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Soil microbial biomass carbon and carbon dioxide response by glucose-C addition in black soil of China

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Abstract

The soil microbial biomass, atmospheric carbon dioxide and abundance of decomposer are influenced by rate and addition pattern of glucose carbon. The present study was conducted to evaluate the effect of single and repeated additions of glucose-C on soil microbial biomass carbon (SMBC) and CO_2 response in black soil of China. The incubator study comprising of 116-days was conducted in different fertility levels of black soil of Jilin province of China, to determine the effects of glucose addition patterns viz single addition (2% glucose-C once application) and repeated addition (2% glucose-C in five splits) on soil microbial biomass carbon and CO_2 accumulation. Forty-gram air dried soil was filled into 250 ml Schott bottle and bottles were arranged in CRD-factorial design with 5 repeats. Factor (A), included glucose addition patterns (single & repeated additions). Factor (B), consisted of soil fertility levels: low, medium and high on the basis of soil organic carbon. Thereafter glucose-C (2%) solution was added drop wise to soil. The addition patterns showed positive response on SMBC, CO_2 evolved and CO_2 accumulation. Over all mean (low, medium and high fertility soils) of repeated addition depicted 32% and 0.92% higher values of SMBC than control and single additions, respectively. The CO_2 emission of repeated addition was 21.3% higher in low fertility soil. The mean CO_2 accumulation showed higher values in low fertility soil by single addition than repeated and control in all soils. Single glucose-C addition in combination with different soil fertility levels augmented the microbial biomass and triggered carbon mineralization for shorter period (up to 3 weeks). The repeated addition of glucose in combination with different soil fertility levels also enhanced soil microbial biomass carbon and CO_2 in longer incubation period. It is concluded from this study that microbial starvation for organic carbon was very high hence; repeated addition may be suggested to meet C demand of microbes.

Keywords: Microbial biomass carbon, CO_2, glucose, single addition, repeated addition

Introduction

Soil organic matter (SOM) is chief constituent of C and nutrient cycling; it is also major carbon reservoir of the biosphere atmosphere system (Falkowski *et al*., 2000). Soil organic C is a leading factor in many microbial processes such as soil respiration and mineralization, but both the processes are dependent on quality and quantity of C, because it contains energy for enzyme production (Kuzyakov *et al*., 2000). Single addition is the application of glucose or fresh organic material (straw), farmyard manure at initial time or at the beginning of experiment. Repeated addition is the application of organic substrates per week, fortnightly, monthly throughout the experimental duration, the same amount of substrate is applied to each treatment over study period.

Organic material net mineralization-immobilization patterns in soil are affected by environmental factors (Saccone *et al*., 2013) the nature and abundance of the microbes/decomposers (Kristiansen *et al*., 2004) and chemical composition of organic substrates (Thomas and asakawa, 1993). Microorganisms satisfy their nutrient demand with low C:N ratio organic amendment, because microbes have low C:N ratio (< 20) and result the early net mineralization. In contrast, organic-C input with high C:N ratio temporarily decrease net mineralization of microbial biomass (Moritsuka *et al*., 2004). Decomposition rate of organic material/substrates is affected by nature of organic C. Glucose-c or water-soluble organic C is rapidly decomposed, because it is mostly in the form of simple compounds and readily accessible to soil microbes. Degradation and decomposition of complex organic carbon requires the production of extracellular enzymes that can only be prepared by subset of microbes (Nannipieri *et al*., 2012).

Soil microbes influence the availability of nutrients, when organic substrate is added to soil (Gichangi *et al*., 2009

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and Khan 2009). Higher the microbial biomass more macronutrients (N & P) are taken up by microbes, when, organic-C availability is high. In these conditions' microbes compete with plant for nutrients. Simultaneously P fixation in clay colloids or leaching is limited due P uptake by microbes (Gichangi *et al*., 2009 & Khan 2009). On, the contrary soil microbial biomass nitrogen (SMBN) and P become readily available to plant as microbial biomass turns over, when easily available carbon depletes (Gichangi*et al*., 2009; Malik *et al*., 2012). So, the microbes can be deliberated as slow release fertilizers that store nutrients particularly N and P, when concentration of these nutrients is high and then release them (Ryazanova *et al*., 2009; Docampo *et al*., 2010). Therefore, for better understanding of nutrient management, it is essential to study nutrient dynamics in response to organic amendment or organic-C input and relationship between nutrient uptake by plants and soil microbial biomass. Keeping in view, this present study was designed to evaluate the effect of single and repeated additions of glucose-C on soil microbial biomass carbon (SMBC) and CO_2 response in black soil of China.

