



Case research on the effects of drought on carbon assimilation within the plant-soil system

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Abstract

Global warming is one of the biggest challenges, the world is facing at the moment and with the current rate of warming, serious consequences to climate have been predicted. Climate change may affect the ecosystem functioning through increased temperatures or changes in precipitation patterns. Water availability is an important driver for various ecosystem processes that may affect the supply of carbon in below-ground pools and alter important ecosystem processes involved in carbon cycling. The objective of this study was to determine the effect of drought stress in the allocation of carbon in different pools within the plant-soil system with *Zea mays*. The conceptual approach included repetitive ¹⁴C₂ pulse labeling of plants grown under drought and optimum moisture levels in order to follow above and below-ground C allocation. After ¹⁴C pulse labeling, ¹⁴C allocation in different pools such as shoots, roots, soil and soil respiration was traced. Incorporation of fresh assimilates in different soil aggregate fractions were also determined by tracing ¹⁴C in different soil aggregates. The study found the drought conditions increased the release of root exudates enhancing the rhizomicrobial respiration (¹⁴CO₂). Also, an increase in fresh carbon assimilates (¹⁴C) into the micro-aggregate fractions compared with optimum moisture levels was found. Thus, maize plants performed quick adaptive response to drought stress by maintaining their biomass and translocating higher photosynthates towards roots for efficient water uptake.

Keywords: Drought, ¹⁴C pulse labeling, *Zea mays*, soil respiration, soil aggregates

Introduction

Global warming is possibly the biggest challenge the world is facing at the moment (Lovelock, 2006) ^[44]. Serious consequences to climate are predicted with the current rate of warming resulting in increased sea level, drought and/or flood prevalence, behavioral change in plants/animals and other associated changes (Carlsson-Kanyama, 1998) ^[11]. Observational and simulation studies on impact of climate change on precipitation suggest that moist regions are getting wetter and dry regions drier (Chou *et al.*, 2007) ^[17] but with a high spatial variability (Allen and Ingram, 2002) ^[1]. One of the comprehensive effects of global warming and climate change is drought and it is likely to increase during the 21st century in many parts of the world (Arnell, 2008) ^[3]. Global warming is likely to exacerbate droughts in many semi-arid, snow-fed, and coastal basins (Kallis, 2008) ^[38].

Drought causes serious alterations in the terrestrial carbon cycle. In some drought-affected areas in China, droughts modified the former carbon sinks into carbon sources and substantially reduced the net primary productivity and net ecosystem productivity (Xiao *et al.*, 2009) ^[61]. Similarly due to severe droughts a huge reduction in the carbon stocks of the Amazon rainforest was found coupled with significant decline of aboveground biomass and productivity (Brando *et al.*, 2008) ^[8]. Elevated CO₂ and climate change also have an impact on global carbon cycle (Friedlingstein *et al.*, 2003) ^[26]. Plant properties have a vital role to play in the C allocation in plant and soil. Carbon allocation pattern varies according to species. Perennial plants allocate higher C in roots while the annual plants allocate more C in the above-ground parts (Kuzyakov and Domanski, 2002) ^[41].

