CARBON ENRICHMENT IN TREES IN RESPONSE TO ELEVATED CO_2, NITROGEN TEMPERATURE, DROUGHT, AND OZONE: A META-ANALYSIS

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A thesis submitted in partial fulfillment of the requirement for the degree of Master of Science in Environmental Sciences

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Dedication

This research is dedicated to my loving, caring, and industrious parents and my siblings whose efforts and sacrifice have made my dream of having this degree reality. Words cannot adequately express my deep gratitude to them.

"O My Sustainer, bestow on my parents your mercy even as they cherished me in my childhood".

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List of Abbreviations

LMF	Leaf Mass Fraction
RMF	Root Mass Fraction
SMF	Stem Mass Fraction
OTC	Open Top Chambers
FACE	Free Air Carbon Enrichment
eCO_2	Elevated carbon dioxide
aCO_2	Ambient carbon dioxide
N	Nitrogen
O_3	Ozone
Temp	Temperature
SE	Standard Error
SD	Standard Deviation

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ABSTRACT

The era of 1700 saw industrial and societal development. However, it adversely contributed to the depletion of natural resources and increased emissions of carbon dioxide (CO_2). Since CO_2 is a greenhouse gas, it became a global cause of concern. In some studies, CO_2 is known for its adverse effects on human health and the ecosystem. While other studies show increased CO_2 concentration in the environment promote greater amount of CO_2 being absorbed by the plants (termed as eCO_2). This eCO_2 facilitates photosynthesis and supplements the biomass growth in trees. The positive impacts of eCO_2 are largely dependent upon factors such as plant species, genetic makeup, absorption site conditions, drought conditions, ozone levels and concentrations of nitrogen. Despite preliminary stimulation of photosynthesis that the literature focuses on, under the outcome of eCO_2, the plant incurs a down-regulation known as photosynthetic acclimation. The aim of this study was to analyze the allocation of biomass in forest species under eCO_2 effect and to assess the different environmental factors that contribute to biomass. For this purpose, a meta-database using woody species of plants was developed (timeline 2000 to 2021). Two values for dry biomass were extracted: the ambient levels and the elevated levels of CO_2. Standard error for the dry biomass was recorded respectively. The data was presented in either table or in the form of figures. The digitization of the figures was done using the software "GetData" and then incorporated into the meta-database. To understand the way elevated levels of $CO_{2,}$ affect the assimilation of carbon into biomass in the tree species is the main goal of meta-analysis. As well as how there is a shift of biomass allocation at the eCO_2. The meta-analysis was done in a multi-factorial way where other treatments *viz.* drought, nitrogen, ozone, and temperature effect the carbon allocation pattern was also studied. In the current study, it was concluded that there is an overall positive response to carbon treatment which results in overall increase of tree mass as well its tissues like leaves, stems, and roots. Nitrogen, drought, temperature, and ozone also have a positive effect on CO_2 enrichment demonstrated by the positive change in percentage of biomass in the total biomass, leaf biomass, root biomass and stem biomass of the trees. Although the results suggest that CO_2 enrichment is most likely to be enhanced if there is more availability of nutrients for the trees which is evident by the increase in biomass as compared to low nutrient availability.

CHAPTER 1

1. INTRODUCTION

In the late 1700s, substantial industrial growth led to the expansion of the society, but out of control use of resources and consumption was followed by harmful consequences. From pre-industrial times, the atmospheric carbon dioxide content (CO_2) has increased by about up to 44%, and the main causes are unsustainable land use change and burning of fossil fuels (Moss et al., 2010; Stocker, 2014). Scientists from all over the world are alarmed by the fact that CO_2 is a greenhouse gas with a radiative forcing of 1.37 105 W/m^2 (Stocker, 2014). According to Moss et al. (2010), the concentration of CO_2 has the potential to rise further until 2050 and 2100, respectively, up to 500 ppm and 900 ppm. Although most studies focused on the detrimental effects of rising CO_2 on human health and the environment, certain experiments also showed that the higher CO_2 concentration can result in plants or trees growing at a faster rate as a response to the elevated levels of CO_2 that they are exposed to. This eCO2 promotes photosynthesis and raises the amount of biomass produced by plants and forests. (Norby *et al.,* 2005).

1.1 Factors affecting the impact of eCO_2 on plants.

While increased CO_2 levels are known to have positive effects that largely depends on the species that have absorbed CO_2 (Wang et al., 2012), the state of the area where the absorption occurred (McCarthy et al., 2010; Bader et al., 2013; Kitao et al., 2015; Ellsworth et al., 2017), the age of the tree etc. (Voelker et al., 2006; Norby. Also, the findings show that genotype has a significant role in the productivity response of trees to eCO_2 across a variety of species (Dickson *et al.,* 1998; Isebrands *et al.,* 2001; Mohan *et al.,* 2004; Cseke *et al.,* 2009). Based on this research, it is acknowledged that intraspecific variations in the genotypic make-up of the tree species have a crucial role in affecting the phenotypic response, such as these trees response in terms of growth to higher levels of CO_2 (Nicotra *et al.,* 2010).

The evolutionary and ecological advantages among various trees can be obtained from these intraspecific variations in responses towards eCO_2 whereby the genotypic nature of one species may render more advantage in terms of fitness and growth than the other individuals within the same species (Ward et al., 2000; Ward and Kelly, 2004). Nonetheless, there is immense evidence of evolution within and among the plants' species related to changing concentrations of atmospheric CO_2 and associated eCO_2 on geographic timescale (Ward and Kelly, 2004; Becklin *et al.,* 2014). However, the role of genotypic makeup from the practical standpoint is critical. The genotypic makeup among the individuals of the same species which enhance the productivity of the individual in relation to eCO_2 as compared to the productivity in other individuals of the same species is crucial in determining the sustenance capacity and the productivity of the managed forests in future under the changing climate (Aspinwall *et al.,* 2012,2015). Despite this, we know very little about the functional factors that contribute to intraspecific variance in tree production responsiveness to eCO_2. The effects of intraspecific diversity in plant production adaptations to eCO_2 are being studied experimentally, mostly with agronomic traits (Bishop *et al.,* 2015, Sanz-Sáez *et al.,* 2017). These studies, however, are uncommon in forest tree species, have limited genotypic variation, and rarely combine physiological responses to eCO_2 with assessments of tree growth or biomass output. Emission of CO_2 increases in situ rates of leaf photosynthetic activity (A_{net}) at the size of the leaf by boosting carboxylation and reducing photorespiration (i.e., oxygenation). Long-term increases in A_{net} frequently result in the accumulation of nonstructural polysaccharides in leaves, an increase in leaf dry mass per unit area (LMA), and a decrease in leaf nitrogen (N) levels. (Curtis 1996, Curtis and Wang 1998, Tjoelker *et al.*, 1999, Medlyn et al., 1999, 2001, Leakey *et al.,* 2009). Enhanced photosynthetic N-use productivity is the consequence of an elevation in A_{net} and a reduction in leaf N at eCO_2 (PNUE). Although higher leaf carbohydrates and lower leaf N at eCO_2 have been linked to reduced photosynthetic ability, commonly known as photosynthetic "down-regulation." Down-regulation occurs when the supply of photosynthate outweighs the demand for it or the capacity of carbon (C) sinks (defense, growth, respiration, and maintenance). Mesophyll cells notice the imbalance as a result, and they decrease the amount or activity of photosynthetic enzymes (Ainsworth and Long, 2005, Ainsworth and Rogers, 2007). A_{net} stimulation is restricted at eCO_2 due to decreased photosynthetic efficiency that could also limit plants' capability to respond productively to eCO_2. Because genotypic variations in physiological responses to eCO_2, and notably photosynthetic down-regulation, have not yet been thoroughly investigated in relation to tree genome biomass output responsiveness to eCO_2, this information is crucial.

It is likely that genomes that prevent the photosynthetic activity at eCO_2 from being downregulated also exhibit the highest productivity improvements there. Modification in C distribution may be significant, while genotype biomass production adaptations to eCO_2 may be influenced by photosynthetic processes. Pritchard *et al.* (1999) theoretically explained how different responses to eCO_2 in plant productivity are influenced by photosynthesis and C distribution. For instance, if eCO_2 lowers the C constraint of photosynthesis, trees may allocate more C to tissues or activities involved in the absorption of other scarce resources, such as nutrients (Iverson *et al.,* 2008, Pritchard *et al.,* 2008). Additionally, the results of the meta-analysis by Resco de Dios et al. (2016a) suggested that root- and other C sinks, including metabolism, root secretion, and defense, may be more important for genotypic variability in productivity responsiveness to eCO_2 than leaf-scale photosynthetic activities. Further research has revealed that variations in genotype production reactions to eCO_2 are correlated to genotype-specific adjustments in whole-tree organic matter distribution (Isebrands *et al.,* 2001, Zhang *et al.,* 2010). Changes in C distribution and photosynthetic reactions likely influenced genotype efficiency adaptations to $eCO_{2.}$

Genotypes with adequate down-regulation of photosynthesis and consistent increases in biomass output under eCO_2 are able to maintain high rates of carbohydrate synthesis and export for growth or storage in sink tissues (such as roots) and low rates of carbohydrate accumulation in leaves (Davey et al., 2006). A loop between genotype production and photosynthetic responses to eCO_2 is also conceivable where sink capacity (growth) impacts source activity (photosynthesis) (Körner, 2003; Fatichi et al., 2014). For instance, no change in Rubisco concentration (measured as total leaf N) or down-regulation of photosynthetic activity may be anticipated if growth is the primary C sink and growth responses to eCO_2 keep pace with C absorption via photosynthesis.

