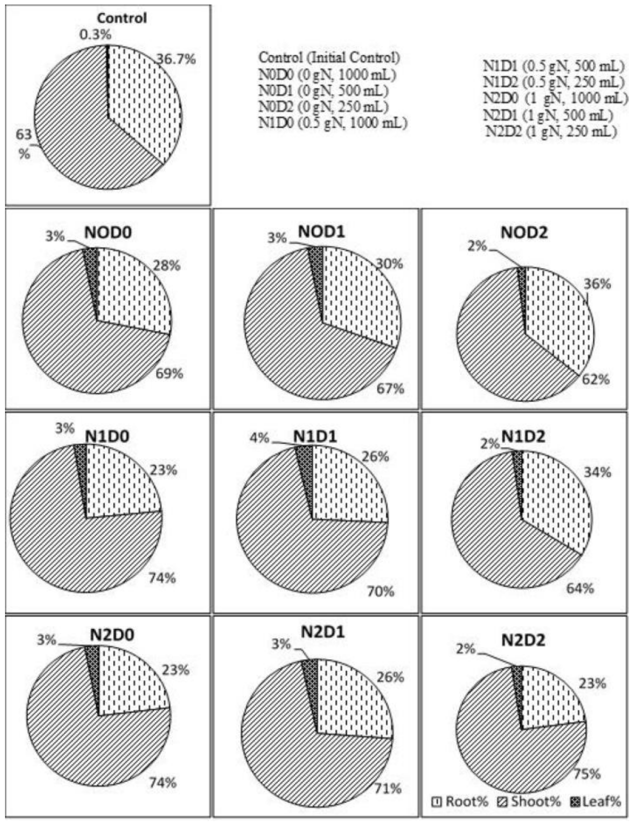

Fig. 8. Carbon content percentages of *P. deltoides* in all 9 treatments.

Biomass production

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 were observed which showed variation in readings (Fig. 11). A 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 a 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.

Root biomass: N1 maintained root biomass in *P. deltoides* but was less observed in *E. camaldulensis* (Fig. 12). Effect of N, W and NxW were significantly different in *E. camaldulensis* and W and NxW effect in *P. deltoides.* Moreover, N2 of *E. camaldulensis* restricted the 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).

Leaf biomass: *E. camaldulensis* seedlings showed a much better response in leaf biomass measurement in N1D2 (4.72g) as stress increased (Fig. 13). 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 incorporates in leaves to grow in severe drought in comparison to N0. Leaves of *P. deltoides* were less in biomass, as the growth was restricted during water stress conditions.

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 Tables 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.

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}) (Fig. 14). 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 the leaf was highest among seedlings where there was no N application (in N0D2 48%) (Fig. 15). RWC was 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 exhibited correlation values for *P. deltoides* in which RWC and RCC showed positive correlation values for WUE, SLA, SCC, and LCC. WUE was significantly increased with an increase in SCC and RCC respectively.

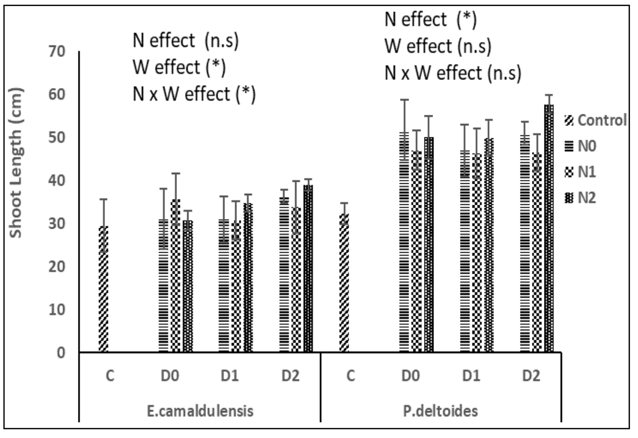

Fig. 9. Shoot length measurements in *E. camaldulensis* and *P. deltoides* after one-month fertilizer application. Control (C) placed along with treatments. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing $p>0.05$, $*p<0.05$ and $**p<0.01$ (Bars indicate SD).