Materials and Methods

Soils

Soil properties such as soil organic carbon (SOC), total N, total P and total K of top 20 cm layer were 18.6 g kg^{-1}, 2.1 g kg^{-1}, 1.46 g kg^{-1} and 25.3 g kg^{-1}, respectively. Alkali hydrolysable N, Olsen-P and 1 M, ammonium acetate was 112, 29 and 186 µg g^{-1}. Soil clay content was 28.8% (< 2 µm) with pH of 7.5. Soil used in this incubation study was collected from top 20 cm from Jilin province of China. Before using the soil samples for incubation study, the sample was air dried, homogenized and sieved through 2 mm sieve. The stones, roots and other materials were removed from soil. The black soil having characteristics clay content (<2 µm) 28.8%, pH ranged from 6.8 to 7.5, water holding capacity (WHC) 59.2 to 61.8% and maintained at 60% throughout the experiment. Soil organic carbon varied from 16.25 to 31.32 g kg^{-1} and total nitrogen varied from 1.46 to 2.82 g kg^{-1} (Table 1).

Experimental design

A pot study in incubators was conducted by using CRD-factorial design with 5 repeats. Factor (A), included glucose addition patterns like control, single and repeated additions. Single addition received all amount of glucose-C at the start of experiment just after one week of incubation, repeated addition received same amount of glucose (as in single addition) in five splits and all subsequent weekly addition was with water only while control received water every week. Factor (B), consisting of soil fertility levels: low, medium and high fertility, was selected to observe response of glucose addition. The sterilized soil was used in control treatment to check microbial contamination.

Forty-gram (40 g) air dried, 2 mm sieved, homogenized soil was filled into 250 mL Schott bottle. The soil water holding capacity was maintained 60% with distilled water. The soil filled Schott bottles used in incubation were pre-incubated in dark at 20 ^{0}C for a week. Then 1 mL of glucose-C (2%) solution or 1 mL water was added to soil

Table 1: Soil properties of different nutrient levels of black soil (Means ± SE, n= 3)

Soil	WHC (%)	pH	SOC (g kg^{-1})	Total N (g kg^{-1})
Low fertile soil	59.2 ± 0.029	7.29 ± 0.012	16.2 ± 0.015	1.468 ± 0.028
Medium fertile soil	58.5 ± 0.053	7.31 ± 0.016	26.5 ± 0.007	2.316 ± 0.031
High fertile soil	61.8 ± 0.042	6.85 ± 0.37	31.3 ± 0.018	2.825 ± 0.025

Table 2: F-values from analysis of variance of parameters as influenced by different glucose additions in black soil

Parameter	Fertility Status	Addition pattern (A)	Time in weeks (T)	Addition pattern × Time
Microbial Biomass Carbon (mg/g C)	Low fertility soil	29.59**	34.11**	2.34**
	Medium fertility soil	15.7**	97.65**	3.66**
	High fertility soil	77.16**	34.85**	10.66**
CO_2 evolved rate (mg/g C)	Low fertility soil	566.7**	292.3**	173.5**
	Medium fertility soil	465.56**	213.86**	166.24**
	High fertility soil	519.9**	270.4**	271.5**
CO_2 accumulation rate (mg/g C)	Low fertility soil	18241.6**	4618.1**	273.3**
	Medium fertility soil	11966.2**	4107.4**	271.3**
	High fertility soil	14714.2**	4413.9**	399.0**

Note: * = significant, ** = highly significant and *** = very highly significant at $p< 0.05$ (High fertility soil showed more CO_2 than added glucose-C because high fertility soil already contained soil organic-C).

drop wise by using the pipette to obtain equal distribution in each Schott bottle. Five milliliters of 1*M* sodium hydroxide was kept in bottles to capture CO_2 and exchanged of every week. The CO_2 captured samples were analysed every week. One gram of calcium chloride was placed in incubation bottle to absorb water vapor and avoid soil moisture from subsequent water additions.

