

Exploring the effects of CO₂ elevation on seedlings' growth of Acacia senegal in the era of changes

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Abstract:

Acacia senegal is a priority and important C₃ tree species in drylands of Sudan and across the gum belt. Investigation of its seedlings response to elevated carbon dioxide (eCO₂) is important as atmospheric ([CO₂]) has increased and predicted to continue to rise. Many studies showed that eCO₂ causes increased photosynthesis in plants, which leads to greater production of carbohydrates and biomass, and increased soil organic matter and carbon content. This study investigated the effects of eCO₂ on *A. senegal* seedlings grown in sand and silt soils under irrigation intervals of every day and every two days. Seven days old seedlings were assigned to the treatments in Split - split plot design for 4 weeks. The main plot is eCO₂ (600-800 ppm) and ambient (≤ 400 ppm) under Free Air CO₂ Enrichment (FACE) system. Subplots are irrigation intervals and soil types. Seedling height and number of leaves were measured weekly, and seedlings were harvested after 4 weeks where growth parameters and soil properties were measured. The eCO₂ showed no effect on the measured parameters except the significant increase in tap-root length. However, the irrigation every day showed significant increase than every two days in seedling's height, number of leaves, root length and seedling's dry weight but not seedling's and soil C% & N%. Soil treatment showed effects on stem height, leaf number, seedling's dry weight, leaves and root N% and soil C% but not root length, seedling C% and soil N%. The results indicate the importance of soil moisture, physical and chemical properties that reflects adaptation of the species to its dry land environment.

Keywords:

Acacia senegal; climate change; drylands; elevated CO₂; Savanna; Sudan

1. INTRODUCTION

Atmospheric carbon dioxide (CO₂) emission and concentration has risen since the start of the industrial revolution [1] and continued to rise [2-4]. Trees and plants may respond to rising CO₂ as elevated atmospheric (eCO₂) acts to increase photosynthetic activity [5], plant growth and plant biomass production of many plant species [6,7]. Drylands vegetation include C₃ and C₄ trees, shrubs and grasses [8]. In some of eCO₂ experiments, C₄ plants showed little or no enhancement of growth (dry matter production) in contrast, C₃ species showed 3 times of that experienced by C₄ plants in stimulation of photosynthesis by eCO₂ [9].

The acacias are important C₃ dryland species [10]. *A. senegal* is a multi-purpose tree producing gum Arabic a high-value export commodity from Sudan and some African countries, and important component of traditional dryland agroforestry resilience system and source of livelihoods in the Sudan [11]. The tree also provides animal fodder, multiple timber products, intercropping, firewood, food and medicines [12,13]. Furthermore, it is one of the most important sub-Saharan African trees inhabiting Savanna systems that are under threat of ongoing anthropogenic and climate-mediated degradation and that have led to substantial losses of natural habitats [14].

Seedlings are most responsive to eCO₂ where the early growth enhancement under eCO₂ accelerates ontogeny and pattern of growth [15,16]. The effect of eCO₂ on acacias as important components of the dryland natural plant communities' needs to be studied. Soil types and soil moisture content are important determinants to *A. senegal* response [17]. The methodological and experimental developments such as the Free-Air Carbon Enrichment (FACE) are effective

way to quantify the effects of eCO₂ on trees in field settings [18,19]. The FACE designs provide means for studies without any direct perturbation of microclimate [20]. Therefore, the objective of the paper is to experimentally examine the responses of *A. senegal* seedlings to eCO₂ under varying water and soil conditions using the FACE design.

2. MATERIALS AND METHODS

2.1. Study site & settings:

The experiment was conducted in the nursery as Split-split plot design with the CO₂ treatments as main plots, and the water interval and soil types as subplots. The nursery of the Faculty of Forestry, University of Khartoum at Shambat, Khartoum North-Sudan (15° 40' 5" North, 32 32' 1" East). Shambat has a subtropical desert / low-latitude arid hot climate. The experiment was conducted using FACE system for eCO₂ in the range of 600 to 800 and ambient treatments. The watering intervals were every day and every two days, while the soil treatments are sand and silt. Bulk seeds collected from El-Damazeen forests was obtained from the National Tree Seed Center and germinated in polymer bags of 10×20cm filled with silt or sand soils) and irrigated daily. After one week 60 seedlings per soil type were selected with minimum morphological variation (i.e. almost same size & branching pattern) among them. They were then assigned randomly in the experimental plots. The plot was divided into 4 subplots of five seedlings. Sixty seedlings of equal size from each soil type were assigned randomly to irrigation treatments. Three pairs of 1m×2m plots were prepared and each one assigned randomly for the eCO₂ and the other for the ambient CO₂ treatments. Then each plot was divided into two parts of 1×1m, and assigned randomly for the irrigation treatments every day and every two days. Then 5 seedlings

