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Age Effects of Millet Crops on Phytolith Carbon Sequestration

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Abstract Carbon sequestration within the phytoliths plays an effective long-term carbon sequestration mechanism. This study explores the potential of carbon sequestration among the millet crops such as Pearl millet, Sorghum, Little millet, Finger millet and Foxtail millet at three different growth ages. The crop samples were divided into three growth ages: 30th day, 60th day and 90th day after sowing and divided into aboveground biomass (AGB) and belowground biomass (BGB) for analysis of carbon occluded within phytoliths (phytOC). This study revealed that the maximum accumulation of phytoliths and occlusion of carbon were observed on the 60th day. Among the millet crops, Finger millet showed the highest phytOC yield, flux and production rate. Therefore, cultivating high phytOC crops such as Finger millet could certainly contribute to terrestrial carbon sequestration in mitigation of global warming and climate change.

Keywords: *biomass, millets, phytolith occluded carbon, terrestrial carbon sequestration, growth stages*

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1. Introduction

The recent concern has been the rise in carbon dioxide (CO_2) concentration in the atmosphere and this has led to finding ways to increase long-term carbon sequestration [1]. Terrestrial biogeochemical carbon (C) sequestration has been mentioned as a potential stable C sink strategy to reduce atmospheric CO_2. The C stored in the terrestrial biomass decomposes quickly and releases CO_2 into the atmosphere. Biogeochemical C sequestration within phytoliths is one of the most promising, secure, and efficient long-term C sequestration mechanisms identified in recent research on carbon sequestration [2,3,4].

Phytoliths are microscopic silicified structures formed in or between the cells of the plants [5]. Plant dry weight phytolith content varies significantly in the plant kingdom, ranging from 0.1% to 10% [6]. Phytoliths are released into the soil, and other sediments when a plant dies, and they remain stable over a wide pH range (pH 3-9) in various environments and climatic conditions [7,8,9]. During plant growth, phytoliths can occlude the small amount of organic carbon (0.2% to 5.8 %) absorbed from atmospheric CO_2 at the time of photosynthesis [1,10]. Hence this carbon occluded within phytoliths has been identified as a significant long term terrestrial carbon sink, capturing around 1.5 billion tonnes of CO_2 each year [1].

Phytoliths are found in all types of plants throughout the plant kingdom. Monocotyledons absorb and deposit more silicon than dicotyledons. Certain monocotyledonous families such as the Restionaceae, Arecaceae, Zingiberaceae, Bromeliaceae, Orchidaceae and Cyperaceae are particularly prone to phytolith deposition. However, Poaceae, the most ecologically evolved and flourishing grass family deposits the heaviest and most distinctive phytoliths [11,12]. The accumulation and production of phytoliths vary from species to species. Similarly, the occlusion of carbon within phytoliths varies within the species, such as in sugarcane, which varies from 3.88% to 19.26% [13,14]. Environmental factors played an essential role in absorbing silica from the soil and depositing it into plant parts [15].

Millets are one of the important crops grown in India. They are cultivated in all seasons since they can withstand high temperature as well as short growing season. India is the largest millet producer in the world (41.04%). The main millets grown in India are Pearl millet, Sorghum, Little millet, Foxtail millet, Finger millet, Kodo millet, Proso millet and Barnyard millet [16].

There is limited research on the impact of age on the accumulation of phytoliths and the occlusion of carbon within phytoliths in grass species. Many research studies have shown phytolith content in different grass species such as bamboo and crops (millets, wheat, maize, sugarcane and rice) [17]. Furthermore, several studies have focused on cellular and molecular studies of phytolith formation to investigate the role of phytoliths in biogeochemical cycling [18]. The objective of this study was to test: (1) to determine phytolith content of selected millets at different ages of the crops, and (2) carbon occlusion in these millet species.