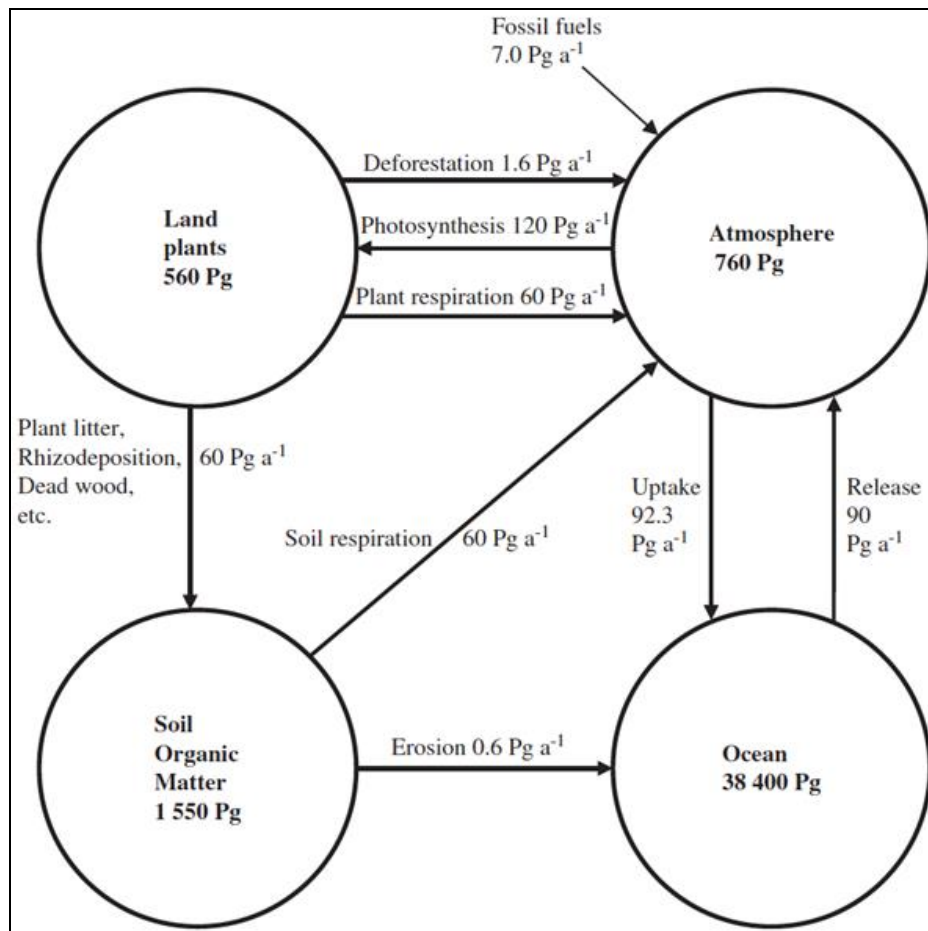


Fig 1: Schematic diagram of the global carbon cycle (Hester and Harrison, 2010) ^[34].

Drought effects on plants are apparent in all phenological stages and the effects range from morphological to molecular levels (Pace *et al.*, 1999) ^[47]. The initial effect of drought is impaired germination and poor stand establishment (Harris *et al.*, 2002) ^[33]. Drought induces stomatal closure, disturbed enzyme activities, reduced CO₂ assimilation, reduced leaf water potential, relative water content, turgor potential, stomatal conductance and transpiration rate (Siddique *et al.*, 2000) ^[54]. It also reduces total nutrient uptake and creates an imbalance in carbon resource utilization (Farooq *et al.*, 2009) ^[24]. One of the major effects of drought is reduced photosynthesis linked with declined leaf expansion, impaired photosynthetic machinery, premature leaf senescence and decrease in food production (Wahid *et al.*, 2005) ^[60]. There is a probability that in the future, plants will experience increase in acute heat and drought stress that would exert serious impacts on ecosystem productivity and biodiversity (Ciais *et al.* 2005) ^[18]. Plant responses to drought vary according to species and their specific adaptations (Chaves *et al.*, 2009) ^[13]. Locally adapted populations from drier habitats and foliage of evergreen species are supposed to withstand drought more efficiently than the deciduous species due to their exposure to wider environmental variations (Hallik *et al.*, 2009) ^[32]. The major mechanisms to withstand drought stress by plants include curtailed water loss by increased diffusive resistance, enhanced water uptake with prolific and deep root systems and its efficient use, and smaller and succulent leaves to reduce the transpirational loss (Pace *et al.*, 1999)

^[47]. Phenological responses to drought include escaping dry periods by shortening the life cycle or the growing season and allowing plants to reproduce before the environment gets dry (Farooq *et al.*, 2009) ^[24] or by harmonizing the growth and development with periods of soil moisture availability (Araus *et al.*, 2002) ^[2]. Some tackle drought physiologically by performing osmotic adjustment, osmoprotection, antioxidant and scavenging defense system or by triggering changes in gene expressions at the molecular level (Farooq *et al.*, 2009) ^[24].

The present study uses a C₄ plant (*Zea mays* L.) for studying the effects of drought on carbon allocation in plants and soil. C₄ plants are known to fix carbon more effectively during moisture stress, high temperature and during limiting levels of carbon dioxide and nitrogen. They do so by using extra energy to bind more CO₂ in their mesophyll cells using the Phospho-enol-pyruvate (PEP) carboxylase (Slack and Hatch, 1967) ^[55]. In a global scale, maize is cultivated on nearly 100 million hectares of land (Shiferaw *et al.*, 2011) ^[53]. Maize is considered to be a large C-sink due to its higher net primary productivity and it is found to effectively capture root-derived C into the soil organic matter 1.5 times more compared to leaves and sheaths (Shen *et al.*, 2018) ^[52]. Maize is characterized by its high-water use efficiency. Maize can survive prolonged drought periods by preventing xylem tensions developed by stomatal closure (Cochard, 2002) ^[19]. Some maize varieties with reduced leaf area, short thick stems, small tassels, erect leaves, delayed senescence, smaller root biomass and a deep root system with little

lateral root branching have also been developed which are better suited to drought stress (Ribaut *et al.*, 2009) ^[49]. Certain properties like rapid leaf folding to avoid water loss, early closing of stomata under water stress, maintaining photosynthesis and growth at lower water potentials and the development of plasticity of both the main stem and tiller allow maize to perform better under moisture stress conditions (Fischer *et al.*, 1982) ^[25].

The general objective of the study was to investigate the effects of drought on carbon translocation in plant-soil system. The specific objectives of the study were as follows:

- To study the effect of drought stress on the distribution of C in different pools.
- To elucidate the effects of drought in soil respiration.
- To determine the change in carbon allocation in different soil fragments due to drought stress.

2. Materials and Methods

2.1 Site Description

The soil samples were taken from the top 25 cm of Haplic Luvisol developed under C₃ vegetation, located on a terrace plain of the river Leine in the northwest of the city of Göttingen, Germany. The collected soil represents a temperate climatic zone with mean annual temperature of 8.7 °C and mean annual precipitation of 645 mm. Collected soil was slightly acidic (pH 6.0 ± 0.1) with a silty texture (7% sand, 87.2% silt and 5.8% clay). Bulk density of this soil was 1.4 g cm⁻³, with organic carbon and total nitrogen content of 12.6 gkg⁻¹ and 1.3 gkg⁻¹ of soil, respectively. The δ¹⁵N and δ¹³C contents of the soil were 8‰ and -27.4‰, respectively. After sampling the soil was air-dried, mixed and passed through a 5-mm sieve.