from those raised in silt soil and 5 ones from sandy soil were assigned randomly to each of the watering treatments (total of 120 seedlings, 60 silt and 60 sand).

2.2. Measured variables:

Seedlings' height was measured and new leaves were counted weekly. After four weeks the plants were harvested, length of the tap root was measured and dry weight of the leaves, stems and roots was weighed for each plant separately. Seedling's C% and N% were determined using CHNS-O Analyzer and applying Standard Test Method (ASTM International, model D 5291-02. 2002, USA) for instrumental determination of carbon and nitrogen of plant.

Soil C% measurement is based on the oxidation of organic C with dichromate in acid medium [21] and soil N% was measured by using Bremner's method [22].

2.3. Data analysis:

The Analysis of Variance (ANOVA) procedures and Duncan's Multiple Range Test to separate means of the same factor at significance were carried out using SAS. The model is Split-split plot with three blocks, CO₂ (main plot), watering interval and soil type within the watering interval. The model used in the experiment was:

Y (dependent variable) = B (block effect) + C (CO₂ concentration) + $B \times C$ + W (irrigation interval) + $B \times C \times W$ + S (soil type) + $C \times S$ + $W \times S$ + $C \times W \times S$.

3. RESULTS

3.1. Effects of eCO₂ concentration on seedlings' growth parameters:

The eCO₂ concentration had no effect on seedling's height, number of leaves per seedling after 1 week, 2 weeks, 3 weeks and 4 weeks from the start of the experiment. In addition to leaves, stem, root, total dry weight, C% and N% were not affected (Table 1). Similarly, eCO₂ had not significantly affected soil media C%, N% and C/N. While tap root length was significantly affected by CO₂ elevation (P=0.002) as eCO₂ had decreased tap root length by 16% (P≤ 0.05; Table 1).

3.2. Effects of irrigation interval on seedlings' growth parameters:

Irrigation interval had significant effect on seedling's height, leaf number and tap root length at the end of the first, second, third and fourth week (p≤ 0.050 -< 0.0001; Table 2). Irrigation every day had resulted in increase in seedling's height by 42%, 36%, 37% and 46% by the end of first, second, third and fourth weeks, respectively. Also, It increased number of leaves per seedling at the end of each week (45, 40, 37 and 61% respectively). Similarly irrigation every day had increased tap root length by 24% at the end of fourth week (p≤ 0.05; Table 2).

Irrigation interval had affected leaves, stem, root and seedling weights (P ≤ 0.035-0.0001). Irrigation every day, as compared to irrigation every two days, had increased weight of leaves by 43%, stem by 15%, root by 21% and seedling by 15% (p≤ 0.05; Table 2).

Irrigation interval had not affected seedling's C%, N% and its compartments, soil C%, N% and soil C/N. Except root N% was affected (P=0.040) as irrigation every day had decreased root N by 6%. However, the daily irrigation was numerically lower C/N than two days irrigation but not significant (p≤ 0.05; Table 2).

3.3. *Effects of soil type on seedlings' growth parameters:*

Soil type showed significant effects on seedling's height (first, third and fourth week) and leaf number (second, third and fourth week) but not length of tap root ($P=0.027-0.0001$; Table 3).

The silt soil had increased seedling's height by 66% for the first week, by 29% for the third and by 31% for the fourth week. Also, silt soil had enhanced leaf number by 22% for the second, by 26% for the third and by 33% for the fourth week ($P \leq 0.05$; Table 3). Silt soil gave longer root (9%) but not significant.