1.2 Linking partitioning of eCO_2 and biomass accumulation.

Partitioning is the term given to how the biomass is assigned in different parts of the tree i.e., stem, roots, branches, and leaves. Partitioning assists in understanding how biomass distribution will be done in future when there will be eCO_2 in the atmosphere due to climate change. The biomass partitioning helps in creating a standard data for the models to run their analysis in creating a future trend of how different factors will affect the biomass accumulation in future under different scenarios like eCO_2 levels because of climate change. It also helps to predict whether the species will adapt to the changing environment as loss of biodiversity is at stake. Suitable precautions can be made to save certain species from perishing in the future and preserve biodiversity.

With other factors, ozone concentration and the levels of nitrogen also prominently affect the C allocation in the trees. Particularly in China, rapid industrialization and agricultural growth over the past few decades have resulted in a notable surge in anthropogenic emissions of reactive nitrogen (N) and ground-level ozone (O_3) (Feng *et al.,* 2015; Liu *et al.,* 2013, 2017; Lu *et al.,* 2018; Yu *et al.,* 2019). Ozone pollution and N deposition co-occur and may have an impact on biogeochemical processes and ecosystem services (Mills et al., 2016; Brewster et al., 2018; Kou et al., 2019; Li et al., 2019; Zeng et al., 2019). A significant deposit of nitrogen may cause the primary output of forests to increase (Pregitzer et al., 2008; Thomas et al., 2010; Du and De Vries, 2018; Tian et al., 2018). Increased O_3 levels have the potential to have negative effects on plant growth, photosynthesis, and the buildup of biomass in some tree species. Greater N availability may mitigate these effects (Wittig *et al.,* 2009; Ainsworth *et al.,* 2012; Li *et al.,* 2017; Feng *et al.,* 2019).

Increased N availability may aid plants in detoxifying O_3 damage and healing it, according to study (Andersen, 2003; Thomas *et al.,* 2005; Mills *et al.,* 2016). By doing this, the negative impacts of O_3 on plants would likewise be diminished (Sanz *et al.,* 2007; Handley and Grulke, 2008; Marzuoli *et al.,* 2016, 2018). Yet, a number of studies have found that higher stomatal conductance, elevated leaf N content, and improved photosynthetic rate result in more O_3 damage (Grulke *et al.,* 2005; Bassin *et al.,* 2007; Yamaguchi *et al.,* 2007; Azuchi *et al.,* 2014; Brewster *et al.,* 2018). Several studies found that nitrogen supply or addition had a negligible effect on O_3 responses (Feng *et al.,* 2011; Niu *et al.,* 2011; Harmens *et al.,* 2017; Dai *et al.,* 2019). While most previous studies focus on above-ground function and structure, a small amount of work has examined the interplay between O_3 and N on below-ground function and structure using a variety of O_3 levels and N addition levels (Mills *et al.,* 2016; Dai *et al.,* 2019). Because roots have a very high sensitivity to increasing O_3 concentrations, understanding how roots respond to growing N inputs and O_3 concentration is essential for improving estimates and predictions of carbon stocks and primary productivity of plants (Wittig *et al.,* 2009; Wang *et al.,* 2016; Li *et al.,* 2017).

Distribution of biomass across various organs is the key ecological mechanism used by plants to adapt to altering surroundings (Agathokleous et al., 2016, 2019; Poorter and Nagel, 2000; Grantz et al., 2006; Poorter et al., 2012). The distribution of biomass in consequence to the higher O_3 and N addition, however, is still poorly understood. Lower root: shoot (R:S) ratio mostly accompanies reduced carbon distribution to underground tissues due to ozone pollution. Due to a variety of influencing variables, such as exposure parameters, inter-species variance, ontogenetic alterations, and interplaying climate factors, reactions of R:S ratio are, nevertheless, very varied (Grantz *et al.,* 2006; Agathokleous *et al.,* 2016, 2019). Because it encourages carbon buildup in ground biomass more than root biomass, increased N input also lowers the R:S ratio (Li *et al.,* 2015; Peng and Yang, 2016). The size of a plant's root system in comparison to its shoot may have an impact on how exposed it is to environmental pressures like winds and drought (Agathokleous *et al.,* 2016, 2019). The optimal partitioning hypothesis and the allometric biomass partitioning theory are commonly used to explain how organic material is distributed in response to environmental changes (Enquist and Niklas, 2002; McCarthy and Enquist, 2007). When attempting to understand how organic material is dispersed in response to environmental changes, the optimum partitioning hypothesis and the allometric biomass partitioning theory are frequently used (Enquist and Niklas, 2002; McCarthy and Enquist, 2007). According to the optimum partitioning theory, plants adapt to environmental changes by dividing their biomass across several organs to best capture light, water, and nutrients for development (Bloom *et al.,* 1985; Chapin *et al.,* 1987). According to this model, plants would selectively allocate more organic matter to the roots in nutrient-limited situations such as soil N, (Li *et al.,* 2015; Kobe *et al.,* 2010) and more biomasses move to the leaves and stems in nutrient-rich settings (Poorter *et al.,* 2012; Chen *et al.,* 2018). Whether the N-induced decrease in the R:S ratio is the result of alternate allometric methods, or the optimum potential biomass partitioning is still up for debate (Shipley and Meziane, 2002; Fortunel *et al.,* 2009; Luo *et al.,* 2016; Peng and Yang, 2016). Furthermore, it is not yet understood how the addition of soil nitrogen may affect the processes for allocating biomass in the presence of higher O_3 concentrations.

1.3 Objectives of the study

The objectives formed after studying the past literature and research are as following:

1. Meta-analysis to understand the impact of elevated CO_2 on carbon enrichment and biomass allocation.
2. To assess biomass partitioning in trees exposed to ambient *vs.* elevated levels of $CO_{2.}$
3. To assess the combined effect of elevated CO_2 and nitrogen, temperature, drought or ozone in biomass allocation and its partitioning.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Photorespiration and its role in elevated carbon distribution

According to research, photosynthesis in C3 plants tends to increase when they are exposed to higher levels of CO_2, despite the fact that photosynthesis is a critical process for plant growth and one of the process' regulatory variables (Ainsworth and Long, 2005; Wang et al., 2012). The increase in CO_2 has an impact on photosynthesis in higher plants in both good and negative ways. Significant variations in physiological, biochemical, and molecular reactivity to the eCO_2 environment exist. Elevated CO_2 concentration effects plant development, source-sink balance as well as interactive mechanism including other environmental factors (Reddy and Raghavendra, 2010). On the other hand, the process of photorespiration is severe and is relatively an inefficient process. The plant requires additional energy in exchange for slight gains in terms of carbon or energy (Peterhansel *et al.*, 2010). However, under eCO_2 state, the levels of Rubisco increase and the ratio of CO_2: O_2 shifts dramatically. This type of change causes the rate of carboxylation to increase while the rate of oxygenation decreases (Makino and Mae, 1999).

In both chamber experiments and FACE investigations (Ainsworth & Long, 2005), plants undergo a down-regulation of photosynthesis when exposed to CO_2 over an extended period not withstanding initial stimulation (Warren et al., 2014). This event serves as a marker for photosynthetic adaptation. The benefits of eCO_2 on photosynthesis are not always totally excluded by photosynthetic acclimation. For instance, in a study on white clover, photosynthesis was observed to improve by 37% following acclimation. White clover was grown for 8 years in an environment with high (600 ppm) [CO_2] (Ainsworth et al., 2003). The results imply that the final growth response to eCO_2 is established through plant acclimatization to it.

2.2 Causes of photosynthetic acclimation in plants

Reduced leaf nitrogen (N) is one of the explanations for photosynthetic acclimation, which has various other causes. One such explanation is decreased leaf nitrogen (N) levels. In a study on rice, the fall in N allocation into leaf blade caused by

eCO_2 resulted in a reduction in Rubisco and other protein synthesis (Seneweera *et al.,* 2011). This is supported by a 12-year study that found no acclimation response between the periods that leaf N sufficed for photosynthetic requirements (Warren *et al.,* 2014). In the absence of sufficient N to use up in Rubisco, the capacity of photosynthesis in leaves falls. Low soil nitrate availability increases the severity of photosynthetic acclimation, and it also appears to be linked to a reduction in leaf nitrate uptake (Vicente *et al.,* 2016). Moreover, leaf nitrate absorption is inhibited by eCO_2 (Bloom et al., 2014). It is unclear if the decline in Rubisco synthesis at eCO_2 is directly linked to poorer N assimilation or if Rubisco is simply controlled to maintain a balance between sources and sink activity.