2. Material and Methods

2.1. Study Site Selection

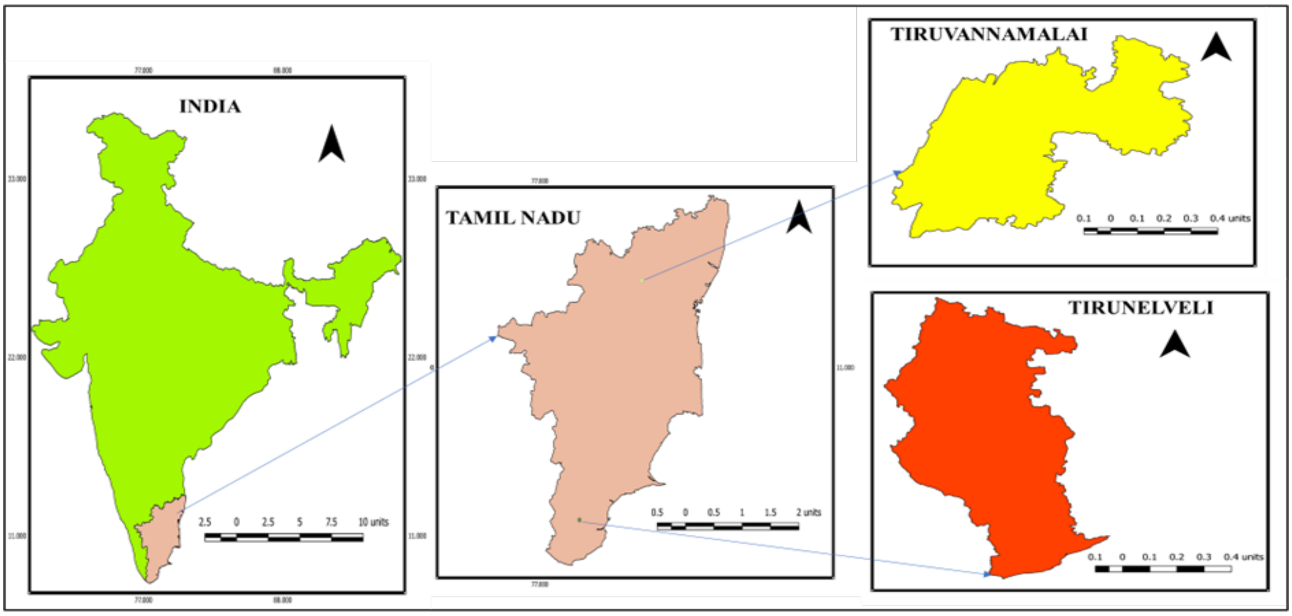

Figure 1. Map showing the location of sampling sites in Tamil Nadu, India

The study was conducted in two places of typical millet lands. One study site is located around 36 km west of Thiruvannamalai city, Tamil Nadu, India (Chengam, latitude 12°3068′N and longitude 78°7958′E). This site experiences mean annual temperature of 33.77°C in the daytime and 23.68°C in the night hours. Average annual precipitation is 75.94mm with warmest month is May and coldest month is January. The soil is a mix of red loam and black soil. The dominant vegetation is all varieties of millets. The second site selected was close to a famous South Indian city called Tirunelveli, India (8°4014′N, 77°6174′E). This region experiences mean annual temperature of 30°C in the daytime and 26°C in the night hours. Annual average precipitation is 163.28mm. The warmest month is April and coldest month is January. The soil is a red sandy and soil. Though the dominant vegetation is paddy, the other vegetation such as varieties of millets, banana plantations, and maze can be seen widely (Figure 1).

2.2. Sampling of Crops and Experimental Design

Three replicates of the five millet varieties at different growth ages, i.e., 30th day, 60th day, and 90th day after sowing, were randomly sampled. The samples were collected by digging up each individual plant to a depth of 20 cm below ground level at the sites. The plants were cut into two parts: above ground parts, and below ground parts that is buried below the soil surface. The above ground sample is used to calculate AGB (Above Ground Biomass) and below ground sample is used to calculate BGB (Below ground Biomass). This crop samples were carefully washed with running water and rinsed with distilled water. The cleaned samples were oven-dried at 75°C to a constant weight. The biomass per unit was calculated, and the crop samples were ground and passed to a 0.25 mm sieve before analyzed for Phytolith and PhytOC content.

2.3. Soil Sampling and Characterization

Soil samples were collected from the top layer (0-15 cm) of the soil each time crop samples are collected from the sites for further studies. 1kg of soil samples was sealed in an airtight bag and transferred to the laboratory. Soil samples were thoroughly mixed before sieving through a 2.0 mm mesh screen for further analysis. The soil samples collected from different sites were assessed total organic carbon by Walkley-Black Method and silicon dioxide by Gravimetric method.