Analysis of soil microbial biomass carbon

After incubation, the chloroform fumigation/extraction method modified by Vance *et al*., (1987) was used for quantification of SMBC. About 12.5 g of fresh soil was fumigated with ethanol chloroform for 24 hours, additional 12.5 g soil was kept un-fumigated for 24 hours and then extracted with 50 mL of 0.5 mol/L K_2SO_4, the total concentration in K_2SO_4 extracted in fumigated – non-fumigated soils. Soil microbial biomass carbon was analyzed after every three weeks throughout the experiment, week 3, 6, 9, 12 and 15, respectively. The SMBC were determined by Analytic jena Multi N/C 3100 (TOC/TN).

Analysis of CO_2

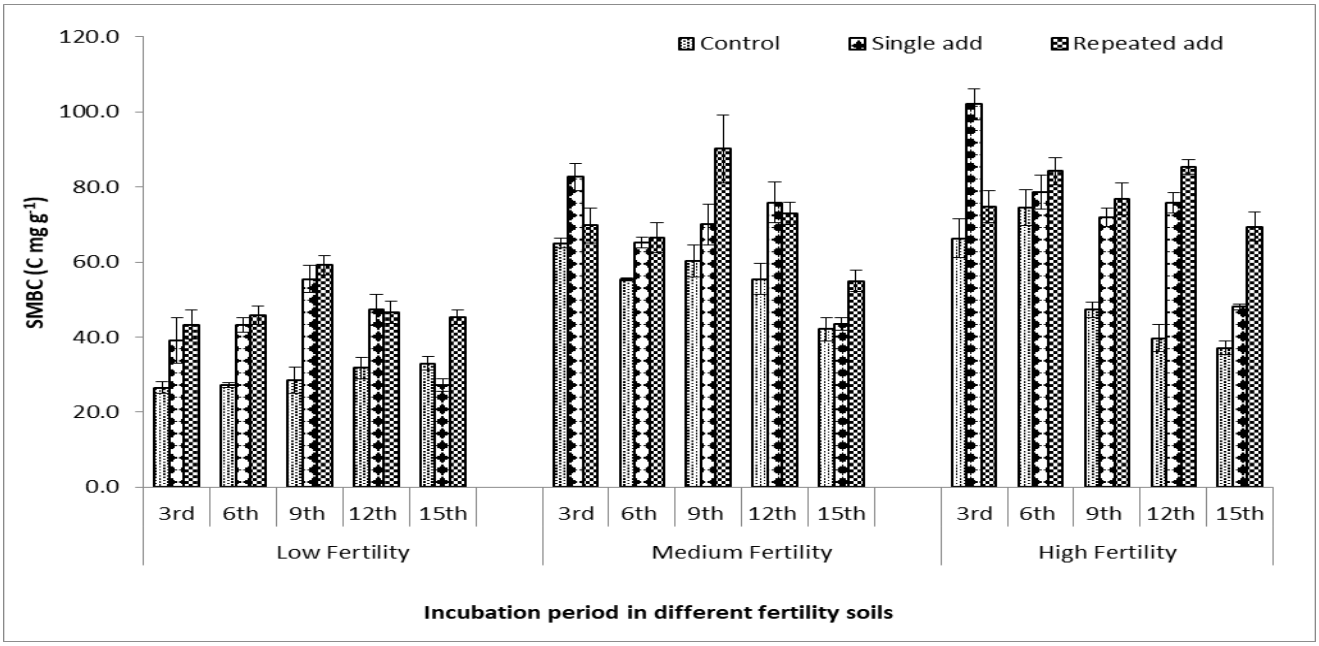
Carbon dioxide (CO_2) was measured as precipitate carbonate absorbed in NaOH. The NaOH was kept in bottles to trap CO_2 and replaced every week. Carbon dioxide-C of samples was captured in incubation bottle and quantified for CO_2, after every week. The CO_2 concentrations were analyzed by Analytic jena Multi N/C 3100 while change its mode on IC, CO_2 evolved rate (mg/g C) were calculated per week.

Statistical analysis

Every treatment contained five replicates. The data of SMBC and CO_2 evolved and CO_2 accumulation at the end of the experiment was analyzed by three-way ANOVA (analysis of variance) with glucose- C addition patterns (control, single and repeated additions), fertility levels (low, medium and high) and time (weeks) as (glucose additions × fertility levels × weeks). Tukey HSD test was applied to assess significant difference among the treatments by using software Statistix® Version 8.1. The data were plotted using non-metric multi-dimensional scaling (MDS) plot. Significant differences in soil microbial biomass carbon, CO_2 evolved and CO_2 accumulation among the treatments were determined at probability HSD (≤ 0.05).