An explanation for the adaptation of plants to eCO_2 is the increase in sugar production, which orders the source-sink balance and causes more sugar to be produced in source tissues to be consumed in sink tissues. This also occurred in the FACE experiment by Ainsworth et al. (2004), where single gene alterations were employed to test the theory that photosynthetic acclimation happens because of insufficient sink capacity. A soybean cultivar and the line with the mutation were compared in the study to assess growth for an unidentified growth feature. Besides, no increase in photosynthesis is shown by the soybean cultivar (ELF) determinate by mutation. This can be explained in a way that Elf is cultivar kept ignoring sink limitation, whereas photosynthetic acclimation is caused by single gene mutation (Ainsworth *et al.,* 2004). With high-risk capacity, plants continue to utilize the greater CO_2 availability. Nevertheless, for the maintenance of source activity plants must reduce photosynthesis with restricted carbon sink capacity. Hence, when high CO_2 induces excessive photosynthesis (Ainsworth et al., 2004) and is followed by feedback inhibition of photosynthesis, non-structural carbohydrates build up.

As chemical messengers, NSCs could influence gene transcription (Mishra et al., 2009; de Jong et al., 2014). In this way, feedback inhibition results in photosynthetic adaptation while suppressing photosynthesis.

Rubisco, a crucial enzyme in the photosynthetic pathway, is known to be decreased in leaves that have accumulated carbohydrate, or "carbohydrate accumulation" (Aranjuelo et al., 2008). Despite the evidence, a study by Ludewig and Sonnewald (2000) refuted the idea that sugar accumulations lead to photosynthetic acclimation when they found that increased [CO_2] accelerated the senescence of leaves in *Nicotiana tabacum* by down-regulating genes involved in leaf photosynthetic processes. Only senescent leaves showed down-regulation of photosynthetic genes, with no increase in sugar levels. The result reached was that leaf senescence causes photosynthetic adaptation but not sugar buildup. Yet, photosynthesis was suppressed before the plants reached senescence. As a result, there is no single explanation for photosynthetic acclimation; rather, a number of processes play a role to a different extent.

2.3 Carbon allocation and carbohydrate synthesis under eCO_2

eCO_2 boosts the generation of carbohydrates by promoting photosynthesis. In pea plants subjected to eCO_2 in growth chambers, the primary result of photosynthesis is sucrose, which rises in all organs while glucose concentrations remain mostly unaltered (Aranjuelo *et al.*, 2013). The ratio of hexose to sucrose will change depending on when glucose levels are monitored. When measuring glucose during a time when blood glucose levels are naturally high, the hexose to sucrose ratio will be higher than when measuring glucose during a time when blood glucose levels are low. Sucrose levels increased by an average of one third in castor oil plants cultivated in growth chambers with 700 ppm CO_2 compared to 350 ppm CO_2. (Grimmer *et al.,* 1999). Sucrose levels are higher than hexose in both chamber and field tests with eCO_2 (Rogers et al., 2004), but as the growing season comes to a conclusion, the ratio of hexose-carbon to sucrose-carbon in leaves increases with contact to eCO_2, reaching a five-fold higher ratio. (Rogers *et al.,* 2004). Potentially, changes in the hexose: sucrose ratio during plant growth can have an impact on the source and sink activities of plants. It is possible that the plant that prefers to generate one type of carbohydrate has genes that are regulated by that carbohydrate. For instance, if sucrose is needed to initiate the repression of a specific gene, producing more glucose than sucrose would be worthless.

The main carbohydrate found in plants that grow under eCO_2 is starch. (Aranjuelo *et al.,* 2008). Increased starch was seen with eCO_2 and the conversion of starch to sucrose leads to the high level of sucrose. This conversion is crucial for regular plant growth under natural circumstances (Smith et al., 2005), but it could also lead to sucrose buildup. The starch content increases during day and vanishes overnight in plants grown in ambient conditions. Not all of the starch from the plants reserve is washed out during night, shown by increased production of starch in eCO_2 (Grimmer *et al.,* 1999). The length of daylight influences the carbon portioning degree among starch and sucrose. Carbon partitioning shifts to synthesis in smaller period of light with decreased sucrose consumption and synthesis (Pokhilko *et al.,* 2014). Sucrose synthesis is increased, and little starch is collected during day time and vice versa (Pokhilko *et al.,* 2014). When the length of the day shortens, so does the amount of sucrose (Sulpice et al., 2014). During night, the amount of trhalose-6-phosphate (T6P) affects how starch breaks down. Increased T6P in Arabidopsis plants, which increases the starch reserves by night's end, inhibits starch breakdown at night (Martins et al., 2013). Martins et al. (2013) also discovered that T6P is responsible for the little increase in starch production. These results suggested a paradigm for nocturnal starch metabolism, with starch breakdown limited by the circadian rhythm of plants (Martins *et al.,* 2013; Lunn *et al.,* 2014). Low T6P results from high sucrose demand, which reduces the inhibition of starch breakdown and raises the concentration of sucrose. T6P rises and prevents starch breakdown when there is less desire for sucrose. The circadian clock of the plant limits the amount of starch that can degrade at night in order to prevent total starch depletion (Martins *et al.,* 2013).

While extra carbs are given to the plant in varying amounts, some tissues receive more than others. Under eCO_2, carbohydrate allocations differ between species. In other species, more carbon is devoted to the seeds and shoots (Sasaki *et al.*, 2007; Aljazairi *et al.,* 2014; Butterly *et al.,* 2015). In the case of rice, for instance, eCO_2 promotes the translocation of carbohydrates stored in vegetative tissues by allocating freshly fixed carbohydrates to the panicle, which also promotes the starch, in between the stage of grain filling (Sasaki *et al.,* 2007). The carbon allocation between durum wheat and bread wheat differs under eCO_2. According to Aljazairi et al. (2014), the bread wheat cultivar Yitpi allocates more carbon to shoots than the durum cultivars Blanqueta and Sula do (Butterly *et al.,* 2015). Moreover, more carbon is allocated into spikes in Sula than in Blanqueta (a modern cultivar). There is different yield potential of the two durum cultivars. The ten-fold increase in starch seen by a root with eCO_2 increasing the growth of roots and shoots of tepery bean (Salsman *et al.,* 1999). Maintaining the balance of nutrients in plants can be done by assigning more carbon in roots under eCO_2 that will allow nutrients and water to be taken up.

2.4 Mechanism of Nitrogen fixation and CO_2 concentration in plants

Carbohydrate partitioning is influenced by a variety of environmental factors other than carbon content. In an experiment conducted by Aranjuelo et al. (2013), it was discovered that the sucrose content of N_2-fixing, and NO_3-fed plants altered significantly when exposed to increased CO_2. The assimilation of nitrogen (N) in plants is impacted by eCO_2 levels (Bloom et al., 2014; Vicente et al., 2015), which could suggest a connection between N uptake and the enormous 366% rise in sucrose observed in NO_3 fed plants compared to N_2 fixing plants. Plant growth techniques also have an impact on carbon allocation (glasshouse, field, etc). Elevated carbon dioxide causes perennial ryegrass roots to allocate more carbon, which leads to an increase in root dry matter. Such results do not occur in controlled environment chambers (Suter *et al.,* 2002). The difference is attributed to shoot sink strength and plant age, N availability difference is the outcome in ryegrass. Results from Aranjuelo *et al.* (2013) carbon allocation is affected by sink strength, N_2 fixing plant let more storage of carbohydrates that in return blocks photosynthesis inhibition by more carbohydrates. This can result in the availability of carbon sink affected by carbon allocation. Another factor is that this might affect carbohydrate allocation under elevated carbon dioxide that affects leaf area. Compared to others, plant that has less response to leaf area under elevated carbon dioxide might fix greater carbohydrates to roots because the leaf sink capacity is unchangeable to larger production of carbohydrates. For some plants, high carbon dioxide stimulates root development (George et al., 2003), which would increase its sink capacity and enable more carbohydrate allocation. Plants that grow in low pH environments with high carbon dioxide levels have altered carbon allocation (Hachiya *et al.,* 2014).

Experiments have been conducted by several studies that apply exogenous carbohydrates in roots of the plants with creating conditions for increase in root sugar that reflects greater root sugar that results from the increase in photosynthesis under elevated carbon dioxide but lack of understanding regarding the effect elevated carbon dioxide have on sugar sensing. Most of the research are on the role of carbohydrate in plant roots that focuses on sucrose entirely. This work is mostly limited to *A. thaliana*, but sugar plays diverse role in functioning of roots that might be discovered between different plant species. the possible outcomes for plants roots that grows in elevated carbon dioxide, altered gene expression is achieved with extra carbohydrates that are transported to roots from leaves. The sugar sensing effects that are in roots receive more attention in shoots as compared to roots which happens under elevated carbon dioxide (Feng *et al.*, 2011).