2.4. Estimation of Organic Carbon in Crops

One gram of ground samples was taken in pre-weighed crucibles and placed inside the muffle furnace at 550ºC for an hour. The crucibles with ash were weighed after cooling in a desiccator [19]. Organic carbon was calculated as below:

$$Ash(\%) = \frac{(W3 - W1)}{(W2 - W1)} \times 100$$

Where,
W1= Weight of empty crucibles,
W2 = Weight of oven-dried grind samples + Crucibles,
W3 = Weight of ash + Crucibles.

$$C(\%) = (100 - Ash\%) \times 0.58$$

(Considering 0.58% carbon in ash-free plant materials)
Where C is the Organic Carbon.

2.5. Extraction of Phytoliths from Crops

The Phytolith was extracted from plant material using a dry ashing and acid extraction method. 1g of dry plant material was placed in a silica crucible and heated to 500°C for 8 hours in a muffle furnace. It was cooled before being transferred to test tubes. 20 ml of 10% HCl was added and kept in the water bath at 70°C for 20 minutes before centrifuging at 3500 rpm for 5 minutes and discarding the supernatant. The remaining material was rinsed with distilled water and centrifuged at 3500 rpm for 5 minutes before discarding the supernatant. After that, 20 ml of 15% H_2O_2 was added, and the mixture was kept in a water bath at 70°C for 20 minutes. The supernatant was discarded after centrifugation at 3500 rpm for 5 minutes. Furthermore, the remaining material was rinsed with distilled water and centrifuged at 3500 rpm for 5 minutes before discarding the supernatant. Finally, 1 mL of 100% ethanol was added and allowed to dry [20].

2.6. Estimation of PhytOC

Dried extracted phytolith samples were treated with one mol/L HF at 55°C for 60 minutes to dissolve Phytolith. After HF treatment, the organic carbon released from phytoliths was dried at 45°C [21] and phytOC was analyzed by TOC Analyzer (SHIMADZU SSM-5000A) [22].

2.7. Data Analysis

The data was analysed using ANOVA, Duncan's Multiple Range Test ($p < 0.05$), and the means were compared using the LSD test using SPSS (Version 21, SPSS Inc., Wacker Drive, Chicago, USA) to examine the mean difference in phytolith, and phytOC contents in the millet crop samples. Carried out Correlation analysis to examine phytolith content, C concentration, and phytOC in the crop samples. The following are the related formulae [23]:

1. Phytolith concentration (g/Kg) = phytolith weight (g)/dry biomass (Kg)
2. C concentration in phytolith (g/Kg) = C content in phytolith(g)/phytolith weight(Kg)
3. PhytOC concentration (g/Kg) = C content in phytolith(g)/ dry biomass (Kg)
4. PhytOC flux Production (Kg/ha/yr) = PhytOC concentration (g/Kg) × NPP (Kg/ha/yr) × 10^{-3}
5. PhytOC production rate (Kg/yr) = PhytOC production flux (Kg/ha/yr) × area (ha)

3. Results and discussion

3.1. Soil Characteristics

Soil organic carbon is essential in increasing crop yield and sequestering atmospheric carbon [24]. Total organic carbon (TOC) ranged from 0.96% to 1.65% at various growth stages (Figure 2). TOC of soil was analysed for each millet variety growing stage, and there was no significant difference ($p>0.05$) in Pearl Millet, Foxtail Millet, Finger millet and Little millet. However, in Sorghum, there was significant difference at different growth stages. The variations in soil organic carbon imply no proper soil management in agricultural land. Research has shown that improved agricultural methods and management techniques that might offer carbon input higher than the above necessary level are likely to sustain the soil organic carbon level and preserve good soil health [25].