Soil type had significant effects of leaves' dry weight ($P < 0.0001$), stem ($P=0.016$) and seedling ($P=0.0019$) but it had slightly insignificant effects on root's dry weight ($P=0.061$). Consequently, silt soil had higher dry weights in leaves by 30%, stem by 15% and seedling by 24% ($P \leq 0.05$; Table 3).

Soil type had no effects on seedling's, leaves', stem's and root's C and N content. Similarly, soil N% and C/N were not affected by soil type, but soil C% was affected by soil type ($P=0.003$). However, the N% and C/N of silt were numerically higher than sand soil.

4. DISCUSSION

4.1. *Effects of eCO₂ concentration on growth parameters:*

The insignificance of eCO₂ on growth parameters (height, leaf number, root length, seedling dry weight and seedling C%, N%) is in line with many other literatures [23-25]. On the other hand, eCO₂ is known to increase plant growth productivity and consequently has stimulated overall forests biomass growth [26-29]. Such discrepancy might be due to differences in genetic characteristics of the studied species, duration of studies, CO₂ elevation (exposure) techniques, and sites microclimates. Our results can be explained as, less photosynthetic machinery that

made by N deficiency or other nutrients causing lower rates of photosynthesis resulted in no increasing in net primary productivity (NPP).

4.2. Effects of eCO_2 concentration on soil carbon and nitrogen content:

The irresponsive of soil C%, N% and C/N to eCO_2 is in agreement with various studies [30,31]. On the other hand, the results contrast the findings of [32,33]. The difference of these results can be attributed to the adverse response through down regulation of photosynthesis when plants exposed to higher CO_2 concentrations beyond the certain thresholds, or the rapid rate of CO_2 assimilation requires correspondingly other nutrients specially foliar N which is experienced to be declined under elevation CO_2 .

5. CONCLUSION:

The irresponsive of most measured variables of *A. senegal* to eCO_2 concentration and the high significant effects of water and soil factors can be attributed to the long time adaptation of the species in drylands of Sudan to water and to some extent soil type. Generally, the growth parameters of the *A. senegal* seedlings were more responsive to the environmentally limiting factors in its natural habitat, such as soil moisture content and soil chemical and physical properties.

Nevertheless, our study was limited in a number of aspects including sample size, duration & design of the experiment. Further studies on incorporating some or all of these factors will give a better picture about responses of *acacias* to eCO_2 in dryland settings.

According to the results and general trends of this study, it is recommended that:

1- Water availability is the most important factor of eCO_2 for seedling growth and hence water harvesting and management will play a key role in the context of elevation of CO_2 .

3- In drylands where it characterized by low level of precipitation, planting seedling in silt soil is recommended for greatest growth and productivity.

4- More long-term experiments on *A. senegal* are recommended to evaluate the effects of eCO₂ for long term periods as some measured variables seems to be affected by time (e.g. biomass).

5- Other interacted factors with CO₂ like thermal stress and nutrient limitation should be investigated for better understanding of *Acacias*' response to CO₂.

6- The responses of the most important C₃ tree species in drylands Sudan like *Acacia nilotica*, *Acacia seyal*, ..etc to CO₂ elevation need to be evaluated for long and short terms.

7- FACE systems (are being constructed in the USA, and now widely used in other places in the world) can be used sufficiently to study effects of CO₂ elevation on other trees and crops under natural conditions in Africa.

8- With regard to the net photosynthesis and stomatal responses it would be advantageous to monitor them under CO₂ elevation to understand up-regulation or down-regulation of photosynthesis and to show *A. senegal* stomatal response to eCO₂ and under what conditions this occurs. The responses of stomatal conductance and canopy leaf area to eCO₂ are important specially in drylands to determine both the short and long-term risk of exposure to drought.

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TABLES

Table 1: Effects of CO₂ concentration on seedling's height, number of leaves, root length, seedling's biomass weight and seedling and soil carbon & nitrogen contents after 4 weeks.