2.2 Experimental design and growth conditions

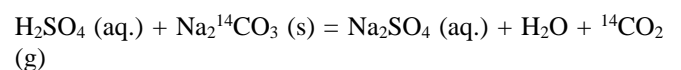
In order to study the effects of water availability on belowground C allocation and root-derived CO₂, a randomized block design experiment was established. Maize (*Zea mays* L.) seeds were soaked for two days in petri dishes for rapid germination. The germinated plants were then sown in the experimental pots containing 3 kg of soil. The experimental setup included planting of 4 maize plants in each pot. To assure three replicates for Maize planted soils and three replicates for unplanted soils at two different water levels, i.e. in total 12pots: 6 pots with planted soil and 6 pots with unplanted soil (control) were incubated for 60 days. The plants were grown at a uniform temperature of 20 °C (day and night) with a day-length of 14 h and light intensity of approximately 400 mmol.m⁻²s⁻¹ at the top of canopy. Macro-nutrients (N, P and K) were added to the soil in the ratio of 2:1:1.

During the first 30 days of plant growth, optimum water level (70% of the available field capacity) was maintained for all plants. After one month of plant development, the soils were adjusted to two water levels: (1) optimum conditions (70% of the field capacity) and (2) drought conditions (30% of the field capacity) for 30 days. The unplanted soil control was also maintained for both moisture levels.

2.3 ¹⁴C Pulse labeling

Pulse labeling with the C isotope is one of the ways to follow the allocation of recent assimilates into respiration,

growth and storage pools. To determine the impact of drought stress on C allocation patterns, repeated ¹⁴C pulse labeling with a week interval were done. The detailed procedure for plant ¹⁴C pulse labeling is given in previous studies (Kuzyakov and Siniakina, 2001) ^[43]. Briefly, the labeling apparatus consisted of two compartments. The lower compartment was used for soil and plant roots and the upper compartment for the shoots and for ¹⁴CO₂ generation. One day before labeling, the hole from which the plants were emerging was sealed with silicon paste. Two hours before first labeling, pots were flushed with CO₂-free air to remove CO₂ evolved prior to labeling. Only the planted pots were labeled. 100 kBq of ¹⁴C was provided to each pot. This activity was mixed in 0.2 M Na₂CO₃ solution and was put in a test tube in the upper compartment of the chamber. After this the chamber was closed and 3 ml of 5NH₂SO₄ was added to the Na₂¹⁴CO₃ solution in the test tube through a Teflon tube. This allowed the complete evolution of ¹⁴CO₂ into the chamber atmosphere. The chemical reaction that took place evolving radioactive CO₂ is as follows:



The plants were allowed to perform photosynthesis in the atmosphere containing ¹⁴CO₂ for 2 hours. After the labeling period of 2 hours, trapping of CO₂ from the upper compartment was started to remove the remaining unassimilated ¹⁴CO₂ by pumping the air through 15 ml of 1M NaOH solution for 30 minutes.

2.4 Soil Respiration Trapping

For trapping CO₂ evolving from soil respiration, each pot was connected to a membrane pump. The pipe connected to the top of the pot brought the respired labeled CO₂ into a test tube containing 20 ml of 1M NaOH solution. Here most of the CO₂ got trapped and the remaining was pumped back into the pot with the help of the tube connected to the lower portion of the pot (Fig. 2). There was no loss of CO₂ due to incomplete absorption by NaOH solution because of the closed circulation. The NaOH solution was replaced after 1, 2, 3, 5 and 7 days of each labeling. This trapped CO₂ in NaOH solution was analyzed for ¹⁴C activity and total C-CO₂ contents.

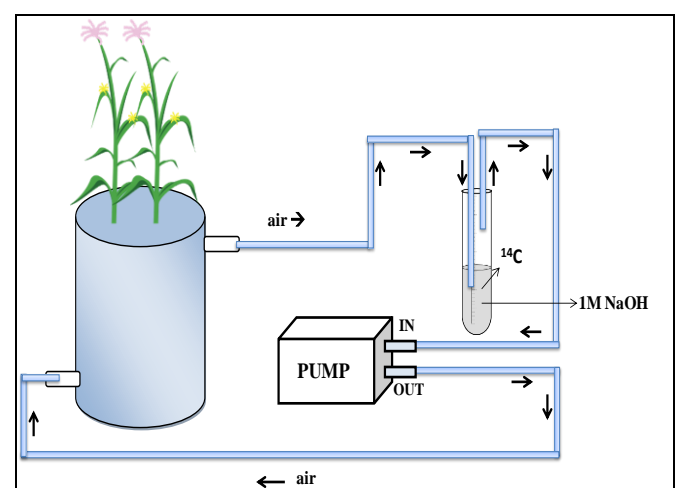


Fig 2: Schematic diagram representing soil respiration trapping

2.5 Plant harvesting

After 60 days of plant growth, the soil-root chamber was destructively sampled. Shoot material was separated from roots and roots were separated from soil and washed by dipping them into water. Thereafter, roots and shoots were dried at 60 °C and the soil was placed into a ventilation box at room temperature. Weights of wet and dried root, shoot and soil samples were measured.