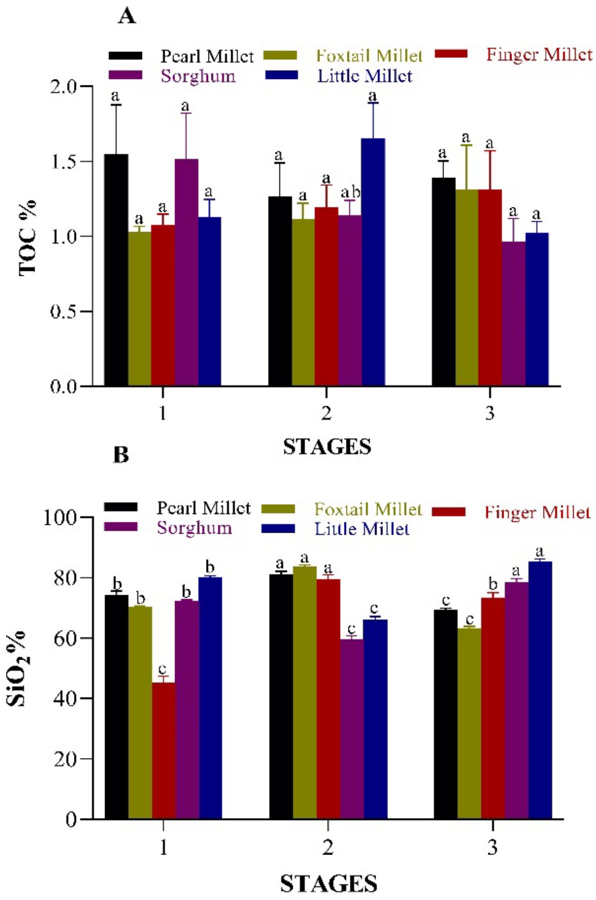

Figure 2. Total organic carbon of soil at different growth stages of crops grown. 1 = 30th day, 2= 60th day and 3 = 90th day. Based on the least significant difference (LSD) test, various lowercase letters indicate significant differences at the P = 0.05 level; error bars represent standard error (n = 3)

Silicon dioxide (SiO_2) is commonly found in nature in the form of quartz, which is also the most common source of silica used in agriculture. Silicon, a mineral element, is not considered necessary. It is neither macro-elements nor micro-elements in plant mineral nutrition. Scientists regard it as a facilitating element at the agricultural level. Its accumulation in the epidermis provides rigidity and support to crops, protecting them from biotic and abiotic stresses [26]. In this study, SiO_2 in soil ranged from 45.38% to 85.45% and varied significantly ($p<0.05$) in each millet variety at different ages (Fig 2). The soil in which Little millet was cultivated had the highest SiO_2 among other soil.

3.2. Plant Biomass and Carbon Stored in Millet Varieties at Different Ages

Accumulation of biomass in crops depends on the photosynthesis process, where carbon is either transported to the growth sites or stored [27]. Plant biomass increased from the initial to 90th day after sowing. The biomass accumulation varied among millet crops ranging from 2478.77 Kg/ha to 28611.03 Kg/ha (Figure 3). Little millet showed the highest biomass accumulation among all the millet crops on the 30th day and 60th day. On the 90th day, Pearl millet showed the highest biomass. The variation of plant biomass could be due to the crops grown under different environmental factors such as temperature and precipitation [28].

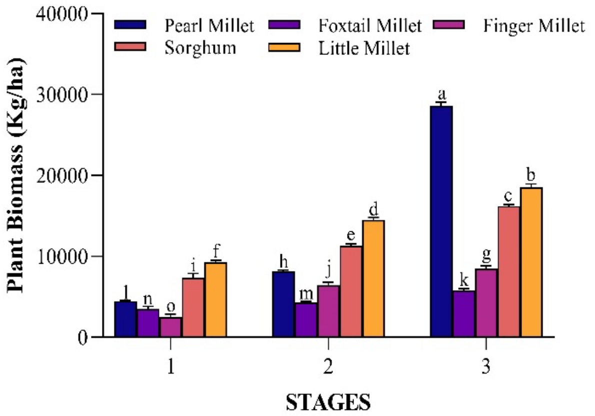

Figure 3. Plant biomass at different growth stages. 1 = 30th day, 2= 60th day and 3 = 90th day. Based on the least significant difference (LSD) test, various lowercase letters indicate significant differences at the P = 0.05 level; error bars represent standard error (n = 3)