Results

Soil microbial biomass carbon (SMBC), as CO_2 evolved, and CO_2 accumulation showed highly significant F values ($p = 0.000$) from the ANOVA (analysis of variance) for the soils, glucose addition patterns and time in weeks, respectively (Table-2). The interaction between soils × addition patterns, soils × time, addition pattern × time and soils × addition pattern × time in table – 2, were also varied with highly significant F-values ($p = 0.000$).



Tukey HSD ≤ 0.05: Low fertility (Glucose add: 3.99, Weeks:6.05, Glucose add x weeks: 13.22): Medium fertility (Glucose add: 6.09, Weeks:9.20, Glucose add x weeks: 20.12): High fertility (Glucose add:5.38, Weeks:8.15, Glucose add x weeks: 17.82).

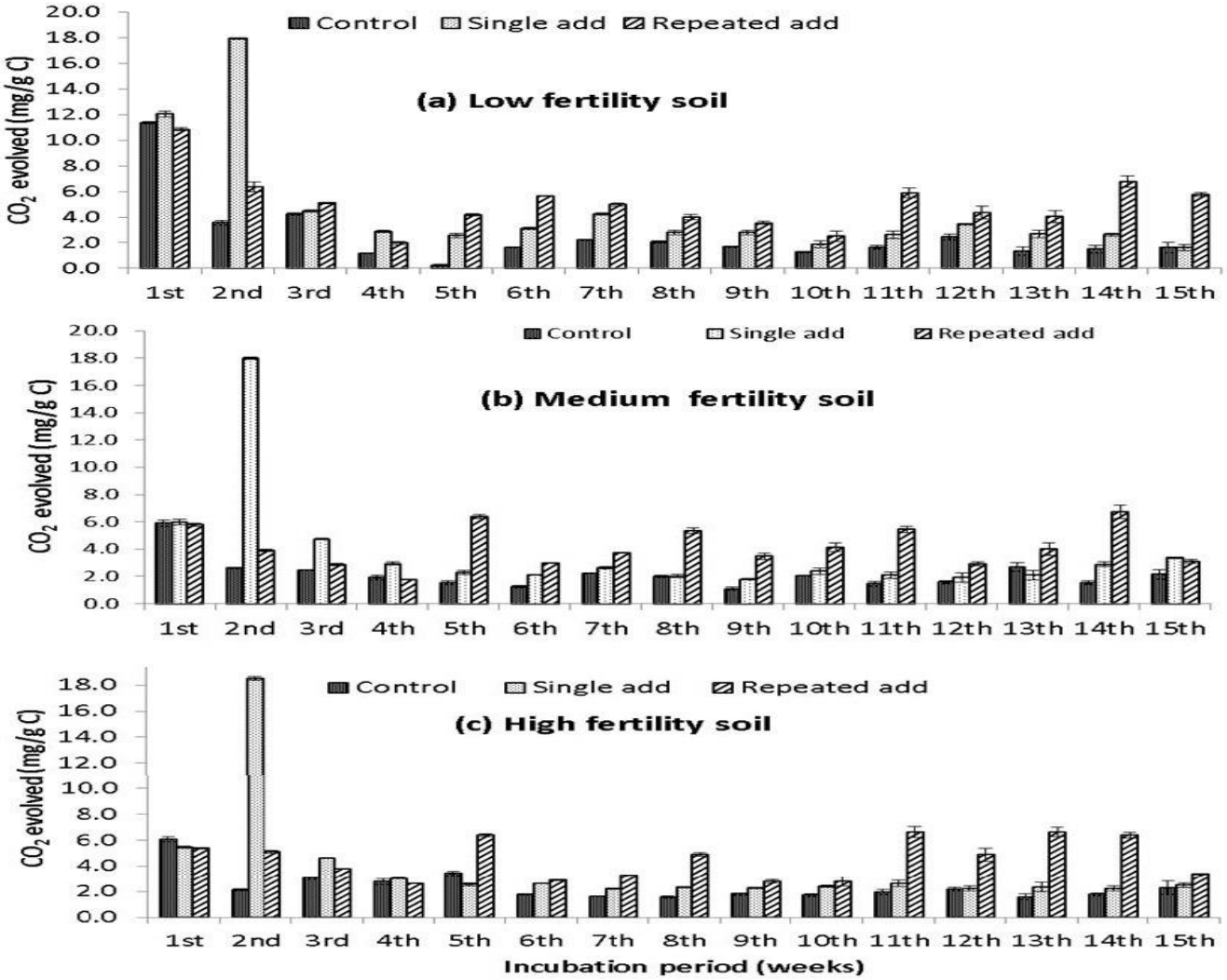
Figure 1: Soil microbial biomass carbon with different glucose-C additions in amended black soils