CHAPTER 3

3. METHODOLOGY

3.1 Database development

Meta-database for the study was developed with fixing the timeline for search from 2000 to 2021. An extensive and comprehensive literature review was carried out to find the suitable literature which met the general requirements for the paper to be added in the meta-database. The meta-database was created using only the woody species of plants i.e., the tree species. The response variables that were extracted from the papers and placed under different headings are as followings; stem/branch biomass, leaf biomass, root biomass (main and fine roots if the distinction between the two is done) and total biomass. Two values for dry biomass were extracted for a species, one for the ambient levels and one for elevated levels of carbon dioxide. Standard deviation or standard error for the dry biomass was recorded respectively for the biomass at the ambient and elevated levels. If the paper had standard error given it was converted into standard deviation using the conversion formula and vice versa was done if standard deviation was given for the biomass. For a paper to be added into the meta-database, the criteria had to be met by the paper which includes the response mean for biomass (X_AMB, X_ELEV), the sample size (N_AMB, N_ELEV), standard error (SE_AMB, SE_ELEV), standard deviation (SD_AMB, SD_ELEV). All these numerical readings which were extracted from the papers were important in calculating the weight of response ratios for each study for the primary results of the current study. Many studies were excluded based on these variables being missing from the papers as well as well the unevenness within units which many of these variables were recorded in the paper by the author. In some cases, where the biomass was given in time intervals of two or three, the harvest values of biomass for the longest period of CO_2 fertilization were taken into consideration from that paper. Only one measurement point per treatment per study was considered to keep the individual observations statistically independent, which is a requirement for most of the methods that were used in the meta-analysis. Other variables that were also kept in consideration apart from response of carbon treatment were ozone treatment, nitrogen (fertilization) treatment, drought treatment and temperature treatment. The factorial design papers which were looking into other variables influence on the response of the woody species to the ambient and elevated CO_2, there interaction was also investigated and measured. Other categorical variables that were recorded for the current study to look for their interaction/influence on the response of the species to CO_2 are as following; plant functional group, duration of CO_2 treatment (Time), type of experiment, type of tree, div 1 (woody), div 2 (angiosperm/gymnosperm). All the variables that are mentioned above were required for a study to be considered eligible to be added into our database for the meta-analysis. The data was presented in either table or in the form of figures, the data was easily available to be extracted for the former while for the latter the figures had to be digitized. The digitization of the figures was done using the software "GetData" and then incorporated into the meta-database.

3.2 Meta-analysis of response ratios

The studies that are based on meta-analysis are dependent on some estimation of treatment effect size (Curtis, 1998). The studies that are more important are given a higher weightage in terms of effect size through the response ration method. Most commonly the treatment effect size is checked by the relativity of the mean of experimental treatment to the mean of control treatment. In this case, the experimental treatment mean is Xe while the control treatment is the Xa. The previous meta-analysis that was carried out have used d-index to estimate the different effect sizes of a particular study. The other much more common effect size metric for the studies on the elevated levels of CO_2 studies is the response ratio, which is used in this study as well to calculate the effect size. The effect size calculations are done through the software 'R studio', a statistical software which is commonly used to do meta-analysis of different studies.

3.3 Calculations

The variation of the data that was gathered was calculated by using the following formula.

$$\boldsymbol{v = \frac{S_e^2}{n_e\bar{X}_e^2} + \frac{S_a^2}{n_a\bar{X}_a^2}} \qquad \textbf{(1)}$$

The independent studies taken were summarized and given weights. For such meta-analysis where, independent studies are used weighted means are used normally since the individual studies that are taken in consideration differ in their statistical precisions. This means that studies which have smaller standard errors have greater statistical precision therefore more weight is given to them. Independent studies which have a greater precision are given more weight which increases the precision for the combined estimate (Gurevitch and Hedges, 1993).

$$\overline{\boldsymbol{lr}}^{*} = \frac{\sum_{\boldsymbol{i=1}}^{\boldsymbol{k}} \boldsymbol{w_i^{*} lr_i}}{\sum_{\boldsymbol{i=1}}^{\boldsymbol{k}} \boldsymbol{w_i^{*}}} \qquad \textbf{(2)}$$

In order to calculate the mass fraction values the simple formula was applied to find the stem, root and leaf mass ration respectively.

$$\textbf{Stem/ Root/ Leaf Mass Fraction =}\frac{\boldsymbol{Biomass\ at\ Elevated\ Levels}}{\boldsymbol{Biomass\ at\ Ambient\ Levels}} \qquad \textbf{(3)}$$

3.4 Partitioning variance between and within groups

Meta-analysis test the homogeneity in between the categorical groups like angiosperms and gymnosperms with respect to effect size, and whether there are other differences in mean responses between the groups (Curtis, 1998). The classification of data was done into angiosperms and gymnosperms. Further classification was done into whether the trees are evergreen broad leaf/needle leaf or deciduous broad leaf/narrow leaf. The response and homogeneity can be tested within these categories as well using effect size. Since all of the characteristics along with the difference in the treatments that they were exposed to had a significant impact on the assimilation of CO_2 into the trees biomass into the different components. The different treatments that were taken in consideration along with the elevated CO_2 were chosen because these treatments have a vital role in tree growth in various different processes. Therefore, it was important to see if the absence or enrichment of these other treatments i.e. nitrogen, ozone, temperature and drought conditions had a significant impact on CO_2 enrichment in trees. Whether these treatments diminished or enhanced the assimilation of CO_2 into the tree and whether these treatments shifted the allocations patterns that were seen in ambient and elevated levels of CO_2 enrichment only.

One of the main goals of the meta-analysis is to understand how the elevated levels of CO_2 influence the assimilation of carbon into biomass in the tree species. As well as how there is a shift of biomass allocation in the elevated levels, whether the allocation stays the same or is there any change in how a tree allocates the carbon into its various parts (i.e., stem, roots, leaves, branches).

The meta-analysis was done in a multi-factorial way where other treatments (i.e., drought, nitrogen, ozone, and temperature) effect on the carbon allocation was also studied. The response to elevated carbon is affected by other treatments that are also under consideration of different studies therefore the meta-analysis also aims to find that how the different treatments.

CHAPTER 4

4. RESULTS AND DISCUSSION

For this research, the analysis was done on R studio and Microsoft excel. The forest plots were created within the R studio using the R script while the bar graphs for mass fractions were generated in MS Excel with statistical treatments applied to them.

4.1 Change in biomass

The research study was set up to know how the trees have gained carbon in terms of biomass allocation in the various parts of the tree that can be categorized as stem, roots and leaves in ambient and elevated levels of CO_2. The difference in biomass allocation or the overall gain in tree biomass in the elevated levels of CO_2 is important for the research to generate results. The change in biomass was calculated and presented in the forest plots in percentage change in biomass with response to the CO_2 and other treatments i.e., nitrogen, temperature, drought, and ozone. The overall effect size was studied to deduce how the trees have responded to the treatment in terms of biomass change.

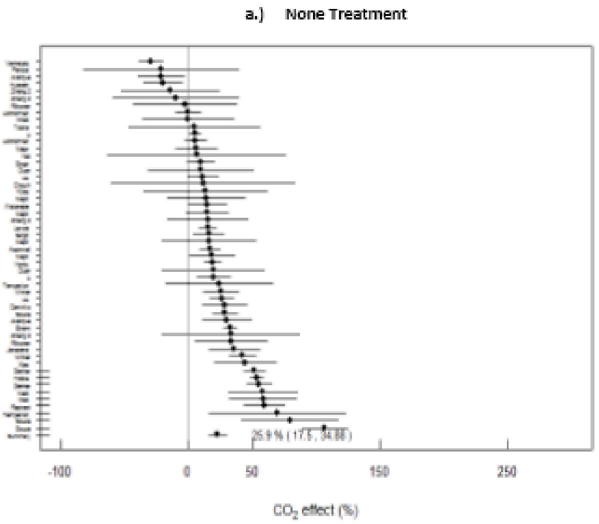
4.1.1 Percentage change in total biomass

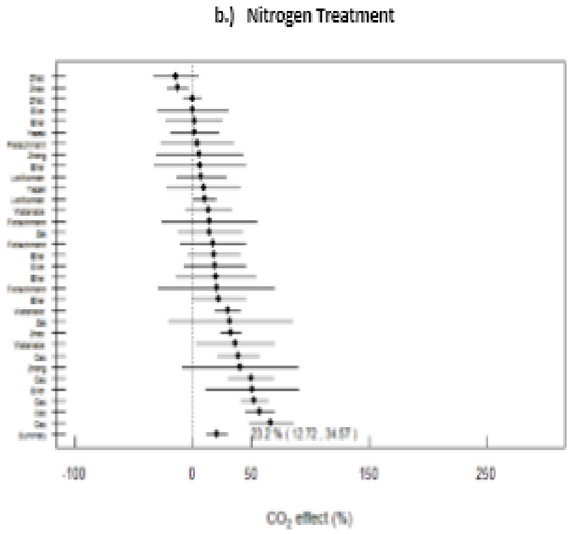
The tree's total biomass is the biomass accumulation in the overall tree that is the accumulative biomass gain in the leaves, stem, and roots of a tree. The total biomass is always equal to the sum of the biomass of leaves, stem, and roots. For every experimental setup for a specific specie of a tree, there were two levels at which the total biomass was recorded i.e., ambient, and elevated level of CO_2, which were then extracted from the papers and used in our data table. To understand how the trees have responded to the change of CO_2 levels the gain in biomass in the different parts of the trees has to be analyzed. For this particular analysis, we measured the gain in biomass in terms of percentage change in the tree's biomass in the elevated levels of CO_2 as compared to the normal levels of CO_2. The total biomass change can be seen in Figure 1 for the different treatments. The studies which only took elevated levels of CO_2 have been placed under the "no treatment" in Fig. 1(a). We can see in the "no treatment" forest plot for total biomass data the total change in plant biomass is 25.9%. This means that studies which only investigated the effect of carbon enrichment under the ambient and elevated levels of CO_2 with no focus on any other treatment like nitrogen, ozone, drought, and temperature treatments were taken in consideration and plotted into this forest plot. The overall change which is the mean of all studies shows that under elevated levels of CO_2 the trees gained 25.9% more biomass as compared to the ambient levels biomass gain. So, for the effect of elevated levels of CO_2 it can be said that the trees show a faster growth in terms of biomass accumulation when they are given optimal growing environment with elevated levels of CO_2.