(1) Seedling growth/cm			
	<i>eCO₂</i>	<i>CO₂</i>	<i>P value</i>
1. Stem height: Week1	4.52	4.43	0.89
Week 2	5.89	5.25	0.25
Week 3	13.71	12.86	0.16
Week 4	16.52	15.86	0.30
2. Leaf number: Week1	6.65	7.74	0.23
Week 2	12.93	12.32	0.54
Week 3	19.38	18.57	0.59
Week 4	25.07	23.81	0.55
3. Root length	9.72a	11.62b	0.002
(2) Biomass weight/g			
1. Leaves dry weight	0.64	0.66	0.423
2. Stem dry weight	0.45	0.43	0.423
3. Root dry weight	9.00	8.65	0.607
4. Seedling dry weight	1.49	1.35	0.072
(3) C&N contents and ratio			
1. Seedlings C%	41	43	0.610

Leaves C%	33.6	42	0.346
Stem C%	44.5	45.9	0.816
Root C%	41.79	39.95	0.696
2. Seedlings N%	3.2	3.3	0.725
Leaves N%	4.57	4.47	0.056
Stem N%	2.4	2.3	0.378
Root N%	2.0	2.4	0.101
1. Soil C%.	0.48	0.58	0.255
2. Soil N%	0.14	0.16	0.457
3. Soil C/N	11.0	11.2	0.962

370 **Table 2:** Effects of irrigation interval on seedling's height, number of leaves, root length,
 371 seedling's biomass weight and seedling & soil carbon and nitrogen contents after 4 weeks.
 372

(1) Seedling growth/cm			
	<i>1d irri.</i>	<i>2 d irri.</i>	<i>P value</i>
1. Stem height: Week1	5.24a	3.70b	0.050
Week 2	6.42a	4.72b	0.011
Week 3	15.34a	11.23b	<0.0001
Week 4	19.20a	13.18b	<0.0001
2. Leaf number: Week1	8.50a	5.88b	0.0157
Week 2	14.73a	10.52b	0.0022
Week 3	21.92a	16.04b	0.0042
Week 4	30.16a	18.72b	0.0005
3. Root length	11.83a	9.51b	0.0007
(2) Biomass weight/g			
1. Leaves dry weight	0.77a	0.54b	<0.0001
2. Stem dry weight	0.47a	0.41b	0.029
3. Root dry weight	9.66a	7.99b	0.035
4. Seedling dry weight	0.47a	0.41b	0.029
(3) C&N contents and ratio			
1. Seedlings C%	43.2	41.6	0.466
Leaves C%	42	33	0.34

Stem C%	46.4	44	0.689
Root C%	39.13	42.61	0.466
2. Seedlings N%	3.4	3.2	0.461
Leaves N%	4.48	4.56	0.648
Stem N%	2.4	2.4	0.432
Root N%	2.0 a	2.12b	0.040
1. Soil C%.	0.56	0.50	0.483
2. Soil N%	0.18	0.12	0.112
3. Soil C/N	11.2	11.1	0.969

Table 3: Effects of soil type on seedling's height, number of leaves, root length, seedling's biomass weight and seedling & soil carbon & nitrogen contents after 4 weeks.

(1) Seedling growth/cm			
	<i>silt</i>	<i>sand</i>	<i>P value</i>
1. Stem height: Week1	5.59a	3.36b	0.0113
Week 2	5.933	5.217	0.205
Week 3	14.95a	11.62b	0.0003
Week 4	18.35a	14.04b	<0.0001
2. Leaf number: Week1	7.81	6.57	0.186
Week 2	13.90a	11.35b	0.027
Week 3	21.18a	16.77b	0.017
Week 4	27.90a	20.99b	0.009
3. Root length	11.14	10.20	0.06
(2) Biomass weight/g			
1. Leaves dry weight	0.74a	0.57b	<0.0001
2. Stem dry weight	0.47a	0.41b	0.016
3. Root dry weight	9.55	8.10	0.061
4. Seedling dry weight	1.57a	1.27b	0.0019
(3) C&N contents and ratio			
1. Seedlings C%	42.8	42	0.722
Leaves C%	42	33.68	0.346

Stem C%	45.7	44.7	0.937
Root C%	40.31	41.43	0.813
2. Seedlings N%	3.4	3.2	0.505
Leaves N%	4.72a	4.32b	0.045
Stem N%	2.5	2.1	0.444
Root N%	2.5a	2.0b	0.0149
1. Soil C%.	0.69a	0.37b	0.003
2. Soil N%	0.17	0.13	0.217
3. Soil C/N	13.3	9.0	0.308