Change in soil microbial biomass carbon (SMBC) by glucose addition patterns

The glucose-C additions (single & repeated additions) showed variable impact on SMBC. Single addition in 3rd week showed the highest SMBC (102.5 mg g^{-1}) in high fertility soil, followed by repeated addition in 9th week (90.1 mg g^{-1}) in medium fertility soil (Figure 1). Repeated addition showed the highest (45.69 mg g^{-1}) SMBC during the 3rd week of incubation period. Over all mean (low, medium and high fertility soils) of repeated addition depicted 32% and 0.92% higher values of SMBC than control and single additions, respectively (Figure 3a). The incubation period of study illustrated variable impact on SMBC. Maximum SMBC (53. 94 mg g^{-1}) was observed during the 3rd week of incubation period. The interactive effect of glucose-C additions × weeks depicted 1179% higher SMBC by repeated addition in 3rd week than control during 5th week of incubation period.

Change in soil carbon dioxide (CO_2) emission by glucose addition patterns

The single glucose-C addition released the maximum CO_2 (20.9 mg g^{-1} C soil $week^{-1}$) in low fertility soil during 2nd week (Figure 2). The average CO_2 emission of single addition in all amended soils was 642% and 280%



Tukey HSD ≤ 0.05: Low fertility (Glucose add: 0.183, Weeks:0.594, Glucose add x weeks: 1.194): Medium fertility (Glucose add: 0.16, Weeks:0.53, Glucose add x weeks: 1.068): High fertility (Glucose add:0.147, Weeks:0.476, Glucose add x weeks: 0.958).

Figure 2: Total CO_2 release from black soil with different glucose-C additions at various interval periods

higher than control and repeated addition during 2nd week (Figure 2).

The repeated addition released higher CO_2 than single addition with consistency during the rest of all weeks. Therefore, mean wise repeated addition released more CO_2 as compared to single addition and control (Figure 3b). The interactive effects amended soils and glucose-C additions showed variable response to CO_2released. The repeated addition ×low fertility soil showed 133% more CO_2 emission than medium fertility soil × control. The interaction of amended soils × time illustrated low fertility soil× 1st week of time (mean wise) released 506% more CO_2 than medium fertility soil × 10th week of study period. The single × 2nd week of time emitted 1036%, the highest CO_2 over to control × 10th week of study period (Figure 2).

Similar trend of CO_2 release was observed by glucose-C additions (single and repeated) as observed in above mentioned CO_2 release, when difference between additions and control values were calculated in amended soils. In repeated addition CO_2 emission gradually increased with increase of time (Figure2 & 3b).