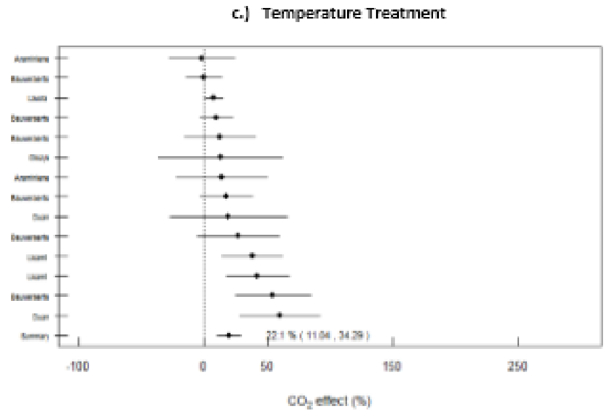
The forest plot 1(b-e) are plots of carbon enrichment studied in trees at ambient and elevated levels of CO_2 along with another treatment variable such as nitrogen, temperature, drought, and ozone. These other variables are taken in consideration to study how the addition of these variables may affect the change in total biomass of the trees since these variables hold an important role in many processes that are linked directly or indirectly to the gaining of the biomass or tree growth. The addition of these variables may or may not affect the tree growth in a positive or a negative way which means that there may be either an increase in the tree growth or may hamper the fast growth of tree that is seen when the tree is grown in the optimum environment that is needed for its growth. When the percentage biomass change value of “no treatment” is compared with the other treatments, we see no as such significant change in values when it comes to nitrogen and temperature, while there is a significant increase in the biomass percentage when compared with drought and ozone. Studies which also investigated nitrogen treatment shows a 23.2% of biomass increase while the studies with temperature treatment shows a 22.1 % gain in biomass. A slight better response in terms of mass gain is seen in treatments which had drought in consideration into them along with fertilization of carbon dioxide which shows a 35.8% of increase in biomass in response to the respective treatments that were observed in these studies.

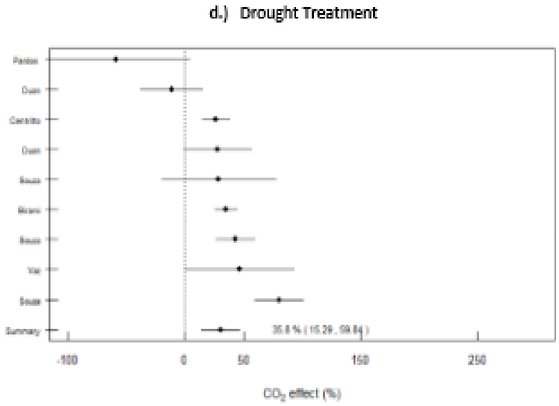
In Fig. 1(b), it can be seen that some studies showed a negative effect towards the nitrogen treatment. The reason for this negative biomass growth percentage is that these studies investigated the CO_2 effect along with low levels of nitrogen. Nitrogen is a very important for the growth of a plant or tree therefore when low levels of nitrogen are there in a study the tree shows a decrease in biomass accumulation as compared to the biomass accumulation that was seen under the ambient levels of CO_2. So, a low source of nutrient availability shows a decline in growth hence proving that along with increase in CO_2 plants need more nutrients as well to show a positive response towards elevation of CO_2. to aid the rapid growth or biomass accumulation in the tree.

The studies that are in account for ozone effect are relatively few due to which the statistical power of the results is low as well therefore it is hard to make any statistically inferred analysis from such results. The difference in the values of biomass change that can be seen in the ozone Fig. 1(e), which is 49.8% significantly more that can be seen with treatments that have taken only elevated levels of CO_2 into account and not ozone. So, ozone has a higher effect in relation with elevated levels of CO_2 in terms of benefitting the trees to gain more mass rapidly in experiments. Although the number of studies that are taken in consideration for ozone are relatively less as compared to the other treatments that is why there could be a significantly higher mean of biomass change in studies which took ozone in consideration.









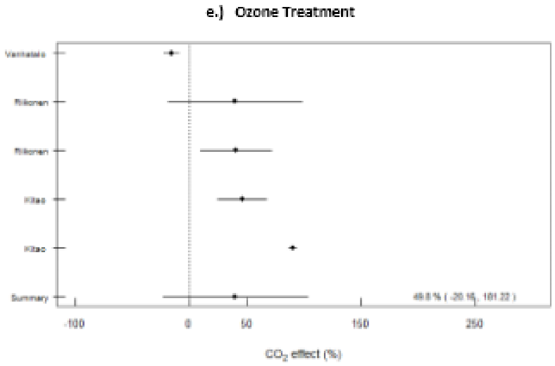
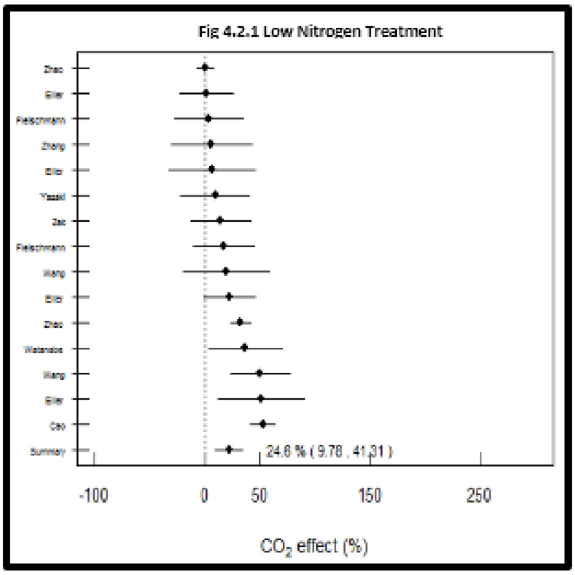


Figure 4.1: Total Data Forest Plots; the x-axis represents the change in biomass accumulation in elevated CO$_2$, the y-axis represents the studies that have been included and plotted against the first author's name. the parentheses define the confidence intervals of the summary of all studies along with the average effect size mentioned towards the left side of the confidence intervals.



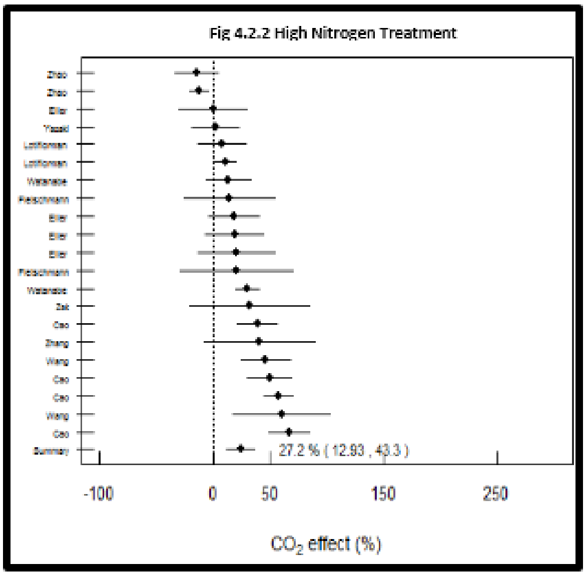
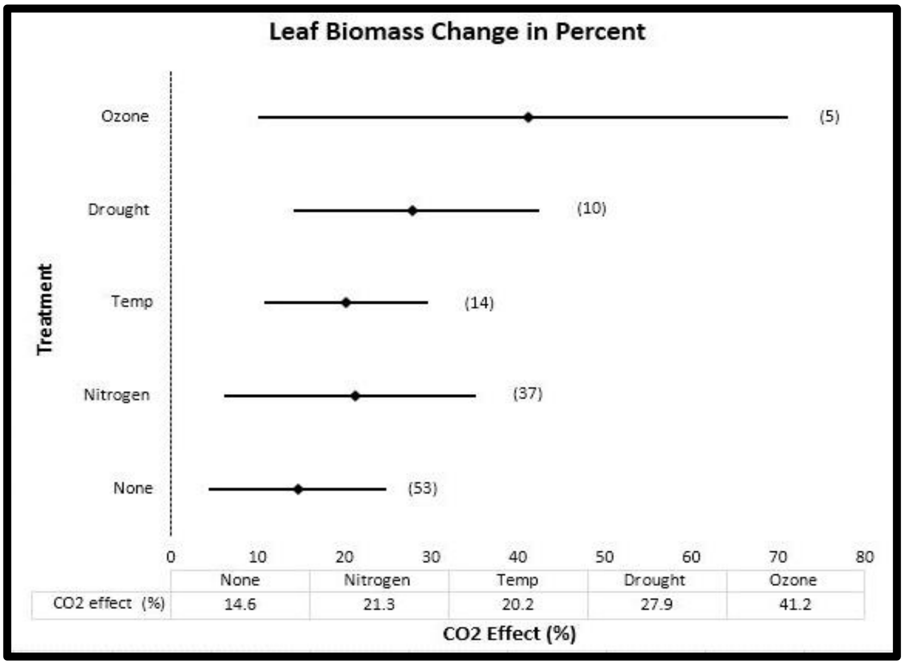


Figure 4.2.1&2: Low Nitrogen Treatment & High Nitrogen Treatment Forest Plots; the x-axis represents the change in biomass accumulation in elevated CO_2, the y-axis represents the studies that have been included and plotted against the first author's name respectively. The parentheses define the confidence intervals of the summary of all studies along with the average effect size mentioned towards the left side of the confidence intervals. The two forest plots are made on studies with high and low nitrogen treatments respectively.