2.6 Soil aggregate size fractionation

A sub sample of soil was used for aggregate size fractionation and was dried to optimal moisture allowing limited mechanical stress to induce maximum brittle failure along natural planes of weakness. When individual soil clods reached the desired condition, they were gently manually crumbled and all visible roots and stones were removed. The bulk soil was then sieved by using automatic sieving system at sieving speed of 70 rpm for 1 minute. Three aggregate size classes: <0.25mm, 0.25-2 mm and >2 mm as micro-aggregate, small macro-aggregate and macro-aggregate size class, respectively, were separated. These soil fractions were then oven dried at 60 °C and were ground for chemical analysis.

2.7 ¹⁴C Determination

To determine ¹⁴C activities of CO₂ dissolved in NaOH from respiration, 1 ml of this solution was well mixed with 2 ml of scintillation cocktail. ¹⁴C measurements were done by putting the mixture into a liquid scintillation counter (Beckmann 6500 LS). For determining ¹⁴C activity in soil, shoots and roots, the ground samples were combusted and the evolving CO₂ was trapped into scintillation cocktail. This mixture was also analyzed for ¹⁴C by feeding the samples into the scintillation counter (Beckmann 6500 LS). ¹⁴C data for each replicate were expressed as percentages of ¹⁴C recovered in the plant/soil system:

$$^{14}\text{C recovered in the plant/soil system} = ^{14}\text{C}_{\text{CO}_2} + ^{14}\text{C}_{\text{soil}} + ^{14}\text{C}_{\text{shoot}} + ^{14}\text{C}_{\text{root}}$$

2.8 Total Carbon Determination

For assessing the total carbon content from soil respiration, titration (TITRONIC basic, Schott Instruments) was performed. Total content of CO₂-C collected in the NaOH

solution was measured by titration against 0.1 M HCl with phenolphthalein, as indicator in excessive 0.1 M BaCl₂ solution. The volume of HCl required to neutralize the NaOH present in the sample was recorded for each sample. The values generated were then converted into milligrams of total carbon per kilogram of soil per hour (efflux).

2.9 Statistical analysis

The differences between the values of planted and unplanted pots and between optimum water condition and drought stress condition were evaluated using Paired t-test. Analysis of effects of drought in soil respiration and in soil aggregates were estimated by two-way ANOVA with Tukey HSD test. The level of significance of statistical tests was $p < 0.05$. The statistical software Statistix (version 8.1) and Statistica (version 10) were used for these analyses.

3. Results and Discussion

3.1 Soil Respiration

Total C-CO₂ efflux for the unplanted control soil was significantly reduced from 2.65 ± 0.35 to 1.76 ± 0.12 mg kg⁻¹ soil h⁻¹ due to drought stress (Fig. 3a). Similarly, there was significant ($p < 0.05$) decrease in cumulative soil respiration due to drought stress under maize planted soils i.e. from 13.86 ± 1.0 to 10.36 ± 1.03 mgkg⁻¹ soil h⁻¹ (Fig. 3b). For the unplanted soils, the values were quite low in both the moisture conditions compared to planted soils as in this case respiration is associated only with the microbial respiration. Droughts are normally supposed to reduce soil respiration; however the degree of reduction can be somewhat species dependent (Ghashghaie, 2001) [28]. In the current study, total C-CO₂ efflux from soil decreased in both unplanted and planted treatments under drought stress compared to optimum moisture contents. This is in line with results from a field experiment that demonstrated a severe reduction in total C-CO₂ efflux from soil under grassland after a drought period and this effect persisted even after rewetting the grassland (Joos *et al.*, 2010) [37]. Further analogous studies resulted in similar reductions in soil respiration conducted under temperate forest ecosystem (Borken *et al.*, 2006) [6]. Similar results were obtained from soils under *Lolium perenne*, *Festuca arundinacea* and *Medicago sativa* subjected to drought stress (Sanaullah *et al.*, 2012) [50].

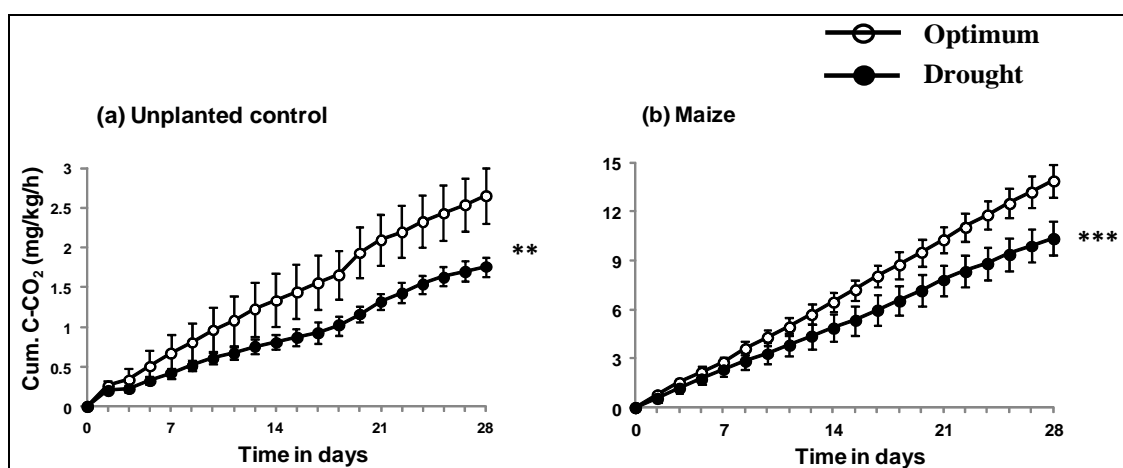


Fig 3: Effect of drought stress on cumulative soil C-CO₂ efflux in (a) unplanted control and (b) maize planted condition. The values represented mean \pm SD of three replicates (n=3)