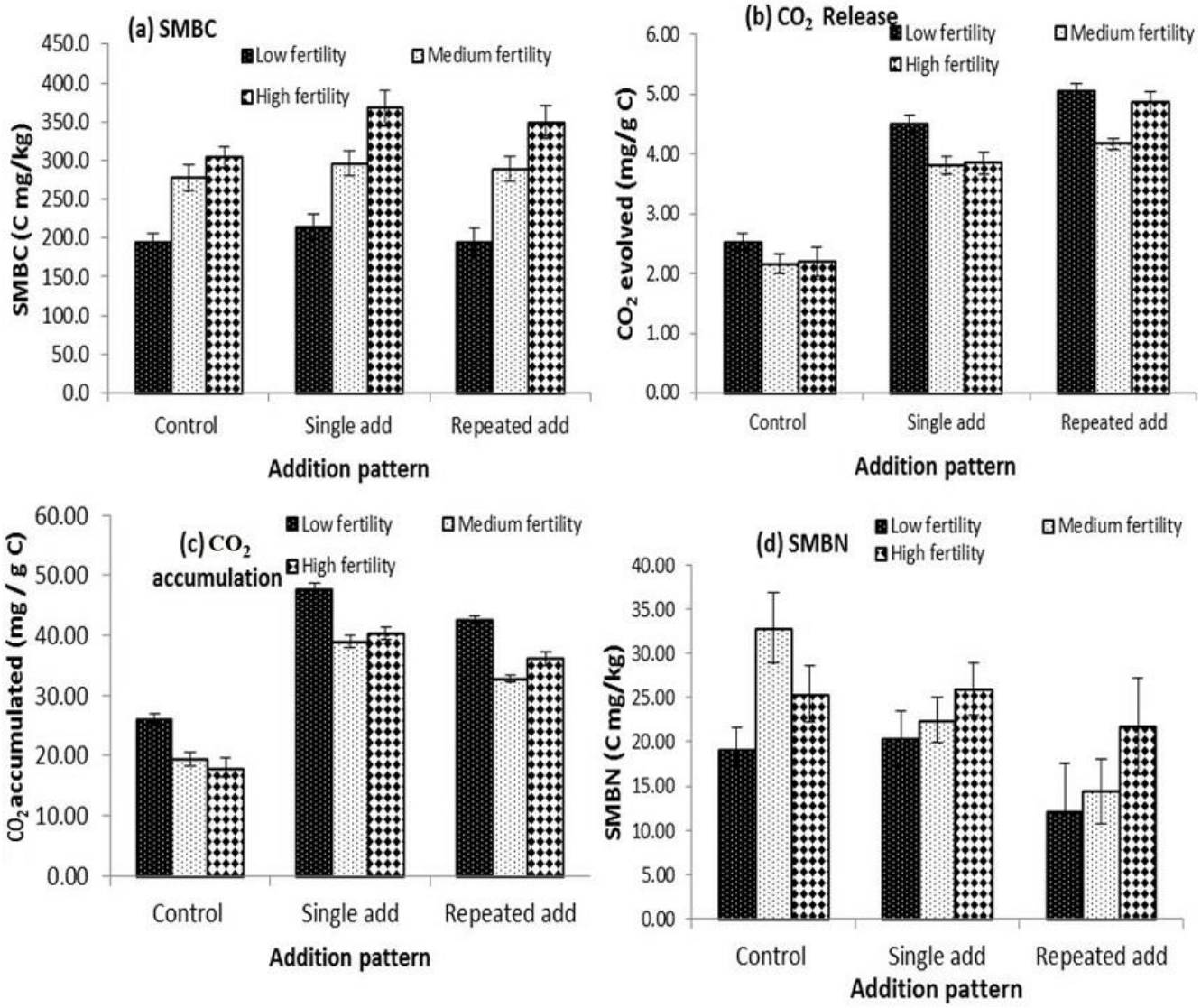
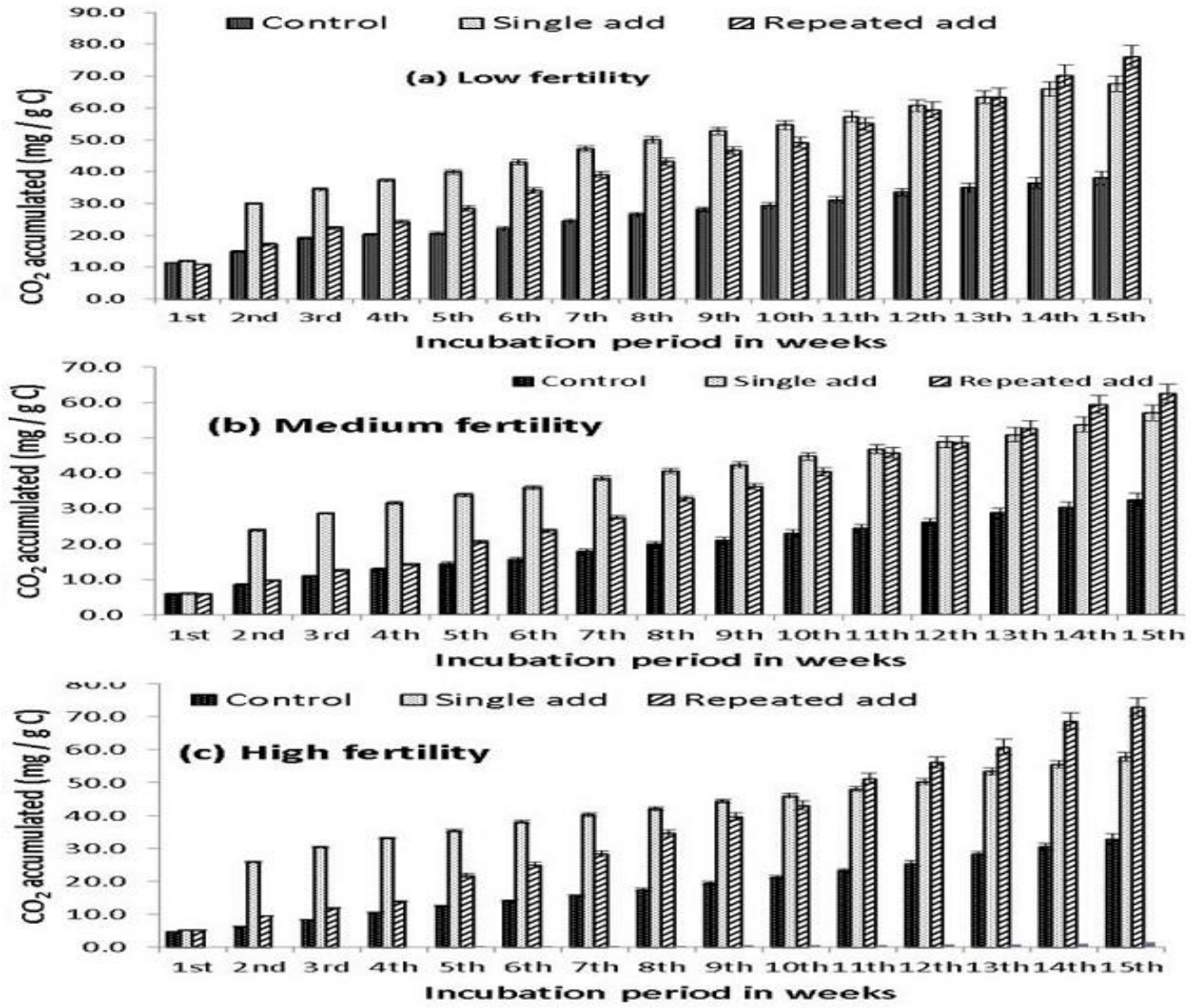