Nitrogen is known as the building block for the plants tissue and is therefore very important for growth. The studies that were taken in consideration looked into two different approaches when it comes to nitrogen being a variable for treatment along with elevated CO_2. The nitrogen is provided to the trees through nutrients that are given to the plants which include fertilization of the soil so therefore the treatments were categorized as low or high nutrients. The trees were provided with either high nutrients in elevated levels of CO_2 or were starved of the nutrients which is termed as low nutrients to study how they impact the growth under ambient and elevated levels of CO_2. For this, we further breakdown the total nitrogen into high and low nitrogen (nutrients) treatments for the total biomass accumulation. The purpose of segmenting the data furthermore on this basis of nitrogen concentration was to determine whether the carbon enrichment is affected positively or negatively by the nitrogen treatment that is given to the trees. Studies that had both treatment of high and low nitrogen monitored over a certain species were added into a subgroup of the database. After which the forest plots as seen above show the effects of nitrogen on carbon enrichment. For the CO_2 effect percent, it is evident that low nitrogen treatment shows a 24.6% CO_2 effect which is lower than the higher nitrogen treatment value which is 27.2%. There is a difference of 2.6%, which points to the fact that higher nitrogen availability leads to significantly more growth in trees under elevated levels of CO_2.

4.1.2 Percentage change in leaf biomass



	None	Nitrogen	Temp	Drought	Ozone
CO2 effect (%)	14.6	21.3	20.2	27.9	41.2

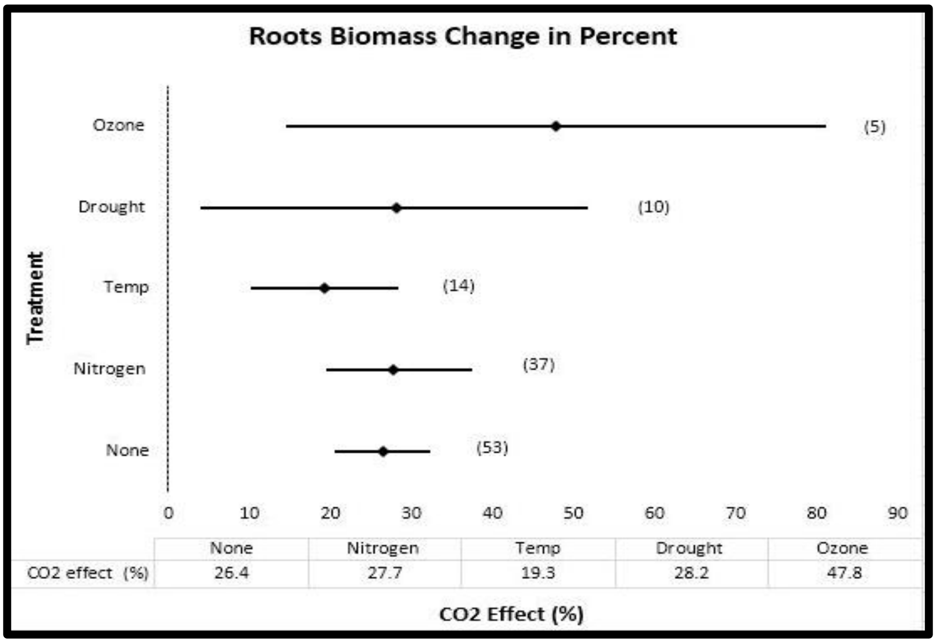
Figure 4. 3: Leaf Biomass Forest Plot; the x-axis represents the change in biomass accumulation in elevated CO_2, the y-axis represents the treatments plotted against the x-axis. Inside the parentheses the number of studies that are incorporated in the specific treatment is written. The table below gives the summary of each treatment in CO_2 effect (%).

Analyzing the percentage gain in leaf biomass is one of the objectives to understand how the leaf biomass accumulation is affected by CO_2 enrichment. Figure 4.3 is the forest plot for the leaf biomass change in percent. The forest plot shows that elevated carbon levels also significantly increase the leaf biomass, which shows an overall positive trend towards the accumulation of biomass in leaf. The results suggest that there is an enhanced response of the other treatments on the tree growth under elevated levels of CO_2. Although the most increase is seen in ozone which can most likely be due to difference in response of the genotypes although due to low power of statistically inference no definite reason can be concluded or given (Kitao *et al.*, 2015). The changes in biomass accumulation that was seen in the studies that also monitored the ozone as a second treatment show that the different rate of biomass accumulation maybe due to the difference of genotype difference between the same species of the tree, The low power of statistical inference is due to the low number of studies that were added into the ozone treatment. As there weren't many studies that we could infer our results from therefore the results concluded from them may not be an accurate picture for the wider database.

4.1.3 Percentage change in root biomass

Similarly, the response of roots to the carbon enrichment treatment is to be assessed by the change of biomass accumulation in the elevated levels when compared to the ambient levels of CO_2. An overall positive trend can be seen towards biomass accumulation in the roots under elevated levels of carbon and other treatments. The biomass increase in roots under temperature is 19.3% which shows that higher levels of temperature significantly affect the roots of a tree therefore slowing down the growth of trees under elevated levels of carbon along with higher levels of temperature treatments. This indicates that with higher levels of temperature there is a chance of trees responding poorly to the CO_2 enrichment.

4.1.4 Percentage change in stem biomass

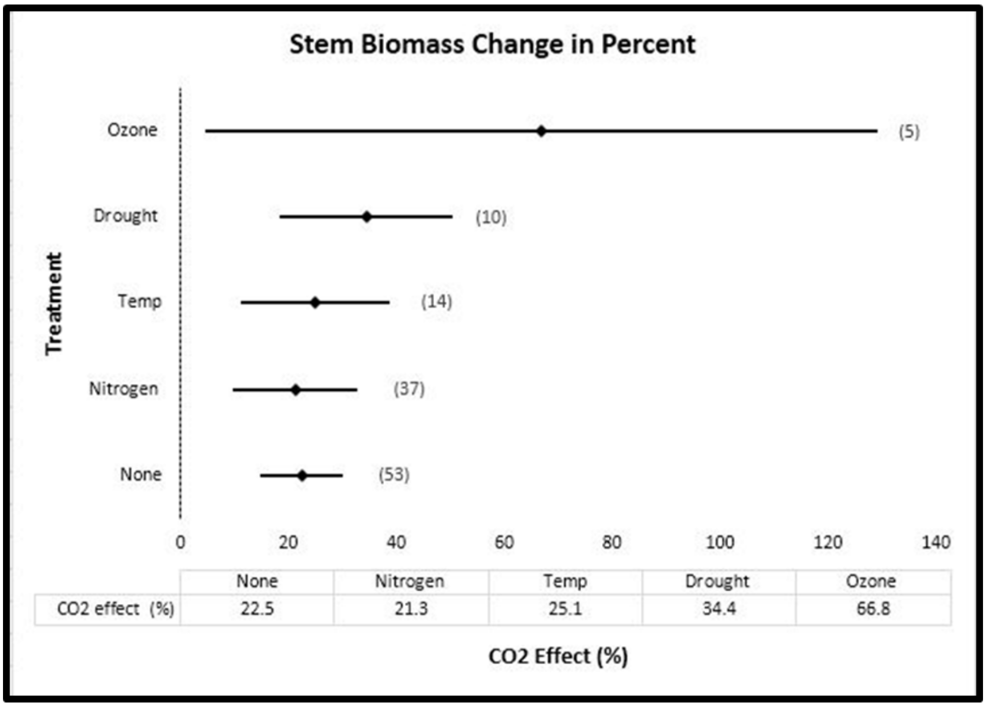


	None	Nitrogen	Temp	Drought	Ozone
CO2 effect (%)	26.4	27.7	19.3	28.2	47.8

Figure 4. 4: Root Biomass Forest Plot; the x-axis represents the change in biomass accumulation in elevated CO_2, the y-axis represents the treatments plotted against the x-axis. Inside the parentheses the number of studies that are incorporated in the specific treatment is written. The table below gives the summary of each treatment in CO_2 effect (%).

The biomass accumulation was assessed in stem for the trees that were taken into account for the study. The aim was to understand how the tree growth has been affected in terms of biomass accumulation in the stem part of the tree the trend was seen in the forest plot that was created that in stem biomass accumulation again an overall positive effect of carbon enrichment along with other treatments as well is observed. Although we see a decrease in stem growth in nitrogen which can be explained by the fact that in nitrogen the shift was moved towards the roots and leaves with a slight less focus on carbon allocation in the stem organ. A higher growth is seen in ozone as compared to the other organs which is 66.8%

which can mean that in ozone the trees focus considerably shifted again in the ozone treatment.



	None	Nitrogen	Temp	Drought	Ozone
CO2 effect (%)	22.5	21.3	25.1	34.4	66.8

Figure 4. 5: Stem Biomass Forest Plot; the x-axis represent the change in biomass accumulation in elevated CO_2, the y-axis represent the treatments plotted against the x-axis. Inside the parentheses the number of studies that are incorporated in the specific treatment is written. The table below gives the summary of each treatment in CO_2 effect (%).