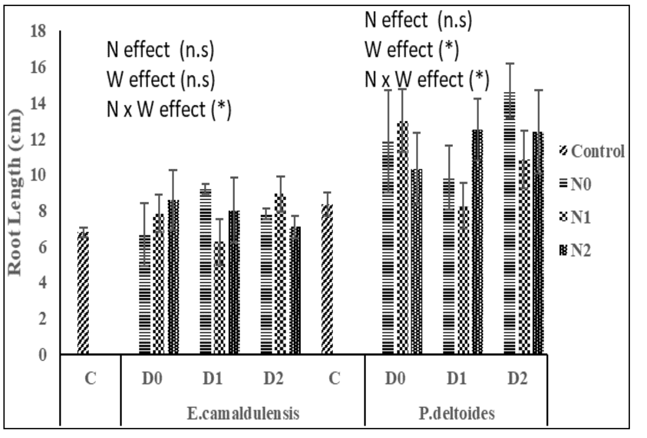

Fig. 10. Root length measurements in *E. camaldulensis* and *P. deltoides* after one-month fertilizer application. Control (C) placed along with treatments. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing $p>0.05$, $*p<0.05$ and $**p<0.01$ (Bars indicate SD).

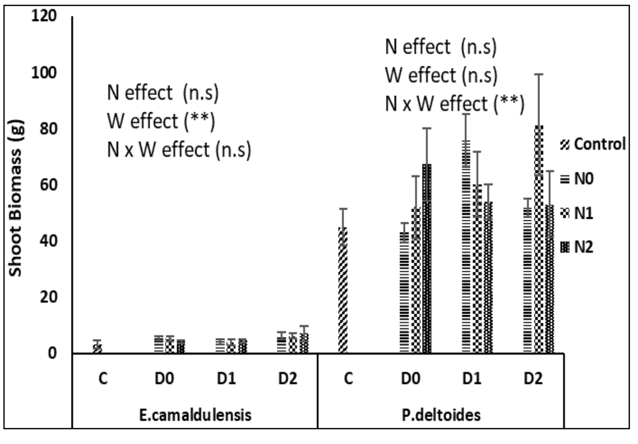

Fig. 11. Shoot biomass measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Control (C) was also placed along with treatments. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) 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).

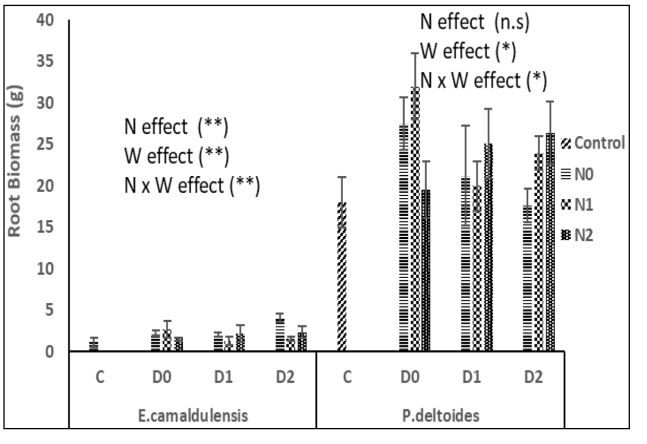

Fig. 12. Root biomass measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Control (C) was also placed along with treatments. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) 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).

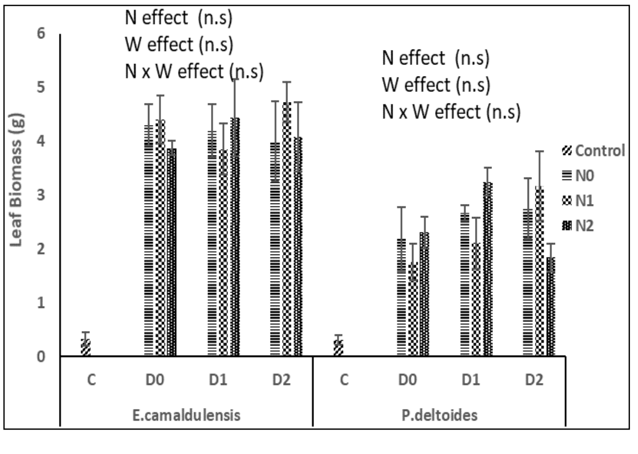

Fig. 13. Leaf biomass measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Control (C) was also placed along with treatments. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) 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).