Figure 3: Mean values of parameters affected by different glucose-C additions in black soils during whole incubation period

Change in soil total carbon dioxide (CO_2) accumulation by glucose addition patterns

The application of glucose-C as repeated addition illustrated the highest accumulation of CO_2 (75. 9 mg g^{-1} C soil), followed by single addition (67.6 mg g^{-1} C soil) and control (38.0 mg g^{-1} C soil), during the 15th week of incubation (Figure 4). The mean CO_2 accumulation of all weeks illustrated the higher CO_2 by single addition than repeated addition and control in all soils (Figure 3c).

The low fertility soil of single addition demonstrated CO_2 accumulation of 22.4 and 17.6% higher than medium and high fertility soils respectively (Figure 3c). The highest average CO_2 accumulation values were observed in 15th and 14th weeks of study period, which were 628% and 589% over the 1st week (Figure 4). The positive interaction between amended soils and glucose-C additions was observed. The single addition ×low fertility soil showed 163% more CO_2 accumulation than Control × high fertility. (Figure 3c). The interaction of repeated addition × 15th week



Tukey HSD ≤ 0.05: Low fertility (Glucose add: 0.411, Weeks:1.32, Glucose add x weeks: 2.67): Medium fertility (Glucose add: 0.301, Weeks:0.975, Glucose add x weeks: 1.96): High fertility (Glucose add:0.324, Weeks:1.05, Glucose add x weeks: 2.11).

Figure 4: Total CO_2accumulation of black soils with different glucose-C additions at various interval periods

showed 828% higher CO_2 accumulation than repeated addition $\times 1^{st}$ week (Figure 4). The difference among single, repeated and control showed similar trend of CO_2 accumulated as observed in above para of CO_2 accumulation. The single addition consistently showed higher values of CO_2 accumulation in all soils upto 10^{th} week of incubation period. After 11^{th} and 12^{th} weeks repeated addition, accumulated more CO_2 than single addition. In repeated addition CO_2 accumulation gradually increased with increase of time (Figure 4).