4.2 Allometric Responses

4.2.1 Allocation pattern under ambient and elevated levels

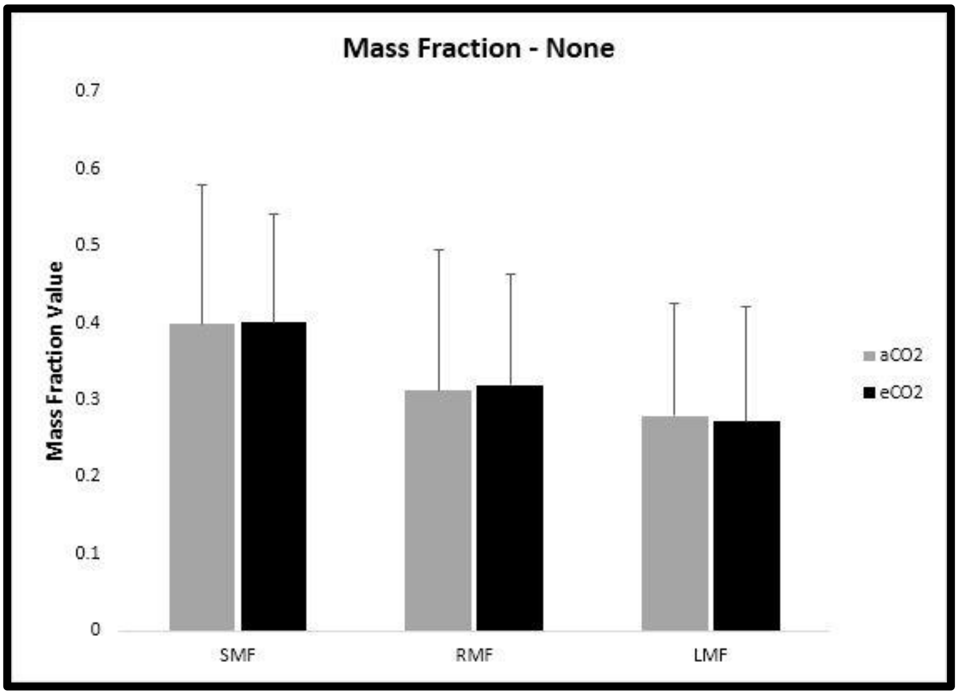


Figure 4. 6. Ambient vs. Elevated CO_2 treatment mass fraction bar graph; the x-axis represents Stem Mass Fraction (SMF), Root Mass Fraction (RMF), Leaf Mass Fraction (LMF) plotted against Mass Fraction Value on the y-axis. The standard error bars are represented on each bar accordingly. The bars in gray represent the mass fractions in ambient levels of CO_2 (aCO_2), while the black bars represent mass fraction values under the elevated levels of CO_2 (eCO_2). None represents that no other treatment was taken in consideration along with elevated CO_2.

Allocation patterns are an important and decisive in this research study since we have to see if allocations patterns are different under the different levels of CO_2 that are used for the carbon enrichment process. Allocation is the way which a tree is using the carbon that is converted into biomass in a specific part of the tree which then contributes to the overall gain in the biomass of the tree. For this research study the breakdwon of thee tree according to allocation patterns was done into stem, roots and leaves. When the tree growth is mentioned it means that the tree has converted the carbon into biomass in the form of leaves, roots and stem. Since most of the studies that were taken into our research studied and conducted the experiments on seedlings and saplings of the trees therefore the terminology of stem is used here instead the trunk of the tree. Looking into the allocation patterns of the trees is therefore important for the research in order to know if there is a shift in how tree will allocate the biomass with the resources that it is provided with. The allocation pattern may differ in a tree under different situations that is the allocation pattern that is seen in ambient levels of CO_2 and after the levels of CO_2 have been increased for the tree. This means that there can be a shift in how a tree allocates the biomass when the trees are exposed to higher levels of CO_2. The best way to look into allocation patterns is by using mass fraction to study how the tree is allocating biomass and how the mass fraction ratio may have changed with the increase of CO_2 that is being provided to the tree.

In the figure 4.6, we see that the mass fraction values at ambient levels are compared with elevated levels of CO_2 with no other treatment. We can see that the SMF, RMF and LMF values are statically non-significant from each other which means that there is no shift in allocation patterns under the elevated levels of CO_2 . In simpler words, the tree biomass allocation patterns are the same in response to elevated levels of carbons as they were when trees were grown in ambient levels of carbon. This shows that under the higher levels of CO_2 the plants response is sginificantly positive towards the mass accummulatin but there is no shift from one part of the tree to another, which means the tree gains mass in the same allocation pattern as it was to normal levels of CO_2.