The carbon allocation between aboveground and belowground biomass varies due to environmental factors [29]. There were significant ($p<0.05$) variations in the organic carbon content among the five millet varieties. The study showed that crops allocated carbon more in AGB. The results indicated that carbon content increased with plant growth from the initial age (30th day) to the final age (90th day). The carbon content of AGB varied from 46.31% to 53.15% and BGB from 10.29% to 52.04% (Table 1). In AGB, a common trend was observed: carbon accumulated highest on the 60th day, except in Sorghum, where carbon was high on the 90th day. On the contrary, carbon in BGB on 60th day showed lesser accumulation except in Finger millet where carbon decreased from 30th to 90th day. The study revealed that Sorghum sequestered carbon high in AGB (52.72%), and Pearl millet high in BGB (52.04%).

3.3. Phytolith and PhytOC Content of Different Millet Varieties

The Poaceae family accumulates more phytoliths than other monocots [30]. The production of phytoliths differs in each plant. Our study showed that phytolith content ranged from 0.1515 g/kg to 11.2922 g/Kg (Figure 4). The study revealed that the production of phytoliths significantly varied ($p<0.05$) among the millet varieties with age. In AGB, the accumulation of phytoliths showed highest on the 60th day, and in BGB, Pearl and Foxtail Millet had highest on the 30th day, whereas Finger, Sorghum and Little millet were high on the 60th day.

Overall, BGB accumulated the highest phytoliths than AGB. Foxtail (5.8770 g/Kg) and Sorghum (11.2922 g/Kg) generally accumulated more phytoliths in AGB and BGB, respectively on the 60th day. The phytolith variation between the species could be due to different locations, fertilizers, temperature, and silicon availability for plants in different soil types [21].

Table 1. Carbon Content of Millet Varieties

	Pearl Millet	Foxtail Millet	Finger Millet	Sorghum	Little Millet
1AGB	46.58±0.22^{b}	46.31±0.97^{a}	47.95±1.68^{ab}	48.09±0.40^{a}	49.56±0.34^{a}
1BGB	28.29±6.51^{c}	44.39±4.77^{a}	41.44±11.35^{bc}	34.78±2.06^{b}	34.67±2.61^{c}
2AGB	53.15±0.16^{a}	49.60±0.98^{a}	51.55±0.17^{a}	51.99±0.74^{a}	52.85±0.20^{a}
2BGB	10.29±3.61^{d}	23.71±3.72^{b}	33.82±0.99^{c}	14.81±2.19^{c}	17.29±1.84^{d}
3AGB	52.40±0.22^{ab}	50.32±0.33^{a}	49.66±0.17^{ab}	52.72±1.21^{a}	49.86±2.53^{a}
3BGB	52.04±2.07^{ab}	45.44±4.79^{a}	15.30±2.15^{d}	36.78±6.20^{b}	44.66±4.29^{b}

Each value is given as the mean standard deviation (n=3). The mean values of different letters in a column differ significantly ($p<0.05$).