The reduction in respired total C-CO₂ has been attributed to the reduced heterotrophic nutrition limited by the moisture deficiency (Borken *et al.*, 2006) [6]. Drought can cause various effects to soil including reductions in microbial and enzyme activities that are eventually responsible for soil respiration (Dijkstra and Cheng, 2007) [23]. Usually when soils get dry, the thickness of water film on soil surfaces is reduced; this slows down the diffusion rates of substrates to microbes resulting in diminished SOM decomposition consequently diminishing soil respiration (Stark and Firestone, 1995) [57]. The amount of CO₂ released from planted soils in the current study was about 5-6 times higher compared to unplanted soils. Respiration fluxes in this regard may be related to enhanced root respiration as well as enhanced microbial respiration of root exudates, which could generate rhizosphere effect on SOM decomposition

(Cheng and Kuzyakov, 2005; Kuzyakov, 2002) [15, 40]. In general, respiration rate is found to decrease during water stress due to the reduction in photosynthate assimilation and growth requirements (Tezara *et al.*, 1999) [58].

3.2 ¹⁴C dynamics in soil-plant system

3.2.1 ¹⁴C incorporation in different pools

Drought caused no effect in the ¹⁴C incorporation within plant shoot, soil and in soil respiration. However, a very significant increase was observed in plant roots under drought stress where the recovery of ¹⁴C increased considerably from 10.83 ± 0.1% to 17.25 ± 0.2% (Fig. 4a). Among different carbon pools of plant-soil system, plant shoots were found to be the main sinks for assimilated ¹⁴C regardless of the moisture treatment. The shoots allocated 72-77% of total ¹⁴C recovered (Fig. 4b).

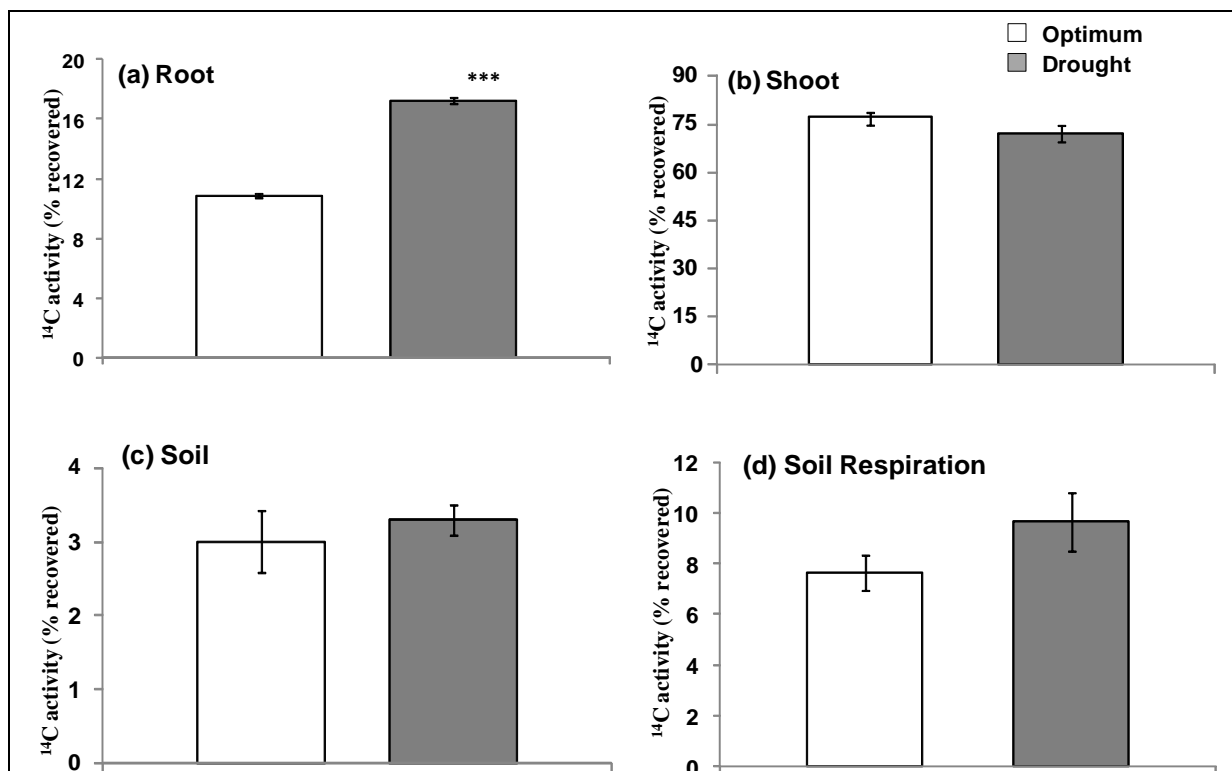


Fig 4: Proportion of ¹⁴C incorporated according to moisture treatment in (a) plant roots, (b) plant shoots, (c) soil and (d) soil respiration. The values represent mean ± SD for three replicates (n=3)