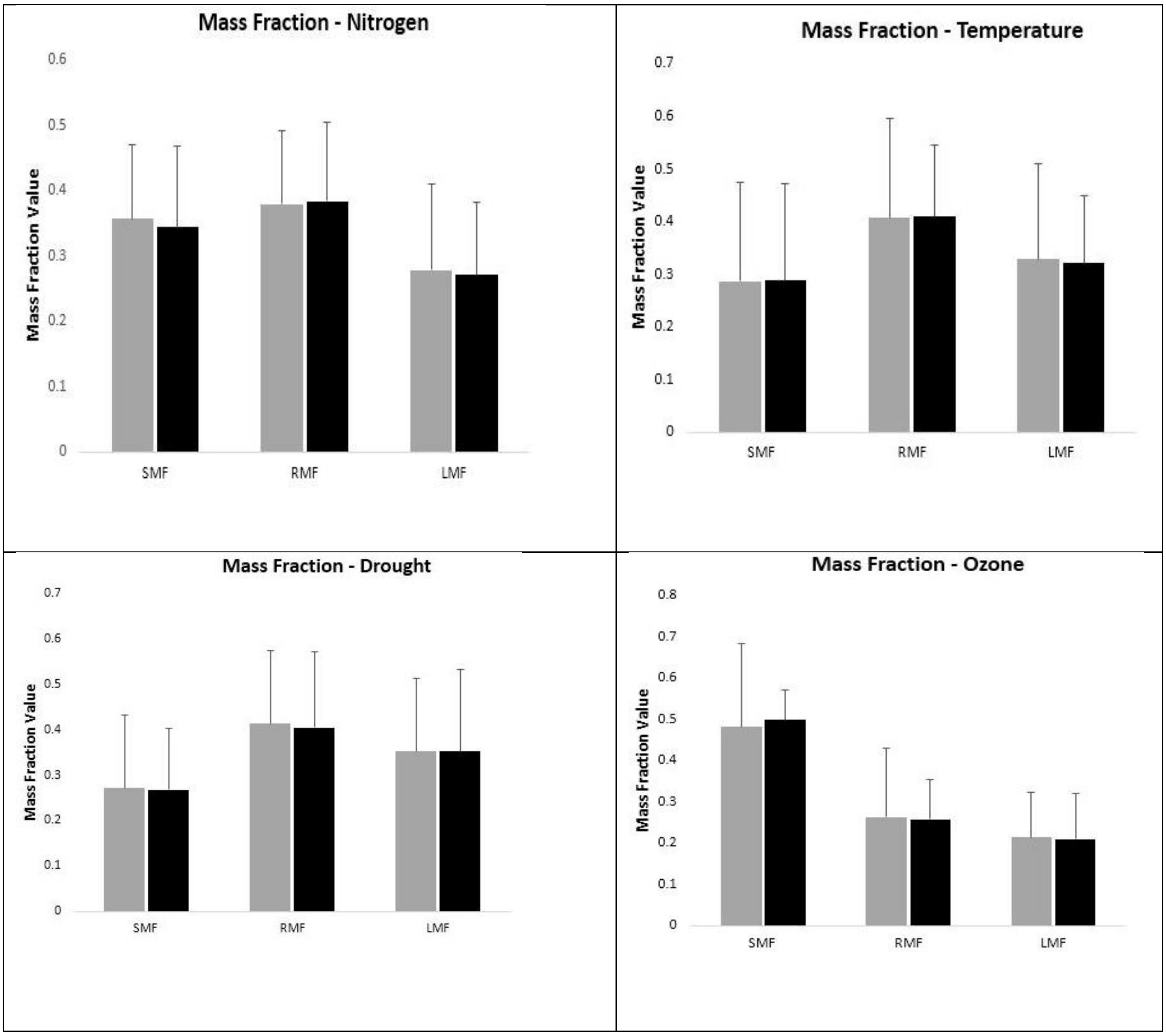


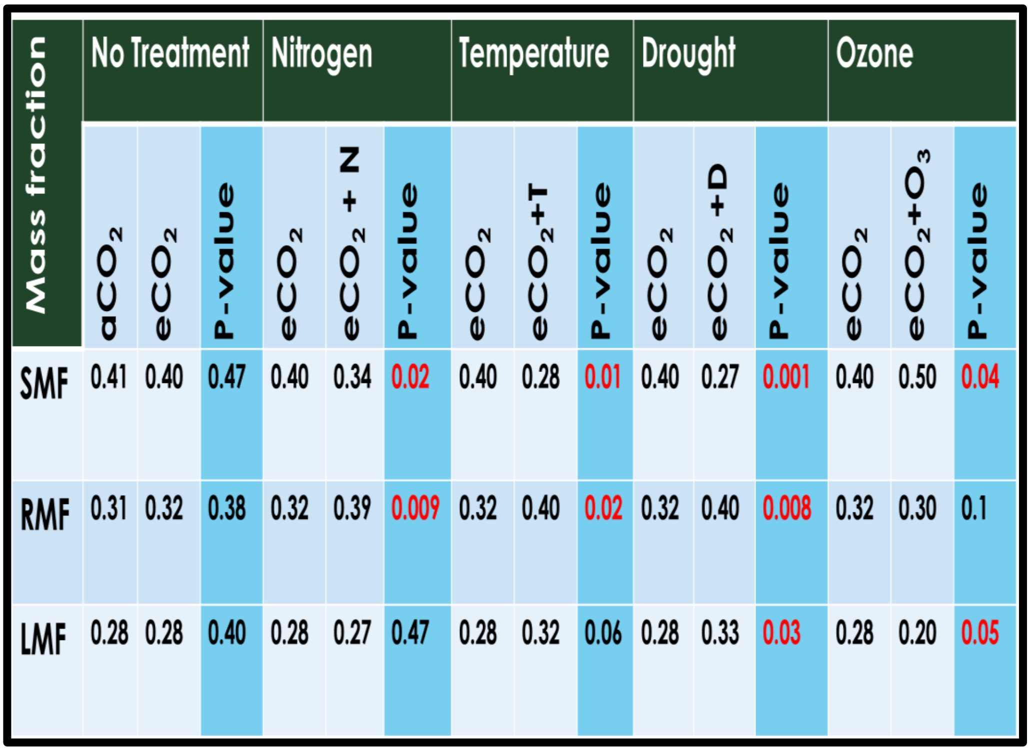
Figure 4.7: Mass Fraction Bar Graphs for Nitrogen, Temperature, Drought, and Ozone under aCO_2 and eCO_2 the x-axis represents Stem Mass Fraction (SMF), Root Mass Fraction (RMF), Leaf Mass Fraction (LMF) plotted against Mass Fraction Value on the y-axis. The standard error bars are represented on each bar accordingly. The treatments mentioned are taken in consideration along with elevated CO_2 in order to study the effect of other treatments.

The research study aimed to look at the impact of other treatments that are selected for this study on the carbon enrichment that is being done under the elevated levels of CO_2. To see if adding another variable into the treatment made the tree assimilate and allocate CO_2 in a different way than it was doing under the elevated and ambient levels of CO_2

with no other treatments. It is important to look into the allocation patterns in trees after another variable was introduced into the system and compare it with the allocations patterns with that of the tree that were seen in the ambient and elevated levels of CO_2 alone. The addition of another treatment into the growing system may affect the allocation patterns resulting in shift of mass accumulation in a tree part which is not normally seen in ambient levels with optimum growing environment for the tree.

In figure 4.7, we can see that when mass fractions of ambient and elevated levels of CO_2 along with other treatments i.e., nitrogen, temperature, drought, ozone we don't see any significant shift of allocation patterns. In order to understand whether the presence of another treatment along with elevated carbon levels had any effect on the carbon allocation patterns in trees these separate forest plots along with CO_2 treatment were also created. The non-significant differences tell us that the patterns that were observed in ambient levels didn't shift in response to elevated CO_2 in synthesis with other treatments. This indicates that the allocation patterns that were seen under the ambient and elevated levels of CO_2 with no other treatment are generally true or the same even after the addition of another treatment. No change or shift of biomass is observed in any of the mass fractions when compared to the ambient and elevated levels of CO_2 with no other treatments. The overall trend that we see is that no changes of allocation pattern were observed in any of the treatment that was taken along with elevated $CO_{2,}$ which shows that carbon allocation isn't affected with the presence of another treatment.

4.2.2 Allometric responses comparison of other treatments with CO_2 enrichment



Mass fraction	No Treatment			Nitrogen			Temperature			Drought			Ozone		
	aCO_2	eCO_2	P-value	eCO_2	eCO_2 + N	P-value	eCO_2	eCO_2+T	P-value	eCO_2	eCO_2 +D	P-value	eCO_2	eCO_2+O_3	P-value
SMF	0.41	0.40	0.47	0.40	0.34	0.02	0.40	0.28	0.01	0.40	0.27	0.001	0.40	0.50	0.04
RMF	0.31	0.32	0.38	0.32	0.39	0.009	0.32	0.40	0.02	0.32	0.40	0.008	0.32	0.30	0.1
LMF	0.28	0.28	0.40	0.28	0.27	0.47	0.28	0.32	0.06	0.28	0.33	0.03	0.28	0.20	0.05

Table 4.1: Comparison of Allometric responses; the table represents the SMF, RMF and LMF in aCO_2 and eCO_2 along with the p-values to determine the significance of changes in the values if there is a considerable shift in the allocation from one part to another. Mass fractions are all reported against the treatments in aCO_2 and eCO_2 respectively.

For the improved understanding of the biomass allocation, we investigated the comparison of mass fractions of trees under eCO_2 with the mass fraction of eCO_2 along with another treatment (eCO_2 + Treatment). The comparison of biomass allocation pattern under elevated levels of CO_2 with the allocation patterns under elevated levels of CO_2 with a secondary treatment is important to determine whether the allocation pattern under the carbon enrichment changes or shifts as compared to the normal allocation pattern that is seen under the elevated levels of CO_2 with no other treatment. The need for this is to determine that the allocation patterns are constant or if there is a shift in them under the elevated levels of CO_2 after another treatment is taken in consideration. The mass fraction values of stem, roots and leaves are statically tested to check whether the difference in those values is there or not. And if there is any difference then statistically how significant those values are is also determined using the p-values that are calculated.

The partitioning theory (Poorter *et al.*, 2012), suggests that the plant will invest its biomass allocation towards that organ which is closest to the limiting resource/treatment. The simple explanation of this theory is that the trees component, which will be in the closest physical interaction with the treatment that is being conducted, is the tree part which will be affected the most by the treatment. This can be either an increase or decrease in the biomass of that specific tree component when compared to the rest of the tree parts.

The roots and leaves can be seen getting more allocation under drought conditions increased allocation to roots can improve the uptake of water and nutrients (Duan, 2018). This is due to the fact during drought conditions the trees adapt by expanding roots length to reach down further to find moisture and water content. The surface area of the roots is also increased to intake more water content through the roots. The leaves similarly see an expansion in size to capture more moisture from the atmosphere. Therefore, we can see an increased mass fraction in roots and leaves, simultaneously a decrease in the stem mass fraction, which supports the optimal partitioning theory of organs discovering limiting substrates which are invested by plants with greater proportion (Poorter et al., 2012). In the above Figure we see how the more availability of nutrients considerably affect the shift of mass fraction from SMF to RMF, while LMF remains undisturbed. The reason for such an allometric response is that when more nutrients are present in the soil for the trees to uptake, the roots surface area and the root hair, which is termed as the fine roots, significantly increases in nitrogen enrichment (Thomas et al., 2001). Therefore, the larger value of RMF can be seen as compared to the “none treatment” with only CO2 enrichment.

In temperature treatment along with the elevated CO2 we can see a considerable shift again from the stem towards the roots along with a slight increase in the leaf mass fraction. The slight increase that can be seen in the leaf mass fraction is due to the fact in higher temperatures there is an increase in stomatal openings which in return increases the photosynthetic activity (Urban et al., 2017). The increase of photosynthetic activity increases the dry biomass for the leaf due to which we can see a non-significant slight increase in LMF. Similarly, with the increase in temperature the soil temperature is likely to increase as well due to which the microbial root cells hence the increase in allocation to RMF under the elevated levels of temperature (Duan et al., 2018).

In ozone treatment we can see there is a considerable shift of mass fraction value from LMF towards the stem this is due to the fact that ozone is damaging to the plant, specifically to the leaf organ of a plant (Riikonen et al., 2004). The damaging property that the ozone holds towards the leaves is the reason why the biomass decreased in the leaves and trees shift towards the stem can be seen under such a circumstance, where it starts allocating more of it carbon into making biomass for the stem. The ozone enters the stomata that is found on the leaves and oxidizes the plant tissue while it is respiring (Kitao et al., 2015). The partitioning theory here thus stands true as ozone is in close interaction with the tree leaves therefore having a negative impact on the biomass of the leaves.

CHAPTER 5

5. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, an overall positive response to carbon treatment was observed which can be seen in terms of overall increase in growth of tree mass as well its organs like leaf, stem, and roots. Nitrogen, drought, temperature, and ozone also have a positive effect on CO_2 enrichment which can be seen in the positive change in percentage of biomass in the total biomass, leaf biomass, root biomass and stem biomass of the trees. Although the results suggest that CO_2 enrichment is most likely to be enhanced if there is more availability of the nutrients for the trees which is evident by the more increase in biomass as compared to low nutrient availability. The effect of temperature also showed a positive growth effect but with the increase of temperature along with the atmospheric carbon dioxide. Similarly, drought treatment along with carbon showed that trees responded positively towards it and the biomass increased significantly faster than it did in ambient conditions. For ozone, not much can be said because of limited data available to make any significant analysis on ozone treatment but overall trees also responded positively towards it. For allometric responses, it can be concluded that the theory for partitioning was backed up by the allometric responses that were seen in the mass fractions. Accumulation of mass increased significantly towards the organ that was closest in interaction or getting affected by the specific treatment. The shift of allocation is in line with the partitioning which was seen in our results.

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