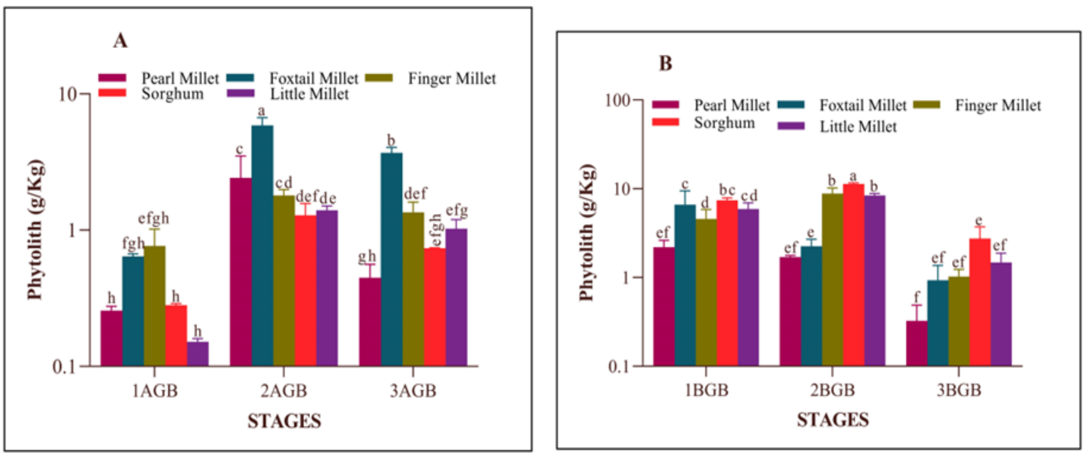

Figure 4. Comparison of Phytolith in AGB and BGB at different growing stages of millet varieties. 1 = 30th day, 2= 60th day and 3 = 90th day. A= AGB, B=BGB. Based on the least significant difference (LSD) test, various lowercase letters indicate significant differences at the P = 0.05 level; error bars represent standard error (n = 3)

In general, AGB had higher phytOC content than BGB. phytOC content varied at different ages. No common trend was observed in both AGB or BGB. In AGB, most phytOC contents were high on the 60th day. However, in BGB, phytOC content decreased from 30th to the 90th day. The phytOC concentration varied from 0.013 g/Kg to 0.09 g/Kg (Figure 5).

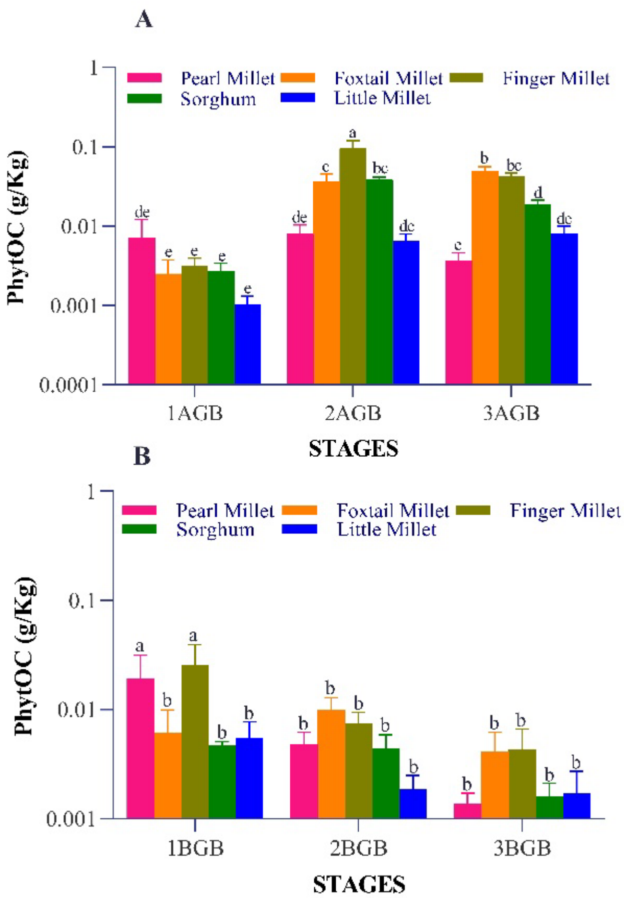

Figure 5. Comparison of PhytOC in AGB and BGB at different growing stages of millet varieties. (A) PhytOC in AGB and (B) PhytOC in BGB.1 = 30th day, 2= 60th day and 3 = 90th day. Based on the least significant difference (LSD) test, various lowercase letters indicate significant differences at the P = 0.05 level; error bars represent standard error. (n = 3)

Finger millet showed the highest phytOC in AGB and BGB on 60th and 30th days, respectively. The factors influencing the variations of phytOC in each millet crop variety are unknown. Further study is required to identify the factors influencing the production of phytOC and to accurately estimate phytOC production based on the pattern of phytOC content of millet crops grown under the same environmental conditions.

The correlations between phytolith content, carbon in phytoliths, and phytOC content in each millet variety at total growth were investigated. The findings show no significant (p>0.05) relationship between phytolith content in plants and phytOC in all the millet varieties (Table 2). This pattern agrees with a recent study on eight millet species that showed no correlation between phytolith yield and percentage phytOC content on a dry weight basis (P = 0.071, R^2 = 0.105) [30]. Similarly, previous work on rice, wheat and bamboo indicated the same results [18,31,32]. These results imply the deposition of phytoliths and the efficiency of carbon trapping within the phytoliths rather than the amount of Phytolith taken up by the plant that determines the relative phytOC yields.