The significant allocation of recently fixed C in plant roots under drought stress was also found in a study where *Lolium perenne*, *Festuca arundinaceae* and *Medicago sativa* were incubated in monoculture and in mixture (Sanaullah *et al.*, 2012) [50]. Similar results were obtained in other studies where the recovery of ¹⁴C in plant roots significantly increased when subjected both to direct drought and the after-effects of drought (Grzesiak *et al.*, 1991; Grzesiak *et al.*, 1999) [30, 31]. Similar results were obtained in a study for colonial bentgrass, creeping bentgrass and velvet bentgrass following a 12 day-drought treatment which was attributed as a mechanism adapted in order to maintain higher water content during prolonged periods of drought (DaCosta and Huang, 2006) [22]. This greater carbon allocation to roots than to shoots under drought condition resulted from lower reductions in root growth compared to shoot growth (Palta and Gregory, 1997) [48]. It has been reported that soil drying

leads to increased proportion of carbon partitioned to roots for drought tolerance (Huang and Gao, 2000) [35]. This increase of carbon partitioning to roots has been correlated to the capability of roots to continue growing under drought stress (Nandwal *et al.*, 1996) [46]. Roots are one of the most actively growing plant organs during the vegetative stage, thus during moisture deficiency a major portion of recently labeled carbon gets mobilized from shoot towards root (Nandwal *et al.*, 1996) [46]. This accumulated carbon assimilates are used not only to perform respiratory and tissue build-up process but also to release exudates into the surrounding soil (Kuzyakov and Domanski, 2002) [41]. For plant shoots, there was no any effect of drought in the recovered ¹⁴C content. Similar result was obtained in a study where no effect in carbon allocation was observed in shoots of *Lolium perenne* under drought stress (Sanaullah *et al.*, 2012) [50]. However in the same study, ¹⁴C content in the

shoots of *Festucia arundinacea* and *Medicago sativa* were significantly reduced. Carbon allocated in the shoot portion is used up for shoot respiration (Kuzyakov and Domanski, 2002) [41] and a part of it is translocated below ground incorporating into the roots (Keutgen *et al.*, 1995) [39]. The photosynthetic assimilation is reduced as the moisture deficiency triggers the stomatal closure in plant leaves (Chaves, 1991; Cornic and Massacci, 1996) [12, 20]. This reduction plays an important role in optimizing carbon assimilation in relation to water supply which restricts the change in carbon allocation under moisture stressed condition (Cowan, 1982) [21].

The ^{14}C content in the soil after labeling of plants represents the C amount transferred into the soil and stored there for a longer period of time which suggests that the exudates and root residues would be humified and transformed into some fractions of soil organic matter (Kuzyakov and Domanski, 2002) [41]. The ^{14}C content in the soil (Fig. 4c) was unchanged under drought stress for the current study. Similar results were obtained for soils under *Medicago sativa* subjected to drought stress (Sanaullah *et al.*, 2012) [50]. This was described as the attenuation of extreme drought responses exhibited by the plant that prevented the allocation of recently fixed ^{14}C from shoot towards the soil. This is apparent as the shoot ^{14}C allocation under drought stress was also found to be unaffected in the present study. However, the results are comparable to a study where ^{14}C content in soil didn't undergo any change after a two-month long dry period (Gorissen *et al.*, 2004) [29]. Analogous results were obtained in a study where ^{14}C activity in the soil was not found to be affected by moisture treatment (Chen *et al.*, 2014) [14]. The proportion of ^{14}C recovered in soil in this study was only a small fraction (about 3%) of the total ^{14}C recovered from the plant-soil system irrespective of the moisture treatment. This coincides with a study where only 1.5% of the total ^{14}C was recovered in the planted soil (Cheshire *et al.*, 1981) [16]. The uptake of $^{14}\text{CO}_2$ by heterotrophic microorganisms doesn't contribute greatly to incorporate the same into soil ^{14}C content (Sparling *et al.*, 1982) [56].

For soil respiration too, there was no effect in the ^{14}C incorporation due to drought effects (Fig. 4d). ^{14}C allocation responses to soil under drought condition is found to differ from species to species as was seen in a study where the allocation was reduced in soils under *Lolium perenne* and *Festucia arundinacea* while increased in soils under *Medicago sativa* (Sanaullah *et al.*, 2012) [50]. When soils get dried up under drought environment, the water film on the soil surface lose their thickening resulting in reduced diffusion of substrate to microbes (Stark and Firestone, 1995) [57]. This suggests that the root-respiration was not affected by drought and the water limitation applied to the soil in this study was not strong enough to influence the microbial decomposition of assimilates.

The ability of roots to continue growing even during dry and harsh period suggests the increase in sink strength in roots under drought stress. The maintenance of turgor by plant roots increase their sink strength and allow for greater allocation of carbon under water stressed condition.