Discussion

The study was carried out to affirm the hypothesis that single addition may produce more microbial biomass carbon and CO_2 in short period than repeated additions, because initially it contains large amount of substrate, while repeated addition will produce in larger period ranging from weeks to months. The findings of the study are discussed as under:

The glucose-C additions (single & repeated additions) showed variable impact on SMBC. The observations of SMBC were recorded on 3^{rd}, 6^{th}, 9^{th}, 12^{th} and 15^{th} week of study, respectively. Single addition on an average basis produced more microbial biomass carbon than repeated additions during the 3^{rd} week of study period as expected according to hypothesis (Figure 1). The important driving force for microbial processes is soil carbon, especially for soil respiration and mineralization processes. Soil mineralization and respiration may be carried because of microbes which change quantity and availability of soil carbon (Jenkinson *et al*. 1985; Kuzyakov *et al*. 2000). Even though earlier findings have been illustrated that SMBC (soil microbial biomass carbon) can be triggered by little amounts of glucose-C (Nobili *et al*., 2001; Mondini *et al*., 2006). The single addition contributed more SMBC in initial incubation phase (3^{rd} week) due to availability of high substrate content; it decreased immediately in 6^{th} week due to high C demand by microbes or it may be the substrate pressure on soil biota. The continuous pressure on soil biology may check microbial biomass from achieving full recovery (Allison *et al*. 2008). The study further indicated that mineralization of glucose as single addition decreased with time. In contrast, repeated addition enhanced the glucose mineralization with time and ultimately contributed to microbial biomass. Similar findings were reported by Hamer and Marschner (2005), reported that repeated substrate increased mineralization with time.

In our study response of glucose as single and repeated additions with different fertility levels were used to maintain optimal microbial activity. The highest (90%) SMBC was observed in bottles that contained the high fertility soil over the low fertility soil, similar findings were reported by (Malik *et al.*, 2013), who reported that organic amendments stimulated microbial community, mineral nutrition of plants. Soil microbes enhanced plant nutrition by encouraging availability of nutrients through microbial biomass.

Change in CO_2 release is depending on amount of substrate addition (glucose or FYM). This study, single and repeated glucose-C additions were used to investigate the CO_2 released from soil organic matter and microbial biomass. Single glucose C addition showed the highest CO_2 over the repeated addition during 2^{nd} week of incubation (Figure 2). Single addition included the maximum glucose – C addition at a time or in one application which reflected in the highest CO_2 release. The results are in agreement with the findings of Conde *et al*. (2005) and Hamer and Marschner (2005) were reported that the decomposition of glucose –C (easily available C source) showed greater effects than the addition of manure (low available substrates) to soil. The quantity of more CO_2 emission gave key clues regarding the sources (Nottingham *et al*., 2009; Blagodatskaya *et al*., 2010). The highest CO_2 concentration was observed in 2^{nd} week of study period by single addition and it decreased subsequently in the rest of all weeks till the 15^{th} week of study. In contrast, repeated addition initially released very less amount of CO_2 while it enhanced in later weeks of study period. Considering the cumulative CO_2 release for 116 days of incubation study, 12.9% and 88.1% higher CO_2 was released by single addition than repeated addition and control, respectively, in low fertility soil. (Figure 4). Similar trend in total cumulative CO_2 release of whole incubation study illustrated as CO_2 released in individual weeks. Glucose addition significantly enhanced the cumulative CO_2 in comparison to control. Hammer and Marschner (2005) reported the triggering effects after substrate addition occurring during the intensive substrate decomposition. Single addition showed higher accumulation of CO_2 than repeated addition. This would be because of small amount of glucose-C addition could not be enough to activate microbes and this could be resulted by depletion of microbial carbon or energy deficit, that leads activity of microbes and their nutrient demand (Cheng and Kuzyakov, 2005).

Conclusion

The findings of our study showed that scope of glucose additions on low, medium and high fertility soils. Single glucose-C addition in combination with different soil fertility levels augmented the microbial biomass and triggered C mineralization for shorter period (up to 3 weeks). The repeated addition of glucose in combination with different soil fertility levels also enhanced soil microbial biomass, CO_2 release and C mineralization in longer incubation period. So, it can be suggested that

repeated addition of glucose may be studied for longer incubation period and in more splits for better response of microorganisms and carbon mineralization. Moreover, our results suggest that activation of microbes with glucose addition in different soil fertility levels, addition patterns (single & repeated) and localization of microorganisms to added glucose are key factors that control carbon mineralization.

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