Table 2. Correlation Matrix Between the Phytolith, PhytOC and Carbon Content in Phytolith in Millet Varieties

Samples		Phy	PhytOC	C
Pearl Millet	Phy	1		
	PhytOC	0.6235	1	
	C	-0.3721	-0.2925	1
Foxtail Millet	Phy	1		
	PhytOC	0.4117	1	
	C	0.2584	0.9778**	1
Finger Millet	Phy	1		
	PhytOC	-0.2268	1	
	C	-0.26848	0.164298	1
Sorghum	Phy	1		
	PhytOC	-0.28555	1	
	C	-0.54339	0.218402	1
Little Millet	Phy	1		
	PhytOC	-0.14986	1	
	C	-0.52033	0.799287*	1

* Correlation is significant at the 0.05 level (1 tailed), ** Correlation is significant at the 0.01 level (1 tailed)

Furthermore, the study also revealed a significant correlation between phytOC and C content within Phytolith in Foxtail Millet (R^2 = 0.9778; p<0.01) and Little Millet (R^2 = 0.7992; p<0.05). The results agree with the previous work on rice, millet and bamboo [33]. Our findings suggest that phytOC concentrations in Foxtail and Little millet are closely related to phytolith content and the efficiency of C deposition during phytolith formation.

The phytOC production flux was observed to be high on the 60th day. The average phytOC production flux contributed by millet varieties ranged from 0.0628 Kg/ha/yr to 1.313 Kg/ha/yr (Figure 6). Moreover, the phytOC production rate ranged from 18.85 Kg/yr to 394.17 Kg/yr (Table 3). Finger millet contributed the highest phytOC production flux and rate.

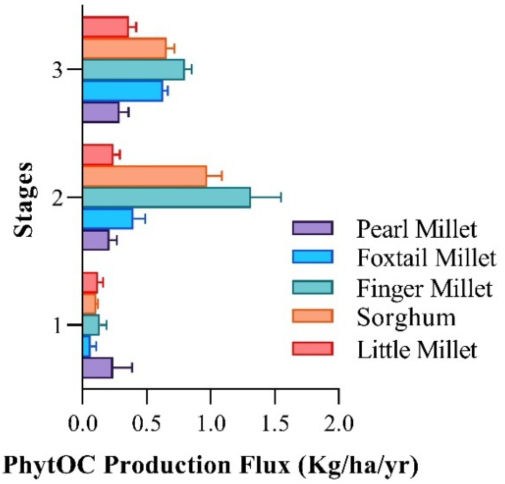

Figure 6. Estimated PhytOC Production Flux of Millet crop Varieties at different age. 1 = 30th day, 2= 60th day and 3 = 90th day. error bars represent standard error. (n = 3)

Table 3. Estimated PhytOC Production Rate in Millet Varieties

PhytOC production rate (Kg/yr)					
Stages	Pearl Millet	Foxtail Millet	Finger Millet	Sorghum	Little Millet
1	71.69	18.85	40.96	32.65	36.39
2	63.18	119.98	394.17	291.76	73.06
3	87.14	188.99	240.57	197.30	108.17

1 = 30th day, 2= 60th day and 3 = 90th day. *The area of the millet cultivated land is assessed for 300ha.

4. Conclusion

The study revealed that the carbon stored from the initial to their full maturity stage varies from species to species in above and belowground biomass. The crop biomass increased from the 30th day to the 60th day. The soil properties of TOC and SiO_2 were high on the Little millet soil. However, it did not yield high Phytoliths and phytOC content. The accumulation of phytoliths was high on the 60th day, and the same was observed in phytOC content, phytOC production flux and rate. The amount of carbon sequestrated within the phytoliths during the growth phase showed that Finger millet had high phytOC yield than other millet varieties. Therefore, the phytOC available in Finger millet contributes to the long-term terrestrial carbon sequestration when released into the soil during the death of the crop. Practices such as managing soil nutrients and regularly adding silicon fertilizers to agricultural lands can improve phytolith production, increase the phytOC accumulation to contribute to terrestrial carbon sequestration, and assist in mitigating climate change.

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