3.2.2 $^{14}\text{CO}_2$ activity in soil respiration

The total $^{14}\text{CO}_2$ efflux from soil respiration was determined

in order to assess the involvement of recently fixed carbon in soil respiration (Fig. 5). A very significant increase in the proportion of ^{14}C recovered was obtained under drought stress.

The result presented here is in agreement with an experiment that too demonstrated a significant increase in ^{14}C contribution to root respiration under drought stress for soils cultivated under *Medicago sativa* (Sanaullah *et al.*, 2012) [50]. The results presented here show low CO_2 concentrations and significantly high radiocarbon ($^{14}\text{CO}_2$) values under drought stress which coincides with a study conducted in order to determine the drought effects on soil respiration under temperate forest (Borken *et al.*, 2006) [6]. Respiration fluxes from the rhizosphere is related to enhanced root respiration as well as enhanced microbial respiration of root exudates that generate a rhizosphere effect on SOM decomposition (Cheng and Kuzyakov, 2005) [15]. Our results suggest that during the drought period, the portion respired from recently fixed carbon increased and the portion respired from older carbon decreased in the experimental pots. This increase has also been attributed to the death of root and rhizosphere organisms and subsequent decomposition of labeled materials (Sparling *et al.*, 1982) [56]. This indicates that the soil must have released somewhat younger carbon with increasing drought. This also agrees with a study that indicated the release of higher amounts of recently fixed radiocarbon (^{14}C) from root respiration (Gaudinski *et al.*, 2000) [27] and explains the relatively active status of rhizosphere during dry periods (Caldwell *et al.*, 1998) [10].

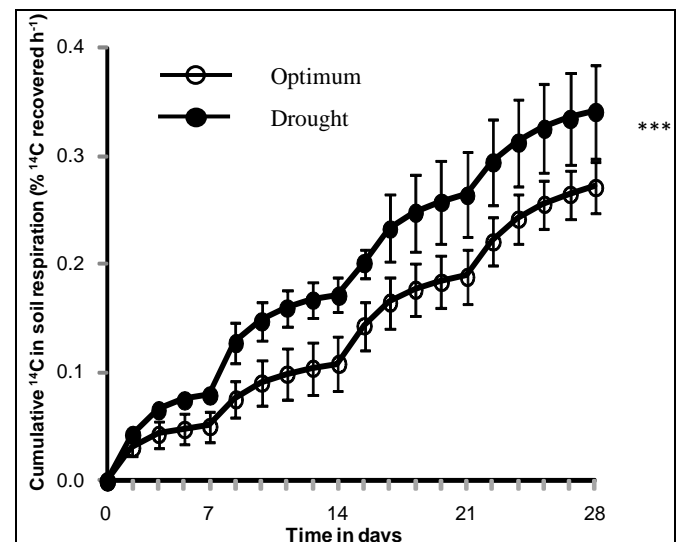


Fig 5: Proportion of ^{14}C recovered from soil respiration. The values represented mean \pm SD (n=3).

The result presented here can be correlated with the decrease of total CO_2 under drought stress. Drought reduced the enzymatic activity necessary for the decomposition of soil organic matter which caused significant reduction in the total soil respiration under drought stress. On the other hand, the appearance of $^{14}\text{CO}_2$ in soil solution was mainly related to rhizomicrobial respiration of recently fixed ^{14}C assimilates that is transferred via shoots to roots (Bhupinderpal-Singh *et al.*, 2005) [4]. The use of assimilates by microbial organisms may be very rapid due to their close

association with the roots which may have contributed to enhanced rhizomicrobial respiration under drought stress (Kuzuyakov and Gavrichkova, 2010) [42]. Our study shows a rapid allocation in the recently fixed ^{14}C toward roots from the shoots. The root derived CO_2 efflux is controlled by the rate of substrate supply to roots (Kuzuyakov and Gavrichkova, 2010) [42]. Thus, it is apparent that the C assimilates allocated to roots was used up in the rhizomicrobial respiration. The rhizomicrobial respiration in the present study was significantly affected by drought stress. This was attributed to a rapid translocation of the recently fixed ^{14}C assimilates from metabolic pool of the roots as respired $^{14}\text{CO}_2$ (Bhupinderpal-Singh *et al.*, 2005) [4]. The increased $^{14}\text{CO}_2$ under drought indicates the faster decomposition of root-released carbon to CO_2 .

Hence, the ^{14}C allocated in the roots was utilized in root respiration and microbial respiration. Drought increased the release of root exudates which may have stimulated the rhizomicrobial activity. Thus, the plant roots through the production of labile compounds are able to maintain the rhizomicrobial activity.

3.2.3 ^{14}C recovered in soil aggregates

The allocation of recovered ^{14}C in different soil aggregate fractions was determined for identifying the fraction pool where the most recent carbon had been recovered. Among

the aggregate classes, the proportionate allocation of recently labeled ^{14}C was found in the small macro-aggregate fraction (Fig. 6). There was, however, no any significant effect of drought in this fraction. The recovery of ^{14}C in the micro-aggregate fraction (<0.25 mm) increased significantly following drought. ^{14}C contents in this fraction increased from $0.41 \pm 0.02\%$ to $0.67 \pm 0.07\%$. On the other hand, the allocation of ^{14}C in the macro-aggregate (2-5 mm) fraction decreased significantly from $0.56 \pm 0.05\%$ to $0.40 \pm 0.05\%$ following drought. During the optimum moisture condition, the allocation was lowest in the micro-aggregate fraction which shifted to the macro-aggregate fraction with significant changes occurring in both the classes because of drought stress.