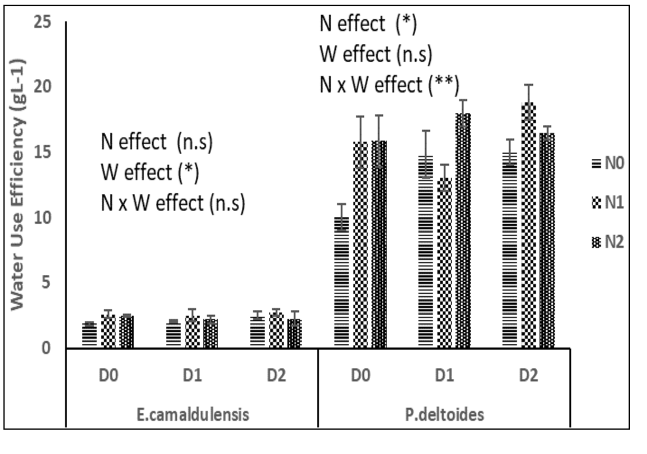

Fig. 14. Water-use efficiency (WUE) measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing $p>0.05$, $*p<0.05$ and $**p<0.01$ (Bars indicate SD).

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

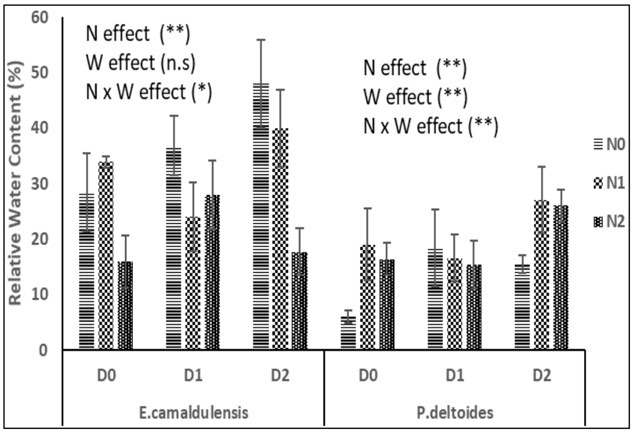

Fig. 15. Relative Water Content (RWC) of leaf measurements in *E. camaldulensis* and *P. deltoides* after one month fertilizer application. Fertilizer applications were 0, 1 and 2 g N kg-1 (N0, N1 and N2) with respective three water levels 1000, 500 and 250mL (D0, D1 and D2) having 3 or 4 replicates for each treatment; where n.s showing $p>0.05$, $*p<0.05$ and $**p<0.01$ (Bars indicate SD).

Discussion

In the present study, the interactive effect of nitrogen and drought had a more increasing 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 a significant response in water stress level and N interaction. These results were in accordance with the results of Kaul *et al*., (2010) in which carbon allocation in the shoot of *Populus deltoides, Eucalyptus tereticornis,* and *Tectona grandis* ranged 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 a positive increase in biomass results in N0 without any nitrogen treatment (Hunter, 2001, Chen *et al.,* 2015) 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. Fortunel *et al*., (2009) demonstrated that Poplar seedlings shifted more biomass to shoots as compared to root and leaf biomass when both N supply and water stress treatments were applied. 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 a major portion of their biomass in roots may cause an 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. Several 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 the limited water supply (Liu *et al*., 2005). However, some others have found that every species have different water- use efficiency depending on their strategy to water use (Clavel *et al*., 2005; Yin *et al*., 2009).

Previous studies showed 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 might cause a change in leaf morphology and high N might restrict leaf growth (Erice *et al*., 2010). Overall, more carbon allocation was observed in the shoot of *P. deltoides* as compared to *E. camaldulensis.* Same results were shown by Saraswathi & Ezhilarasi (2012) in which the highest amount of carbon content was observed in *Pongamia pinnata* under water stress and urea supplementation.

Conclusion

Carbon storage in the shoot of *P. deltoides* increased 75% (N2D2) and in *E. camaldulensis* up to 52% (N0D2) where there were NxD interactions. Biomass production was more in shoot of *P. deltoides* (45 to 81g) as compared to *E. camaldulensis* in N2D2 (3.8 to 7g). 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 in leaves 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.

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

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	

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