The micro-aggregate soil fraction is found to incorporate less amount of recently fixed carbon and usually involve older and more humified carbon compared to the macro-aggregate fractions (Buyanovsky *et al.*, 1994) [9]. The carbon-rich young plant residues form and stabilize the macro-aggregates while the old carbon is occluded in the micro-aggregate fraction (Blanco-Canqui and Lal, 2004) [5]. This statement was found to hold true for the current study as well where the content of recently fixed carbon was found to be higher in the small macro-aggregate and the macro-aggregate fractions during the optimum moisture condition.

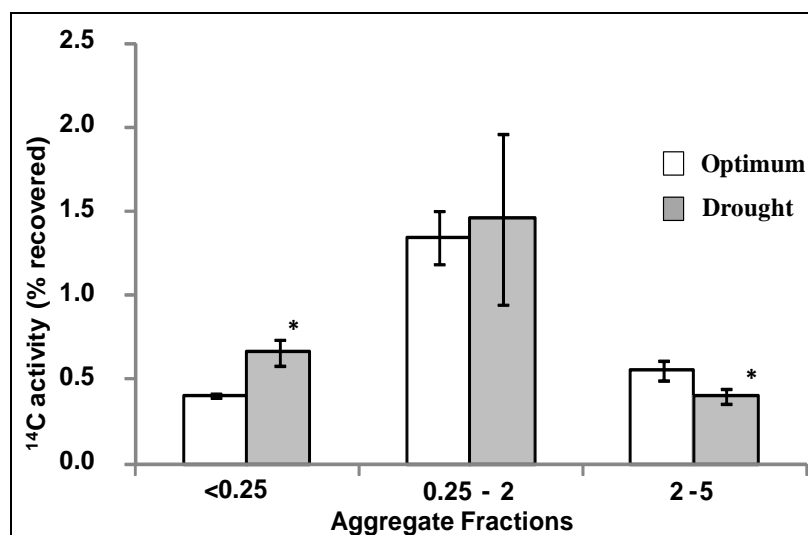


Fig 6: ^{14}C incorporation in different aggregate size fractions at both optimum and drought stress. Data represents mean \pm SD for three replicates (n = 3)

Similar results were found in a field study with ^{14}C -labeled soybean residues where the radiocarbons that were partially processed were found to be incorporated in the macro-aggregates soil fractions (Buyanovsky *et al.*, 1994) [9]. The retention of recent carbon assimilates in the bigger soil aggregate fraction was found to be in contrast with another finding where the recently added carbon was found to be incorporated more in the micro-aggregates (Bossuyt *et al.*, 2002) [7]. This was explained as the tendency of younger carbon to get stabilized in non-disturbed soil where the stabilization was established mainly at the micro-aggregate level. Further in another study, retention of the recent carbon assimilates was also found to be concentrated in the bigger aggregate fractions compared to the micro-aggregates (Majumder and Kuzuyakov, 2010) [45]. This was

explained as the increase in aggregation assisted by the various binding agents that increased the resistance of macro-aggregates against microbial decomposition. The present study shows a significant decline in the macro-aggregate fraction and a corresponding significant increase in the micro-aggregate fraction following drought stress. These changes have previously been interpreted as the characteristic property of smaller soil particle fractions containing the recent carbon assimilates to decompose sluggishly compared to the bigger sized fractions during moisture stress conditions (Jenkinson, 1977) [36]. Further the availability of recently fixed carbon for decomposition is believed to be hindered by the immobilization process at the soil surface (Séguaris *et al.*, 2010) [51]. These changes suggest that the ^{14}C lost from the macro-aggregate fraction

due to drought effect was recovered in the micro-aggregate fraction.

Thus, the increased release of root exudates under the moisture stress condition affected the allocation pattern of newly added ^{14}C in the soil aggregate fractions. ^{14}C released in soil was mostly incorporated in the micro-aggregate fraction.

4. Conclusion

Drought affected the allocation of carbon in the plant-soil system. The source-sink relationship of plants was changed and this decreased total soil respiration due to the suppression of enzymatic activity responsible for soil organic matter decomposition. Drought increased the release of root exudates that enhanced the rhizomicrobial activity and increased the rhizomicrobial respiration ($^{14}\text{CO}_2$). Drought also altered the distribution pattern of the soil aggregate fractions. The proportionate allocation increased in the small macro-aggregate fraction for unplanted control soils with a relative decrease in the micro-aggregate fraction, whereas for the planted soils, the allocation decreased in the small macro-aggregate fraction with a relative increase in the micro-aggregate fraction. The increased release of root exudates under the moisture stress condition affected the allocation pattern of newly added ^{14}C in the soil aggregate fractions. Due to drought stress, there was increase in fresh carbon assimilates (^{14}C) into the micro-aggregate fractions compared with optimum moisture levels.

Hence, it can be concluded that maize plants performed quick adaptive response to drought stress by translocating higher photosynthates towards roots for efficient water uptake. In conclusion, the impact of climate change induced drought may modify the belowground carbon allocation and root-derived respiration processes.

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